frontiers | Research Topics

Combating cancer with natural products: Non-coding RNA and RNA modification

Edited by
Yongye Huang, Yun Dai, Peng Qu and Yue Hou

Published in
Frontiers in Pharmacology
Frontiers in Oncology

FRONTIERS EBOOK COPYRIGHT STATEMENT

The copyright in the text of individual articles in this ebook is the property of their respective authors or their respective institutions or funders. The copyright in graphics and images within each article may be subject to copyright of other parties. In both cases this is subject to a license granted to Frontiers.

The compilation of articles constituting this ebook is the property of Frontiers.

Each article within this ebook, and the ebook itself, are published under the most recent version of the Creative Commons CC-BY licence. The version current at the date of publication of this ebook is CC-BY 4.0. If the CC-BY licence is updated, the licence granted by Frontiers is automatically updated to the new version.

When exercising any right under the CC-BY licence, Frontiers must be attributed as the original publisher of the article or ebook, as applicable.

Authors have the responsibility of ensuring that any graphics or other materials which are the property of others may be included in the CC-BY licence, but this should be checked before relying on the CC-BY licence to reproduce those materials. Any copyright notices relating to those materials must be complied with.

Copyright and source acknowledgement notices may not be removed and must be displayed in any copy, derivative work or partial copy which includes the elements in question.

All copyright, and all rights therein, are protected by national and international copyright laws. The above represents a summary only. For further information please read Frontiers' Conditions for Website Use and Copyright Statement, and the applicable CC-BY licence.

ISSN 1664-8714
ISBN 978-2-83251-750-5
DOI 10.3389/978-2-83251-750-5

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Combating cancer with natural products: Non-coding RNA and RNA modification

Topic editors
Yongye Huang — Northeastern University, China
Yun Dai — First Affiliated Hospital of Jilin University, China
Peng Qu — National Institutes of Health (NIH), United States
Yue Hou — Northeastern University, China

Citation
Huang, Y., Dai, Y., Qu, P., Hou, Y., eds. (2023). *Combating cancer with natural products: Non-coding RNA and RNA modification*. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-83251-750-5

Table of contents

05 Editorial: Combating cancer with natural products: Non-coding RNA and RNA modification
Yongye Huang, Yue Hou, Peng Qu and Yun Dai

09 Riddle of the Sphinx: Emerging Role of Transfer RNAs in Human Cancer
Zhilin Qiu, Qin Wang, Lei Liu, Guozheng Li, Yi Hao, Shipeng Ning, Lei Zhang, Xin Zhang, Yihai Chen, Jiale Wu, Xinheng Wang, Shuai Yang, Yaoxin Lin and Shouping Xu

27 AhR Antagonist Promotes Differentiation of Papillary Thyroid Cancer *via* Regulating circSH2B3/miR-4640-5P/IGF2BP2 Axis
Ri Sa, Meiliang Guo, Danyan Liu and Feng Guan

42 The Role and Application of Exosomes in Gastric and Colorectal Cancer
Qirong Li, Dongxu Wang, Dayong Ding, Ye Feng, Ruizhi Hou, Dianfeng Liu, Chao Lin and Yongjian Gao

48 Ailanthone Inhibits Proliferation, Migration and Invasion of Osteosarcoma Cells by Downregulating the Serine Biosynthetic Pathway
Yawen Zhang, Runze Gong, Yong Liu, Xipeng Sun, Jinrong Liang, Yan Zhou, Yaling Wang, Wenxi Yu, Yonggang Wang, Lina Tang, Aina He, Zan Shen, Yang Yao, Haiyan Hu, Xin Liu and Jianjun Zhang

61 Research Progress of RNA Methylation Modification in Colorectal Cancer
Weizheng Liang, Hongyang Yi, Chenyu Mao, Qingxue Meng, Xueliang Wu, Shanliang Li and Jun Xue

72 Puerarin: A Potential Therapeutic for Colon Adenocarcinoma (COAD) Patients Suffering From SARS-CoV-2 Infection
Weizheng Liang, Xiushen Li, Yue Yao, Qingxue Meng, Xueliang Wu, Hao Wang and Jun Xue

83 The Interaction Between Epigenetic Changes, EMT, and Exosomes in Predicting Metastasis of Colorectal Cancers (CRC)
Meiqi Yang, Mingjun Sun and Huijing Zhang

95 The Role of m6A RNA Methylation in Cancer: Implication for Nature Products Anti-Cancer Research
Na Song, Kai Cui, Ke Zhang, Jie Yang, Jia Liu, Zhuang Miao, Feiyue Zhao, Hongjing Meng, Lu Chen, Chong Chen, Yushan Li, Minglong Shao, Jinghang Zhang and Haijun Wang

110 Solamargine Inhibits the Development of Hypopharyngeal Squamous Cell Carcinoma by Decreasing LncRNA *HOXA11*-As Expression
Ying Meng, Mengli Jin, Dai Yuan, Yicheng Zhao, Xiangri Kong, Xuerui Guo, Xingye Wang, Juan Hou, Bingmei Wang, Wu Song and Yong Tang

123 Non-coding RNAs in skin cancers:Biological roles and molecular mechanisms
Fei Liu and Shenglong Li

141 Dietary stilbenes as modulators of specific miRNAs in prostate cancer
Anait S. Levenson

154 *Salvia miltiorrhiza* in cancer: Potential role in regulating MicroRNAs and epigenetic enzymes
Meng Lu, Xintian Lan, Xi Wu, Xiaoxue Fang, Yegang Zhang, Haoming Luo, Wenyi Gao and Donglu Wu

174 Natural products exert anti-tumor effects by regulating exosomal ncRNA
Shuang Hu, Yi Liu, Shuguang Guan, Zhidong Qiu and Da Liu

189 Plant extracellular vesicles: A novel bioactive nanoparticle for tumor therapy
Zhao-Lin Tan, Jing-Fei Li, Hao-Ming Luo, Yang-Yang Liu and Ye Jin

199 Anti-cancer effect and potential microRNAs targets of ginsenosides against breast cancer
Meiling Fan, Mengyao Shan, Xintian Lan, Xiaoxue Fang, Dimeng Song, Haoming Luo and Donglu Wu

211 Resveratrol activates $CD8^+$ T cells through IL-18 bystander activation in lung adenocarcinoma
Wei Zhang, Ruohao Zhang, Zhiguang Chang and Xiaobo Wang

222 *SLC2As* as diagnostic markers and therapeutic targets in LUAD patients through bioinformatic analysis
Yanli Zhang, Han Qin, Jing Bian, Zhanchuan Ma and Huanfa Yi

240 Non-coding RNA-related antitumor mechanisms of marine-derived agents
Zhixia Zhou, Qianqian Cao, Yujing Diao, Yin Wang, Linhai Long, Shoushi Wang and Peifeng Li

262 Targeting lncRNAs of colorectal cancers with natural products
Woo Jung Sung and Jaewoo Hong

frontiers | Frontiers in Pharmacology

TYPE Editorial
PUBLISHED 10 February 2023
DOI 10.3389/fphar.2023.1149777

Check for updates

OPEN ACCESS

EDITED AND REVIEWED BY
Olivier Feron,
Université catholique de Louvain,
Belgium

*CORRESPONDENCE
Yongye Huang,
huangyongye88@163.com
Yue Hou,
houy20002000@163.com
Peng Qu,
peng.qu@nih.gov
Yun Dai,
daiyun@jlu.edu.cn

SPECIALTY SECTION
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

RECEIVED 23 January 2023
ACCEPTED 06 February 2023
PUBLISHED 10 February 2023

CITATION
Huang Y, Hou Y, Qu P and Dai Y (2023), Editorial: Combating cancer with natural products: Non-coding RNA and RNA modification.
Front. Pharmacol. 14:1149777.
doi: 10.3389/fphar.2023.1149777

COPYRIGHT
© 2023 Huang, Hou, Qu and Dai. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Editorial: Combating cancer with natural products: Non-coding RNA and RNA modification

Yongye Huang[1]*, Yue Hou[1]*, Peng Qu[2]* and Yun Dai[3]*

[1]Key Laboratory of Bioresource Research and Development of Liaoning Province, College of Life and Health Sciences, Northeastern University, Shenyang, China, [2]National Cancer Institute, Frederick, MD, United States, [3]Laboratory of Cancer Precision Medicine, The First Hospital of Jilin University, Changchun, China

KEYWORDS

natural products, cancer, non-coding RNAs, RNA methylation, circRNA

Editorial on the Research Topic
Combating cancer with natural products: Non-coding RNA and RNA modification

Cancer is a type of tumor with the ability of malignant proliferation, whereby cancer cells survive and multiply gradually out of the body's control. The hallmarks of cancer have been clearly and comprehensively summarized by the Hanahan study group. The hallmarks of cancer have been described as sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing/accessing vasculature, activating invasion and metastasis, reprogramming cellular metabolism, avoiding immune destruction, deregulating cellular metabolism and avoiding immune destruction (Hanahan, 2022). Meanwhile, Hanahan (2022) notes that unravelling phenotypic plasticity, non-mutational epigenetic reprogramming, polymorphic microbiomes and senescent cells are likely to be incorporated into the cancer conceptualization of hallmarks. On this basis, the researchers have further revealed that cancer epigenetics, genomic instability and mutations play an important role in the acquisition of cancer hallmarks by cells (Ravi et al., 2022; Zhang et al., 2022).

The emergence of the concept of cancer epigenetics has inspired a wide range of researchers to conduct more in-depth research into cancer mechanisms and cancer treatments. It is generally assumed that epigenetic abnormalities are caused by interactions between multiple protein complexes and their components, such as histone modifications, DNA methylation mechanisms, chromatin remodeling proteins and polycomb (PcG) proteins (Tsai and Baylin, 2011). Eighty per cent of human genome transcripts are non-coding RNAs (ncRNAs) that can regulate gene expression to modulate cancer progression, stemness, migration, and metastasis. According to the localization, length and function, ncRNAs can be classified into several types: rRNA, tRNA, snRNA, snoRNA, siRNA, piRNA, microRNA, lncRNA, and circRNA. Among them, microRNA, lncRNA, and circRNA play a critical role in tumorigenesis and cancer therapy. Alternative splicing defects are often found in cancer metastasis, invasion and the generation of drug resistance.

The crosstalk among lncRNA, microRNA, and circRNA has become a critical regulatory mechanism in tumorigenesis and cancer progression. Long non-coding RNAs are RNA molecules with transcriptional lengths longer than 200 nt and lacking

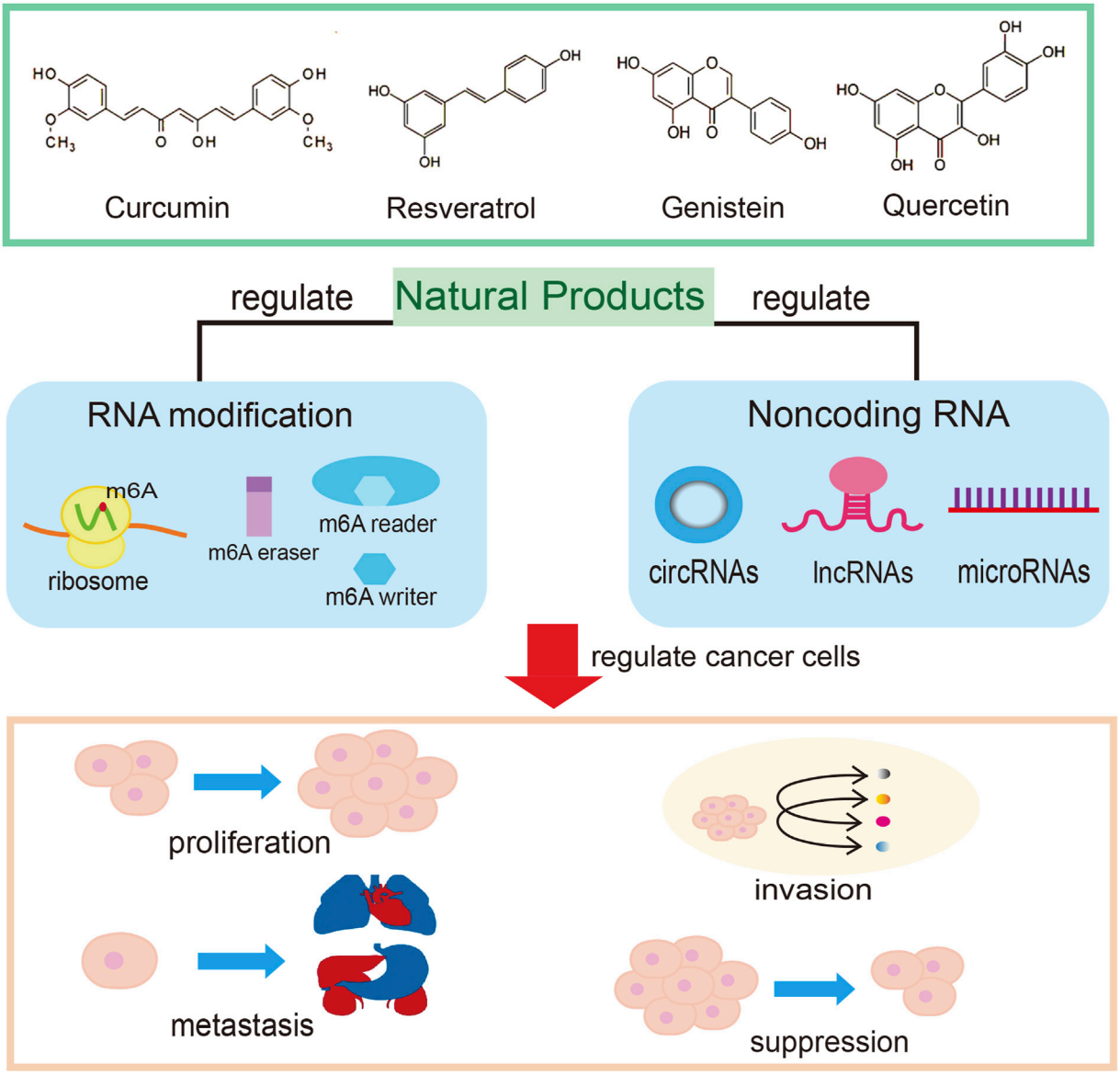


FIGURE 1
ncRNAs and RNA modifications underlying natural products-based cancer treatment.

protein-coding capabilities, which regulate gene expression by interacting with DNA, RNA and proteins (Xing et al., 2021). Epigenetic and non-coding RNA abnormalities can lead to uncontrolled gene expression, including epigenetic silencing of tumor suppressor microRNAs, epigenetic aberrations caused by microRNAs, epigenetic activation of oncogenic microRNAs and abnormalities in natural compounds that regulate microRNA expression by epigenetic mechanisms (Farooqi et al., 2019). CircRNA is an endogenous RNA with a covalent closed loop that plays an important part in the epigenetic regulation of transcriptional and post-transcriptional genes, and its dysregulation is associated with tumorigenesis and metastasis (Huang and Zhu, 2021). As research intensified, lncRNA/circRNA has been described as a sponge for microRNA in the competitive endogenous RNA (ceRNA) regulatory model, which proceeds to regulate the downstream target genes of microRNA (Han et al., 2020). In a ceRNA regulatory network research on circRNA-lncRNA-miRNA-mRNA of Acute myeloid leukemia, it was demonstrated that circRNA and lncRNA participate in the complex post-transcriptional regulation of the ceRNA network (Cheng et al., 2020). The lncRNAs, circRNAs, microRNAs and snRNAs among ncRNAs have been shown to affect cancer progression through the alternative splicing process, thereby regulating the alternative splicing process and generating alternatively spliced isoforms (Liu et al., 2021). Therefore, investigation into the transcriptional regulatory functions of non-coding RNAs is likely to contribute to finding appropriate strategy for cancer therapy.

Natural products have been used in cancer treatment for a long time and some recent mechanistic studies have attracted much attention (Xiang et al., 2019). The research on natural products to combat tumors is abundant, and their functions in regulating the tumor microenvironment and activating the body's immune response to kill cancer cells have been proven in various aspects. Natural medicines have vital regulatory functions at the genetic, epigenetic and signalling pathway transductions (Xiang et al., 2019; Huang et al., 2021; Zhang et al., 2022). The PI3K-Akt-mTOR pathway is one of the critical signaling pathways that may be targeted to inhibt cancer progression; afrocyclamin A, oridonin, salidroside, vitexin, arctigenin, cryptotanshinone, apigenin, and curcumin, are proposed to interfere with this pathway (Tewari et al., 2022). Natural products play a significant part in modulating the immune response against cancer. For example, saponins and flavonoids are two major groups of natural products that have shown excellent efficacy in reversing the tumor immunosuppressive microenvironment in conjunction with cancer immunotherapy (Tewari et al., 2022). Natural products have been shown to selectively trigger an effective host immune reaction against cancer cells, which strongly supports the strategy of turning "cold" tumors into "hot" ones to increase the efficiency of immune checkpoint inhibitor responses for cancer therapy (Atanasov et al., 2021). Natural products such as curcumin, resveratrol, apigenin, quercetin, berberine, genistein, epigallocatechin gallate, and parthenolide play a part in regulating epigenetic abnormalities (Huang et al., 2018; Xiang et al., 2019; Li et al., 2022; Zhang et al., 2022). Increasingly clear knowledge of the epigenetic mechanisms in cancer development has led to a search for more accurate and efficient anticancer agents.

Through continuous attempts to dismantle the threat of epigenetic abnormalities from environmental and dietary factors using natural products, many viable therapeutic options are available for cancer prevention and anti-cancer drug development (Figure 1). However, even though some natural products are currently undergoing clinical evaluation for cancer treatment, a large proportion of natural products are far from being used in a clinical setting. As previously mentioned, ncRNAs and RNA modifications are critically important in cancer development and therapy. Targeting these actors may actually contribute to the anti-tumor effects of natural products through inhibition of proliferation, invasion and metastasis (Homayoonfal et al., 2021). In addition, natural products such as paclitaxel, curcumin, resveratrol and genistein exert their antiproliferative and/or proapoptotic effects by modulating one or more miRNAs, thereby inhibiting cancer cell growth, inducing apoptosis and enhancing the effects of conventional cancer therapy (Xiang et al., 2019; Zhang et al., 2020). Therefore, the prospect of using the regulatory function of natural products on ncRNA to treat cancer is quite encouraging.

Numerous investigations have confirmed that RNA modification has emerged as a primary mechanism in controlling cell transcriptome and proteome during cancer development (Xiang et al., 2019; Zhang et al., 2022). More than one hundred RNA modifications, including mRNA cap modifications, N6-methyladenosine (m6A), and RNA editing. Among them, m6A modification is the most abundant RNA modification and exerts function in various biological processes in eukaryotes. Increasing evidence has confirmed that m6A modification could regulate the expression and function of ncRNAs, subsequently modulating the consequence of tumorigenesis and cancer therapy. The m6A modification is closely associated with cancer metastasis, stemness, drug resistance and microenvironment remodeling. Previously, investigators have suggested that m6A modification is likely to be one of the upstream regulatory mechanisms for lncRNAs differentially expressed in tumors. However, the clinical application of natural products that target RNA modifications for cancer treatment is rare, and research related to the regulation of m6A modifications by natural products is also infrequent. In a natural product screening study of m6A modulators, it was noted that using artificial intelligence-assisted technology combined with database analysis of traditional drugs and natural products holds promise for the development of more effective m6A modification-mediated therapeutic agents to inhibit tumor progression (Deng et al., 2022). The mechanism linking natural drugs and RNA modification should be thoroughly explored by taking full advantage of artificial intelligence, gene editing, RNA interference and other technologies in the future studies. Screening safe and efficient natural drugs as RNA modification modulators will bring new hope for cancer clinical treatment.

Author contributions

YYH drafted the manuscript; YH revised the draft; YH, PQ, and YD made substantial contributions to the work through in-depth discussion. All the authors proposed the Research Topic theme, made a direct and intellectual contribution to the work, and approved the final version for publication.

Funding

This study was funded by the National Natural Science Foundation of China (No. 81502582). Funding was also provided by the Fundamental Research Funds for the Central Universities (N182004002), Natural Science Foundation of Liaoning Province (2021-MS-104, 2022-YGJC-39), Fundamental Scientific Research Fund of Liaoning Provincial Education Department (LJKQZ2021002), and Key Laboratory of Bioresource Research and Development of Liaoning Province (2022JH13/10200026).

Acknowledgments

We would like to thank Wanlu Zhang in our research team for assistance.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., and Supuran, C. T. (2021). Natural products in drug discovery: Advances and opportunities. *Nat. Rev. Drug Discov.* 20 (3), 200–216. doi:10.1038/s41573-020-00114-z

Cheng, Y., Su, Y., Wang, S., Liu, Y., Jin, L., Wan, Q., et al. (2020). Identification of circRNA-lncRNA-miRNA-mRNA competitive endogenous RNA network as novel prognostic markers for acute myeloid leukemia. *Genes* 11 (8), 868. doi:10.3390/genes11080868

Deng, L.-J., Deng, W.-Q., Fan, S.-R., Chen, M.-F., Qi, M., Lyu, W.-Y., et al. (2022). m6A modification: recent advances, anticancer targeted drug discovery and beyond. *Mol. Cancer* 21 (1), 52–21. doi:10.1186/s12943-022-01510-2

Farooqi, A. A., Fuentes-Mattei, E., Fayyaz, S., Raj, P., Goblirsch, M., Poltronieri, P., et al. (2019) "Interplay between epigenetic abnormalities and deregulated expression of microRNAs in cancer," in *Seminars in cancer biology* (Amsterdam, Netherlands: Elsevier), 47–55.

Han, T.-S., Hur, K., Cho, H.-S., and Ban, H. S. (2020). Epigenetic associations between lncRNA/circRNA and miRNA in hepatocellular carcinoma. *Cancers* 12 (9), 2622. doi:10.3390/cancers12092622

Hanahan, D. (2022). Hallmarks of cancer: New dimensions. *Cancer Discov.* 12 (1), 31–46. doi:10.1158/2159-8290.CD-21-1059

Homayoonfal, M., Asemi, Z., and Yousefi, B. (2021). Targeting long non coding RNA by natural products: Implications for cancer therapy. *Crit. Rev. Food Sci. Nutr.* 2021, 1–29. doi:10.1080/10408398.2021.2001785

Huang, Y., Du, J., Mi, Y., Li, T., Gong, Y., Ouyang, H., et al. (2018). Long non-coding RNAs contribute to the inhibition of proliferation and EMT by pterostilbene in human breast cancer. *Front. Oncol.* 8, 629. doi:10.3389/fonc.2018.00629

Huang, Y., Yuan, K., Tang, M., Yue, J., Bao, L., Wu, S., et al. (2021). Melatonin inhibiting the survival of human gastric cancer cells under ER stress involving autophagy and Ras-Raf-MAPK signalling. *J. Cell Mol. Med.* 25 (3), 1480–1492. doi:10.1111/jcmm.16237

Huang, Y., and Zhu, Q. (2021). Mechanisms regulating abnormal circular RNA biogenesis in cancer. *Cancers* 13 (16), 4185. doi:10.3390/cancers13164185

Li, T., Zhang, X., Cheng, L., Li, C., Wu, Z., Luo, Y., et al. (2022). Modulation of lncRNA H19 enhances resveratrol-inhibited cancer cell proliferation and migration by regulating endoplasmic reticulum stress. *J. Cell Mol. Med.* 26 (8), 2205–2217. doi:10.1111/jcmm.17242

Liu, Y., Liu, X., Lin, C., Jia, X., Zhu, H., Song, J., et al. (2021). Noncoding RNAs regulate alternative splicing in Cancer. *J. Exp. Clin. Cancer Res.* 40 (1), 11–14. doi:10.1186/s13046-020-01798-2

Ravi, S., Alencar, A. M., Jr, Arakelyan, J., Xu, W., Stauber, R., Wang, C.-C. I., et al. (2022). Primary middle meningeal artery embolization for a chronic subdural hematoma after non-accidental trauma in a child: A case report. *Cureus* 14 (5), e26399. doi:10.7759/cureus.26399

Tewari, D., Patni, P., Bishayee, A., Sah, A. N., and Bishayee, A. (2022). "Natural products targeting the PI3K-Akt-mTOR signaling pathway in cancer: A novel therapeutic strategy," in *Seminars in cancer biology* (Amsterdam, Netherlands: Elsevier), 1–17.

Tsai, H.-C., and Baylin, S. B. (2011). Cancer epigenetics: Linking basic biology to clinical medicine. *Cell Res.* 21 (3), 502–517. doi:10.1038/cr.2011.24

Xiang, Y., Guo, Z., Zhu, P., Chen, J., and Huang, Y. (2019). Traditional Chinese medicine as a cancer treatment: Modern perspectives of ancient but advanced science. *Cancer Med.* 8 (5), 1958–1975. doi:10.1002/cam4.2108

Xing, C., Sun, S.-g., Yue, Z.-Q., and Bai, F. (2021). Role of lncRNA LUCAT1 in cancer. *Biomed. Pharmacother.* 134, 111158. doi:10.1016/j.biopha.2020.111158

Zhang, B., Tian, L., Xie, J., Chen, G., and Wang, F. (2020). Targeting miRNAs by natural products: A new way for cancer therapy. *Biomed. Pharmacother.* 130, 110546. doi:10.1016/j.biopha.2020.110546

Zhang, W., Li, S., Li, C., Li, T., and Huang, Y. (2022). Remodeling tumor microenvironment with natural products to overcome drug resistance. *Front. Immunol.* 13, 1051998. doi:10.3389/fimmu.2022.1051998

frontiers in Pharmacology

REVIEW
published: 15 December 2021
doi: 10.3389/fphar.2021.794986

Riddle of the Sphinx: Emerging Role of Transfer RNAs in Human Cancer

Zhilin Qiu[1†], Qin Wang[1†], Lei Liu[1†], Guozheng Li[1], Yi Hao[1], Shipeng Ning[1], Lei Zhang[1], Xin Zhang[1], Yihai Chen[1], Jiale Wu[1], Xinheng Wang[1], Shuai Yang[1], Yaoxin Lin[2] and Shouping Xu[1]**

[1]Department of Breast Surgery, Harbin Medical University Cancer Hospital, Harbin, China, [2]CAS Center for Excellence in Nanoscience, CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology, Beijing, China

The dysregulation of transfer RNA (tRNA) expression contributes to the diversity of proteomics, heterogeneity of cell populations, and instability of the genome, which may be related to human cancer susceptibility. However, the relationship between tRNA dysregulation and cancer susceptibility remains elusive because the landscape of cancer-associated tRNAs has not been portrayed yet. Furthermore, the molecular mechanisms of tRNAs involved in tumorigenesis and cancer progression have not been systematically understood. In this review, we detail current knowledge of cancer-related tRNAs and comprehensively summarize the basic characteristics and functions of these tRNAs, with a special focus on their role and involvement in human cancer. This review bridges the gap between tRNAs and cancer and broadens our understanding of their relationship, thus providing new insights and strategies to improve the potential clinical applications of tRNAs for cancer diagnosis and therapy.

Keywords: transfer RNA, human cancer, biomarker, tumorigenesis, therapy

OPEN ACCESS

Edited by:
Yue Hou,
Northeastern University, China

Reviewed by:
YiKun Qu,
Jiamusi University, China
Jie Yang,
Southern University of Science and Technology, China

Correspondence:
Yaoxin Lin
linyx@nanoctr.cn
Shouping Xu
shoupingxu@hrbmu.edu.cn

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

Received: *14 October 2021*
Accepted: *10 November 2021*
Published: *15 December 2021*

Citation:
Qiu Z, Wang Q, Liu L, Li G, Hao Y, Ning S, Zhang L, Zhang X, Chen Y, Wu J, Wang X, Yang S, Lin Y and Xu S (2021) Riddle of the Sphinx: Emerging Role of Transfer RNAs in Human Cancer. Front. Pharmacol. 12:794986. doi: 10.3389/fphar.2021.794986

INTRODUCTION

Cancer is one of the most complex diseases caused by multiple genetic disorders and cellular abnormalities. Its development and progression are regulated by multiple pathological processes (Bhawe and Roy, 2018), including environmental (Pan et al., 2018; Lewandowska et al., 2019), gender (Jara-Palomares et al., 2018), cultural (Tejeda et al., 2017; Lee, 2018), and lifestyle factors (Kerr et al., 2017; Jara-Palomares et al., 2018), as well as genetic mutations (Pak et al., 2015; Tejeda et al., 2017; Liew et al., 2019), epigenetic changes (Zhang and Huang, 2017; Porcellini et al., 2018; Liew et al., 2019), and abnormal signal transduction (Farooqi et al., 2018). Tremendous efforts have been made over the past decades with the aim of searching novel and more efficient tools in cancer therapy (Winn et al., 2016). However, cancer incidence and mortality remain high. Therefore, there is an urgent need to develop new strategies for early identification and more accurate diagnosis of cancer biomarkers for disease.

Recent studies on tRNAs have revealed the unexpected complexity of their structure and function (Schwartz et al., 2018). Besides participating in transcription and translation, tRNAs are also involved adaptive protein synthesis and can function as non-coding RNAs involving multiple

Abbreviations: KLF, Kruppel-like factor; nts, nucleotides; pre-tRNAs, precursor tRNAs; tRFs, tRNA-derived fragments; tRNAs, transfer RNAs; tsRNAs, tRNA-derived small RNAs; tiRNAs, tRNA-derived stress-induced RNAs; YBX-1, Y-box binding protein 1.

regulatory networks (Schimmel, 2018). Moreover, some small RNAs previously thought as miRNAs were actually tRNA-derived small RNA (tsRNAs). Compared with miRNAs, tRNAs, tsRNAs, and tRNA-derived fragments (tRFs) are more stable and richer in biological fluids in solid cancers and blood malignant tumors (Zhang et al., 2009; Li et al., 2012). High expression of plasma exosome tRNAs in patients with lung cancer (Balatti et al., 2017), chronic lymphocytic leukemia (Veneziano et al., 2019), and liver cancer (Zhu et al., 2019) indicate that plasma exosome tRNAs may be involved in cancer development. These findings provide evidence that tRNAs and their derivatives may be potential diagnostic and therapeutic molecular biomarkers of cancer (Schageman et al., 2013; Dhahbi et al., 2014). In addition, mutation of the tRNA itself as well as supplementary proteins produced and modified by tRNA physiologically, are associated with cancer (Suzuki et al., 2011; Yao and Fox, 2013; Blanco and Frye, 2014). However, the exact molecular mechanisms by which tRNAs and tsRNAs are involved in cancer are unclear.

Moreover, in some cancers, the dysregulation of tRNAs can trigger the progression and proliferation of cancer cells by regulating transcription, translation, ribosome biogenesis and functioning as novel epigenetic factors. Although tRNAs and tsRNAs have been receiving increasing research attention, to date, there is no comprehensive summary of these findings, which would greatly be beneficial to future studies exploring tRNAs.

Herein, we detailed the current literature on tRNAs related to cancer. We comprehensively summarize the basic characteristics and functions of tRNAs, focusing on their involvement in various human cancers, particularly breast cancer, lung cancer, and melanoma. This review closes the gap between tRNAs and cancers and deepens our understanding of them, thereby providing new insights and strategies to guide researchers in further exploring the potential clinical applications of tRNAs in cancer diagnosis and treatment.

STRUCTURE AND BIOGENESIS OF TRNAS AND THEIR DERIVATIVES

tRNAs are fundamental biological molecules that complete the flow of genetic information from DNA to protein by reading the cognate codons in the mRNA (Kirchner and Ignatova, 2015). Mature tRNAs in human cells are derived from precursor tRNAs (pre-tRNAs) containing 5′ leader and 3′ trailer sequences and introns in the anticodon loop (Phizicky and Hopper, 2010; Raina and Ibba, 2014). Mechanistically, pre-tRNAs are processed co- and post-transcriptionally to acquire their mature 5′ and 3′ ends, modified nucleosides, and the cloverleaf secondary structure, which contains four domains organized in unpaired and paired regions namely, acceptor arm, D arm, anticodon arm, and TΨC arm.

Upon maturation, tRNAs obtains the L-shaped tertiary structure (Kim et al., 1974; Stout et al., 1976; Moras et al., 1980) by means of base build-up and non-Watson-Crick base pairing between the receptor and TΨC arms, and with the D arm and the anti-coding arm (Madison et al., 1966) at the other end (Madison et al., 1966) (**Figure 1**). This structure allows tRNA to enter the ribosome and convert genetic information into polypeptides. During the translation process, mature tRNAs bind to adenosine covalently at the tail of the unchanging 3′CCA, acting as a connection and can be catalyzed by 20 different aminoacyl-tRNA synthases (Phizicky and Hopper, 2010). Generally, mature tRNAs are highly modified by tRNA-modifying enzymes, which play a vital role in obtaining 3D L-shaped structures and stability, translation start and extension factors, aminoacyl-tRNA synthases and ribosomes, and decoding efficiency and fidelity. Mature tRNAs add amino acids to the two-step reaction through aminoacyl-tRNA synthases, which are activated by ATP to form amino amp, and then add end adenosine to the tRNAs 3′ end (**Figure 1**). Then, the tRNAs charged with the amino acid interact with the enzyme machinery of the ribosome to decode mRNAs into proteins during translation (**Figure 1**). Meanwhile, tsRNAs are is a class of non-coding small RNAs produced by mature tRNAs or pre-tRNAs at different sites that are widely present in prokaryotic and eukaryotic transcriptomes, and produced by mature tRNAs or pre-tRNAs at different sites. tsRNAs refers to specific nucleic acid enzymes such as Dicer and angiogenin, especially cells or specific cleavage of tRNAs under certain conditions, such as stress and hypoxia (Levitz et al., 1990; Thompson and Parker, 2009). There are two main types of tsRNAs: tRFs (Keam and Hutvagner, 2015) and the tRNA-derived, stress-induced RNAs (tiRNAs) (Saikia and Hatzoglou, 2015; Shigematsu and Kirino, 2017). tRFs are 14–30 nucleotides (nts) long, and tRFs can be further divided into four categories, namely tRF-5s, tRF-1s, i-tRF, and tRF-3s, depending on their position on tRNAs. tRF-5s come from the 5′ end of mature tRNAs without D-loop and have three subclasses: 1) tRF-5a about 14–16 bases, a cutting site before the D ring; 2) tRF-5b, containing 22–24 bases and cleavage site behind the D ring; and 3) tRF-5c, about 28–30 bases long, has cleavage site before the anticodon ring (Kumar et al., 2014). tRF-3s are rooted in the 3′ end of mature tRNAs and include a CCA parting without the L-loop. They are divided into two subclasses according to size. tRF-3a usually has a cleavage site before the T-ring, while the tRF-3 cleavage site is in the T-ring, and the tRF-3a is usually made up of 18 bases, while the tRF-3 is 22 bases (Kumar et al., 2016). tRF-1s, the third category, is produced at the 3′ end of the pre-tRNAs, and their 5′ ends begin just after the 3′ end of the mature tRNA sequence. Finally i-tRF comes mainly from the middle region of mature tRNAs (Lee et al., 2009).

tiRNAs (31–40 nts in length) are formed by cleaving the tRNA anticodon loop affected by sex hormones and their receptors (Rashad et al., 2020), and have two types: tiRNA-5s and tiRNA-3s (Anderson and Ivanov, 2014; Saikia and Hatzoglou, 2015). Angiogenin, the nuclease responsible for tiRNA formation, generally in the nucleus of cells but under certain conditions can enter the cytoplasm, belonging to the RNase A superfamily (Fu et al., 2009; Li and Hu, 2012). In response to stress, angiogenin is isolated by RNH1 (an angiogenin inhibitor), enters the cytoplasm from the nucleus, and cleaves tRNAs into tiRNA-5s and tiRNA-3s in the cytoplasm (Li and Hu, 2012) (**Figure 1**).

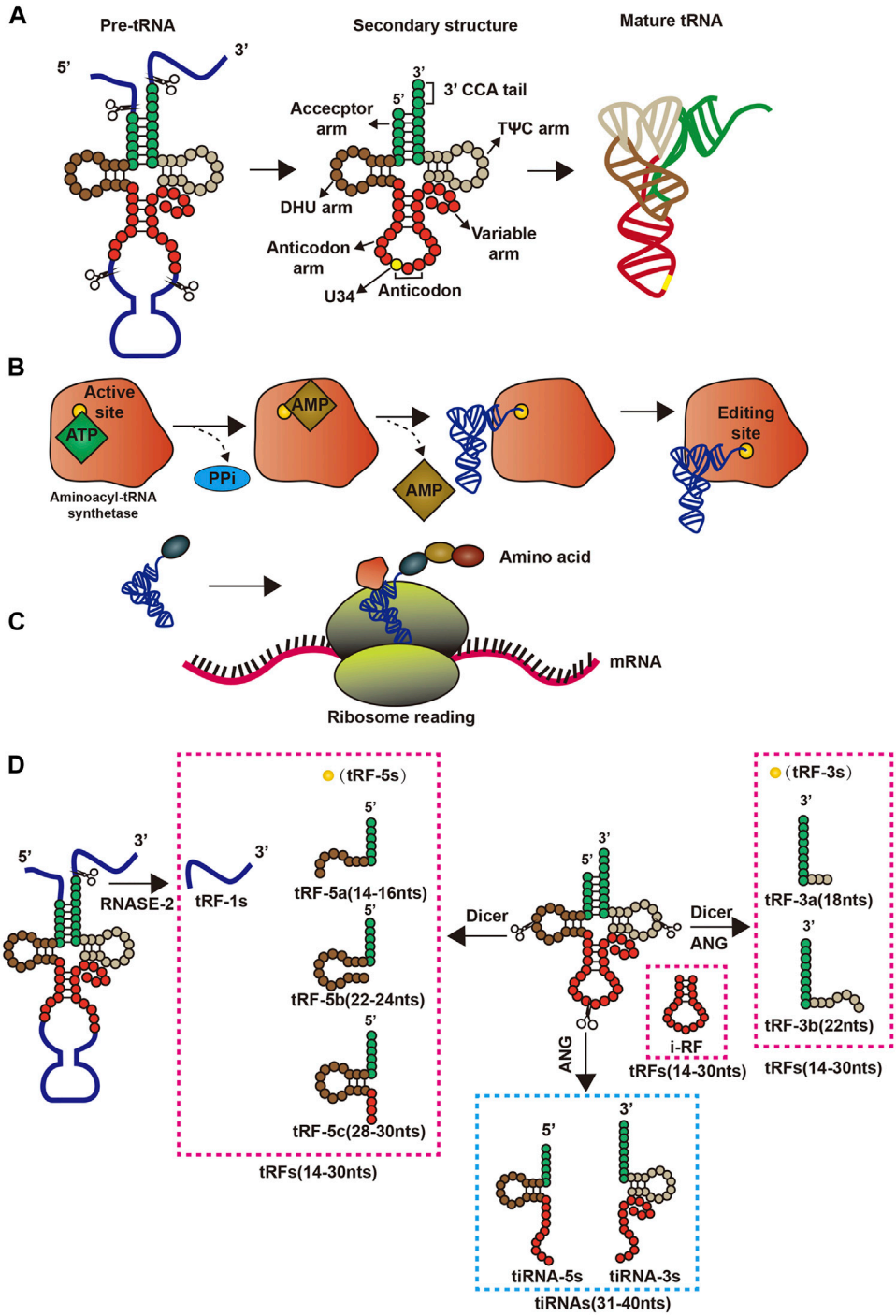


FIGURE 1 | Structure and pre-transcriptional changes in tRNAs. **(A)** Precursor tRNAs forms mature tRNAs; **(B)** Aminoacyl-tRNA synthetase binds amino acids to its corresponding tRNAs by consuming energy; **(C)** tRNAs and ribosomes are involved in the translation process; **(D)** The process by which precursor tRNAs and mature tRNAs form four tsRNAs.

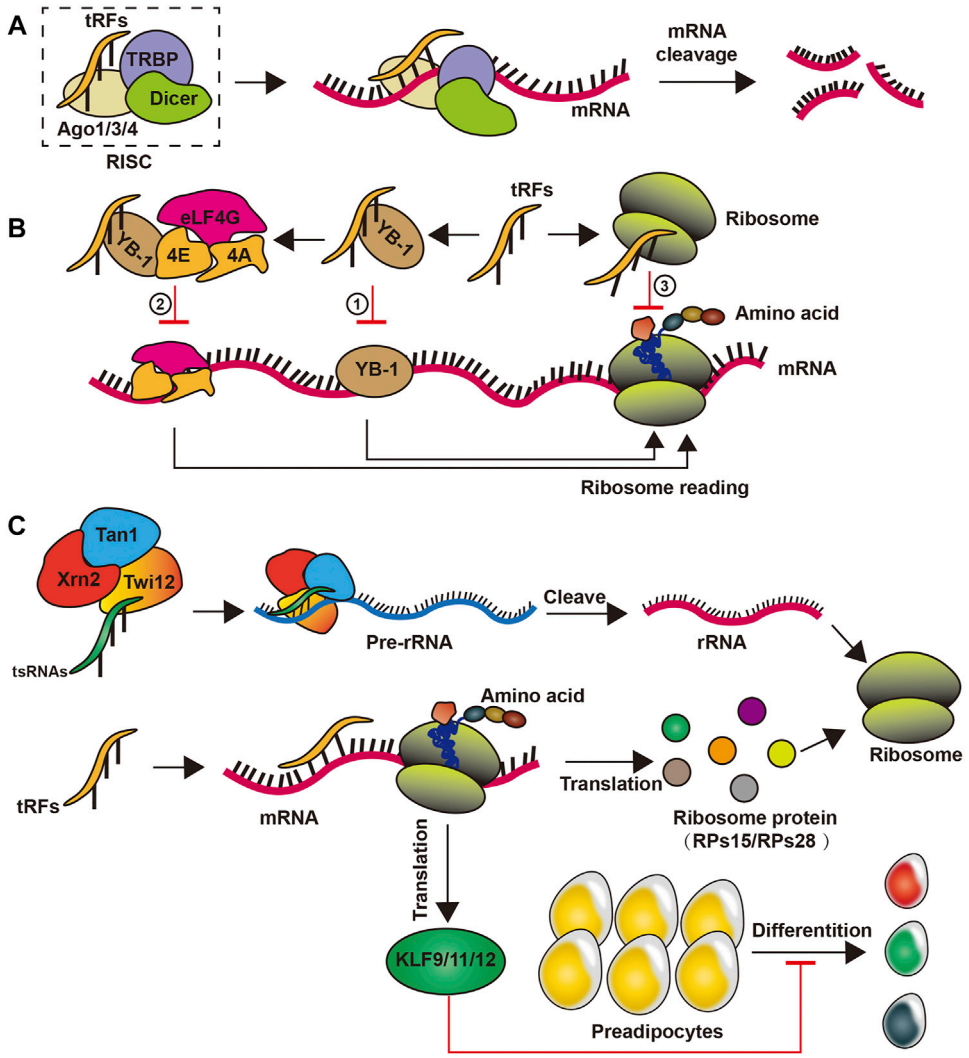


FIGURE 2 | Biogenesis of tsRNAs. **(A)** tsRNAs reduce mRNA stability by binding to a complex such as Ago1/3/4 proteins; **(B)** tsRNAs can inhibit the translation of mRNA by competitive binding of YB-1 or by self-binding of ribosomes; **(C)** tsRNAs promote translation by promoting the formation of rRNAs in ribosomes, while participating in ribosome protein formation to facilitate translation processes and inhibit preadipocytes differentiation.

BIOLOGICAL ROLES AND FUNCTIONS OF TRNAS AND THEIR DERIVATIVES

The functions of tsRNAs in different pathways, such as increasing mRNA stability (Saikia and Hatzoglou, 2015; Shigematsu and Kirino, 2017), repressing translation (Kumar et al., 2014; Kumar et al., 2016), regulating ribosome biogenesis (Lee et al., 2009), functioning as novel epigenetic factors (Rashad et al., 2020), promoting RNA reverse transcription (Anderson and Ivanov, 2014), functioning as immune signaling factors (Fu et al., 2009), have recently emerged.

Regulation of mRNA Stability

As miRNAs, tRFs can reduce mRNA stability by mediating target gene deacetylation, thereby promoting mRNA degradation and instability. tRFs prioritizes the inhibition of ribosome proteins and translational initiation or elongation factors of mRNA translation through antisense pairing in *Drosophila melanogaster* (Karaiskos et al., 2015; Babiarz et al., 2008; Eichhorn et al., 2014). In human cells, The GW182 protein inhibits translation and promotes the degradation of target mRNAs, and the tRF-3 target mRNA pairs in the RNA-induced silencing complex associate with GW182 proteins, which means that tRFs can affect the function of RNA-induced silencing complex by regulating the stability of mRNA (Kuscu et al., 2018; Ren et al., 2019). Furthermore, in mature B lymphocytes, tRF-3s derived from $tRNA^{Gly\text{-}GCC}$ (referred as CU1276) possess miRNA-like structure and function, thereby repressing mRNA transcripts by destabilizing mRNA, and can inhibit protein translation and the cleavage of a partially complementary target site, thereby

suppressing proliferation (Shao et al., 2017a). Although some tRFs have similar functions to miRNAs, the formers have been expressed preferentially bind argonaute1, argonaute3, and argonaute4 to promote RNA-induced silencing complex formation and reduce the stability of mRNA, thus inhibiting mRNA translation, rather than binding argonaute2 like miRNA (Kumar et al., 2014; Haussecker et al., 2010) (**Figure 2**). In addition, tRF-2s blocks the interplay of Y-box binding protein 1 (YBX-1) and YBX-1 mRNAs by competitively binding to YBX-1. This reduces the stability of these mRNAs, which could subsequently reduce the genetic stability of human breast cancer cell metastasis (Goodarzi et al., 2015) (**Figure 2**). Moreover, some tRF-3s chimeras have been related to histone mRNAs and can thus affect mRNA stability by competing with stem-ring binding proteins in human cells (Kumar et al., 2014) (**Figure 2**). Although there is an increased understanding of some of the functions of tRFs in regulating mRNA stability, the functions of tiRNAs remain elusive.

Downregulation of Translation

Some studies have shown that tiRNAs, mainly tiRNA-5s, could decrease translation speed by 10–15% (Yamasaki et al., 2009). For instance, tiRNA-5s from $tRNA^{Ala}$ and $tRNA^{Cys}$ can form a G-quadruplex-like structure that selectively binds to eIF4G/eIF4A in the translation of the starting complex, thereby inhibiting cap-dependent translation of cellular mRNAs rather than traditionally internal ribosome entry site mediated translation (Ivanov et al., 2011; Ivanov et al., 2014). tiRNAs can also selectively repress the housekeeping components' translation under stress conditions, thereby reducing cell energy consumption without affecting the generation of pro-survival proteins (Li and Hu, 2012). These studies suggest that tiRNAs are produced to regulate the translation process under stress conditions and are not intended to reduce the level of functional maturity tRNAs affecting mRNA function (Ivanov et al., 2011; Ivanov et al., 2014).

In addition, some researches have proved that tRFs plays a positive role in reducing protein translation behavior. For example, tRF-5s is supposedly involved in new mechanisms underlying the regulation of small RNA in human cell by repressing protein translation through conserved residues in tRNAs present in tRF-5s without the need for complementary target sites in mRNA (Sobala and Hutvagner, 2013). tRF-5s derived from $tRNA^{Val\text{-}GAC}$ in *Haloferax volcanii* has been revealed to bind the small ribosomal subunit near the mRNA channel, leading to substitution in the initiation complex and thereby attenuating global translation both *in vivo* and *in vitro* (Gebetsberger et al., 2017).

Regulation of Ribosome Biogenesis

tsRNAs have recently emerged as important regulators of ribosome biogenesis. Particularly, in the lower organism *Tetrahymena thermophila*, tsRNAs are composition of the precursor ribosomal RNA splicing complex (Couvillion et al., 2012). In *Drosophila*, tsRNAs restrain global translation by impeding ribosome biogenesis. Mechanistically, *Drosophila* argonaute2-bound tsRNAs preferentially inhibit the mRNA translation of ribosome proteins or translational initiation or elongation factors *via* an RNA-like pathway, thus attenuating overall translation (Couvillion et al., 2012; Dou et al., 2019). In addition, tRF-3s can recruit exonuclease Xrn2 and Tan1 protein to form compounds by specifically binding to the Twi12 protein, which cleaves and processes precursor ribosomal RNA to enhance ribosomal RNA synthesis in physiological conditions in *Tetrahymena* (Couvillion et al., 2012). In mammalian cells, tRF-3s from $tRNA^{Leu\text{-}CAG}$ bind at least two ribosomal protein mRNAs to itself, such as ribosome proteins 28 and ribosome proteins 15, to promote translation (Kim et al., 2017) (**Figure 2**). However, the mechanism by which tsRNAs regulate ribosomal biogenesis in human remains to be explored.

As Novel Epigenetic Factors

Recent studies have unveiled that tsRNAs may function as epigenetic factors to regulate gene expression. Obese rat model under the control of a high-fat diet, increased levels of $tRF^{Glu\text{-}TTC}$ directly targeted the transcription factors from the Kruppel-like factor (KLF) family, such as KLF9, KLF11, and KLF12, which are injected themselves into multiplication, apoptosis, differentiation and progress, and significantly suppressed their target mRNA expression, thus preventing the differentiation of preadipocytes (Shen et al., 2019) (**Figure 2**). Moreover, $tRF^{Glu\text{-}TTC}$ suppressed adipogenesis by inhibiting lipids transcription factors' expression (Shen et al., 2019). tRF-3s of different lengths can block reverse transcription and post-transcription silence by 18 and 22 nts long respectively, thus silence the long terminal repeat reverse transcription transposer (Schorn et al., 2017). Furthermore, Dicer-like 1 processes tRF-5s and then integrates into argonaute1, which, like miRNA, regulates genomic stability by targeting the transcriptional elements in plant *Arabidopsis thaliana* (Martinez et al., 2017). Intriguingly, tsRNAs from high-fat diet male sperm injected into normal fertilized eggs of mature mouse sperm caused gene expression changes in the early embryo and triggered islet metabolic pathways independent of DNA methylation in CpG-enriched regions. This proves that tsRNAs may be a paternal epigenetic factor in the intergenerational inheritance of metabolic diseases affected by a mediated diet (Chen et al., 2016).

Upregulation of RNA Reverse Transcription

tsRNAs can also act as an agonist for viral reverse transcription and promote the viral reverse transcription process in various ways. For example, tRF-3s can combine with the primer-binding site of human T cell leukemia virus type 1 RNA to initiate reverse transcription and promote viral synthesis of HIV-infected host cells (Ruggero et al., 2014). Meanwhile, the respiratory syncytial virus infection induces the angiotensincutting2 tRNAs to produce tiRNAs, thus triggering stress response in host cells. respiratory syncytial virus uses host tiRNAs as primers to promote its replication and improve the infection efficiency (Wang et al., 2013; Deng et al., 2015; Zhou et al., 2017).

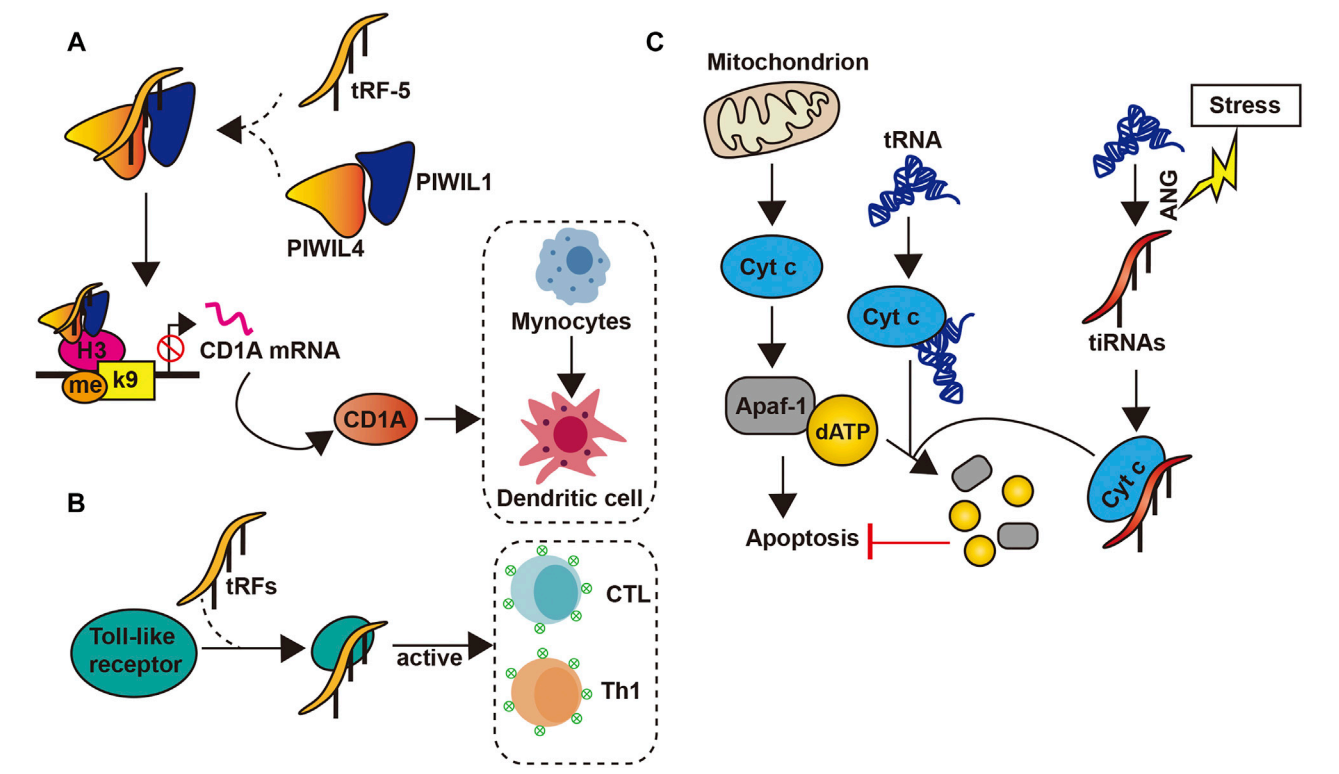


FIGURE 3 | tsRNAs' functions. **(A)** tsRNAs inhibit the transcription of the CD1A gene by forming a complex with PIWIL1/4, thereby inhibiting the conversion of Mynocytes to Thedritic cell; **(B)** tsRNAs can be combined with toll-like receptor to activate CTL and Th1; **(C)** The interaction between tsRNAs and Cty c inhibits the apoptosis process.

Moreover, host cellular proteins can regulate retroviral replication by binding to tRNAs, thereby affecting all steps in the viral life cycle. In certain circumstances, aminoacyl-tRNA synthetases bind tRNAs and link them with their corresponding amino acids and cognate aminoacyl-tRNA synthetases to facilitate tRNA primer selection, thus promoting viral reverse transcription (Jin and Musier-Forsyth, 2019).

Immune Regulation

Studies have also revealed the potential role of tsRNAs as novel immune factors. tsRNAs are highly and stably expressed in hematopoietic and lymphatic organs and blood compared with other tissues (Dhahbi, 2015). This suggests that tsRNAs may participate in the immune process. In addition, when the body is in an acute inflammatory state, tsRNAs levels in the blood increase rapidly, particularly in the sera of mice and monkeys with acute and chronic hepatitis B and active hepatitis B virus infection, as well as chimpanzees with chronic viral hepatitis (Zhang et al., 2014; Selitsky et al., 2015). Likewise, tRF-5s derived from $tRNA^{Glu}$ can lead to the inhibition of CD1A expression by compounding with PIWIL4 and PIWIL1, which was able to promote the maturation of monocytes into dendritic cells (Zhang et al., 2016) (**Figure 3**). Moreover, tsRNAs can also activate the immune response of Th1 and cytotoxic T lymphocyte by interacting directly with toll-like receptors (Wang et al., 2006) (**Figure 3**).

In addition, the specific nucleoside motifs of tRNAs may be a structural determinant of innate immune recognition. For example, the interaction between the human $tRNA^{Ala}$ stem loop and the D and T rings of $tRNA^{His}$ may be epitopes of autoantibodies in the sera of patients with idiopathic inflammatory myopathy (Bunn and Mathews, 1987), and the anti-adenovirus infection-induced tRNAs fungal protective cell therapy (Alvarado-Vásquez et al., 2005). Finally, the major histocompatibility complex contains the largest tRNA gene cluster in human, which also coexist with immune-related functions are co-located. This may imply the role of tRNA in the immune system (Horton et al., 2004). These findings overall support the proposition that tRNAs may act as immune signaling molecules.

Other Mechanisms of Action

Both mitochondrial and cytosolic tRNAs have been shown to bind to cytochrome c. This binding inhibits the interaction between cytochrome c and apoptotic protease activating factor-1, thus blocking activating factor-1 oligomerization and caspase activation, which eventually preventing apoptosis (Mei et al., 2010). Another study revealed that under high osmotic pressure or stress, angiogenin-induced tiRNAs can inhibit apoptotic formation and activity by binding cytochrome c to form a ribonucleoprotein complex (Saikia et al., 2014) (**Figure 3**). Moreover, tsRNAs can also regulate micro-organisms found in

TABLE 1 | Summary of human cancer-associated tRNAs and tsRNA.

tRNAs and tsRNA	Function	Role	Cancer type	References
$tRF^{Glu-YTC}$ $tRF^{Asp-GTC}$ $tRF^{Gly-TCC}$	Destabilization of YB-1 bound oncogenic transcripts then suppresses cell proliferation and cancer metastasis	Tumor suppressor	Breast cancer	Goodarzi et al. (2015)
tRF-1001	Promotes cell proliferation	Tumor promoter	Prostate cancer	Lee et al. (2009)
$tiRNA^{Asp-GUC}$ $tiRNA^{His-GUG}$ $tiRNA^{Lys-CUU}$	Sex hormone-dependent production, promote cell proliferation	Tumor promoter	Breast cancer and prostate cancers	Honda et al. (2015)
$tiRNA^{Leu-CAG}$-5	Promotes cell proliferation and G_0/G_1 cell cycle progression, greatly upregulates in stage III and stages IV cases and relates with the development of stage	Tumor promoter	Non-small cell lung cancer	Shao et al. (2017a)
tRF/miR-1280	Inhibits cell proliferation and tumor growth through inhibiting Notch signaling pathway by targeting JAG2	Tumor suppressor	Colorectal Cancer	Huang et al. (2017)
CU1276/tRF-3018	Associates with Argonaute proteins and represses endogenous RPA1, suppresses proliferation and modulates the molecular response to DNA damage	Tumor suppressor	B cell lymphoma	Maute et al. (2013)
$tRF^{Ser-GCT}$	Unknown	Unknown	Breast cancer	Telonis et al. (2015)
tRF^{Ser}	Cleavage of tRNAs during stress	Unknown	Hepatocellular cancer	Fu et al. (2009)
tRF^{Lys3}	Combine with AGO2 and target HIV primers with binding sites	Unknown	Cervical cancer	Yeung et al. (2009)
$tRF^{His-GTG}$ $tRF^{Leu-CAG}$	Associates with AGO2 and down-regulate target genes by transcript cleavage	Unknown	B cell lymphoma	Li et al. (2012)
$tiRNA^{Ala}$ $tiRNA^{Cys}$	Inhibits protein synthesis and triggers the phospho-eIF2α independent assembly of stress granules	Promoting tumor	Osteosarcoma	Ivanov et al. (2011)
tRF^{Val}	Induces the assembly of cytoprotective stress granules	Unknown	Osteosarcoma	Emara et al. (2010)
tRF^{Gln}	Inhibits the process of protein translation without the need for complementary target sites in the mRNA	Tumor suppressor	Cervical cancer	Sobala and Hutvagner, (2013)
cand14 cand45	Primarily associates with AGO3 and AGO4, RNA silencing by targeting luciferase reporter gene	Unknown	Kidney and colorectal cancer	Haussecker et al. (2010)
$tiRNA^{Val}$	Associates with response to the treatment of DNA methyltransferase inhibitors	Unknown	MDS	Guo et al. (2015)
Multiple tRFs	Overexpresses in metastatic tissues, potential diagnostic and prognostic biomarkers	Unknown	Prostate cancer	Martens-Uzunova et al. (2012)
tRF-544 tRF-315	High expression ratio of tRF-315/tRF-544 predict poor PFS	Unknown	Prostate cancer	Olvedy et al. (2016b)
$tRF^{Val-AAC}$ $tiRNA^{Leu-CAG}$-5 $tiRNA^{Arg-CCT}$-5 $tiRNA^{Glu-CTC}$-5 $tiRNA^{Lys-TTT}$-5	Greater downregulation in advances and less differentiates in ccRCC tissues	Tumor suppressor	Clear cell renal cell carcinoma	Nientiedt et al. (2016), Zhao et al. (2018)
ts-46 ts-47	ts-47: upregulated with KRAS mutation ts-46: upregulated with PIK3CA mutation in breast cancer cells; inhibition effect on colony formation in H1299 and A549 cell lines	Tumor suppressor	Lung cancer, breast cancer	Balatti et al. (2017)
ts-53 ts-101	ts-53: reduce lung cancer colony formation through exogenous expression; ts-53, ts-101: act as miRNAs and piRNAs by their interaction with argonaute and Piwi proteins	Tumor suppressor	Lung cancer, CLL	Pekarsky et al. (2016)
miRNA-1236-3p	Suppresses the proliferation, migration, and invasion capacity of cancer cells	Tumor suppressor	Hepatocellular cancer, ovarian cancer, bladder cancer, gastric cancer	An et al. (2019)
tRF-03357	Promotes cell proliferation, migration, and invasion	Tumor promoter	High-grade serous ovarian cancer	Zhang et al. (2019b)
$tRNA^{Leu}$	Promotes cell proliferation and transformation	Tumor promoter	ER + breast Cancer	Fang et al. (2017)
$tRNA^{Leu}$ $tRNA^{Tyr}$	Initiated tumorigenesis	Tumor promoter	Triple-negative breast cancer	Khattar et al. (2016)
$tRNA^{Leu-CAG}$	Increases protein synthesis and proliferative ability of cancer	Tumor promoter	Her2(ErbB2)-positive breast	Kwon et al. (2018)
mt-$tRNA^{Asp}$	Involved in the carcinogenesis of breast cancer	Tumor promoter	Breast cancer	Meng et al. (2016)
$tRNA^{Arg-CCG}$ $tRNA^{Glu-UUC}$	Promotes metastasis and invasion	Tumor promoter	Breast cancer	Goodarzi et al. (2016)
methionine tRNA	Advances cancer cell migration, invasiveness, and lung colonization capacity	Tumor promoter	Melanoma	Birch et al. (2016)

(Continued on following page)

TABLE 1 | (*Continued*) Summary of human cancer-associated tRNAs and tsRNA.

tRNAs and tsRNA	Function	Role	Cancer type	References
$tRNA^{Arg}$ $tRNA^{Ala}$ $tRNA^{Asn}$ $tRNA^{Cys}$ $tRNA^{Lys}$ $tRNA^{Glu}$ $tRNA^{Thr}$	TERT promotes cancer cell Proliferation by augmenting tRNA expression	Tumor promoter	Melanoma	Khattar et al. (2016)
$tRNA^{Arg}$ $tRNA^{Sec}$	The oncoproteins E6 and E7 stimulates tRNA transcription	Tumor promoter	Cervical cancer	Daly et al. (2005)
High levels of tRNA abundance	Increases translation of highly active proteins	Tumor promoter	Multiple myeloma	Zhou et al. (2009)

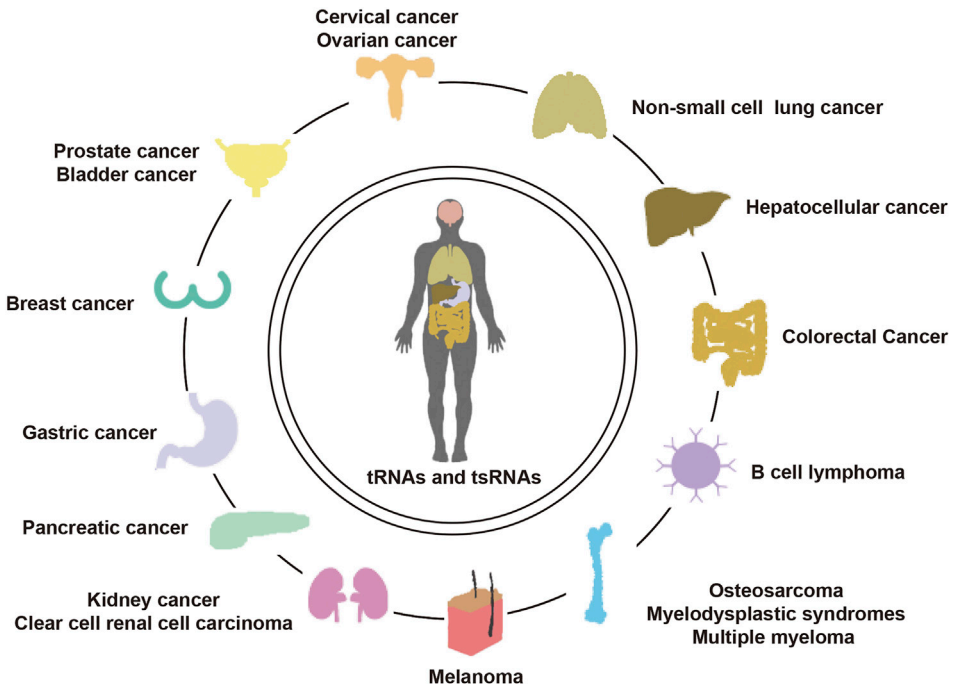


FIGURE 4 | Landscape of tRNAs in human diseases.

human. In the oral cavity, the presence of *Fusobacterium nucleatum* triggers the release of tsRNAs, which may inhibit the growth of the former by interfering with the biosynthesis of bacterial proteins (He et al., 2018).

At present, studies exploring the function of tRNAs are only emerging. Future researches are expected to reveal the further regulatory role of tRNAs and tsRNAs in biological functions.

TRNAS AND TSRNAS IN HUMAN CANCER

tRNAs dysregulation have been detected in a variety of cancers, including breast cancer (Fang et al., 2017), lung cancer (Lu et al., 2009), melanoma (Phizicky and Hopper, 2010), prostate cancer (Olvedy et al., 2016a), and tRNA has been revealed to be regulated by oncogenes and tumor-suppressor genes. Particularly, oncogenes *Ras* (Wang et al., 1997) and *c-myc* (Gomez-Roman et al., 2003) can promote the expression of RNA polymerase III, whereas suppressor genes Rb (White et al., 1996) and p53 (Crighton et al., 2003) can inhibit its transcription. This leads to a serious imbalance of tRNA expression in cancers. Abnormally expressed tRNA can also promote cell proliferation and inhibit cell apoptosis, thus promoting tumor progression (Pavon-Eternod et al., 2013; Kwon et al., 2018).

In addition, the tRNA modification system can initiate tumorigenesis by directly affecting cellular processes associated with characteristic cancer cell phenotypes, such as increased proliferation, metastasis potential, and stem cell

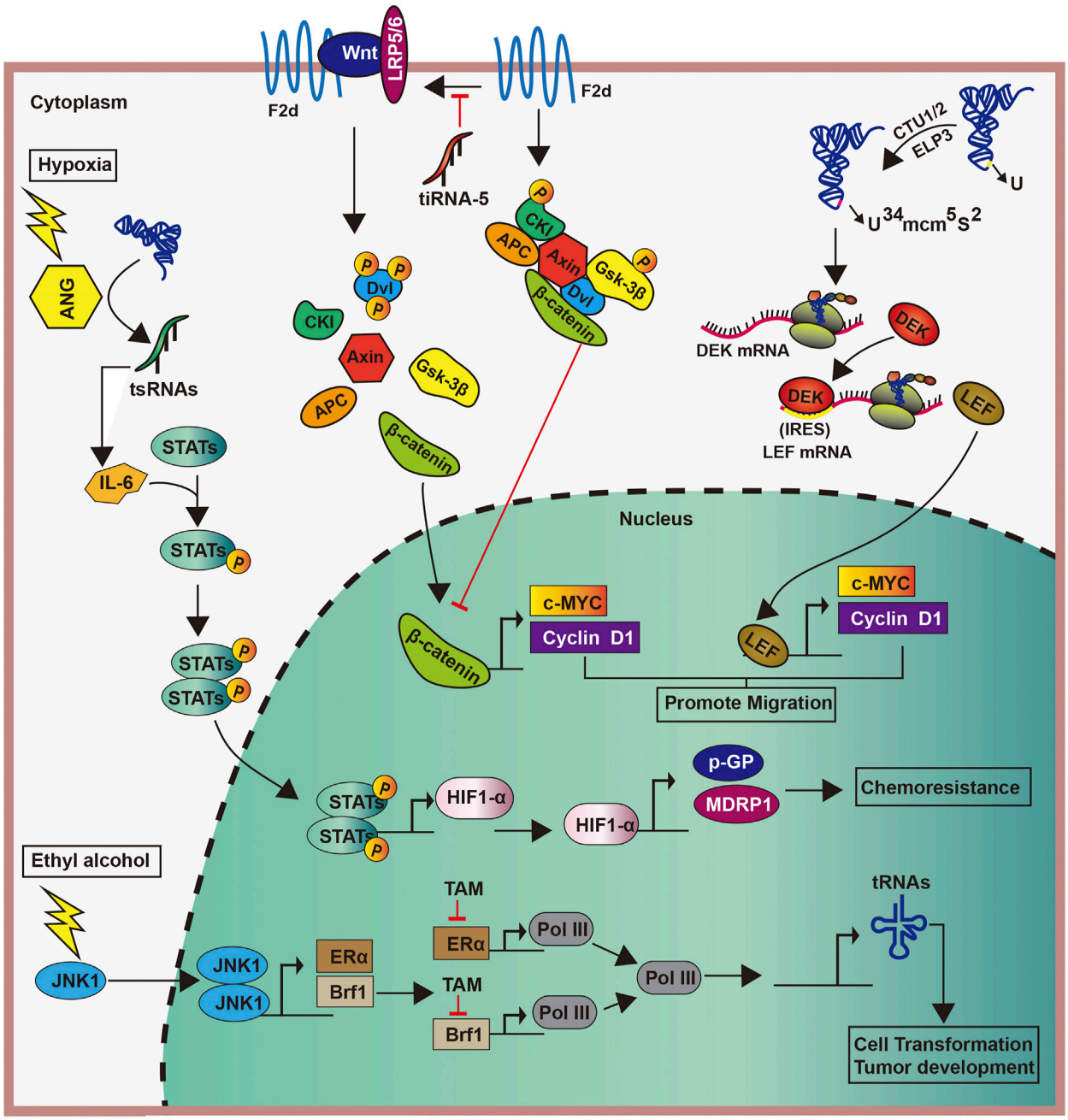


FIGURE 5 | Roles of tRNAs and tsRNAs in breast cancer.

survival (Endres et al., 2019). Further, the tRNA modification system is also a key component of carcinogenic signaling pathways (Endres et al., 2019). In various cancers, the tRNA modifying enzyme increases the modification of a specific tRNA, thereby altering the preference of the tRNA codon. This results in increased levels of proteins corresponding to these mRNAs was found to be rich in a particular subset of the new "preferred" codons (Novoa et al., 2012; Novoa and Ribas De Pouplana, 2012).

Mutations in mitochondrial tRNA (mt-tRNA) have been confirmed that increased tumorigenic relates to the invasive phenotypes (Amuthan et al., 2001). These mutations can seriously affect the tertiary structure of mt-tRNAs, thus severely damaging the synthesis of mitochondrial proteins (Grzybowska-Szatkowska and Slaska, 2012). At present, the involvement of mt-tRNA mutations in the carcinogenesis of breast and lung cancers has been confirmed (Lu et al., 2009; Meng et al., 2016).

Several studies have also reported that tsRNAs, a derivative of tRNA, is dysregulated in a variety of cancers and may play a carcinogenic or anti-cancer role (Pekarsky et al., 2016; Balatti et al., 2017). In breast cancer, many studies have shown that cancer genes can regulate the expression of tsRNAs, which may also be a key effector molecule in cancer gene regulation (Balatti et al., 2017).

While current knowledge of tRNAs and tsRNAs in cancer is still in its infancy, their potential applications in the improvement of fresh biomarkers and original healthful strategies for the diagnosis, monitoring, predicting, and treating cancer cannot be understated. We have summarized the recent literature on the functions and mechanisms of cancer-associated tRNAs in **Table 1** (**Figure 4**).

Breast Cancer

Different factors can induce the abnormal expression of tRNAs in breast cancer, thereby promoting tumor progression. For example, the stimulation of ethanol activates c-Jun N-terminal kinase 1, which promotes the proliferation of Brf1 and ERα. Subsequently, the interaction between Brf1 and ERα upregulates *Pol III* gene transcription to enhance the production of tRNA, ultimately leading to the development of breast cancer. However, tamoxifen can hold in the incident of breast cancer by containing the effects of Brf1 and ERα, also indirectly inhibits the generation of tRNA (Fang et al., 2017) (**Figure 5**). Similarly, the TATA box-binding protein human Maf1 and the oncogene *Ras* can promote the transcription of tRNAs by targeting RNA pol Ⅲ, particularly the Brf1 subunit of TFⅢ B factor, thereby promoting tumor progression (Wang et al., 1997; Shen et al., 1998; Rollins et al., 2007; Johnson et al., 2008).

Enzymes catalyzing tRNA modifications play significant roles in the biological processes in breast cancer (Towns and Begley, 2012; Frye and Watt, 2006; Delaunay et al., 2016). In human, the overexpression of the U34-modifying enzymes Elp3 and Ctu1/2 directly promoted the translation of the oncoprotein DEK by catalyzing the mcm^5s^2-U34 tRNA modification. Increased DEK can then bind the LEF1 internal ribosome entry site sequence, thereby increasing the translation of the oncogenic LEF-1 mRNA and promoting the invasion and metastasis of breast cancer cells (Delaunay et al., 2016) (**Figure 5**). Furthermore, the high expression of $tRNA^{Arg\text{-}CCG}$ and $tRNA^{GIu\text{-}UUC}$ in breast cancer can promote the invasion and metastasis of cancer cells by directly upregulating the expression of EXOSC2 and enhancing that of GRIPAP1 (Goodarzi et al., 2016).

In a study cohort of Polish women with breast cancer, the tertiary structure of mt-tRNAs was affected by genetic mutation, resulting in the severe impairment of the mitochondrial protein synthesis, and thus affecting cell proliferation (Grzybowska-Szatkowska and Slaska, 2012). Further, mutations in mt-tRNAs are participated in the carcinogenesis of breast cancer, such as mt-$tRNA^{Asp}$ (Meng et al., 2016), but the specific mechanism of mtRNA mutation in cancer is unknown.

Similarly, tiRNAs are abnormally expressed in breast cancer and are involved in tumorigenesis (Goodarzi et al., 2015; Honda et al., 2015; Balatti et al., 2017). Decreased abundances of 26 specific circulatory tiRNAs from the $tRNA^{Gly}$, $tRNA^{Glu}$, and $tRNA^{Lys}$ heterogenous receptors was observed in ER-positive breast cancer (Dhahbi et al., 2014). The same study have suggested that inflammatory breast cancer is associated with an increase in $tiRNA^{Ala}$ (Dhahbi et al., 2014). Some tiRNAs, such as $tiRNA^{Asp}$-5 and $tiRNA^{His}$-5, are significantly overexpressed in breast cancer, and the knockout of tiRNA-5s can inhibit tumor proliferation (Honda et al., 2015). These findings provide sufficient evidence of the involvement of abnormal tiRNAs expression in the course of breast cancer.

The connection of tsRNAs in breast cancer progression has also been confirmed by several studies. First, the anomalous demonstration of tiRNAs has been sighted at some stages of the carcinogenesis process [19]. The downregulation of $tiRNA^{Val}$-5 in the serum is positively associated with lymph node metastasis and cancer stage progression, whereas its overexpression inhibits malignant cell activity (Mo et al., 2019). Meanwhile, the expression of tRF-3s are strongly downregulated in invasive advanced breast cancer, whereas that of tRF-1s were raised in an advanced cancer cell, thus suggesting that tRF-3s and tRF-1s may be related to advanced pathological changes in cancer (Balatti et al., 2017). Second, tsRNAs can regulate breast cancer progression by affecting gene transcription. For example, tRFs from $tRNA^{Glu}$, $tRNA^{Asp}$, $tRNA^{Gly}$, and $tRNA^{Tyr}$ contend with YB-1 for the transcription of endogenous cancer genes, thereby undermining the stability of transcriptions of proto-oncogene and reducing their expression. This subsequently inhibits breast cancer progression (Goodarzi et al., 2015). Similarly, in breast cancer cells, tRFs derived from $tRNA^{Tyr}$, $tRNA^{Asp}$, $tRNA^{Gly}$, and $tRNA^{Glu}$ can inhibit tumor progression by displacing the 3′-UTRs of multiple oncogenic transcripts from the RNA-binding protein YBX-1, thus reducing their stability (Goodarzi et al., 2015). Another study showed that $tiRNA^{Val}$-5 leads to the inhibition of *c-myc* and *cyclinD1* by downregulating the FZD3-Wnt/β-catenin axis, which inhibits the progression of breast cancer (Mo et al., 2019) (**Figure 5**). Third, the impairment of the tiRNAs production also affect cancer progression. estrogen and its receptors promote the angiogenin cutting mature tRNA anticodon ring, thus producing large amounts of tiRNAs in ER-positive breast cancer. This accumulation makes for cells proliferating, which may promote tumor occurrence and tumor growth (Honda et al., 2015). Meanwhile, in a hypoxic environment, angiogenin induces the production of tsRNAs, and tsRNAs then interact with interleukin-6 to promote the phosphorylation of signal transducers and activators of transcription proteins. This promotes the transcription of the hypoxia inducible factor-1α, as well as those of multidrug-resistant genes and glycolytic proteins, ultimately leading to cytochemical resistance (Cui et al., 2019) (**Figure 5**). These studies suggest the varied functions of tsRNAs in cancer pathogenesis.

Lung Cancer

mt-tRNA gene mutations have been found to promote the development of lung cancer (Lu et al., 2009). These mutations damage the secondary structure of tRNAs, thereby affecting post-transcriptional modifications and aminoacylation, which can change the specificity, stability, or affinity of tRNAs (Brulé

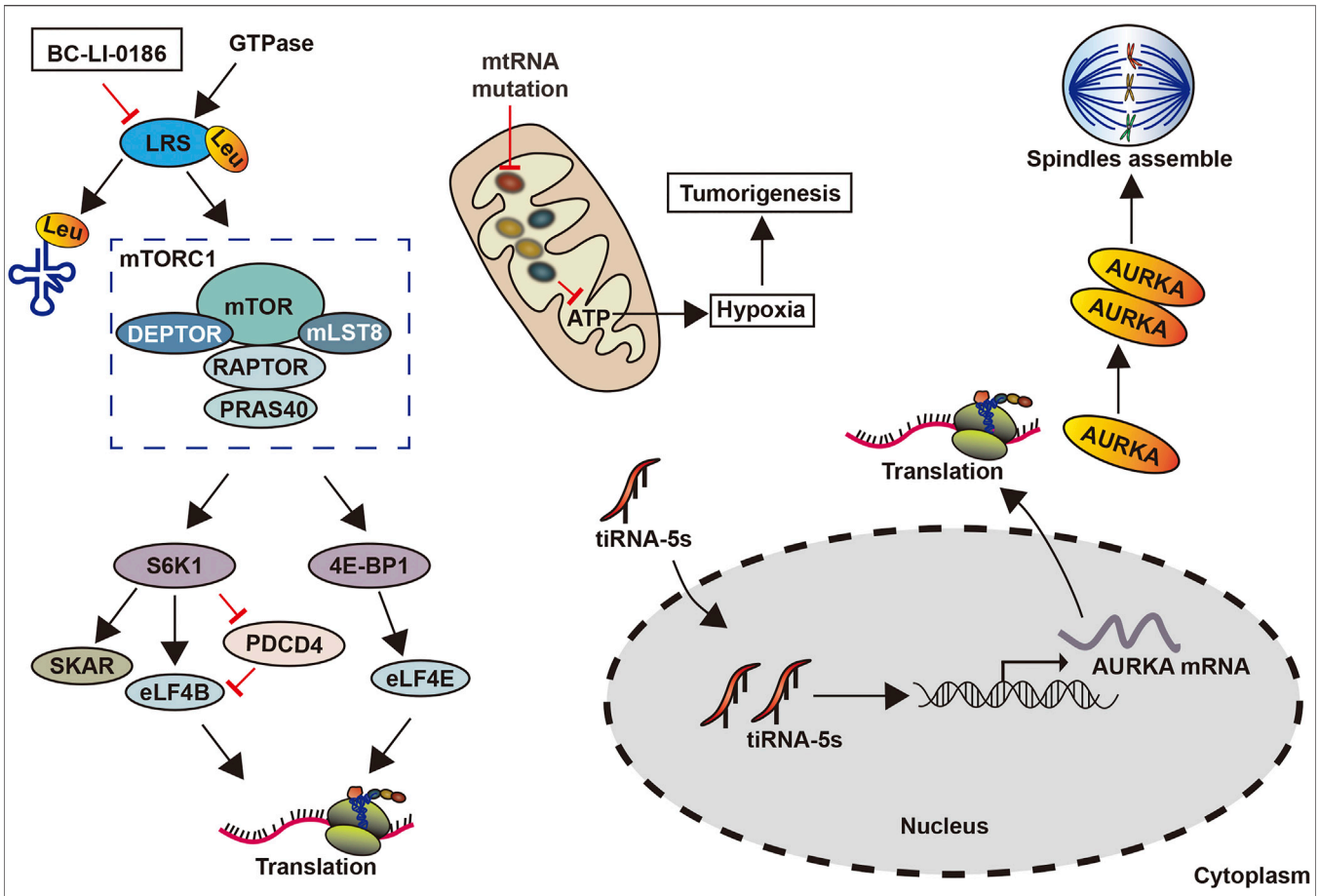


FIGURE 6 | Roles of tRNAs and tsRNAs in lung cancer.

et al., 1998). Further, these mutations caused a decrease in mitochondrial protein synthesis and the cellular inability to reach the respiratory phenotypes and ATP thresholds required by normal cells to promote lung cancer (Lu et al., 2009) (**Figure 6**). The drug BC-Li-0186 when combined with leucyl-tRNA synthetase inhibited its activity, reduced the abundance of tRNA-carrying leucine, and prevented leucyl-tRNA synthetase mediated the non-classical mammalian target of rapamycin complex 1. This ultimately restrains the development of non-small cell lung cancer (Kim et al., 2019) (**Figure 6**).

tsRNAs have also been associated with lung cancer development through its regulation of the biological behavior of cells. For instance, miR-4521, acts as an inhibitor in CLL, has been revealed to be a tsRNA (ts-4521), which is lowered and mutated genes in lung cancer. Meanwhile, a reduction in ts-4521 has been shown to support tumor movement by the cell proliferation-related pathways' activation and inhibiting apoptosis-related pathways in cancer cells (Phizicky and Hopper, 2010; Pekarsky et al., 2016). In addition, tsRNAs can regulate the demonstration of oncogenes. tRF-Leu-CAG is highly expressed in non-small cell lung cancer tissues, promoting tumor cell proliferation and cell cycle progression by upregulating the oncogene *AURKA* (Shao et al., 2017b) (**Figure 6**). However, how to mediate other signaling pathways through *AURKA* remains unclear.

Melanoma

Melanoma is highly malignant and accounts for the majority of skin tumor deaths. The roles of tRNAs and tsRNAs in its melanoma have also been investigated. The overexpression of the promoter methionine tRNA gene promoted tumor growth and angiogenesis in mouse melanoma cells, as well as an increase in cancer cell migration, invasion, and lung colonization, thereby resulting in increased metastasis potential (Phizicky and Hopper, 2010; Clarke et al., 2016). Its upregulation in cancer-associated fibroblasts accelerated the secretion of stromal cells, especially type-II collagen, thus facilitating tumor growth and metastasis (Phizicky and Hopper, 2010) (**Figure 7**).

In a recent study, the accurate translation of hypoxia inducible factor 1 α mRNA in melanoma requires the participation of 34 uridine tRNA-modifying enzymes to adapt to a metabolic environment that is not conducive to growth conditions. The particular translation reprogramming of which relies partly on mTORC2-mediated enzymatic phosphorylation to modify the anti-codon of tRNA. Further, enhanced codon dependence on hypoxia inducible factor 1 α

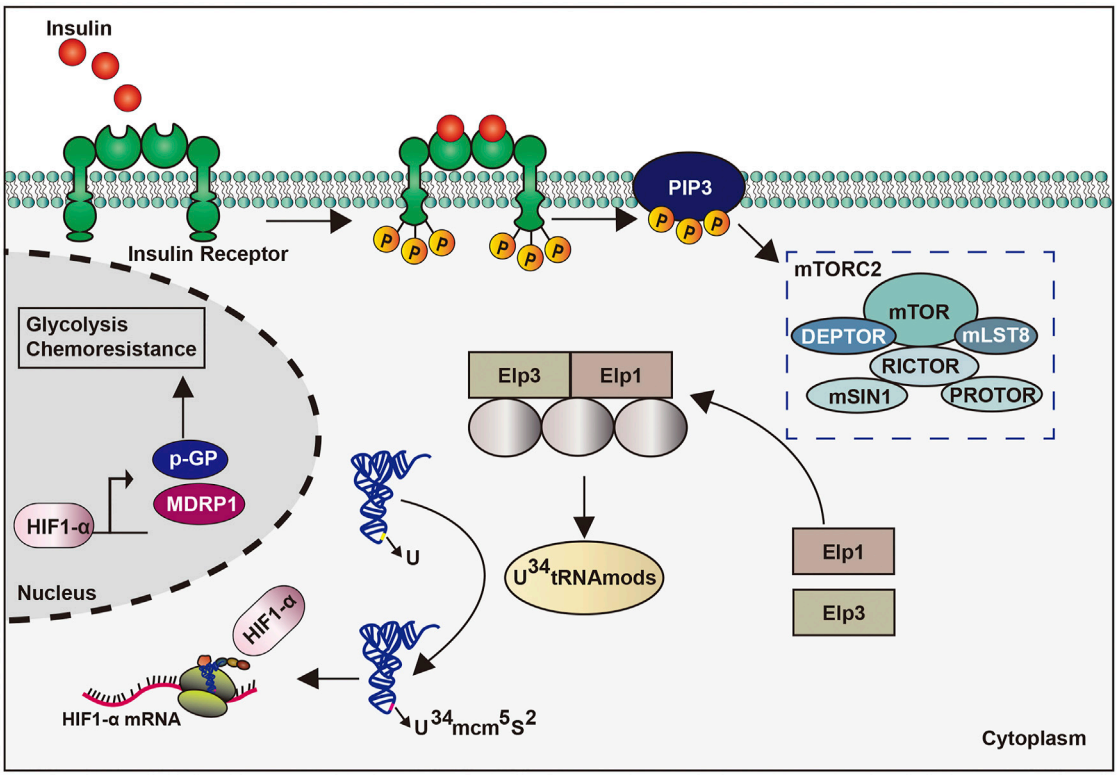


FIGURE 7 | Roles of tRNAs and tsRNAs in melanoma.

translation can promote glycolytic metabolism and the proliferation of melanoma cells (Mcmahon and Ruggero, 2018) (**Figure 7**). However, the specific regulatory mechanism of tRNAs in melanoma needs further validation, whereas the role of tsRNAs in melanoma has yet to be studied.

Other Cancers

tRNAs and tsRNAs have also been implicated in the biological processes of liver (Selitsky et al., 2015) and prostate cancers (Olvedy et al., 2016b). In liver cancer, tsRNAs $tRNA^{Val\text{-}TAC\text{-}3}$, $tRNA^{Gly\text{-}TCC\text{-}5}$, $tRNA^{Val\text{-}AAC\text{-}5}$, and $tRNA^{Glu\text{-}CTC\text{-}5}$ were significantly increased in plasma exosomes (Selitsky et al., 2015) (**Figure 8**). Meanwhile the expression of $tiRNA^{Arg\text{-}CCT}$-5, $tiRNA^{Glu\text{-}CTC}$-5, $tiRNA^{Leu\text{-}CAG}$-5, and $tiRNA^{Lys\text{-}TTT}$-5 was downregulated in clear-cell renal-cell carcinoma, suggesting a potential role as a tumor suppressor (Zhao et al., 2018). Moreover, the relative abundance of $tiRNA^{Gly}$ is 50–60% lower in hepatitis B virus and Hepatitis C virus-related cancers than normal liver tissue (Selitsky et al., 2015). These findings suggest that tRNAs and tsRNAs may have opposite effects in liver cancer and clear-cell renal-cell carcinoma and may thus be used as new diagnostic biomarkers.

tsRNA (CU1276) modulated DNA damage response and suppressed cell proliferation *via* the inhibition of RPA1, which is an endogenous single-stranded DNA binding protein, in B cell lymphoma cells (Maute et al., 2013) (**Figure 8**). Meanwhile, $tRNA^{Leu}$ and pre-miRNA derived from tRF/miR-1280, can suppress the growth and transfer of colorectal cancer by inhibiting Notch signaling pathways. Particularly, tRF/miR-1280 can target the Notch ligand jagged 2 (JAG2) to repress Notch signaling pathways, which in turn inhibits the cancer stem cell phenotypes by inhibiting direct transcription of Gata1/3 and miR200b genes, thus inhibiting tumorigenesis and metastasis (Huang et al., 2017) (**Figure 8**).

In high-grade serous ovarian cancer, upregulated tRFs can promote protein phosphorylation, transcription, cell migration, cancer pathways, MAPK, and Wnt signaling pathways, as well as regulating HMBOX1 to attack human ovarian cancer cells (Zhang et al., 2019a). This proves that tRFs play a role as a regulatory factor for cancer development in serous ovarian cancer (**Figure 8**). In prostate cancer, 589 differentially expressed tRFs have been detected, suggesting its potential as a biomarker (Olvedy et al., 2016b). Similarly, tRF-1001 from $tRNA^{Ser}$ was eminently expressed in prostate cancer, whereas its knockdown suppressed DNA biosynthesis and cell proliferation (Anderson and Ivanov, 2014).

CLINICAL APPLICATION OF TRNAS AND TSRNAS IN CANCER

Although tsRNAs and tRNAs function in the human body are still in the exploratory stage, the potential it has shown as a cancer marker is not to be underestimated. Next, we will discuss its role in clinical diagnosis and treatment.

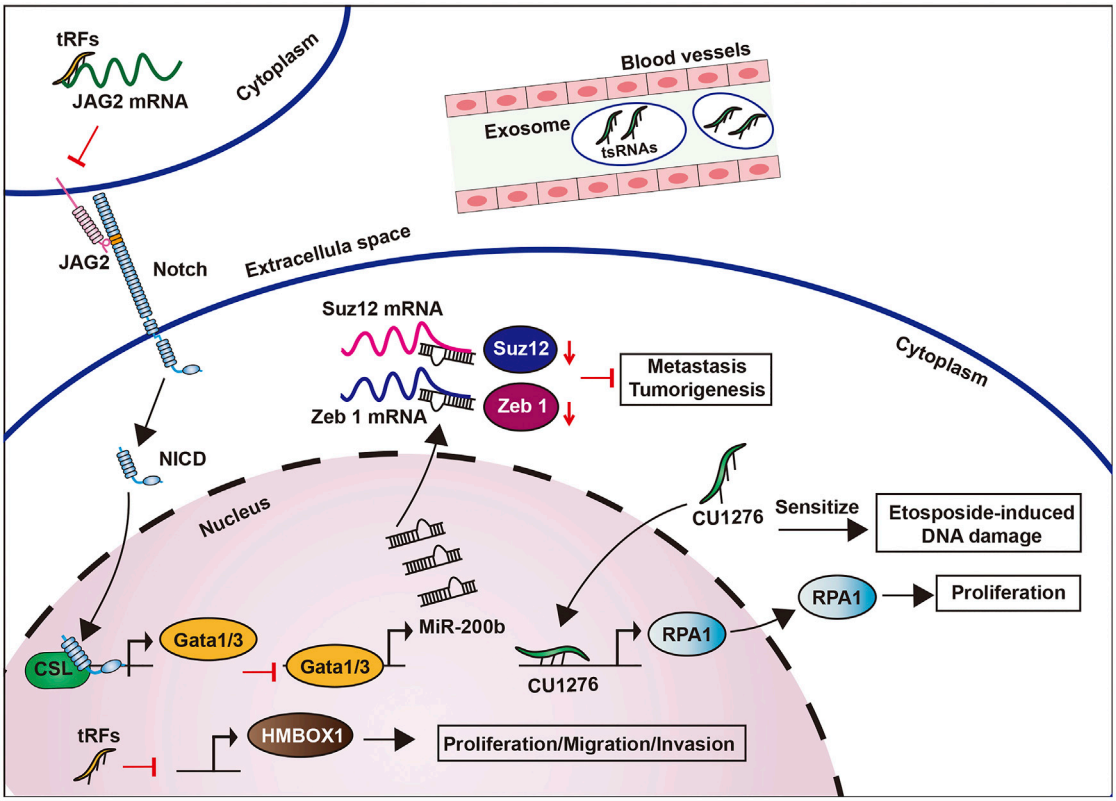


FIGURE 8 | Roles of tRNAs and tsRNAs in other cancers.

As a Diagnostic Biological Marker

Cancer is the most dangerous disease known. In many cases, patients with cancer are often unaware of their early stages, and when clinical symptoms appear, the cancer is in its late stages. Finding reliable and sensitive diagnostic molecular markers is researchers have been struggling to find. Recently, the function of tRNAs and tsRNAs in the human body has gradually received clinical attention and shown great molecular marker potential.

Previous studies have shown that tsRNAs can be detected in serum and urine stabilized (Romaine et al., 2015; Santos et al., 2019). Now, with the continuous development of high-throughput sequencing technology, different types of tsRNAs are constantly detected and isolated in body fluids, and their functions are gradually reflected. For example, there are differences in abundance between prostate cancer and renal transparent cell carcinoma and tsRNAs in normal prostate tissue (Hayes et al., 2014; Zhao et al., 2018). Subsequently, many studies have found tRNAs and tsRNAs disorders may be related to the regulation of tumor genes and tumor suppressor genes, while the abnormal expression of tRNAs and tsRNAs can promote cell proliferation and inhibit cell apoptosis, further promoting cancer progression (Pavon-Eternod et al., 2013; Kwon et al., 2018). In breast cancer, ethanol stimulation can promote the proliferation of Brf1 and ER+, thereby enhancing their interaction, and enhance the production of tRNA by Pol III gene transcription, further promoting tumor progression (Fang et al., 2017). Meanwhile many studies have shown that cancer genes can regulate the expression of tsRNAs and tsRNAs may also be a key effector molecule of cancer gene regulation (Zhu et al., 2019). On the other hand, in hepatocellular carcinoma, tsRNAs $tRNA^{Val\text{-}TAC\text{-}3}$, $tRNA^{Gly\text{-}TCC\text{-}5}$, $tRNA^{Val\text{-}AAC\text{-}5}$, and $tRNA^{Glu\text{-}CTC\text{-}5}$ are significantly elevated in plasma exosomes, which may play a transcriptional role (Selitsky et al., 2015). These characteristics are sufficient to demonstrate the great potential of tsRNAs to become a biomarker of tumors. But there is still less research on tsRNAs, and hopefully more people will look to it in the future so that tsRNAs can really play its clinical role and contribute to the cause of human medicine.

The Role of Targeted Therapy

tRNAs and tsRNAs, as small molecular markers related to tumor, may also play a role in molecular targeted therapy to suggest local therapeutic targets. For example, mt-tRNA mutations in lung cancer inhibit the development of NSCLC by leucyl-tRNA synthase mediated rapamycin complex 1 as a non-classical mammalian target (Kim et al., 2019). In high-grade serous ovarian cancer, tRF-03357 promotes cell proliferation, migration and invasion, partly by modulating HMBOX1. And this phenomenon can be reversed by targeting tRF-03357 (Zhang et al., 2019a). These functions indicate that tRNAs and tsRNAs can be used as therapeutic targets for clinical interventions.

However, as a therapeutic target, more precise mechanisms of action and higher sensitivity specificity are required, which still leaves some unexplored areas.

CONCLUSION AND PERSPECTIVES

Recent studies have revealed they are key role to various human diseases' development, especially cancer. Meanwhile, tsRNAs are increasingly being tapped for its potential role in diseases. This review summarizes recent literature on the biogenesis, structure, and biological characteristics and functions of tRNAs and tsRNAs, with an extraordinary attention to their participation and potential clinical significance in human cancers.

In human cancer, tRNAs and tsRNAs possess carcinogenic roles by promoting cell proliferation, migration, and invasion and inhibiting apoptosis (Santos et al., 2019). Meanwhile, tRNAs and tsRNAs also have anti-tumor effects. Although in clinical settings, fluid screening mainly focuses on miRNAs (Hayes et al., 2014; Romaine et al., 2015). However, tRNAs and tsRNAs are stably enriched in the biofluids in solid cancers and blood malignancies, (Zhang et al., 2009; Li et al., 2012), fluid screening can determine tRNAs and tsRNAs biomarker candidates for cancer diagnosis.

Several studies have confirmed that tRNAs and tsRNAs regulate cancer progression. Indeed, such mechanisms may become suitable targets for novel therapeutic approaches in several tumor types. Although tRNA has already been confirmed as a regulator, it remains unclear whether its dysregulation in many cancers is a trigger for tumor initiation, progress, or metastasis.

Compared with that on other non-coding RNAs, i.e., miRNA and lncRNA, the current state of knowledge on tRNAs and tsRNAs are still in its infancy, and they have not yet been studied in clinical settings. There are several challenges and limitations in the study of tRNAs. First, the molecular mechanism of tRNAs and tsRNAs in cancer development needs to be further confirmed. Second, most studies on tRNAs and tsRNAs and their role in cancer used cancer cells. To advance research in this field, future studies should utilize clinical samples, such as tumor tissues and body fluids. Third, how to utilize tRNAs and tsRNAs against cancer cells effectively and with long-term efficacy should be sufficiently addressed. Fourth, to ensure their safety and efficacy in human, pre-clinical and clinical studies are warranted. Lastly, the relationship between mutations in mitochondrial tRNA and maternal genetic diseases needs to be more clearly studied.

In conclusion, this review bridges the gap between what is known about tRNAs and tsRNAs and their involvement in human cancer, thus providing new insights and strategies for cancer diagnosis, management, and treatment (Hopper and Phizicky, 2003).

AUTHOR CONTRIBUTIONS

SX and YL provided direction and guidance throughout the preparation of this manuscript. ZQ wrote and edited the manuscript. QW and LL generated the figures and made significant revisions to the manuscript. GL, YH, SN, LZ, XZ, YC, JW, XW, and SY compiled the related literature. All authors have read and approved the final version of the manuscript.

FUNDING

This work was funded the National Natural Science Foundation of China (grant numbers 82072904, 81872149, 82103325); the Outstanding Youth Project of Heilongjiang Provincial Natural Science Foundation (grant number YQ2019H027); the Distinguished Young Scholars of Harbin Medical University Cancer Hospital (grant number JCQN2018-03) and the Young Elite Training Foundation Grant of Harbin Medical University Cancer Hospital (grant number JY2016-02); Postgraduate Practice Innovation Project of Harbin Medical University (grant number YJSCX2020-120HYD).

REFERENCES

Alvarado-Vásquez, N., Santiago, J., Alcázar-Leyva, S., Zenteno, E., Negrete-García, C., and Alcázar-Montenegro, H. (2005). A Fungal tRNA of Aspergillus niger Induces IFN-Beta Synthesis in HEp-2 Cells. *Life Sci.* 77, 578–588. doi:10.1016/j.lfs.2004.11.034

Amuthan, G., Biswas, G., Zhang, S. Y., Klein-Szanto, A., Vijayasarathy, C., and Avadhani, N. G. (2001). Mitochondria-to-nucleus Stress Signaling Induces Phenotypic Changes, Tumor Progression and Cell Invasion. *EMBO J.* 20, 1910–1920. doi:10.1093/emboj/20.8.1910

An, J. X., Ma, Z. S., Ma, M. H., Shao, S., Cao, F. L., and Dai, D. Q. (2019). MiR-1236-3p Serves as a New Diagnostic and Prognostic Biomarker for Gastric Cancer. *Cancer Biomark* 25, 127–132. doi:10.3233/CBM-171026

Anderson, P., and Ivanov, P. (2014). tRNA Fragments in Human Health and Disease. *FEBS Lett.* 588, 4297–4304. doi:10.1016/j.febslet.2014.09.001

Babiarz, J. E., Ruby, J. G., Wang, Y., Bartel, D. P., and Blelloch, R. (2008). Mouse ES Cells Express Endogenous shRNAs, siRNAs, and Other Microprocessor-independent, Dicer-dependent Small RNAs. *Genes Dev.* 22, 2773–2785. doi:10.1101/gad.1705308

Balatti, V., Nigita, G., Veneziano, D., Drusco, A., Stein, G. S., Messier, T. L., et al. (2017). tsRNA Signatures in Cancer. *Proc. Natl. Acad. Sci. U S A.* 114, 8071–8076. doi:10.1073/pnas.1706908114

Bhawe, K., and Roy, D. (2018). Interplay between NRF1, E2F4 and MYC Transcription Factors Regulating Common Target Genes Contributes to Cancer Development and Progression. *Cel Oncol (Dordr)* 41, 465–484. doi:10.1007/s13402-018-0395-3

Birch, J., Clarke, C. J., Campbell, A. D., Campbell, K., Mitchell, L., Liko, D., et al. (2016). The Initiator Methionine tRNA Drives Cell Migration and Invasion Leading to Increased Metastatic Potential in Melanoma. *Biol. Open* 5, 1371–1379. doi:10.1242/bio.019075

Blanco, S., and Frye, M. (2014). Role of RNA Methyltransferases in Tissue Renewal and Pathology. *Curr. Opin. Cel Biol* 31, 1–7. doi:10.1016/j.ceb.2014.06.006

Brulé, H., Holmes, W. M., Keith, G., Giegé, R., and Florentz, C. (1998). Effect of a Mutation in the Anticodon of Human Mitochondrial tRNAPro on its post-transcriptional Modification Pattern. *Nucleic Acids Res.* 26, 537–543. doi:10.1093/nar/26.2.537

Bunn, C. C., and Mathews, M. B. (1987). Autoreactive Epitope Defined as the Anticodon Region of Alanine Transfer RNA. *Science* 238, 1116–1119. doi:10.1126/science.2446387

Chen, Q., Yan, M., Cao, Z., Li, X., Zhang, Y., Shi, J., et al. (2016). Sperm tsRNAs Contribute to Intergenerational Inheritance of an Acquired Metabolic Disorder. *Science* 351, 397–400. doi:10.1126/science.aad7977

Clarke, C. J., Berg, T. J., Birch, J., Ennis, D., Mitchell, L., Cloix, C., et al. (2016). The Initiator Methionine tRNA Drives Secretion of Type II Collagen from Stromal Fibroblasts to Promote Tumor Growth and Angiogenesis. *Curr. Biol.* 26, 755–765. doi:10.1016/j.cub.2016.01.045

Couvillion, M. T., Bounova, G., Purdom, E., Speed, T. P., and Collins, K. (2012). A Tetrahymena Piwi Bound to Mature tRNA 3' Fragments Activates the Exonuclease Xrn2 for RNA Processing in the Nucleus. *Mol. Cel* 48, 509–520. doi:10.1016/j.molcel.2012.09.010

Crighton, D., Woiwode, A., Zhang, C., Mandavia, N., Morton, J. P., Warnock, L. J., et al. (2003). p53 Represses RNA Polymerase III Transcription by Targeting TBP and Inhibiting Promoter Occupancy by TFIIIB. *EMBO J.* 22, 2810–2820. doi:10.1093/emboj/cdg265

Cui, Y., Huang, Y., Wu, X., Zheng, M., Xia, Y., Fu, Z., et al. (2019). Hypoxia-induced tRNA-Derived Fragments, Novel Regulatory Factor for Doxorubicin Resistance in Triple-Negative Breast Cancer. *J. Cel Physiol* 234, 8740–8751. doi:10.1002/jcp.27533

Daly, N. L., Arvanitis, D. A., Fairley, J. A., Gomez-Roman, N., Morton, J. P., Graham, S. V., et al. (2005). Deregulation of RNA Polymerase III Transcription in Cervical Epithelium in Response to High-Risk Human Papillomavirus. *Oncogene* 24, 880–888. doi:10.1038/sj.onc.1208031

Delaunay, S., Rapino, F., Tharun, L., Zhou, Z., Heukamp, L., Termathe, M., et al. (2016). Elp3 Links tRNA Modification to IRES-dependent Translation of LEF1 to Sustain Metastasis in Breast Cancer. *J. Exp. Med.* 213, 2503–2523. doi:10.1084/jem.20160397

Deng, J., Ptashkin, R. N., Chen, Y., Cheng, Z., Liu, G., Phan, T., et al. (2015). Respiratory Syncytial Virus Utilizes a tRNA Fragment to Suppress Antiviral Responses through a Novel Targeting Mechanism. *Mol. Ther.* 23, 1622–1629. doi:10.1038/mt.2015.124

Dhahbi, J. M. (2015). 5' tRNA Halves: The Next Generation of Immune Signaling Molecules. *Front. Immunol.* 6, 74. doi:10.3389/fimmu.2015.00074

Dhahbi, J. M., Spindler, S. R., Atamna, H., Boffelli, D., and Martin, D. I. (2014). Deep Sequencing of Serum Small RNAs Identifies Patterns of 5' tRNA Half and YRNA Fragment Expression Associated with Breast Cancer. *Biomark Cancer* 6, 37–47. doi:10.4137/BIC.S20764

Dou, S., Wang, Y., and Lu, J. (2019). Metazoan tsRNAs: Biogenesis, Evolution and Regulatory Functions. *Noncoding RNA* 5, 1. doi:10.3390/ncrna5010018

Eichhorn, S. W., Guo, H., Mcgeary, S. E., Rodriguez-Mias, R. A., Shin, C., Baek, D., et al. (2014). mRNA Destabilization Is the Dominant Effect of Mammalian microRNAs by the Time Substantial Repression Ensues. *Mol. Cel* 56, 104–115. doi:10.1016/j.molcel.2014.08.028

Emara, M. M., Ivanov, P., Hickman, T., Dawra, N., Tisdale, S., Kedersha, N., et al. (2010). Angiogenin-induced tRNA-Derived Stress-Induced RNAs Promote Stress-Induced Stress Granule Assembly. *J. Biol. Chem.* 285, 10959–10968. doi:10.1074/jbc.M109.077560

Endres, L., Fasullo, M., and Rose, R. (2019). tRNA Modification and Cancer: Potential for Therapeutic Prevention and Intervention. *Future Med. Chem.* 11, 885–900. doi:10.4155/fmc-2018-0404

Fang, Z., Yi, Y., Shi, G., Li, S., Chen, S., Lin, Y., et al. (2017). Role of Brf1 Interaction with ERα, and Significance of its Overexpression, in Human Breast Cancer. *Mol. Oncol.* 11, 1752–1767. doi:10.1002/1878-0261.12141

Farooqi, A. A., Jabeen, S., Attar, R., Yaylim, I., and Xu, B. (2018). Quercetin-mediated Regulation of Signal Transduction Cascades and microRNAs: Natural Weapon against Cancer. *J. Cel Biochem* 119, 9664–9674. doi:10.1002/jcb.27488

Frye, M., and Watt, F. M. (2006). The RNA Methyltransferase Misu (NSun2) Mediates Myc-Induced Proliferation and Is Upregulated in Tumors. *Curr. Biol.* 16, 971–981. doi:10.1016/j.cub.2006.04.027

Fu, H., Feng, J., Liu, Q., Sun, F., Tie, Y., Zhu, J., et al. (2009). Stress Induces tRNA Cleavage by Angiogenin in Mammalian Cells. *FEBS Lett.* 583, 437–442. doi:10.1016/j.febslet.2008.12.043

Gebetsberger, J., Wyss, L., Mleczko, A. M., Reuther, J., and Polacek, N. (2017). A tRNA-Derived Fragment Competes with mRNA for Ribosome Binding and Regulates Translation during Stress. *RNA Biol.* 14, 1364–1373. doi:10.1080/15476286.2016.1257470

Gomez-Roman, N., Grandori, C., Eisenman, R. N., and White, R. J. (2003). Direct Activation of RNA Polymerase III Transcription by C-Myc. *Nature* 421, 290–294. doi:10.1038/nature01327

Goodarzi, H., Liu, X., Nguyen, H. C., Zhang, S., Fish, L., and Tavazoie, S. F. (2015). Endogenous tRNA-Derived Fragments Suppress Breast Cancer Progression *via* YBX1 Displacement. *Cell* 161, 790–802. doi:10.1016/j.cell.2015.02.053

Goodarzi, H., Nguyen, H. C. B., Zhang, S., Dill, B. D., Molina, H., and Tavazoie, S. F. (2016). Modulated Expression of Specific tRNAs Drives Gene Expression and Cancer Progression. *Cell* 165, 1416–1427. doi:10.1016/j.cell.2016.05.046

Grzybowska-Szatkowska, L., and Slaska, B. (2012). Polymorphisms in Genes Encoding Mt-tRNA in Female Breast Cancer in Poland. *Mitochondrial DNA* 23, 106–111. doi:10.3109/19401736.2012.660925

Guo, Y., Bosompem, A., Mohan, S., Erdogan, B., Ye, F., Vickers, K. C., et al. (2015). Transfer RNA Detection by Small RNA Deep Sequencing and Disease Association with Myelodysplastic Syndromes. *BMC Genomics* 16, 727. doi:10.1186/s12864-015-1929-y

Haussecker, D., Huang, Y., Lau, A., Parameswaran, P., Fire, A. Z., and Kay, M. A. (2010). Human tRNA-Derived Small RNAs in the Global Regulation of RNA Silencing. *RNA* 16, 673–695. doi:10.1261/rna.2000810

Hayes, J., Peruzzi, P. P., and Lawler, S. (2014). MicroRNAs in Cancer: Biomarkers, Functions and Therapy. *Trends Mol. Med.* 20, 460–469. doi:10.1016/j.molmed.2014.06.005

He, X., Li, F., Bor, B., Koyano, K., Cen, L., Xiao, X., et al. (2018). Human tRNA-Derived Small RNAs Modulate Host-Oral Microbial Interactions. *J. Dent Res.* 97, 1236–1243. doi:10.1177/0022034518770605

Honda, S., Loher, P., Shigematsu, M., Palazzo, J. P., Suzuki, R., Imoto, I., et al. (2015). Sex Hormone-dependent tRNA Halves Enhance Cell Proliferation in Breast and Prostate Cancers. *Proc. Natl. Acad. Sci. U S A.* 112, E3816–E3825. doi:10.1073/pnas.1510077112

Hopper, A. K., and Phizicky, E. M. (2003). tRNA Transfers to the Limelight. *Genes Dev.* 17, 162–180. doi:10.1101/gad.1049103

Horton, R., Wilming, L., Rand, V., Lovering, R. C., Bruford, E. A., Khodiyar, V. K., et al. (2004). Gene Map of the Extended Human MHC. *Nat. Rev. Genet.* 5, 889–899. doi:10.1038/nrg1489

Huang, B., Yang, H., Cheng, X., Wang, D., Fu, S., Shen, W., et al. (2017). tRF/miR-1280 Suppresses Stem Cell-like Cells and Metastasis in Colorectal Cancer. *Cancer Res.* 77, 3194–3206. doi:10.1158/0008-5472.CAN-16-3146

Ivanov, P., Emara, M. M., Villen, J., Gygi, S. P., and Anderson, P. (2011). Angiogenin-induced tRNA Fragments Inhibit Translation Initiation. *Mol. Cel* 43, 613–623. doi:10.1016/j.molcel.2011.06.022

Ivanov, P., O'day, E., Emara, M. M., Wagner, G., Lieberman, J., and Anderson, P. (2014). G-quadruplex Structures Contribute to the Neuroprotective Effects of Angiogenin-Induced tRNA Fragments. *Proc. Natl. Acad. Sci. U S A.* 111, 18201–18206. doi:10.1073/pnas.1407361111

Jara-Palomares, L., Otero, R., Jiménez, D., Praena-Fernández, J. M., Rivas, A., Font, C., et al. (2018). Sex Differences in Patients with Occult Cancer after Venous Thromboembolism. *Clin. Appl. Thromb. Hemost.* 24, 489–495. doi:10.1177/1076029617711805

Jin, D., and Musier-Forsyth, K. (2019). Role of Host tRNAs and Aminoacyl-tRNA Synthetases in Retroviral Replication. *J. Biol. Chem.* 294, 5352–5364. doi:10.1074/jbc.REV118.002957

Johnson, S. A., Dubeau, L., and Johnson, D. L. (2008). Enhanced RNA Polymerase III-dependent Transcription Is Required for Oncogenic Transformation. *J. Biol. Chem.* 283, 19184–19191. doi:10.1074/jbc.M802872200

Karaiskos, S., Naqvi, A. S., Swanson, K. E., and Grigoriev, A. (2015). Age-driven Modulation of tRNA-Derived Fragments in Drosophila and Their Potential Targets. *Biol. Direct* 10, 51. doi:10.1186/s13062-015-0081-6

Keam, S. P., and Hutvagner, G. (2015). tRNA-Derived Fragments (tRFs): Emerging New Roles for an Ancient RNA in the Regulation of Gene Expression. *Life (Basel)* 5, 1638–1651. doi:10.3390/life5041638

Kerr, J., Anderson, C., and Lippman, S. M. (2017). Physical Activity, Sedentary Behaviour, Diet, and Cancer: an Update and Emerging New Evidence. *Lancet Oncol.* 18, e457–e471. doi:10.1016/S1470-2045(17)30411-4

Khattar, E., Kumar, P., Liu, C. Y., Akıncılar, S. C., Raju, A., Lakshmanan, M., et al. (2016). Telomerase Reverse Transcriptase Promotes Cancer Cell Proliferation

by Augmenting tRNA Expression. *J. Clin. Invest.* 126, 4045–4060. doi:10.1172/JCI86042

Kim, E. Y., Lee, J. G., Lee, J. M., Kim, A., Yoo, H. C., Kim, K., et al. (2019). Therapeutic Effects of the Novel Leucyl-tRNA Synthetase Inhibitor BC-LI-0186 in Non-small Cell Lung Cancer. *Ther. Adv. Med. Oncol.* 11, 1758835919846798. doi:10.1177/1758835919846798

Kim, H. K., Fuchs, G., Wang, S., Wei, W., Zhang, Y., Park, H., et al. (2017). A Transfer-RNA-Derived Small RNA Regulates Ribosome Biogenesis. *Nature* 552, 57–62. doi:10.1038/nature25005

Kim, S. H., Suddath, F. L., Quigley, G. J., Mcpherson, A., Sussman, J. L., Wang, A. H., et al. (1974). Three-dimensional Tertiary Structure of Yeast Phenylalanine Transfer RNA. *Science* 185, 435–440. doi:10.1126/science.185.4149.435

Kirchner, S., and Ignatova, Z. (2015). Emerging Roles of tRNA in Adaptive Translation, Signalling Dynamics and Disease. *Nat. Rev. Genet.* 16, 98–112. doi:10.1038/nrg3861

Kumar, P., Anaya, J., Mudunuri, S. B., and Dutta, A. (2014). Meta-analysis of tRNA Derived RNA Fragments Reveals that They Are Evolutionarily Conserved and Associate with AGO Proteins to Recognize Specific RNA Targets. *BMC Biol.* 12, 78. doi:10.1186/s12915-014-0078-0

Kumar, P., Kuscu, C., and Dutta, A. (2016). Biogenesis and Function of Transfer RNA-Related Fragments (tRFs). *Trends Biochem. Sci.* 41, 679–689. doi:10.1016/j.tibs.2016.05.004

Kuscu, C., Kumar, P., Kiran, M., Su, Z., Malik, A., and Dutta, A. (2018). tRNA Fragments (tRFs) Guide Ago to Regulate Gene Expression post-transcriptionally in a Dicer-independent Manner. *RNA* 24, 1093–1105. doi:10.1261/rna.066126.118

Kwon, N. H., Lee, M. R., Kong, J., Park, S. K., Hwang, B. J., Kim, B. G., et al. (2018). Transfer-RNA-mediated Enhancement of Ribosomal Proteins S6 Kinases Signaling for Cell Proliferation. *RNA Biol.* 15, 635–648. doi:10.1080/15476286.2017.1356563

Lee, S. Y. (2018). Factor Structure and Internal Reliability of Cultural Belief Scales about Colorectal Cancer Screening Among Koreans in the Republic of Korea. *BMC Public Health* 18, 1328. doi:10.1186/s12889-018-6240-9

Lee, Y. S., Shibata, Y., Malhotra, A., and Dutta, A. (2009). A Novel Class of Small RNAs: tRNA-Derived RNA Fragments (tRFs). *Genes Dev.* 23, 2639–2649. doi:10.1101/gad.1837609

Levitz, R., Chapman, D., Amitsur, M., Green, R., Snyder, L., and Kaufmann, G. (1990). The Optional *E. coli* Prr Locus Encodes a Latent Form of Phage T4-Induced Anticodon Nuclease. *EMBO J.* 9, 1383–1389. doi:10.1002/j.1460-2075.1990.tb08253.x

Lewandowska, A. M., Rudzki, M., Rudzki, S., Lewandowski, T., and Laskowska, B. (2019). Environmental Risk Factors for Cancer - Review Paper. *Ann. Agric. Environ. Med.* 26, 1–7. doi:10.26444/aaem/94299

Li, S., and Hu, G. F. (2012). Emerging Role of Angiogenin in Stress Response and Cell Survival under Adverse Conditions. *J. Cel Physiol* 227, 2822–2826. doi:10.1002/jcp.23051

Li, Z., Ender, C., Meister, G., Moore, P. S., Chang, Y., and John, B. (2012). Extensive Terminal and Asymmetric Processing of Small RNAs from rRNAs, snoRNAs, snRNAs, and tRNAs. *Nucleic Acids Res.* 40, 6787–6799. doi:10.1093/nar/gks307

Liew, P. L., Huang, R. L., Wu, T. I., Liao, C. C., Chen, C. W., Su, P. H., et al. (2019). Combined Genetic Mutations and DNA-Methylated Genes as Biomarkers for Endometrial Cancer Detection from Cervical Scrapings. *Clin. Epigenetics* 11, 170. doi:10.1186/s13148-019-0765-3

Lu, J., Sharma, L. K., and Bai, Y. (2009). Implications of Mitochondrial DNA Mutations and Mitochondrial Dysfunction in Tumorigenesis. *Cell Res* 19, 802–815. doi:10.1038/cr.2009.69

Madison, J. T., Everett, G. A., and Kung, H. (1966). Nucleotide Sequence of a Yeast Tyrosine Transfer RNA. *Science* 153, 531–534. doi:10.1126/science.153.3735.531

Martens-Uzunova, E. S., Jalava, S. E., Dits, N. F., Van Leenders, G. J., Møller, S., Trapman, J., et al. (2012). Diagnostic and Prognostic Signatures from the Small Non-coding RNA Transcriptome in Prostate Cancer. *Oncogene* 31, 978–991. doi:10.1038/onc.2011.304

Martinez, G., Choudury, S. G., and Slotkin, R. K. (2017). tRNA-derived Small RNAs Target Transposable Element Transcripts. *Nucleic Acids Res.* 45, 5142–5152. doi:10.1093/nar/gkx103

Maute, R. L., Schneider, C., Sumazin, P., Holmes, A., Califano, A., Basso, K., et al. (2013). tRNA-derived microRNA Modulates Proliferation and the DNA Damage Response and Is Down-Regulated in B Cell Lymphoma. *Proc. Natl. Acad. Sci. U S A.* 110, 1404–1409. doi:10.1073/pnas.1206761110

Mcmahon, M., and Ruggero, D. (2018). A Wobbly Road to Drug Resistance in Melanoma: tRNA-Modifying Enzymes in Translation Reprogramming. *EMBO J.* 37, 15. doi:10.15252/embj.201899978

Mei, Y., Yong, J., Liu, H., Shi, Y., Meinkoth, J., Dreyfuss, G., et al. (2010). tRNA Binds to Cytochrome C and Inhibits Caspase Activation. *Mol. Cel* 37, 668–678. doi:10.1016/j.molcel.2010.01.023

Meng, X. L., Meng, H., Zhang, W., Qin, Y. H., and Zhao, N. M. (2016). The Role of Mitochondrial tRNA Variants in Female Breast Cancer. *Mitochondrial DNA A. DNA Mapp. Seq. Anal.* 27, 3199–3201. doi:10.3109/19401736.2015.1007332

Mo, D., Jiang, P., Yang, Y., Mao, X., Tan, X., Tang, X., et al. (2019). A tRNA Fragment, 5'-tiRNAVal, Suppresses the Wnt/β-Catenin Signaling Pathway by Targeting FZD3 in Breast Cancer. *Cancer Lett.* 457, 60–73. doi:10.1016/j.canlet.2019.05.007

Moras, D., Comarmond, M. B., Fischer, J., Weiss, R., Thierry, J. C., Ebel, J. P., et al. (1980). Crystal Structure of Yeast tRNAAsp. *Nature* 288, 669–674. doi:10.1038/288669a0

Nientiedt, M., Deng, M., Schmidt, D., Perner, S., Müller, S. C., and Ellinger, J. (2016). Identification of Aberrant tRNA-Halves Expression Patterns in clear Cell Renal Cell Carcinoma. *Sci. Rep.* 6, 37158. doi:10.1038/srep37158

Novoa, E. M., Pavon-Eternod, M., Pan, T., and Ribas De Pouplana, L. (2012). A Role for tRNA Modifications in Genome Structure and Codon Usage. *Cell* 149, 202–213. doi:10.1016/j.cell.2012.01.050

Novoa, E. M., and Ribas De Pouplana, L. (2012). Speeding with Control: Codon Usage, tRNAs, and Ribosomes. *Trends Genet.* 28, 574–581. doi:10.1016/j.tig.2012.07.006

Olvedy, M., Scaravilli, M., Hoogstrate, Y., Visakorpi, T., Jenster, G., and Martens-Uzunova, E. S. (2016). A Comprehensive Repertoire of tRNA-Derived Fragments in Prostate Cancer. *Oncotarget* 7, 24766–24777. doi:10.18632/oncotarget.8293

Olvedy, M., Scaravilli, M., Hoogstrate, Y., Visakorpi, T., Jenster, G., and Martens-Uzunova, E. S. (2016). A Comprehensive Repertoire of tRNA-Derived Fragments in Prostate Cancer. *Oncotarget* 7, 24766–24777. doi:10.18632/oncotarget.8293

Pak, K., Suh, S., Kim, S. J., and Kim, I. J. (2015). Prognostic Value of Genetic Mutations in Thyroid Cancer: a Meta-Analysis. *Thyroid* 25, 63–70. doi:10.1089/thy.2014.0241

Pan, J. L., Gao, J., Hou, J. H., Hu, D. Z., and Li, L. (2018). Interaction between Environmental Risk Factors and Catechol-O-Methyltransferase (COMT) and X-Ray Repair Cross-Complementing Protein 1 (XRCC1) Gene Polymorphisms in Risk of Lung Cancer Among Non-smoking Chinese Women: A Case-Control Study. *Med. Sci. Monit.* 24, 5689–5697. doi:10.12659/MSM.908240

Pavon-Eternod, M., Gomes, S., Rosner, M. R., and Pan, T. (2013). Overexpression of Initiator Methionine tRNA Leads to Global Reprogramming of tRNA Expression and Increased Proliferation in Human Epithelial Cells. *RNA* 19, 461–466. doi:10.1261/rna.037507.112

Pekarsky, Y., Balatti, V., Palamarchuk, A., Rizzotto, L., Veneziano, D., Nigita, G., et al. (2016). Dysregulation of a Family of Short Noncoding RNAs, tsRNAs, in Human Cancer. *Proc. Natl. Acad. Sci. U S A.* 113, 5071–5076. doi:10.1073/pnas.1604266113

Phizicky, E. M., and Hopper, A. K. (2010). tRNA Biology Charges to the Front. *Genes Dev.* 24, 1832–1860. doi:10.1101/gad.1956510

Porcellini, E., Laprovitera, N., Riefolo, M., Ravaioli, M., Garajova, I., and Ferracin, M. (2018). Epigenetic and Epitranscriptomic Changes in Colorectal Cancer: Diagnostic, Prognostic, and Treatment Implications. *Cancer Lett.* 419, 84–95. doi:10.1016/j.canlet.2018.01.049

Raina, M., and Ibba, M. (2014). tRNAs as Regulators of Biological Processes. *Front. Genet.* 5, 171. doi:10.3389/fgene.2014.00171

Rashad, S., Niizuma, K., and Tominaga, T. (2020). tRNA Cleavage: a New Insight. *Neural Regen. Res.* 15, 47–52. doi:10.4103/1673-5374.264447

Ren, B., Wang, X., Duan, J., and Ma, J. (2019). Rhizobial tRNA-Derived Small RNAs Are Signal Molecules Regulating Plant Nodulation. *Science* 365, 919–922. doi:10.1126/science.aav8907

Rollins, J., Veras, I., Cabarcas, S., Willis, I., and Schramm, L. (2007). Human Maf1 Negatively Regulates RNA Polymerase III Transcription *via* the TFIIB Family Members Brf1 and Brf2. *Int. J. Biol. Sci.* 3, 292–302. doi:10.7150/ijbs.3.292

Romaine, S. P., Tomaszewski, M., Condorelli, G., and Samani, N. J. (2015). MicroRNAs in Cardiovascular Disease: an Introduction for Clinicians. *Heart* 101, 921–928. doi:10.1136/heartjnl-2013-305402

Ruggero, K., Guffanti, A., Corradin, A., Sharma, V. K., De Bellis, G., Corti, G., et al. (2014). Small Noncoding RNAs in Cells Transformed by Human T-Cell Leukemia Virus Type 1: a Role for a tRNA Fragment as a Primer for Reverse Transcriptase. *J. Virol.* 88, 3612–3622. doi:10.1128/JVI.02823-13

Saikia, M., and Hatzoglou, M. (2015). The Many Virtues of tRNA-Derived Stress-Induced RNAs (tiRNAs): Discovering Novel Mechanisms of Stress Response and Effect on Human Health. *J. Biol. Chem.* 290, 29761–29768. doi:10.1074/jbc.R115.694661

Saikia, M., Jobava, R., Parisien, M., Putnam, A., Krokowski, D., Gao, X. H., et al. (2014). Angiogenin-cleaved tRNA Halves Interact with Cytochrome C, Protecting Cells from Apoptosis during Osmotic Stress. *Mol. Cel Biol* 34, 2450–2463. doi:10.1128/MCB.00136-14

Santos, M., Fidalgo, A., Varanda, A. S., Oliveira, C., and Santos, M. A. S. (2019). tRNA Deregulation and its Consequences in Cancer. *Trends Mol. Med.* 25, 853–865. doi:10.1016/j.molmed.2019.05.011

Schageman, J., Zeringer, E., Li, M., Barta, T., Lea, K., Gu, J., et al. (2013). The Complete Exosome Workflow Solution: from Isolation to Characterization of RNA Cargo. *Biomed. Res. Int.* 2013, 253957. doi:10.1155/2013/253957

Schimmel, P. (2018). The Emerging Complexity of the tRNA World: Mammalian tRNAs beyond Protein Synthesis. *Nat. Rev. Mol. Cel Biol* 19, 45–58. doi:10.1038/nrm.2017.77

Schorn, A. J., Gutbrod, M. J., Leblanc, C., and Martienssen, R. (2017). LTR-retrotransposon Control by tRNA-Derived Small RNAs. *Cell* 170, 61–e11. doi:10.1016/j.cell.2017.06.013

Schwartz, M. H., Wang, H., Pan, J. N., Clark, W. C., Cui, S., Eckwahl, M. J., et al. (2018). Microbiome Characterization by High-Throughput Transfer RNA Sequencing and Modification Analysis. *Nat. Commun.* 9, 5353. doi:10.1038/s41467-018-07675-z

Selitsky, S. R., Baran-Gale, J., Honda, M., Yamane, D., Masaki, T., Fannin, E. E., et al. (2015). Small tRNA-Derived RNAs Are Increased and More Abundant Than microRNAs in Chronic Hepatitis B and C. *Sci. Rep.* 5, 7675. doi:10.1038/srep07675

Shao, Y., Sun, Q., Liu, X., Wang, P., Wu, R., and Ma, Z. (2017). tRF-Leu-CAG Promotes Cell Proliferation and Cell Cycle in Non-small Cell Lung Cancer. *Chem. Biol. Drug Des.* 90, 730–738. doi:10.1111/cbdd.12994

Shao, Y., Sun, Q., Liu, X., Wang, P., Wu, R., and Ma, Z. (2017). tRF-Leu-CAG Promotes Cell Proliferation and Cell Cycle in Non-small Cell Lung Cancer. *Chem. Biol. Drug Des.* 90, 730–738. doi:10.1111/cbdd.12994

Shen, L., Tan, Z., Gan, M., Li, Q., Chen, L., Niu, L., et al. (2019). tRNA-Derived Small Non-coding RNAs as Novel Epigenetic Molecules Regulating Adipogenesis. *Biomolecules* 9, 7. doi:10.3390/biom9070274

Shen, Y., Kassavetis, G. A., Bryant, G. O., and Berk, A. J. (1998). Polymerase (Pol) III TATA Box-Binding Protein (TBP)-associated Factor Brf Binds to a Surface on TBP Also Required for Activated Pol II Transcription. *Mol. Cel Biol* 18, 1692–1700. doi:10.1128/mcb.18.3.1692

Shigematsu, M., and Kirino, Y. (2017). 5'-Terminal Nucleotide Variations in Human Cytoplasmic tRNAHisGUG and its 5'-halves. *RNA* 23, 161–168. doi:10.1261/rna.058024.116

Sobala, A., and Hutvagner, G. (2013). Small RNAs Derived from the 5' End of tRNA Can Inhibit Protein Translation in Human Cells. *RNA Biol.* 10, 553–563. doi:10.4161/rna.24285

Stout, C. D., Mizuno, H., Rubin, J., Brennan, T., Rao, S. T., and Sundaralingam, M. (1976). Atomic Coordinates and Molecular Conformation of Yeast Phenylalanyl tRNA. An Independent Investigation. *Nucleic Acids Res.* 3, 1111–1123. doi:10.1093/nar/3.4.1111

Suzuki, T., Nagao, A., and Suzuki, T. (2011). Human Mitochondrial tRNAs: Biogenesis, Function, Structural Aspects, and Diseases. *Annu. Rev. Genet.* 45, 299–329. doi:10.1146/annurev-genet-110410-132531

Tejeda, S., Gallardo, R. I., Ferrans, C. E., and Rauscher, G. H. (2017). Breast Cancer Delay in Latinas: the Role of Cultural Beliefs and Acculturation. *J. Behav. Med.* 40, 343–351. doi:10.1007/s10865-016-9789-8

Telonis, A. G., Loher, P., Honda, S., Jing, Y., Palazzo, J., Kirino, Y., et al. (2015). Dissecting tRNA-Derived Fragment Complexities Using Personalized Transcriptomes Reveals Novel Fragment Classes and Unexpected Dependencies. *Oncotarget* 6, 24797–24822. doi:10.18632/oncotarget.4695

Thompson, D. M., and Parker, R. (2009). Stressing Out over tRNA Cleavage. *Cell* 138, 215–219. doi:10.1016/j.cell.2009.07.001

Towns, W. L., and Begley, T. J. (2012). Transfer RNA Methytransferases and Their Corresponding Modifications in Budding Yeast and Humans: Activities, Predications, and Potential Roles in Human Health. *DNA Cel Biol* 31, 434–454. doi:10.1089/dna.2011.1437

Veneziano, D., Tomasello, L., Balatti, V., Palamarchuk, A., Rassenti, L. Z., Kipps, T. J., et al. (2019). Dysregulation of Different Classes of tRNA Fragments in Chronic Lymphocytic Leukemia. *Proc. Natl. Acad. Sci. U S A.* 116, 24252–24258. doi:10.1073/pnas.1913695116

Wang, H. D., Trivedi, A., and Johnson, D. L. (1997). Hepatitis B Virus X Protein Induces RNA Polymerase III-dependent Gene Transcription and Increases Cellular TATA-Binding Protein by Activating the Ras Signaling Pathway. *Mol. Cel Biol* 17, 6838–6846. doi:10.1128/mcb.17.12.6838

Wang, Q., Lee, I., Ren, J., Ajay, S. S., Lee, Y. S., and Bao, X. (2013). Identification and Functional Characterization of tRNA-Derived RNA Fragments (tRFs) in Respiratory Syncytial Virus Infection. *Mol. Ther.* 21, 368–379. doi:10.1038/mt.2012.237

Wang, Z., Xiang, L., Shao, J., and Yuan, Z. (2006). The 3' CCACCA Sequence of tRNAAla(UGC) Is the Motif that Is Important in Inducing Th1-like Immune Response, and This Motif Can Be Recognized by Toll-like Receptor 3. *Clin. Vaccin. Immunol* 13, 733–739. doi:10.1128/CVI.00019-06

White, R. J., Trouche, D., Martin, K., Jackson, S. P., and Kouzarides, T. (1996). Repression of RNA Polymerase III Transcription by the Retinoblastoma Protein. *Nature* 382, 88–90. doi:10.1038/382088a0

Winn, A. N., Ekwueme, D. U., Guy, G. P., Jr., and Neumann, P. J. (2016). Cost-Utility Analysis of Cancer Prevention, Treatment, and Control: A Systematic Review. *Am. J. Prev. Med.* 50, 241–248. doi:10.1016/j.amepre.2015.08.009

Yamasaki, S., Ivanov, P., Hu, G. F., and Anderson, P. (2009). Angiogenin Cleaves tRNA and Promotes Stress-Induced Translational Repression. *J. Cel Biol* 185, 35–42. doi:10.1083/jcb.200811106

Yao, P., and Fox, P. L. (2013). Aminoacyl-tRNA Synthetases in Medicine and Disease. *EMBO Mol. Med.* 5, 332–343. doi:10.1002/emmm.201100626

Yeung, M. L., Bennasser, Y., Watashi, K., Le, S. Y., Houzet, L., and Jeang, K. T. (2009). Pyrosequencing of Small Non-coding RNAs in HIV-1 Infected Cells: Evidence for the Processing of a Viral-Cellular Double-Stranded RNA Hybrid. *Nucleic Acids Res.* 37, 6575–6586. doi:10.1093/nar/gkp707

Zhang, J., and Huang, K. (2017). Pan-cancer Analysis of Frequent DNA Co-methylation Patterns Reveals Consistent Epigenetic Landscape Changes in Multiple Cancers. *BMC Genomics* 18, 1045. doi:10.1186/s12864-016-3259-0

Zhang, M., Li, F., Wang, J., He, W., Li, Y., Li, H., et al. (2019). tRNA-derived Fragment tRF-03357 Promotes Cell Proliferation, Migration and Invasion in High-Grade Serous Ovarian Cancer. *OncoTargets Ther.* 12, 6371–6383. doi:10.2147/ott.s206861

Zhang, M., Li, F., Wang, J., He, W., Li, Y., Li, H., et al. (2019). tRNA-derived Fragment tRF-03357 Promotes Cell Proliferation, Migration and Invasion in High-Grade Serous Ovarian Cancer. *Onco Targets Ther.* 12, 6371–6383. doi:10.2147/OTT.S206861

Zhang, S., Sun, L., and Kragler, F. (2009). The Phloem-Delivered RNA Pool Contains Small Noncoding RNAs and Interferes with Translation. *Plant Physiol.* 150, 378–387. doi:10.1104/pp.108.134767

Zhang, X., He, X., Liu, C., Liu, J., Hu, Q., Pan, T., et al. (2016). IL-4 Inhibits the Biogenesis of an Epigenetically Suppressive PIWI-Interacting RNA to Upregulate CD1a Molecules on Monocytes/Dendritic Cells. *J. Immunol.* 196, 1591–1603. doi:10.4049/jimmunol.1500805

Zhang, Y., Zhang, Y., Shi, J., Zhang, H., Cao, Z., Gao, X., et al. (2014). Identification and Characterization of an Ancient Class of Small RNAs Enriched in Serum Associating with Active Infection. *J. Mol. Cel Biol* 6, 172–174. doi:10.1093/jmcb/mjt052

Zhao, C., Tolkach, Y., Schmidt, D., Kristiansen, G., Müller, S. C., and Ellinger, J. (2018). 5'-tRNA Halves Are Dysregulated in Clear Cell Renal Cell Carcinoma. *J. Urol.* 199, 378–383. doi:10.1016/j.juro.2017.07.082

Zhou, J., Liu, S., Chen, Y., Fu, Y., Silver, A. J., Hill, M. S., et al. (2017). Identification of Two Novel Functional tRNA-Derived Fragments Induced in Response to Respiratory Syncytial Virus Infection. *J. Gen. Virol.* 98, 1600–1610. doi:10.1099/jgv.0.000852

Zhou, Y., Goodenbour, J. M., Godley, L. A., Wickrema, A., and Pan, T. (2009). High Levels of tRNA Abundance and Alteration of tRNA Charging by Bortezomib in Multiple Myeloma. *Biochem. Biophys. Res. Commun.* 385, 160–164. doi:10.1016/j.bbrc.2009.05.031

Zhu, L., Li, J., Gong, Y., Wu, Q., Tan, S., Sun, D., et al. (2019). Exosomal tRNA-Derived Small RNA as a Promising Biomarker for Cancer Diagnosis. *Mol. Cancer* 18, 74. doi:10.1186/s12943-019-1000-8

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Qiu, Wang, Liu, Li, Hao, Ning, Zhang, Zhang, Chen, Wu, Wang, Yang, Lin and Xu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 23 December 2021
doi: 10.3389/fphar.2021.795386

AhR Antagonist Promotes Differentiation of Papillary Thyroid Cancer *via* Regulating circSH2B3/miR-4640-5P/IGF2BP2 Axis

*Ri Sa[1], Meiliang Guo[2], Danyan Liu[3] and Feng Guan[1]**

[1]Department of Nuclear Medicine, The First Hospital of Jilin University, Changchun, China, [2]Department of Dermatology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China, [3]Department of Radiology, The First Hospital of Jilin University, Changchun, China

Abnormally high expression of aryl hydrocarbon receptor (AhR) has been implicated in dedifferentiation of radioiodine-refractory papillary thyroid cancer (RR-PTC). This study aimed to evaluate the differentiation effect of AhR antagonist in PTC, and to explore the potential mechanism of it. Results showed that AhR antagonists promoted differentiation of PTC, as shown as increase in ^{125}I uptake and Na/I symporter (NIS) expression level. CircRNA microarray in K1 cells treated with StemRegenin 1(SR1) revealed that hsa_circ_0006741 (circSH2B3) was down-regulated in SR1 treated K1 cells. Downregulation of circSH2B3 increased ^{125}I uptake and NIS expression levels. CircSH2B3 acted as an endogenous sponge of hsa-miR-4640-5p and modulated IGF2BP2 expression. IGF2BP2 overexpression induced dedifferentiation of PTC, while silencing IGF2BP2 accelerated differentiation of PTC cells. Rescue studies showed that the dedifferentiation activity of AhR was modulated by the circSH2B3/miR-4640-5p/IGF2BP2 axis. Our findings confirmed for the first time that AhR antagonists promote differentiation of PTC via inhibiting the circSH2B3/miR-4640-5p/IGF2BP2 axis, offering a novel therapeutic approach and a potential marker for differentiation of PTC.

Keywords: AhR antagonist, papillary thyroid cancer, differentiation, circSH2B3, IGF2BP2

OPEN ACCESS

Edited by:
Yongye Huang,
Northeastern University, China

Reviewed by:
Rajwali Khan,
University of Agriculture, Peshawar, Pakistan
Mahmoud Omidi,
Hormozgan University of Medical Sciences, Iran

***Correspondence:**
Feng Guan
guanfeng@jlu.edu.cn

Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

Received: *15 October 2021*
Accepted: *23 November 2021*
Published: *23 December 2021*

Citation:
Sa R, Guo M, Liu D and Guan F (2021) AhR Antagonist Promotes Differentiation of Papillary Thyroid Cancer via Regulating circSH2B3/miR-4640-5P/IGF2BP2 Axis. Front. Pharmacol. 12:795386. doi: 10.3389/fphar.2021.795386

INTRODUCTION

According to the 2020 global cancer statistics, thyroid cancer is one of the most common endocrine malignancies in the worldwide, with responsible for approximately 58,600 cases, ranking in ninth place for incidence in 2020 (Sung et al., 2021). Papillary thyroid cancer (PTC) is the most prevalent subtype of thyroid cancer, accounting for 80% of all thyroid cancers. Radioiodine (^{131}I) therapy has been the standard of care for unresectable ^{131}I -avid metastatic PTC, and ^{131}I uptake is a good prognostic marker (Ahn, 2016). Although most patients do well, the biology of PTC is extremely diverse, ranging from well ^{131}I-responsive indolent lesions to ^{131}I-refractory locally advanced or metastatic disease. The mean life span of ^{131}I refractory disease is less than 5 years and the 10-years-survival rate is often less than 10% (Durante et al., 2006).

Differentiation therapy followed by ^{131}I is a promising alternative therapy for radioiodine refractory thyroid cancer (RR-PTC). To date, several differentiation therapies including retinoic acid (Schmutzler and Köhrle, 2000), tyrosine-kinase inhibitors (Oh et al., 2020), histone deacetylase inhibitors (Jang et al., 2015), peroxisome proliferator-activated receptor (PPAR)-γ agonists etc.

(Park et al., 2005), have been adopted to enhance Na/I symporter (NIS) function to reverse the dedifferentiation of RR-PTC and support ^{131}I therapy. It is feasible, theses differentiation strategies have already brought significant clinical benefits (Buffet et al., 2020). However, only a limited number of patients got benefit from these therapeutic strategies, necessitating the development of other new agents to aid the differentiation of PTC, as well as further research to look into the underlying potential mechanism of dedifferentiation of PTC.

More recent work suggests that the aryl hydrocarbon receptor (AhR), a cytosolic ligand-activated transcription factor, is expressed at aberrantly low or high levels in several malignancies, integrating with promotion or inhibition of cell differentiation (Zhao et al., 2019). Thus, AhR agonists and antagonists have been used to mediate cell differentiation. In melanoma, AhR agonists, for example, can activate the AhR-dependent resistant program to induce dedifferentiation, whereas AhR antagonists can abrogate deleterious AhR sustained-activation to overcome dedifferentiation and drug resistance (Corre et al., 2018). In leukemic stem cells, the AhR pathway is suppressed (Ly et al., 2019). By down-regulating the stem cell transcription factor Oct4, AhR enhanced retinoic acid induced differentiation of myeloblastic leukemia cells (Bunaciu and Yen, 2011). Given that AhR contributes to cell differentiation in several malignancies, there is a strong likelihood that AhR might be involved in differentiation of PTC.

Herein, we confirmed the differentiation effect of AhR antagonists in PTC. *Via* circRNA microarray, we discovered a previously undescribed circRNA hsa_circ_0006741 (termed as circSH2B3) contributing to AhR antagonist induced differentiation of PTC. Therefore, this study was designed to explore the role of circSH2B3 in AhR antagonist induced differentiation of PTC and elucidate the underlying mechanism.

MATERIAL AND METHODS

Cell Culture and Agents

A normal thyroid follicular epithelial cell line (Nthy-ori 3-1) and PTC cell lines (K1 and BCPAP) were purchased from the Chinese Academy of Sciences. All cell lines had been tested and authenticated by DNA analysis. The cells were cultured in RPMI 1640 supplemented with 20% fetal bovine serum (FBS), 100 units/ml of penicillin, and 100 μg/ml streptomycin at 37°C and 5% CO2. AhR antagonists, StemRegenin 1 and CH-223191, were purchased from Selleck.

Plasmid Construction and Transfection

Human short hairpin RNA (shRNA) and overexpressed circSH2B3 were designed and synthesized by Genomeditech (Shanghai, China). Hsa-miR-4640-5p mimics or inhibitors, as well as their controls were synthesized by Genomeditech (Shanghai, China). Cell transfection was performed using Lipofectamine 2000 (Invitrogen, NY, United States) according to the manufacturer's instruction. shRNAs and overexpressed of IGF2BP2 lentivirus plasmid (HanBio, Shanghai, China) were transfected into the polybrene treated PTC cells. In the presence of 5 μg/ml puromycin, the transfection lentivirus content was according to the individual multiplicity of infection. Empty vector was used as a negative control. qRT-PCR was used to assess transfection efficiency.

Cell Viability Assay

Cells were grown in the presence of different doses of StemRegenin 1 (0, 62.5, 125, 250, 500, 1,000, 2000, 4,000 nM) or CH-223191 (0, 7.8, 15.6, 31.3, 62.5, 125, 250, 500 nM) for 48 h. 10 μL of CCK8 reagent (Yeasen, Shanghai, China) was added, incubate for 2 h, and the absorbance measured at 450 nm. Using GraphPad Prism v. 8.0 (GraphPad Software, CA, United States), the half-maximal inhibitory concentration (IC_{50}) was determined based on the relative survival curve.

^{125}I Uptake Assay

Cells (1.5×10^5) were seeded in six-well plates, treated with DMSO, AhR antagonist, or transfected with shRNA or plasmid, and incubated for 48 h. Cells were incubated in 1 ml serum-free RPMI 1640 which contains 74 kBq $Na^{125}I$ at 37°C for 1 h. The $Na^{125}I$ containing medium was withdrawn, and the cells were washed twice in PBS before being lysed on ice with 0.3 M sodium hydroxide. The radioactivity of cell lysates was counted by a γ counter. To normalize the cell counts, each group had one well without $Na^{125}I$ treatment for counting cell numbers.

^{131}I Colony Formation Assay

Cells (5×10^2) were seeded into six-well plates, and treated with DMSO, AhR antagonist or transfected with shRNA or plasmid, and incubated for 48 h. Then, the medium was discarded, and cells were washed twice in PBS. One mL of culture medium with or without 20 μCi $Na^{131}I$ was added for 6 h, and the radioactive medium was discarded. Cells were cultured in regular medium approximately 1 week. Cell colonies were fixed by 4% paraformaldehyde and stained with crystal violet. The number of cell colonies was determined by counting.

RNase R and Actinomycin D Treatment, and Quantitative Real-Time PCR (qRT-PCR) Assay

Total RNA was extracted using the RNA-Quick Purification Kit (Vazyme, Shanghai, China). For the RNase R treatment, 2 μg of total RNA was incubated with or without 3 U/mg RNase R (Geneseed, Guangzhou, China) for 15 min at 37°C. For the Actinomycin D treatment, transcription was prevented by the addition of 2 mg/ml Actinomycin D. One μg of total RNA was transcribed into cDNA on an ABI Veriti™ 96-Well Thermal Cycler (Thermo Fisher) using HiScript II Q RT SuperMix for qPCR (R223-01, Vazyme) for mRNA and circRNA analysis, and miRNA 1st Strand cDNA Synthesis Kit (MR101-01, Vazyme) for miRNA analysis. The qRT-PCR reaction was carried out in a Thermofisher 7500 Real-Time PCR system using AceQ Universal SYBR qPCR Master Mix for mRNA and circRNA (Q511-02, Vazyme) and miRNA Universal SYBR qPCR Master Mix (MQ101-01/02, Vazyme). All sequences were presented in **Supplementary Table S1**.

Western Blotting Analysis

The cells were lysed in RIPA buffer. Equal amounts of total protein were resolved, transferred to PVDF membranes (Millipore) and immunoblotted with the specified primary antibodies. Membranes were hybridized with the following primary antibodies: AhR (Abcam, #ab190797, 1:1000), NIS (Abcam, #ab242007, 1:1000), IGF2BP2 (Abcam, #ab124930, 1:2000), Histone 3 (Abcam, # ab267372, 1:1000) and β-actin (Abcam, #ab8227, 1:1000). Species-specific HRP-conjugated antibodies (1:5000; Cell Signaling Technology) were used to hybridize membranes. The cytoplasm and nuclear proteins were recovered and separated using the nuclear and Cytoplasmic Protein Extraction Kit (Yeasen, Shanghai, China), following cell lysis. The Potent ECL kit (Yeasen, Shanghai, China) was used to visualize the bands.

Immunofluorescent Localization of Na/I Symporter

Cells were seeded in six-well chamber slides and treated with StemRegenin 1 (100 nM). After 48 h, the cells were washed twice with PBS and fixed for 15 min with 4% paraformaldehyde. The cells were blocked with 1% BSA for 1 h. The cells were then treated with rabbit anti-NIS (1:100; Abcam), and goat Anti-Rabbit IgG H&L (Alexa Fluor® 647) (Abcam) diluted at 1:100, followed by DAPI.

Dual-Luciferase Reporter Assay

The wild-type sequences of cicrSH2B3 and IGF2BP2 3′-UTR as well as their corresponding mutant sequences targeting hsa-miR-4640-5p binding sites were synthesized. These sequences were subcloned into the luciferase reporter vector cicrSH2B3 (Genomeditech, Shanghai, China) respectively. Vectors were co-transfected into K1 cells by HG transgene reagent (Genomeditech, Shanghai, China) according to the manufacturer's instructions. The relative luciferase activities were determined using the Dual-luciferase Assay Kit (Genomeditech, Shanghai, China) following the manufacturer's instructions.

Immunohistochemistry (IHC)

For IHC, 5-μm thyroid cancer sections were treated with xylene, 100% alcohol, 95% alcohol, 85% alcohol, 75% alcohol and then Tris/EDTA buffer was used for antigen retrieval. Then the samples were incubated with anti-AhR (1:500 dilution in PBS) for 2 h. After PBS washing, HRP-conjugated secondary (1:500 dilution in PBS) and 3, 3′-diaminobenzidine were used for visualization. All staining was scored using a semiquantitative method in terms of the staining intensity and the positive rate. In detail, the staining intensity was determined as 0 = negative, 1 = weak, 2 = moderate, and 3 = strong, while the positive rate was defined as 0, < 1%; 1, 1–25%; 2, 26–50%; 3, 51–75%; and 4, 75–100%. IHC scoring > 6 and ≤ 6 were defined as high and low expression, respectively (Xu et al., 2019).

Statistical Analysis

The data is represented as means ± standard deviation. SPSS 22.0 (SPSS, Chicago, IL, United States) was used to perform all the statistical analyses. A one-way analysis of variance was used to assess differences across groups, while the Student's *t*-test was used to assess differences between groups. The statistical significance level was set at $p < 0.05$.

TABLE 1 | Clinicopathological features of patients with metastatic papillary thyroid cancer according to the expression of aryl hydrocarbon receptor (*N* = 32).

Characteristic	High (*n* = 18)	Low (*n* = 14)	*p* value
Age	—	—	0.928
≥55 years	8	6	—
<55 years	10	8	—
Sex	—	—	0.957
Female	14	11	—
Male	4	3	—
Size	—	—	0.301
≤2.0 cm	9	10	—
2.0 ~ 4.0 cm	5	2	—
>4.0 cm	4	2	—
Metastatic site(s)	—	—	0.667
Lymph node-only	6	4	—
Lung-only	4	3	—
Bone-only	2	1	—
Others or combined	6	6	—
Rx-WBS after initial ^{131}I therapy	—	—	0.011[a]
^{131}I-avid	4	10	—
Non-^{131}I-avid	14	4	—
Tg_{on} (ng/ml)	53.7 ± 13.2	48.9 ± 23.5	0.338
Tg_{off} (ng/ml)	374.7 ± 79.1	294.2 ± 109.3	0.673

^{131}I, radioiodine; Rx-WBS, post-therapeutic whole body scan; Tg_{on}, suppressed thyroglobulin; Tg_{off}, stimulated thyroglobulin.
[a]Statistical significant difference.

RESULTS

AhR Antagonists Induce Differentiation of PTC

Previously published findings have indicated that AhR was up-regulated in thyroid cancer, but the role of AhR in the differentiation of PTC was still unclear (Moretti et al., 2020). According to AhR levels, clinicopathological data deriving from a total of 32 PTC patients with lymph node or distant metastases were analyzed, in which only 4 (22.2%) patients in high AhR group showed ^{131}I positive lesions on post-therapeutic whole body scan (Rx-WBS) after the initial ^{131}I treatment, while 10 (71.4%) patients in low AhR group showed ^{131}I positive lesions on Rx-WBS after the initial ^{131}I treatment. AhR was correlated with condition of ^{131}I uptake of lesions after the initial ^{131}I treatment ($p = 0.011$) (**Table 1**), indicating that AhR might play an important role in the differentiation of PTC. Representative cases were shown in **Figure 1**. Further, we used AhR antagonists, StemRegenin 1 (SR1) and CH-223191 (CH) in PTC cells (BCPAP and K1) to evaluate the differentiation of PTC. The IC_{50} value for SR1 and CH were 225.3 and 43.1 nM in BCPAP, while 274.4 and 50.3 nM in K1, respectively (**Figure 2A**). SR1 or CH was administered to stimulate the differentiation of PTC cells for 48 h. ^{125}I uptake of BCPAP cells treated with SR1 (100 nM) or CH (20 nM) was 3.32-, and 3.46-fold higher than those of control cells (both $p < 0.05$), and 3.02- and 2.67-fold higher in K1 cells (both $p < 0.05$) (**Figure 2B**). ^{131}I colony formation assay showed that the cell colonies in PTC cells treated with SR1 (100 nM) or CH (20 nM) were reduced (**Figure 2C**). Since NIS is the critical factor which is

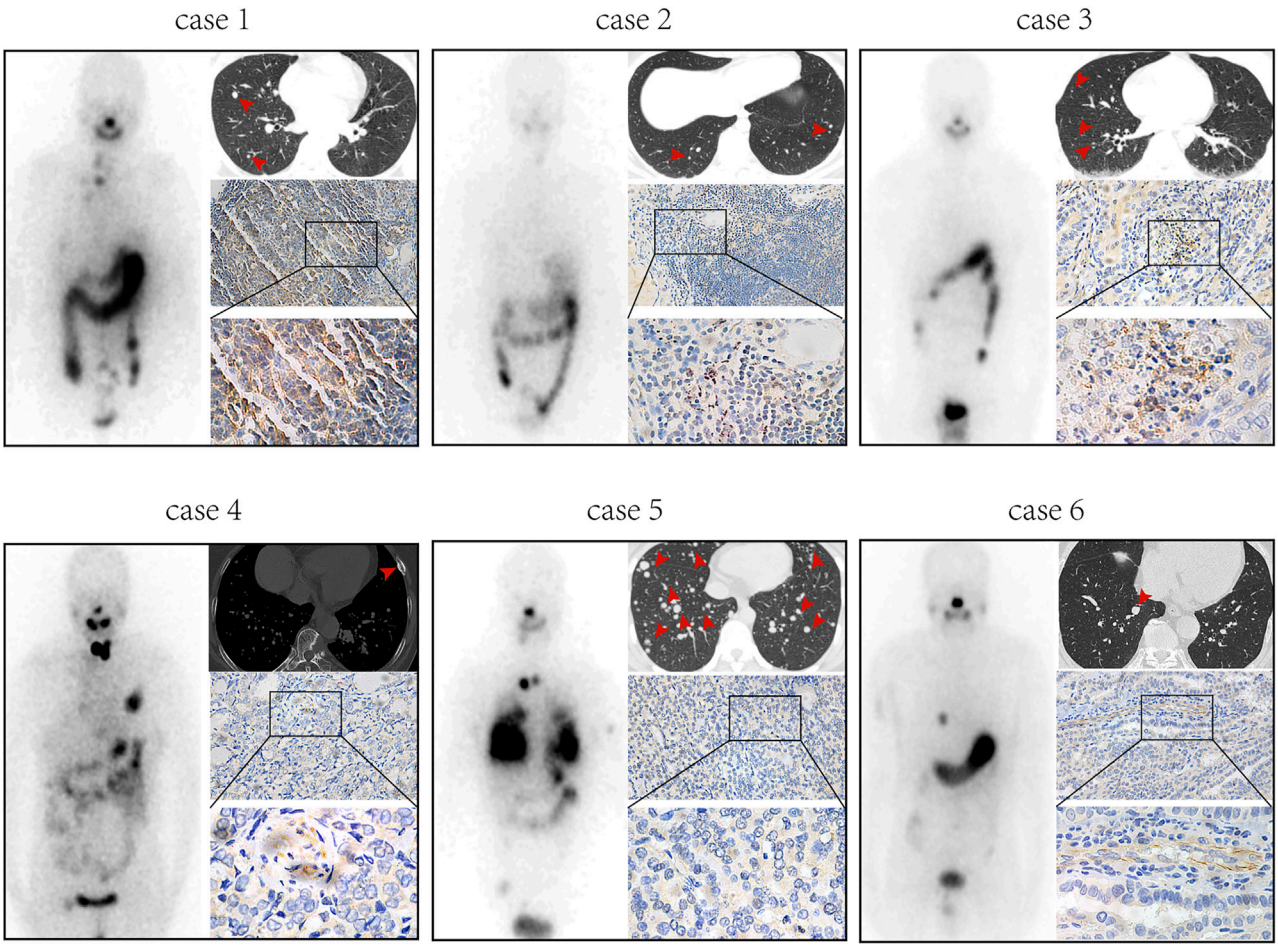


FIGURE 1 | AhR is associated with ^{131}I uptake of lesions in PTC patients with lymph node or distant metastases. Post-therapeutic whole body scan, CT, and immunohistochemistry of AhR in patients with high expression of AhR (cases 1-3) and patients with low expression of AhR (cases 4-6).

responsible for differentiation of PTC, the mRNA and protein levels of NIS in PTC cells treated with AhR antagonists are determined. The mRNA expression of NIS was distinctly enhanced in PTC cells treated with SR1 (100 nM) or CH (20 nM) (**Figure 2D**). With regard to the protein level, various concentration of SR1 (0, 12.5, 25, 50, 100, 200 nM) were used. The protein levels of NIS were significantly increased in PTC cells treated with 50 or 100 nM SR1 (**Figure 2E**). Furthermore, NIS protein was found to be distinctly localized on the membrane of PTC cells after treated with SR1 (100 nM) (**Figure 2F**). These results suggested that AhR antagonists promote the differentiation of PTC cells.

Differentiation Associated hsa_circ_0006741 is Down-regulated in AhR Antagonist Treated PTC Cells

Using circRNA microarray, we examined the differentially expressed circRNAs in K1 control and SR1 treated K1 cells to determine the potential circRNAs that contributed to the differentiation effect of AhR antagonists. By comparing SR1 treated K1 cells to control cells, we found 23 circRNAs with significant up-regulation and 21 circRNAs with significant down-regulation. Fold change filtering was used to identify differential expression patterns of circRNAs, and a heatmap of these circRNAs was generated (**Figure 3A**). Among these differentially expressed circRNAs, hsa_circ_0006741 was associated with cell differentiation and was down-regulated in AhR antagonist treated K1 cells. Therefore, we selected hsa_circ_0006741 for further analysis.

Hsa_circ_0006741 is formed by the circularization of exons 2 of the SH2B3 gene with 759 spliced lengths (hereafter referred to as circSH2B3) and located at chr12:111405107_111451623. Sanger sequencing was conducted to confirm the predicted head-to-tail splicing junction in the qRT-PCR product of circSH2B3 identified by its expected size using well-designed divergent primers (**Figure 3B**). The circSH2B3 expression was higher in multiple PTC cells than in normal thyroid cells (Nthy-ori 3-1), and BCPAP and K1 cells were chosen for further investigation (**Figure 3C**). Following that, RNase R was applied to treat PTC cells to rule out the possibility that head-to-tail splicing may be produced by genomic rearrangement or

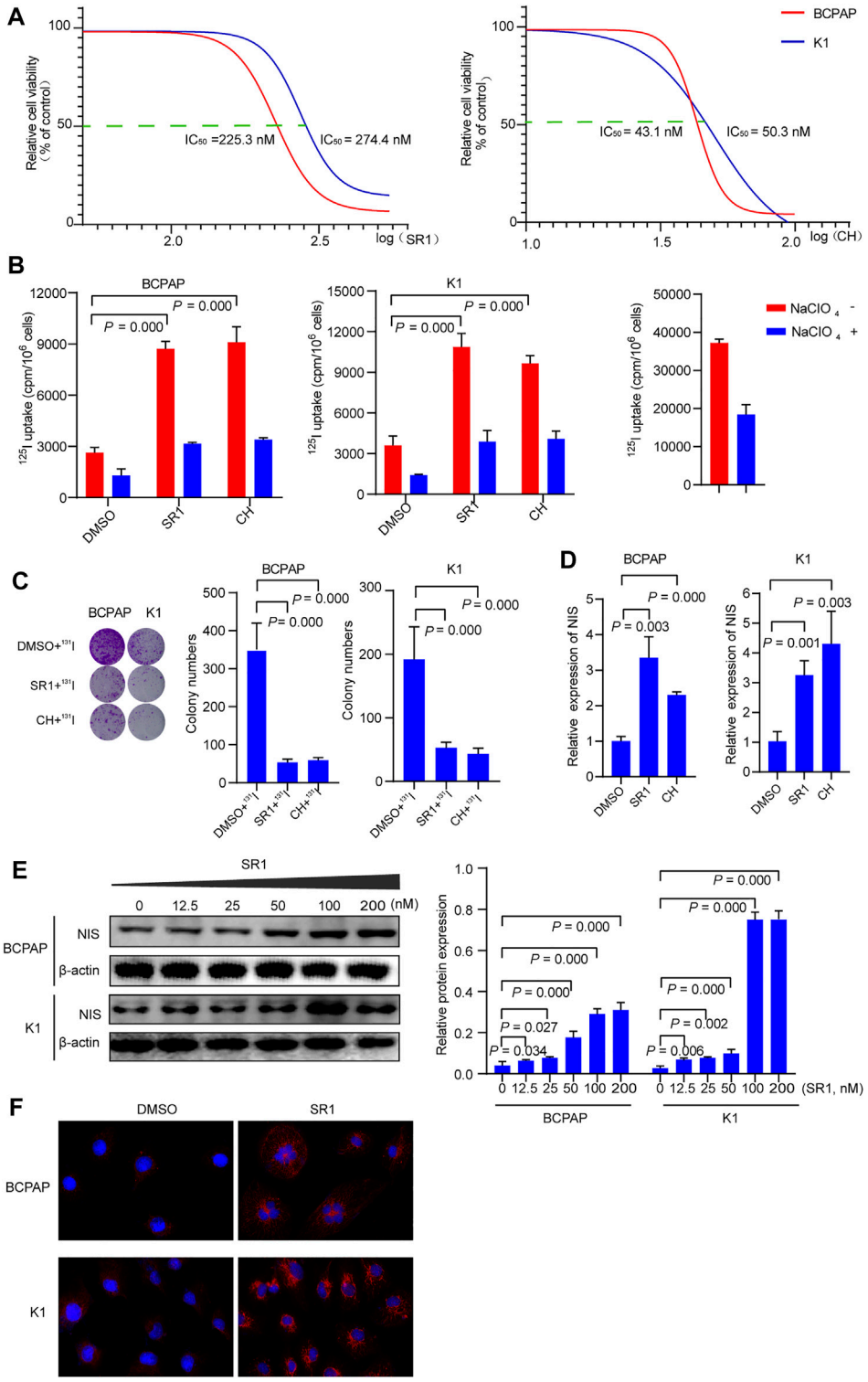


FIGURE 2 | AhR antagonists promote differentiation of PTC cells. **(A)** IC_{50} of SR1 and CH in BCPAP and K1 cells. **(B)** ^{125}I uptake in BCPAP and K1 cells treated with SR1 and CH. **(C)** ^{131}I colony formation in BCPAP and K1 cells treated with SR1 and CH. **(D)** Relative expression of NIS in BCPAP and K1 cells treated with SR1 and CH. **(E)** The protein levels of NIS in BCPAP and K1 cells treated with SR1. **(F)** Localization of NIS in BCPAP and K1 cells treated with SR1. SR1, StemRegenin 1; CH, CH-223191.

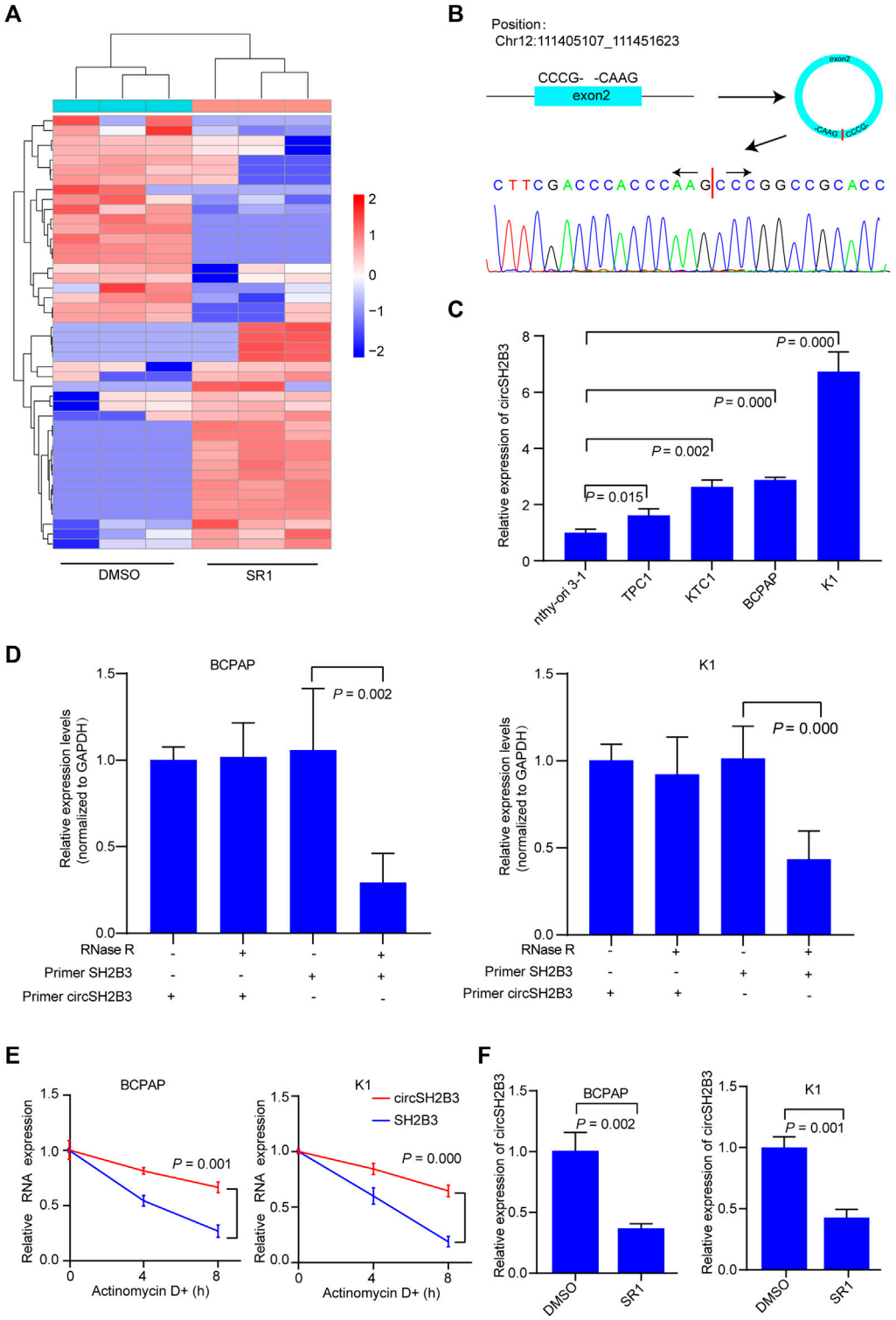


FIGURE 3 | CircSH2B3 is down-regulated in SR1 treated PTC cells. **(A)** Heatmap of different expressed circRNAs in SR1 treated K1 cells and control cells. Red indicated up-regulated while green indicated down-regulated. **(B)** Schematic illustration demonstrating SH2B3 exon 2 circularization to form circSH2B3. The presence of circSH2B3 is validated by Sanger sequencing. **(C)** Relative expression of circSH2B3 in PTC cell lines. **(D)** Relative expression of circSH2B3 and SH2B3 mRNA in BCPAP and K1 cells treated with or without RNase R. **(E)** Relative expression of circSH2B3 and SH2B3 mRNA in BCPAP and K1 cells treated with Actinomycin D. **(F)** Relative expression of circSH2B3 in BCPAP and K1 cells treated with SR1. SR1, StemRegenin 1.

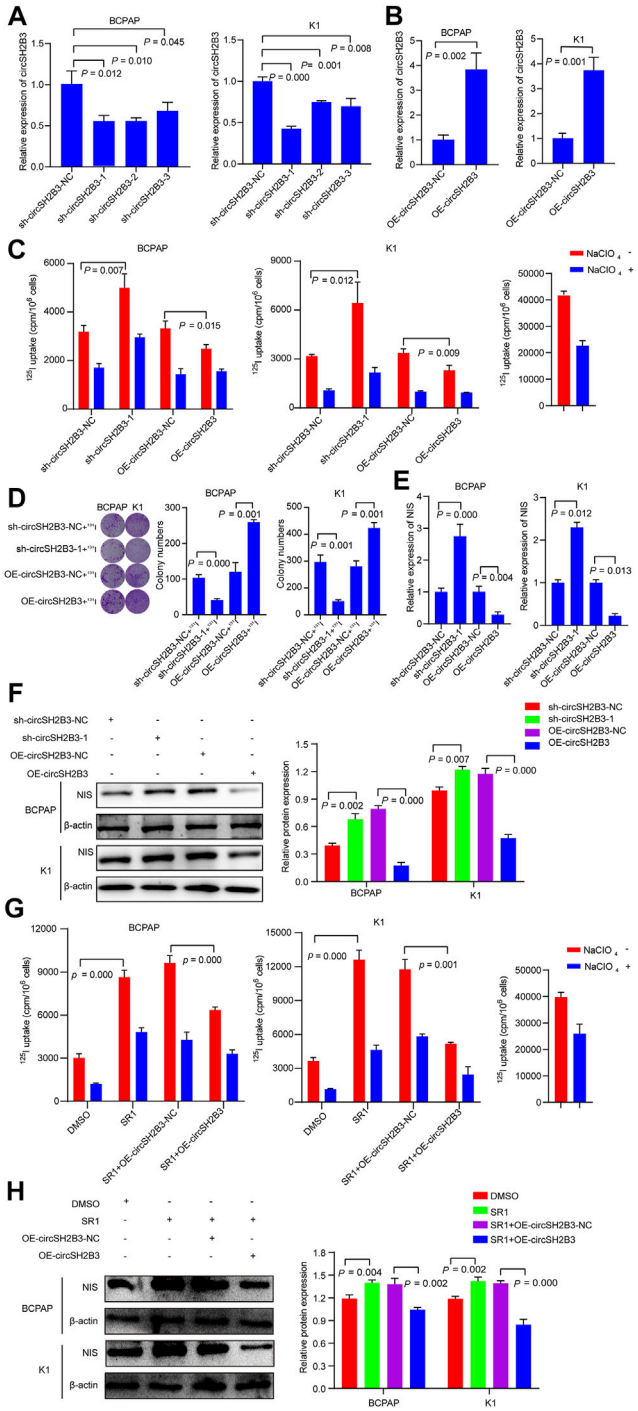


FIGURE 4 | CircSH2B3 plays a key role in SR1 induced differentiation of PTC. **(A,B)** Relative expression of circSH2B3 in BCPAP and K1 cells transfected with circSH2B3 shRNA (sh-circSH2B3) and overexpression (OE-circSH2B3) plasmids. **(C)** ^{125}I uptake in BCPAP and K1 cells transfected with sh-circSH2B3 or OE-circSH2B3. **(D)** ^{131}I colony formation in BCPAP and K1 cells transfected with sh-circSH2B3 or OE-circSH2B3. **(E)** Relative expression of NIS in BCPAP and K1 cells transfected with sh-circSH2B3 or OE-circSH2B3. **(F)** The protein levels of NIS in BCPAP and K1 cells

(Continued)

FIGURE 4 | transfected with sh-circSH2B3 or OE-circSH2B3. **(G)** ^{125}I uptake in SR1 treated BCPAP and K1 cells co-transfected with OE-circSH2B3-NC or OE-circSH2B3. **(H)** The protein levels of NIS in SR1 treated BCPAP and K1 cells co-transfected with OE-circSH2B3-NC or OE-circSH2B3. SR1, Stem-Regenin 1.

trans-splicing. In the RNase R treatment assay, we found that circSH2B3 was resistant to RNase after digestion of RNA (**Figure 3D**). Also, under the treatment of Actinomycin D, the level of linear SH2B3 was decreased rapidly than that of circSH2B3 over time (**Figure 3E**). qRT-PCR was used to validate the relative expression of circSH2B3 in SR1 treated BCPAP and K1 cells (**Figure 3F**). Taken together, these findings suggested that circSH2B3 might be a potential target for SR1.

CircSH2B3 Plays a Key Role in the Dedifferentiation of PTC

To further investigated the dedifferentiation of circSH2B3 in PTC cells, the circSH2B3 silencing (sh-cicrSH2B3) and overexpression (OE-circSH2B3) were constructed using the plasmids transfection (**Figures 4A,B**). We then performed ^{125}I uptake assay and ^{131}I colony formation assay to determine the dedifferentiation effect of circSH2B3. The ^{125}I uptake assay revealed that when the expression of circSH2B3 was inhibited, the ^{125}I uptake was increased, while when the expression of circSH2B was enhanced, the ^{125}I uptake was significantly reduced as compared to control groups (**Figure 4C**). Similarly, colony formation in circSH2B3 knockdown cells was inhibited but enhanced in circSH2B3 overexpression cells (**Figure 4D**). In addition, mRNA and protein levels of NIS were increased in sh-circSH2B3 PTC cells but down-regulated in OE-circSH2B3 PTC cells (**Figures 4E,F**). We also investigated whether acquisition of circSH2B3 could affect the differentiation of SR1, the circSH2B3 was overexpressed in SR1 treated PTC cells. The increase of cirSH2B3 expression decreased ^{125}I uptake and the protein levels of NIS in SR1 treated PTC cells (**Figures 4G,H**). These results indicated that SR1 promotes differentiation of PTC cells via inhibiting circSH2B3.

CircSH2B3 Acts as a Sponge for hsa-miR-4640-5p

One of the main biological roles of circRNAs is to sponge to miRNA and affect the mRNA targeted by the corresponding miRNA (Meng et al., 2017). To investigate the underlying molecular mechanisms of circSH2B3 in dedifferentiation of PTC, we screened the target miRNAs of circSH2B3 via bioinformatics analysis. Circbank (http://www.circbank.cn), circAtlas (http://circatlas.biols.ac.cn), CircInteractome (https://circinteractome.nia.nih.gov/) and ENCORI (http://starbase.sysu.edu.cn/), were used to predict targets miRNAs of circSH2B3. The overlap across the databases revealed a total of 6 miRNAs (hsa-miR-6720-5p, hsa-miR-615-5p, hsa-miR-4731-

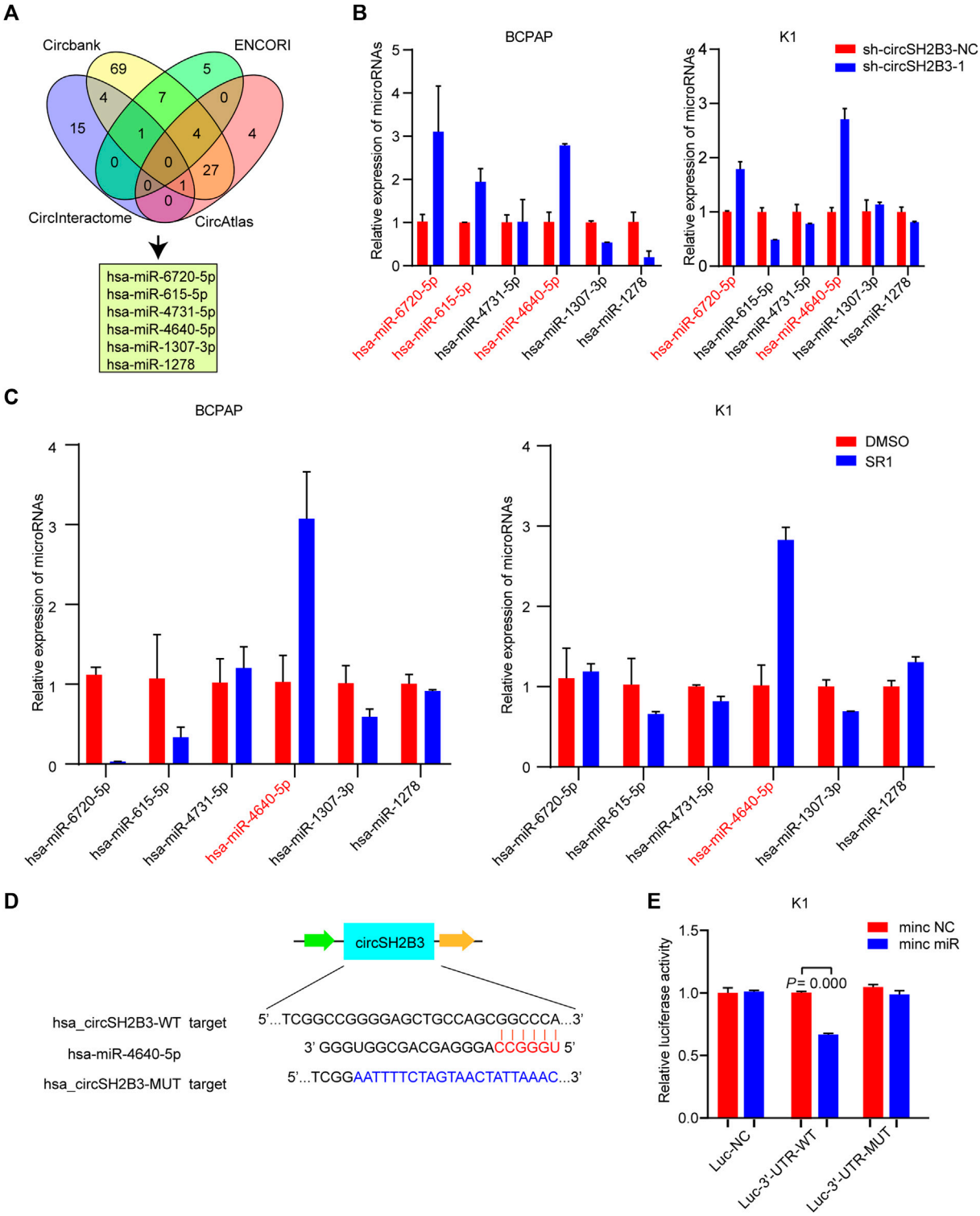


FIGURE 5 | circSH2B3 acts as a sponge for hsa-miR-4640-5p in PTC cells. **(A)** Venn diagram of hsa-miR-6720-5p, hsa-miR-615-5p, hsa-miR-4731-5p, hsa-miR-4640-5p, hsa-miR-1307-3p and hsa-miR-1278. **(B)** Relative miRNA expression in BCPAP and K1 cells transfected with sh-circSH2B3. **(C)** Relative miRNA expression in BCPAP and K1 cells treated with SR1. **(D)** The bioinformation analysis showing the binding site of circSH2B3 and hsa-miR-4640-5p. **(E)** The luciferase activity assay showing the combination of circSH2B3 and hsa-miR-4640-5p. SR1, StemRegenin 1.

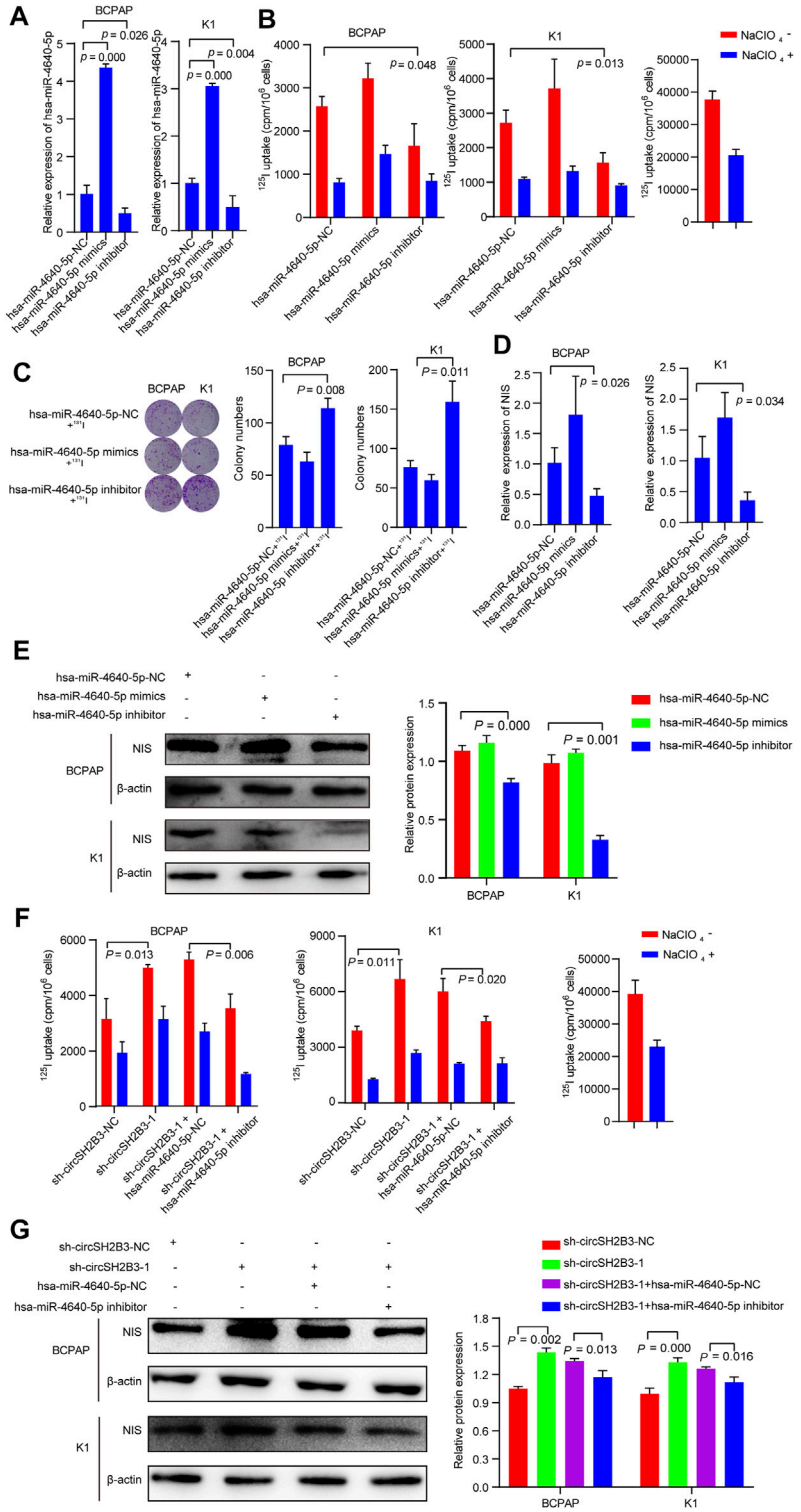


FIGURE 6 | hsa-miR-4640-5p silencing reverses the sh-circSH2B3 induced differentiation of PTC cells. **(A)** Relative expression of hsa-miR-4640-5p in BCPAP and K1 cells transfected with hsa-miR-4640-5p mimics or inhibitor. **(B)** ^{125}I uptake in BCPAP and K1 cells transfected with hsa-miR-4640-5p mimics or inhibitor. **(C)** ^{131}I colony formation in BCPAP and K1 cells transfected with hsa-miR-4640-5p mimics or inhibitor. **(D)** Relative expression of NIS in BCPAP and K1 cells transfected with hsa-miR-4640-5p mimics or inhibitor. **(E)** The protein levels of NIS in BCPAP and K1 cells transfected with hsa-miR-4640-5p mimics or inhibitor. **(F)** ^{125}I uptake in sh-circSH2B3 BCPAP and K1 cells co-transfected with inhibitor NC or hsa-miR-4640-5p inhibitor. **(G)** The protein levels of NIS in sh-circSH2B3 BCPAP and K1 cells co-transfected with inhibitor NC or hsa-miR-4640-5p inhibitor.

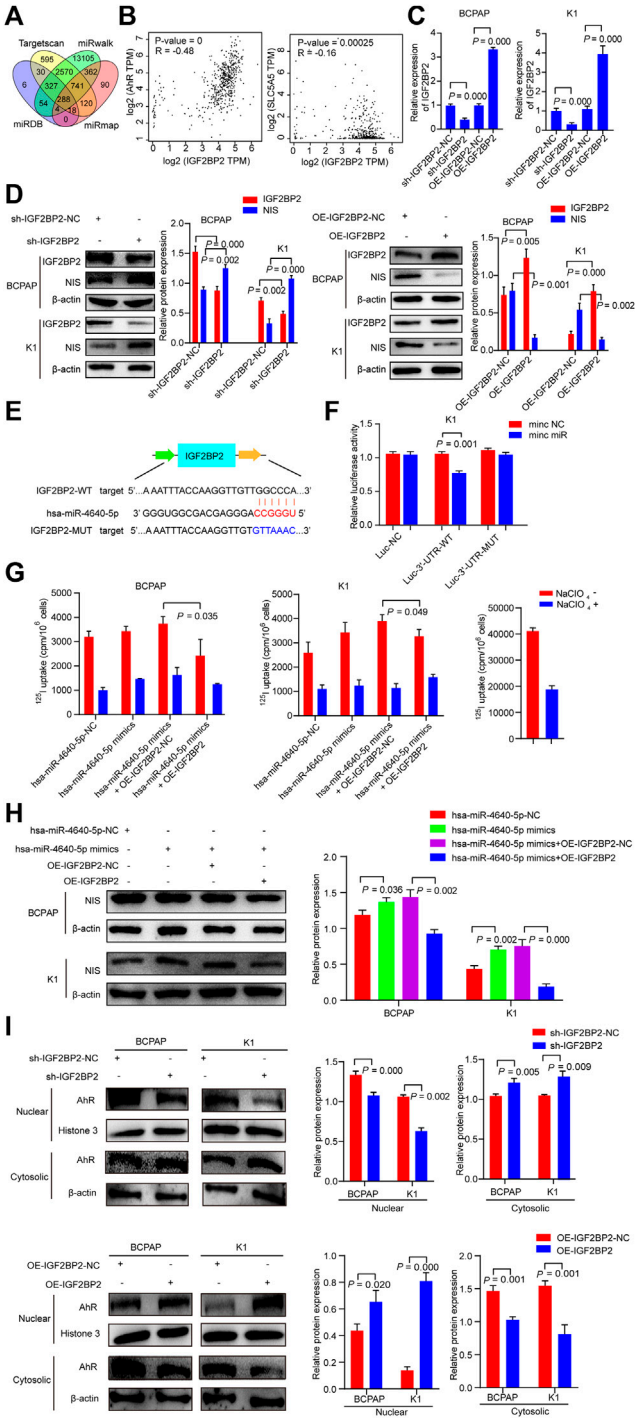


FIGURE 7 | IGF2BP2 is a direct target of hsa-miR-4640-5p. **(A)** Bioinformatic analysis of the potential target gene by prediction software (miRmap, miRwalk, Targetscan, miRDB). **(B)** Pearson analyses showing the correlation between IGF2BP2 and AhR, IGF2BP2 and NIS based on GEPIA (http://gepia.cancer-pku.cn/index.html) databases. **(C)** Relative expression of IGF2PB2 in BCPAP and K1cells transfected with IGF2BP2 shRNA (sh-IGF2BP2) and overexpression (OE-IGF2BP2) plasmids. **(D)** The protein levels of NIS and IGF2BP2 in BCPAP and K1cells transfected with sh-IGF2BP2 or OE-IGF2BP2. **(E)** The bioinformation analysis showing the

(Continued)

FIGURE 7 | binding site of hsa-miR-4640-5p and IGF2BP2. **(F)** The luciferase activity assay showing the combination of hsa-miR-4640-5p and IGF2BP2. **(G)** ^{125}I uptake in hsa-miR-4640-5p mimics treated BCPAP and K1 cells co-transfected with OE-IGF2BP2-NC or OE-IGF2BP2. **(H)** The protein levels of NIS in hsa-miR-4640-5p mimics treated BCPAP and K1 cells co-transfected with OE-IGF2BP2-NC or OE-IGF2BP2. **(I)** The protein levels of AhR in nuclear and cytosolic in BCPAP and K1 cells transfected with sh-IGF2BP2 or OE-IGF2BP2.

5p, hsa-miR-4640-5p, hsa-miR-1307-3p and hsa-miR-1278) (**Figure 5A**). The expression of these miRNAs in circSH2B3 knockdown PTC cells was then confirmed. Hsa-miR-6720-5p and hsa-miR-4640-5p were increased in PTC cells transfected with sh-circSH2B3 (**Figure 5B**). Furthermore, we selected the hsa-miR-4640-5p as the target miRNA via comparing miRNA expression in PTC cells treated with SR1 to control cells (**Figure 5C**). To confirm the interaction between circSH2B3 and hsa-miR4640-5p, we constructed dual-luciferase reporter vectors containing wild (WT) and mutant (Mut) circSH2B3 sequences capable of binding hsa-miR-4640-5p (**Figure 5D**). After transfecting vectors into K1 cells with or without a hsa-miR-4640-5p mimic and circSH2B3, dual-luciferase assays revealed that hsa-miR-4640-5p suppressed luciferase activity in WT cells but not in Mut cells (**Figure 5E**). These results indicated that hsa-miR-4640-5p is a direct downstream target of circSH2B3, involving in cell differentiation of PTC.

Hsa-miR-4640-5p Silencing Reverses the Differentiation Effect of sh-circSH2B3

To determine the differentiation effect of hsa-miR-4640-5p in PTC cells, we transfected BCPAP and K1 cells with the hsa-miR-4640-5p mimics or inhibitor and the transfection efficiency was detected by qRT-PCR (**Figure 6A**). Hsa-miR-4640-5p inhibitor decreased the ^{125}I uptake in PTC cells, while hsa-miR-4640-5p mimics only slightly increased ^{125}I uptake in both BCPAP and K1 cells (**Figure 6B**). The ^{131}I colony formation assay showed increased colony numbers in PTC cells transfected with hsa-miR-4640-5p inhibitor, and slightly decreased colony numbers in PTC cells transfected with hsa-miR-4640-5p mimics (**Figure 6C**). Furthermore, the qRT-PCR and western blot were performed to evaluate the relative mRNA and protein levels of NIS in PTC cells transfected wih hsa-miR-4640-5p mimics or inhibitor. As shown in **Figures 6D,E**, the relative mRNA and protein levels of NIS in PTC cells were reduced following transfection with hsa-miR-4640-5p inhibitors but increased slightly after transfection with hsa-miR-4640-5p mimics. These results suggested that hsa-miR-4640-5p inhibitors act as suppressors in differentiation of PTC.

As we hypothesized that silencing circSH2B3 promotes differentiation of PTC primarily by sponging hsa-miR-4640-5p, it was necessary to determine whether a hsa-miR-4640-5p inhibitor might reverse the sh-circSH2B3 induced differentiation of PTC cells. Rescue experiments were performed by co-transfecting sh-circSH2B3-NC or sh-circSH2B3 in PTC cells with a hsa-miR-4640-5p inhibitor or its control. As shown by the ^{125}I uptake assay, the hsa-miR-4640-5p inhibitor partially weakened the sh-

circSH2B3 induced differentiation effect of PTC cells (**Figure 6F**), as well as protein levels of NIS (**Figure 6G**). These results suggested that hsa-miR-4640-5p slightly promotes differentiation of PTC, and the hsa-miR-4640-5p suppression partially inhibited the sh-circSH2B3 induced differentiation of PTC.

IGF2BP2 is a Direct Target of Hsa-miR-4640-5p

The target genes with the potentiality of hsa-miR-4640-5p were picked out by the prediction software (miRmap, miRwalk, Targetscan, miRDB). Based on the bioinformatic analysis, the potential target gene was identified as Insulin-like growth factor 2 (IGF2) mRNA-binding protein 2 (IGF2BP2) (**Figure 7A**). According to the GEPIA (http://gepia.cancer-pku.cn/index.html) databases, the Pearson analysis revealed that IGF2BP2 was positively associated with AhR, and negatively correlated with NIS (**Figure 7B**). Stably suppressed and overexpressed IGF2BP2 (sh-IGF2BP2 and OE-IGF2BP2) were constructed by lentivirus transfection (**Figure 7C**). Western blot was performed to validate the involvement of IGF2BP2 in the dedifferentiation of PTC, which revealed that the protein levels of NIS were up-regulated in stably knockdown IGF2BP2 PTC cells, but down-regulated in stably overexpression IGF2BP2 PTC cells (**Figure 7D**). Following the discovery of the binding site of hsa-miR-4640-5p and IGF2BP2 through bioinformatics analysis, the luciferase WT and Mut type plasmids were constructed based on the binding site (**Figure 7E**). The luciferase activity assay was conducted to address the interaction between hsa-miR-4640-5p and IGF2BP2, revealing that hsa-miR-4640-5p binds to the IGF2BP2 3′-UTR (**Figure 7F**). In addition, ^{125}I uptake assay and western blot analyses revealed that the slightly enhanced differentiation effect of hsa-miR-4640-5p could be obviously inhibited by IGF2BP2 overexpression (**Figures 7G,H**).

Cytoplasmic AhR translocates to the nucleus, and heterodimerizes with aryl hydrocarbon receptor nuclear translocator (ARNT), and mediates a variety of biological and toxicological effects by inducing the transcription of AhR-responsive genes. IGF2BP2, as an N6-methyladenosine (m6A) reader, it might participate in AhR translocation to promote the function of AhR. Thus, we extracted the protein of the nucleus and cytoplasm of sh-IGF2BP2 or OE-IGF2BP2 PTC cells. The results showed that nucleus AhR protein levels in the sh-IGF2BP2 group were down-regulated, while that in the OE-IGF2BP2 group was up-regulated compared to the control groups (**Figure 7I**). These data above indicated that IGF2BP2 is a direct target of hsa-miR-4640-5p, involving in the dedifferentiation of PTC, as well as activation of AhR.

sh-circSH2B3 and SR1 Promote Differentiation of PTC by Inhibiting IGF2BP2

To investigate whether sh-circSH2B3 promoted PTC differentiation by targeting IGF2BP2, the impact of IGF2BP2 overexpression on circSH2B3-deficient PTC cells was assessed using the ^{125}I uptake assay (**Figure 8A**) and ^{131}I colony formation assay (**Figure 8B**), which demonstrated that high levels of IGF2BP2 could restrict the differentiation effect of circSH2B3-deficient cells. The relative mRNA and protein levels of NIS were also examined in sh-circSH2B3 PTC cells co-transfected with OE-IGF2BP2-NC or OE-IGF2BP2. The relative mRNA and protein levels of NIS increased in sh-circSH2B3 PTC cells but decreased in sh-circSH2B3 PTC cells co-transfected with OE-IGF2BP2 (**Figures 8C,D**). These findings suggested that IGF2BP2 inhibits the sh-circSH2B3 induced differentiation of PTC.

Considering our previous findings indicated that SR1 results in differentiation of PTC cells via inhibiting circSH2B3, and inhibiting circSH2B3 facilitates differentiation of PTC cells via regulating miR-4640-5p/IGF2BP2 axis, we hypothesized that IGF2BP2 might be a target of SR1. As we expected, the use of SR1 (100 nM) reduced the protein levels of IGF2BP2 (**Figure 8E**). We further hypothesized that suppressing IGF2BP2 would synergize SR1 to obtain a robust differentiation of PTC, therefore, PTC cells were treated with SR1 (100 nM) and transfected with sh-IGF2BP2. The ^{125}I uptake assay and the protein levels of NIS revealed that targeting IGF2BP2 enhanced the differentiation effect of SR1 in PTC cells, as anticipated (**Figures 8F,G**). These findings imply that SR1 induces differentiation of PTC by targeting IGF2BP2.

DISCUSSION

Strategies for increasing NIS expression and restoring ^{131}I are of critical for RR-PTC management (Ravera et al., 2017). In this work, we initially confirmed that AhR level was associated with differentiation level of PTC and AhR antagonists promoted differentiation of PTC. Mechanistically, the AhR antagonists suppressed the activity of circSH2B3/miR-4640-5p/IGF2BP2 axis to enhance the differentiation of PTC, which may be of great value for providing new targets for the differentiation therapy of PTC. **Figure 9** shows the functioning model.

AhR antagonists prevent the receptor from translocating into the nucleus, where it releases the chaperone complex and heterodimerizes with the ARNT. AhR antagonists have been proven to be antioncogenic roles in several cancers, including lung, head and neck, and oral cancers etc. (DiNatale et al., 2011; DiNatale et al., 2012; Kolluri et al., 2017). Previous research found that AhR was overexpressed in thyroid cancer, particularly in BRAF^{V600E} mutant ones, and it participated in thyroid cancer initiation and progression by regulating the establishment of an immunosuppressive tumor microenvironment and the epithelial to mesenchymal transition (Moretti et al., 2020). BRAF^{V600E}-mediated MAPK/ERK signaling may up-regulate AhR expression, thus enhancing CYP2S1 transcription, whereas CYP2S1 in turn enhances transcriptional activity of AhR through its metabolites. This AhR/CYP2S1 feedback loop strongly amplifies oncogenic role of BRAF^{V600E} in thyroid cancer cells (Li et al., 2020). The high expression of AhR in thyroid cancer, as well as its oncogenic function in thyroid cancer, prompted us to investigate whether inhibiting AhR nuclear translocation by AhR antagonists induces differentiation of

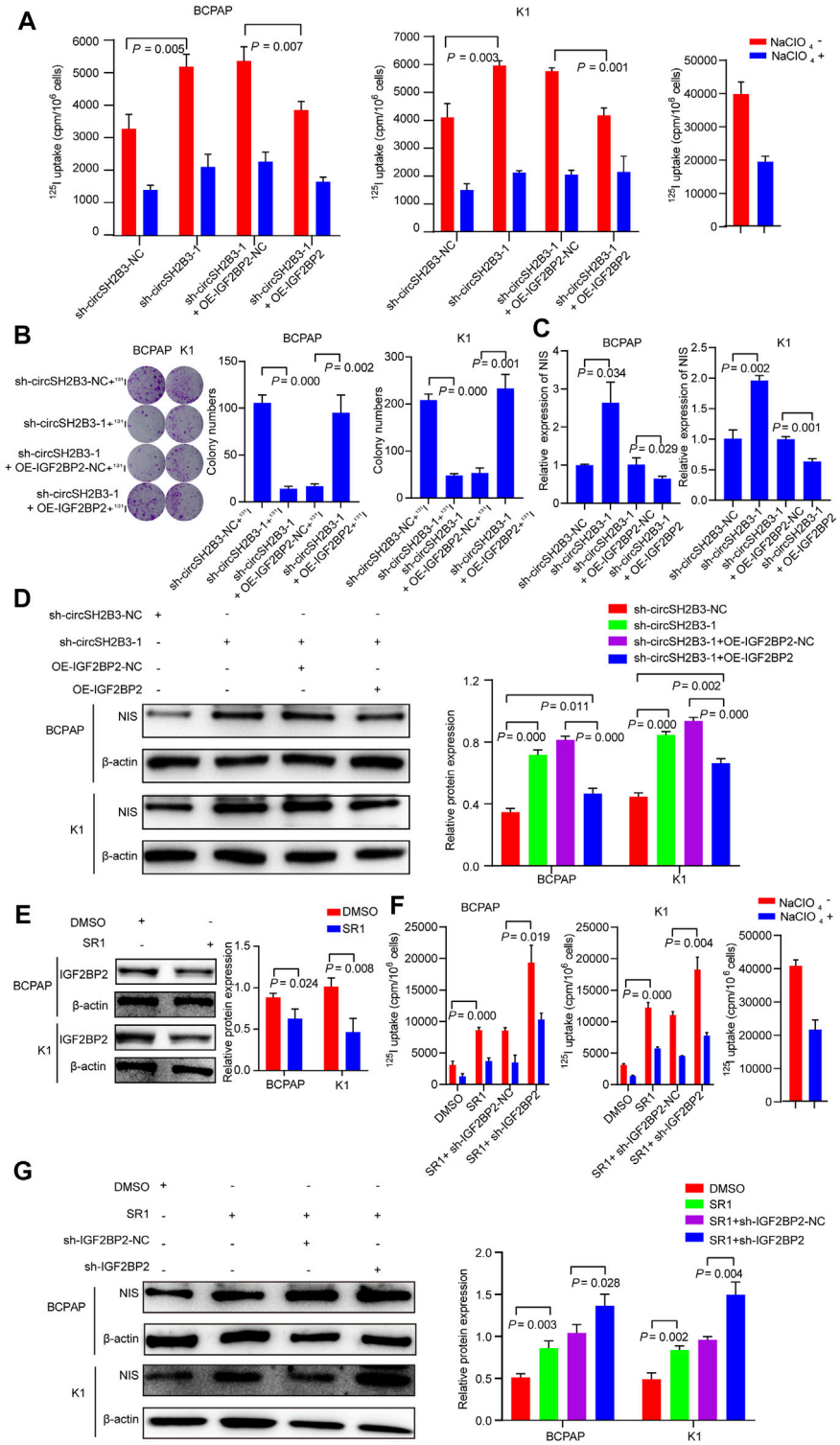


FIGURE 8 | sh-circSH2B3 and SR1 promote differentiation of PTC via targeting IGF2BP2. **(A)** ^{125}I uptake in sh-circSH2B3 BCPAP and K1 cells co-transfected with OE-IGF2BP2-NC or OE-IGF2BP2. **(B)** ^{131}I colony formation in sh-circSH2B3 BCPAP and K1 cells co-transfected with OE-IGF2BP2-NC or OE-IGF2BP2. **(C)** Relative expression of NIS in sh-circSH2B3 BCPAP and K1 cells co-transfected with OE-IGF2BP2-NC or OE-IGF2BP2. **(D)** The protein levels of NIS in sh-circSH2B3 BCPAP and K1 cells co-transfected with OE-IGF2BP2-NC or OE-IGF2BP2. **(E)** The protein levels of IGF2BP2 in BCPAP and K1 cells treated with SR1. **(F)** ^{125}I uptake in SR1 treated BCPAP and K1 cells co-transfected with sh-IGF2BP2-NC or sh-IGF2BP2. **(G)** The protein levels of NIS in SR1 treated BCPAP and K1 cells co-transfected with sh-IGF2BP2-NC or sh-IGF2BP2. SR1, StemRegenin 1.

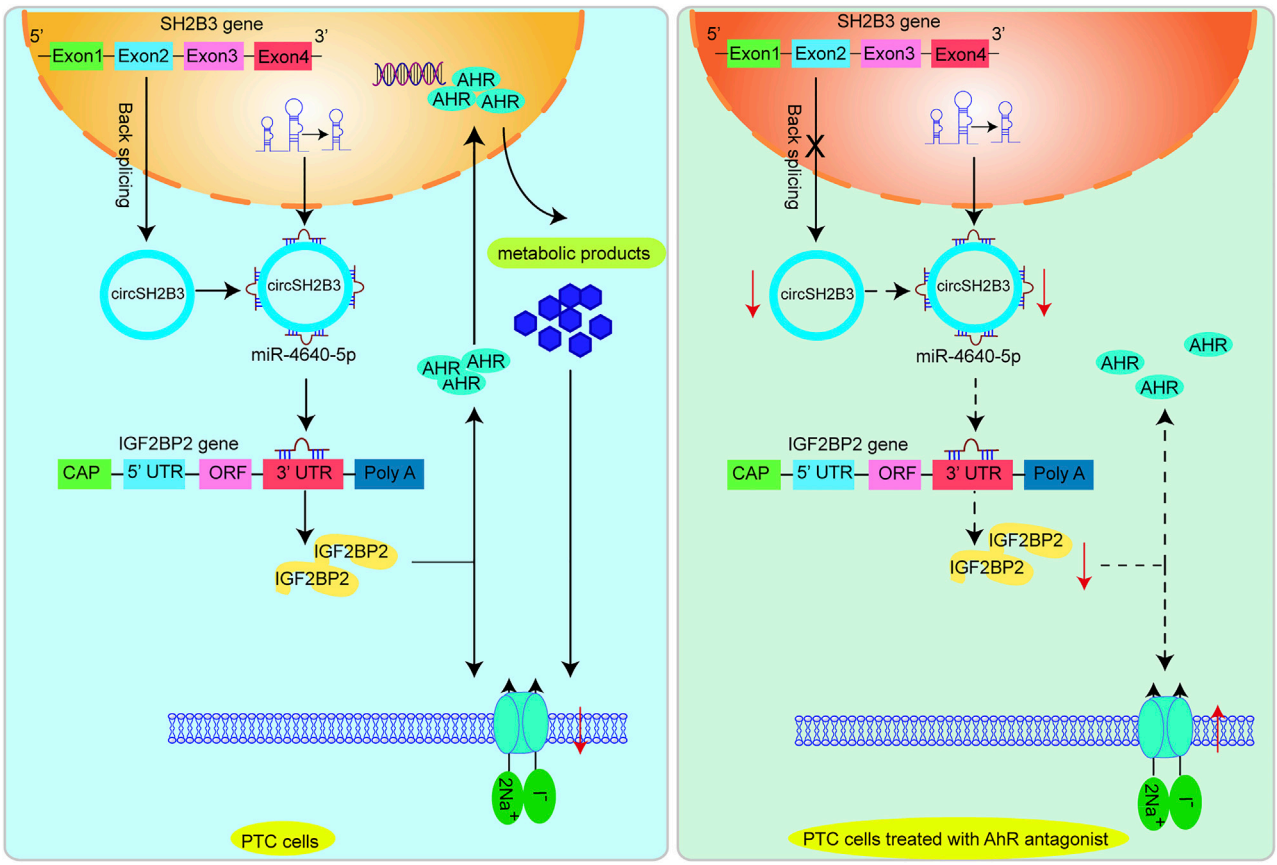


FIGURE 9 | A proposed model that AhR antagonist promotes differentiation of PTC *via* regulating circSH2B3/ miR-4640-5p/IGF2BP2 axis.

PTC. As anticipated, AhR level was closely associated with the ^{131}I uptake of lesions. Furthermore, inhibiting AhR activity with particular antagonists offered a possible direction of differentiation therapy in PTC cells, as shown by increased ^{125}I uptake and NIS expression. Although our findings were obtained *in vitro*, we propose AhR-impairment as a novel approach for inducing differentiation of PTC.

The biological process of AhR is explained by a variety of underlying processes. In the present study, we focused on the differentially expressed circRNAs that contributed to cell differentiation to investigate the AhR antagonist induced differentiation of PTC. CirRNAs are a novel class of endogenous RNAs that are generated by non-canonical back-splicing events, exerting a role in gene transcription and translation (Lei et al., 2020). Several circRNAs, such as circRUNX1 (Chu et al., 2021), circDOCK1 (Cui and Xue, 2020), circFNDC3B (Wu et al., 2020) etc. are implecated in PTC progression. In this study, we discovered that the down-regulated circRNA—circSH2B3, derived from SH2B3, is the primary contributor to the AhR antagonist induced differentiation of PTC.

CircRNAs execute their biological function by competitively reducing the account of active miRNA in a ceRNA-dependent manner (Kristensen et al., 2019). miRNAs are a large family of posttranscriptional regulators of gene expression that regulate numerous developmental and cellular processes in eukaryotic organisms (Krol et al., 2010). In this study, hsa-miR-4640-5p exhibited a high binding capacity with circSH2B3, which was validated by qRT-PCR and luciferase, and was found to play a significant role in AhR antagonist induced differentiation. Previously, hsa-miR-4640-5p was shown to directly bind to the 3′-UTR region of eIF5A mRNA, inhibiting the expression of eIF5A in non-small cell lung cancer (Liu et al., 2021). In our study, IGF2BP2 was identified as a target gene of hsa-miR-4640-5p via bioinformatics analysis, qRT-PCR and luciferase. Accumulating evidence highlights the significance of IGF2BP2 as a key regulator of cancer.

IGF2BP2 is an m6A reader, serving as a posttranscriptional regulatory factor (Hu et al., 2020). It predominantly localizes in the cytoplasm, where it can bind to the target mRNAs to enhance their mRNA stability (Bell et al., 2013), and mediate their translation spatially and temporally (Cao et al., 2018). Based on the TGCA database, IGF2BP2 expression was notably up-regulated in thyroid cancer relative to normal tissues (Wang et al., 2020). IGF2BP2 knockdown suppressed cell proliferation, migration, invasion, and induced cell apoptosis of thyroid cancer *via* by reducing the expression of long non-coding RNA HAGLR (Dong et al., 2021). In this study,

differentiation of PTC was enhanced after IGF2BP2 knockdown which was shown as increase of ^{125}I uptake and NIS expression. Conversely, IGF2BP2 overexpression hampered the differentiation of PTC. Importantly, IGF2BP2 knockdown synergized the differentiation effect of AhR antagonist. Additionally, controlled by the nuclear export signals in the KH domains, IGF2BP2 can transport target transcripts out of the nucleus (Nielsen et al., 2003). We discovered IGF2BP2 suppression decreased the amount of AhR protein levels in nucleus, whereas, IGF2BP2 overexpression increased AhR protein levels in nucleus. The nuclear translocation of AhR in response to ligands may be directly linked to transcriptional activation of target genes. The released metabolic products after AhR activation may further influence the differentiation of PTC, which needs research in the future study.

CONCLUSION

Our findings firstly demonstrated that AhR antagonist can promote the differentiation of PTC *via* inhibiting differentiation associated circRNA circSH2B3. As a sponge of hsa-miR-4640-5p, circSH2B3 could up-regulate expression of IGF2BP2, and induce dedifferentiation of PTC. Collectively, our findings provide a new treatment strategy for differentiation of PTC by AhR antagonist, and circSH2B3/miR-4640-5p/IGF2BP2 network may be a potential target for differentiation therapy of PTC and deserves further clinical research.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/geo/, accession ID: GSE188657.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the First Hospital of Jilin University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

RS and FG contributed to conception and design of the study. RS and MG performed the experiment. DL performed the statistical analysis. RS wrote the draft of the manuscript. FG revised the manuscript. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGMENTS

We would like to thank colleagues in our research team for their assistance.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.795386/full#supplementary-material

REFERENCES

Ahn, B. C. (2016). Personalized Medicine Based on Theranostic Radioiodine Molecular Imaging for Differentiated Thyroid Cancer. *Biomed. Res. Int.* 2016, 1680464. doi:10.1155/2016/1680464

Bell, J. L., Wächter, K., Mühleck, B., Pazaitis, N., Köhn, M., Lederer, M., et al. (2013). Insulin-like Growth Factor 2 mRNA-Binding Proteins (IGF2BPs): post-transcriptional Drivers of Cancer Progression? *Cell Mol Life Sci* 70 (15), 2657–2675. doi:10.1007/s00018-012-1186-z

Buffet, C., Wassermann, J., Hecht, F., Leenhardt, L., Dupuy, C., Groussin, L., et al. (2020). Redifferentiation of Radioiodine-Refractory Thyroid Cancers. *Endocr. Relat. Cancer* 27 (5), R113–r132. doi:10.1530/erc-19-0491

Bunaciu, R. P., and Yen, A. (2011). Activation of the Aryl Hydrocarbon Receptor AhR Promotes Retinoic Acid-Induced Differentiation of Myeloblastic Leukemia Cells by Restricting Expression of the Stem Cell Transcription Factor Oct4. *Cancer Res.* 71 (6), 2371–2380. doi:10.1158/0008-5472.can-10-2299

Cao, J., Mu, Q., and Huang, H. (2018). The Roles of Insulin-like Growth Factor 2 mRNA-Binding Protein 2 in Cancer and Cancer Stem Cells. *Stem Cell Int* 2018, 4217259. doi:10.1155/2018/4217259

Chu, J., Tao, L., Yao, T., Chen, Z., Lu, X., Gao, L., et al. (2021). Circular RNA circRUNX1 Promotes Papillary Thyroid Cancer Progression and Metastasis by Sponging MiR-296-3p and Regulating DDHD2 Expression. *Cell Death Dis* 12 (1), 112. doi:10.1038/s41419-020-03350-8

Corre, S., Tardif, N., Mouchet, N., Leclair, H. M., Boussemart, L., Gautron, A., et al. (2018). Sustained Activation of the Aryl Hydrocarbon Receptor Transcription Factor Promotes Resistance to BRAF-Inhibitors in Melanoma. *Nat. Commun.* 9 (1), 4775. doi:10.1038/s41467-018-06951-2

Cui, W., and Xue, J. (2020). Circular RNA DOCK1 Downregulates microRNA-124 to Induce the Growth of Human Thyroid Cancer Cell Lines. *Biofactors* 46 (4), 591–599. doi:10.1002/biof.1662

DiNatale, B. C., Schroeder, J. C., and Perdew, G. H. (2011). Ah Receptor Antagonism Inhibits Constitutive and Cytokine Inducible IL6 Production in Head and Neck Tumor Cell Lines. *Mol. Carcinog* 50 (3), 173–183. doi:10.1002/mc.20702

DiNatale, B. C., Smith, K., John, K., Krishnegowda, G., Amin, S. G., and Perdew, G. H. (2012). Ah Receptor Antagonism Represses Head and Neck Tumor Cell Aggressive Phenotype. *Mol. Cancer Res.* 10 (10), 1369–1379. doi:10.1158/1541-7786.mcr-12-0216

Dong, L., Geng, Z., Liu, Z., Tao, M., Pan, M., and Lu, X. (2021). IGF2BP2 Knockdown Suppresses Thyroid Cancer Progression by Reducing the Expression of Long Non-coding RNA HAGLR. *Pathol. Res. Pract.* 225, 153550. doi:10.1016/j.prp.2021.153550

Durante, C., Haddy, N., Baudin, E., Leboulleux, S., Hartl, D., Travagli, J. P., et al. (2006). Long-term Outcome of 444 Patients with Distant Metastases from Papillary and Follicular Thyroid Carcinoma: Benefits and Limits of Radioiodine Therapy. *J. Clin. Endocrinol. Metab.* 91 (8), 2892–2899. doi:10.1210/jc.2005-2838

Hu, X., Peng, W. X., Zhou, H., Jiang, J., Zhou, X., Huang, D., et al. (2020). IGF2BP2 Regulates DANCR by Serving as an N6-Methyladenosine Reader. *Cell Death Differ* 27 (6), 1782–1794. doi:10.1038/s41418-019-0461-z

Jang, S., Yu, X. M., Odorico, S., Clark, M., Jaskula-Sztul, R., Schienebeck, C. M., et al. (2015). Novel Analogs Targeting Histone Deacetylase Suppress Aggressive Thyroid Cancer Cell Growth and Induce Re-differentiation. *Cancer Gene Ther.* 22 (8), 410–416. doi:10.1038/cgt.2015.37

Kolluri, S. K., Jin, U. H., and Safe, S. (2017). Erratum to: Role of the Aryl Hydrocarbon Receptor in Carcinogenesis and Potential as an Anti-cancer Drug Target. *Arch. Toxicol.* 91 (9), 3209. doi:10.1007/s00204-017-2026-6

Kristensen, L. S., Andersen, M. S., Stagsted, L. V. W., Ebbesen, K. K., Hansen, T. B., and Kjems, J. (2019). The Biogenesis, Biology and Characterization of Circular RNAs. *Nat. Rev. Genet.* 20 (11), 675–691. doi:10.1038/s41576-019-0158-7

Krol, J., Loedige, I., and Filipowicz, W. (2010). The Widespread Regulation of microRNA Biogenesis, Function and Decay. *Nat. Rev. Genet.* 11 (9), 597–610. doi:10.1038/nrg2843

Lei, M., Zheng, G., Ning, Q., Zheng, J., and Dong, D. (2020). Translation and Functional Roles of Circular RNAs in Human Cancer. *Mol. Cancer* 19 (1), 30. doi:10.1186/s12943-020-1135-7

Li, Y., Su, X., Feng, C., Liu, S., Guan, H., Sun, Y., et al. (2020). CYP2S1 Is a Synthetic Lethal Target in BRAFV600E-Driven Thyroid Cancers. *Signal. Transduct Target. Ther.* 5 (1), 191. doi:10.1038/s41392-020-00231-6

Liu, X., Niu, N., Li, P., Zhai, L., Xiao, K., Chen, W., et al. (2021). LncRNA OGFRP1 Acts as an Oncogene in NSCLC via miR-4640-5p/eIF5A axis. *Cancer Cel Int* 21 (1), 425. doi:10.1186/s12935-021-02115-3

Ly, M., Rentas, S., Vujovic, A., Wong, N., Moreira, S., Xu, J., et al. (2019). Diminished AHR Signaling Drives Human Acute Myeloid Leukemia Stem Cell Maintenance. *Cancer Res.* 79 (22), 5799–5811. doi:10.1158/0008-5472.can-19-0274

Meng, S., Zhou, H., Feng, Z., Xu, Z., Tang, Y., Li, P., et al. (2017). CircRNA: Functions and Properties of a Novel Potential Biomarker for Cancer. *Mol. Cancer* 16 (1), 94. doi:10.1186/s12943-017-0663-2

Moretti, S., Nucci, N., Menicali, E., Morelli, S., Bini, V., Colella, R., et al. (2020). The Aryl Hydrocarbon Receptor Is Expressed in Thyroid Carcinoma and Appears to Mediate Epithelial-Mesenchymal-Transition. *Cancers (Basel)* 12 (1), 145. doi:10.3390/cancers12010145

Nielsen, J., Adolph, S. K., Rajpert-De Meyts, E., Lykke-Andersen, J., Koch, G., Christiansen, J., et al. (2003). Nuclear Transit of Human Zipcode-Binding Protein IMP1. *Biochem. J.* 376 (Pt 2), 383–391. doi:10.1042/bj20030943

Oh, J. M., Baek, S. H., Gangadaran, P., Hong, C. M., Rajendran, R. L., Lee, H. W., et al. (2020). A Novel Tyrosine Kinase Inhibitor Can Augment Radioactive Iodine Uptake through Endogenous Sodium/Iodide Symporter Expression in Anaplastic Thyroid Cancer. *Thyroid* 30 (4), 501–518. doi:10.1089/thy.2018.0626

Park, J. W., Zarnegar, R., Kanauchi, H., Wong, M. G., Hyun, W. C., Ginzinger, D. G., et al. (2005). Troglitazone, the Peroxisome Proliferator-Activated Receptor-Gamma Agonist, Induces Antiproliferation and Redifferentiation in Human Thyroid Cancer Cell Lines. *Thyroid* 15 (3), 222–231. doi:10.1089/thy.2005.15.222

Ravera, S., Reyna-Neyra, A., Ferrandino, G., Amzel, L. M., and Carrasco, N. (2017). The Sodium/Iodide Symporter (NIS): Molecular Physiology and Preclinical and Clinical Applications. *Annu. Rev. Physiol.* 79, 261–289. doi:10.1146/annurev-physiol-022516-034125

Schmutzler, C., and Köhrle, J. (2000). Retinoic Acid Redifferentiation Therapy for Thyroid Cancer. *Thyroid* 10 (5), 393–406. doi:10.1089/thy.2000.10.393

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., et al. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.* 71 (3), 209–249. doi:10.3322/caac.21660

Wang, X., Fu, X., Zhang, J., Xiong, C., Zhang, S., and Lv, Y. (2020). Identification and Validation of m6A RNA Methylation Regulators with Clinical Prognostic Value in Papillary Thyroid Cancer. *Cancer Cel Int* 20, 203. doi:10.1186/s12935-020-01283-y

Wu, G., Zhou, W., Pan, X., Sun, Z., Sun, Y., Xu, H., et al. (2020). Circular RNA Profiling Reveals Exosomal Circ_0006156 as a Novel Biomarker in Papillary Thyroid Cancer. *Mol. Ther. Nucleic Acids* 19, 1134–1144. doi:10.1016/j.omtn.2019.12.025

Xu, X., Yu, Y., Zong, K., Lv, P., and Gu, Y. (2019). Up-regulation of IGF2BP2 by Multiple Mechanisms in Pancreatic Cancer Promotes Cancer Proliferation by Activating the PI3K/Akt Signaling Pathway. *J. Exp. Clin. Cancer Res.* 38 (1), 497. doi:10.1186/s13046-019-1470-y

Zhao, H., Chen, L., Yang, T., Feng, Y. L., Vaziri, N. D., Liu, B. L., et al. (2019). Aryl Hydrocarbon Receptor Activation Mediates Kidney Disease and Renal Cell Carcinoma. *J. Transl Med.* 17 (1), 302. doi:10.1186/s12967-019-2054-5

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Sa, Guo, Liu and Guan. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Pharmacology

MINI REVIEW
published: 17 January 2022
doi: 10.3389/fphar.2021.825475

The Role and Application of Exosomes in Gastric and Colorectal Cancer

*Qirong Li[1,2†], Dongxu Wang[2†], Dayong Ding[1], Ye Feng[1], Ruizhi Hou[1], Dianfeng Liu[2], Chao Lin[3] and Yongjian Gao[1]**

[1]Department of Gastrointestinal Colorectal and Anal Surgery, China-Japan Union Hospital of Jilin University, Changchun, China, [2]Laboratory Animal Center, College of Animal Science, Jilin University, Changchun, China, [3]School of Grain Science and Technology, Jilin Business and Technology College, Changchun, China

Gastric cancer and colorectal cancer are malignant tumors found in the human gastrointestinal tract. Bidirectional communication between tumor cells and their microenvironment can be realized through the transmission of exosomes—small, cell-derived vesicles containing complex RNA and proteins. Exosomes play an important role in the proliferation, metastasis, immune response, and drug resistance of cancer cells. In this review, we focus on the role and application of exosomes in gastric and colorectal cancer. We also summarize the role of exosomes secreted by different types of cells in tumor development and as drug carriers in cancer treatment.

Keywords: gastric cancer, colorectal cancer, ncRNA, exosomes, cancer therapy

OPEN ACCESS

Edited by:
Yue Hou,
Northeastern University, China

Reviewed by:
Wan Zhuo,
Fourth Military Medical University, China
Pan Liu,
Northwestern University, United States

****Correspondence:***
Yongjian Gao
gaoyj123@jlu.edu.cn

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

Received: *30 November 2021*
Accepted: *27 December 2021*
Published: *17 January 2022*

Citation:
Li Q, Wang D, Ding D, Feng Y, Hou R, Liu D, Lin C and Gao Y (2022) The Role and Application of Exosomes in Gastric and Colorectal Cancer. Front. Pharmacol. 12:825475. doi: 10.3389/fphar.2021.825475

INTRODUCTION

Gastric and colorectal cancer are globally-important diseases with high molecular and phenotypic heterogeneity (Smyth et al., 2020). Gastric cancer can be caused by a variety of genetic and epigenetic mutations, and *Helicobacter pylori* is also an important pathogenic factor (Uemura et al., 2001). The tumor microenvironment has a strong influence on the survival and treatment response of gastric cancer patients (Quail and Joyce, 2013). At present, early diagnosis of gastric cancer remains problematic because clinical symptoms often only appear in the late stages of cancer development, which significantly limits treatment options (Maconi et al., 2008). Colorectal cancer is the fourth most deadly cancer in the world; its etiologies include eating habits, old age, and smoking (Dekker et al., 2019). Colorectal cancer is normally treated with adjuvant therapy after surgical resection. However, the risk of subsequent cancer recurrence and metastasis remains high, and it is often related to resistance to traditional therapies such as chemotherapy and radiation (Jänne and Mayer, 2000). As gastric and colorectal cancers are associated with high morbidity and mortality, research into new targeted therapies is urgent.

Recent studies have shown that exosomes can be used as targeted drug carriers. Exosomes are small endocytic vesicles secreted by most cells (Théry et al., 2002), the diameters of which range between 40 and 100 nm. Exosomes have been found to be able to deliver bioactive molecules or other substances to specific recipient cells for intercellular communication (**Figure 1**). Increasing numbers of studies have indicated that exosomes are important nanomaterials that can regulate essential biological behaviors through intercellular transmission (Yang et al., 2019). They also play an important role in the pathogenesis of many diseases, including cancer, as they are involved in the apoptosis of tumor cells, the proliferation and migration of cancer cells, regulation of the tumor microenvironment, and angiogenesis (Nabariya et al., 2020). Because of these characteristics, exosomes can also be used as an efficient targeted drug delivery system in cancer treatment.

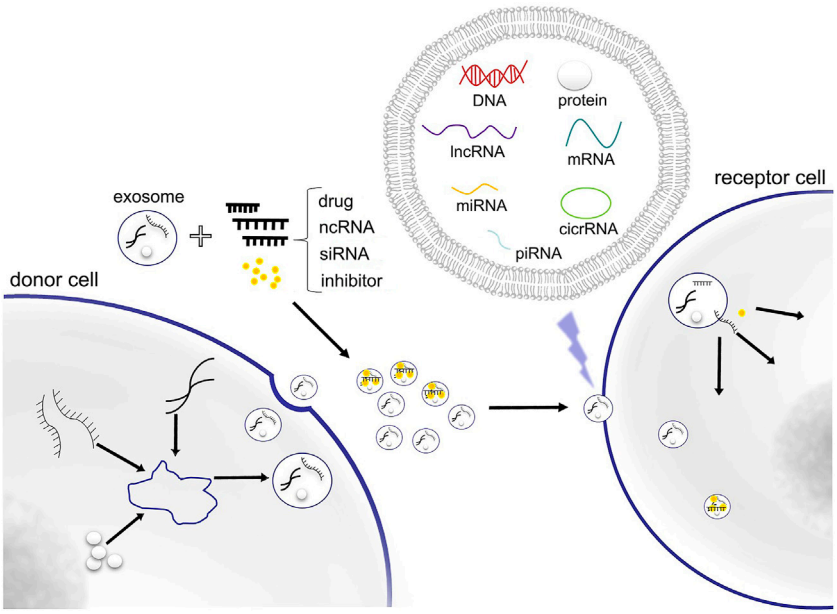


FIGURE 1 | Exosome contains DNA, RNA, protein and other substances for cellular component communication. Exosomes can also be used as delivery vectors of drugs, ncRNA, siRNA and inhibitor.

REGULATION OF EXOSOMES DERIVED FROM CANCER CELLS ON GASTRIC CANCER AND COLORECTAL CANCER

Exosomes play an important role in the communication between tumor cells themselves and between the tumor and its microenvironment. Exosomes can transfer many types of biomolecules, including DNA, RNA, and protein. Exosomes derived from cancer cells affect the biological characteristics of recipient cells and change the tumor microenvironment by transmitting these bioactive molecules to regulate the tumor's development process. Noncoding RNA (ncRNA), including microRNA, circRNA, and lncRNA, is one of the most important bioactive molecules which does not encode protein and perform their biological functions at the RNA level.

Studies have shown that miR-15b-3p is highly expressed in exosomes secreted by gastric cancer cells and miR-15b-3p can be transferred by exosomes to enhance migration, invasion, and proliferation; it also inhibit the apoptosis of gastric cancer through the DYNLT1/*caspase-3*/*caspase-9* signaling pathway (Wei et al., 2020). Exosomal miR-25-3p from colorectal cancer promotes cancer development by inducing vascular permeability and angiogenesis (Zeng et al., 2018). In addition, exosomal circSHKBP1 regulates the miR-582-3p/HUR/VEGF pathway, thereby promoting the progression of gastric cancer (Xie M. et al., 2020). Studies have also shown that exosomal lncRNA *91H* promotes the proliferation of colorectal cancer by changing HNRNPK expression (Gao et al., 2018).

Exosomes also deliver cancer-related molecules that can influence the chemotherapeutic resistance of recipient cells. Cisplatin is one of the most effective and commonly used of the basic chemotherapy drugs for advanced gastric cancer treatment (Keehn and Higgins, 1981). Studies have shown that exosomes from cisplatin-resistant gastric cancer can enhance gastric cancer cells resistance to cisplatin by delivering miR-500a-3p. Therefore, exosomal miR-500a-3p could potentially be used to predict and eliminate chemotherapy-resistant gastric cancer cells (Lin et al., 2020). Research has also shown that p-STAT3-containing exosomes contribute to 5-FU resistance in colorectal cancer cells (Zhang et al., 2019).

Protein is an important component of exosomes, and exosomes have been shown to regulate the liver microenvironment by delivering EGFR to promote liver metastasis of gastric cancer (Zhang et al., 2017). Furthermore, exosomal ANGPTL1 has been shown to alleviate liver metastasis in colorectal cancer by reprogramming Kupffer cells and reducing MMP9 expression (Jiang et al., 2021).

In addition to acting as cell-to-cell transporters, cancer-derived exosomes have also been widely used as biomarkers in cancer diagnosis. Tumors of unknown primary origin can be classified by extracting tumor-type specific proteins that are contained in exosomes from tissues and plasma. Therefore, exosomal proteins can be used as reliable biomarkers for cancer detection and classification (Hoshino et al., 2020). Other research has revealed that exosomal piRNAs in serum may be a biomarker for the diagnosis and monitoring of gastric cancer metastasis (Ge et al., 2020). LncRNA *HOTTIP* has been found to be significantly up-regulated in gastric cancer cell exosomes; it could be used as a new biomarker for determining the diagnosis and prognosis of gastric cancer (Zhao et al., 2018). Further to this, studies have indicated that exosomal circRNA-PNN is a potential biomarker of colorectal cancer (Xie Y. et al., 2020).

These results suggest that tumor-derived exosomes can promote cancer progression and influence drug resistance through ncRNA transmission. Moreover, tumor-derived

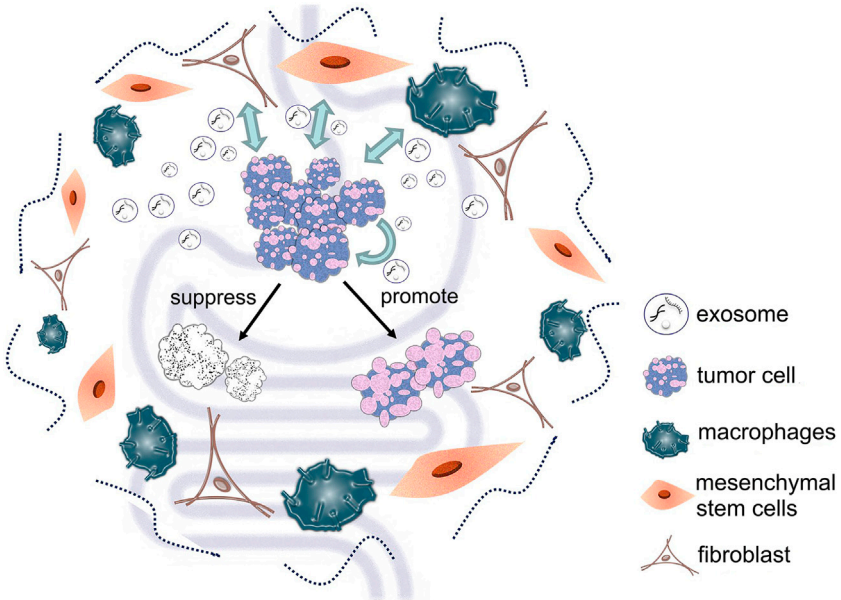


FIGURE 2 | Different types of cell-derived exosomes in the tumor microenvironment can deliver various substances to tumor cells, thereby affecting tumor cell development.

exosomes can be used as biomarkers for cancer; this indicates that tumor-derived exosomes may be used to diagnose and treat gastric and colorectal cancer.

REGULATION OF EXOSOMES FROM OTHER CELLS ON GASTRIC CANCER AND COLORECTAL CANCER

Exosomes derived from cancer cells play an important role in intracellular communication, treatment and diagnosis of gastric and colorectal cancers. Similarly, other types of cell-derived exosomes in the tumor microenvironment are important for cancer progression (**Figure 2**). Cancer-associated fibroblasts (CAFs) are the main components of tumor stroma; they can influence tumor development and drug resistance by secreting exosomes. Studies have shown that exosomal miR-139 from CAFs can inhibit the progression and metastasis of gastric cancer by reducing MMP11 (Xu et al., 2019). Furthermore, miR-34 in exosomes secreted by CAFs can inhibit gastric cancer cell proliferation and invasion both *in vivo* and *in vitro* (Shi et al., 2020). Other research has found that exosomes delivery of miR-590-3p by CAFs can improve radioresistance of colorectal cancer by regulating the CLCA4-dependent PI3K/Akt signaling pathway (Chen X. et al., 2021). Moreover, CAFs can also enhance colorectal cancer drug resistance through exosomes delivery of lncRNA *H19* (Ren et al., 2018).

Tumor-associated macrophages (TAMs) are macrophages that infiltrate tumor tissue; they are the most abundant immune cells within the tumor microenvironment (Zhang Y. et al., 2018). Studies have shown that exosomes down-regulate PTEN and inhibit apoptosis by delivering TAMs-derived miR-21; they also improve cisplatin resistance in gastric cancer cells (Zheng et al., 2017). Research has also indicated that exosomes derived from M1 macrophages carry miR-16-5p; this activates the T-cell immune response through PD-L1 and inhibits gastric cancer proliferation (Li et al., 2020). In addition, exosomes derived from mouse TAMs have been shown to be associated with Th1/M1 polarization, inflammation, and enhancement of immune response in colorectal tumors (Cianciaruso et al., 2019). In M2 macrophage-derived exosomes, miR-21-5p and miR-155-5p were highly expressed, which mediated the migration and invasion of colorectal cancer cells (Lan et al., 2019).

Mesenchymal stem cells (MSCs) have the ability to self-replicate and they have strong differentiation potential, which means they can contribute to the formation of the tumor microenvironment and they can interact with cancer cells (Poggi and Giuliani, 2016). Exosomes derived from human umbilical cord mesenchymal stem cells (hUMSCs) that contain miR-6785-5p inhibit both gastric cancer angiogenesis and metastasis by inhibiting INHBA expression (Chen Z. et al., 2021). Moreover, exosomes derived from MSCs in gastric cancer tissues can deliver miR-221 to gastric cancer cells, thereby promoting their proliferation and migration (Wang et al., 2014). Studies have revealed that GARP knockdown MSCs inhibit the proliferation and invasion of mouse colorectal cancer cells through exosomes (Xing et al., 2020). It has also been shown that exosomes from bone marrow-derived mesenchymal stem cells (BMSCs) can promote stem cell-like characteristics of colorectal cancer through miR-142-3p (Li and Li, 2018).

Proteins delivered by CAFs, TAMs, and MSCs through exosomes are also important mediators of tumor and tumor microenvironment regulation. Studies that used proteomic analysis of CAFs and serum-derived exosomes have identified QSOX1 as a marker for non-invasive colorectal cancer detection

(Ganig et al., 2021). TAM-derived exosomes have also been found to promote migration of gastric cancer cells by delivering functional apolipoprotein E (Zheng et al., 2018), and BMSCs exosomes with p53 deletion can regulate the Wnt/β-catenin pathway by transferring UBR2 to target cells, which promotes the growth and metastasis of gastric cancer (Mao et al., 2017). As can be seen from the above studies, CAFs, TAMs, and MSCs are important components of the tumor microenvironment; they cooperate with the cancer cells to regulate the tumor's growth environment.

THERAPEUTIC EFFECTS OF EXOSOME-CARRYING DRUGS ON GASTRIC CANCER

Recent studies have indicated that exosomes are the promising carrier of anticancer drugs and that exosome-based therapy may be an efficient and beneficial approach during cancer treatment. Exosomes have good biocompatibility, specificity, and low immunogenicity, and since they can be easily internalized by cells, they can effectively deliver drugs to cancer cells. The proteins and lipid compositions of tumor-derived exosomes are similar to those of the cells that secret them. (Sun W. et al., 2018), and report shows that exosomes from cancer cells are specifically taken up by the same type of tumor tissue or cell. If the tumor-derived exosomes are injected into the body, the exosomes will eventually return to the original tumor tissue. The tumor-targeting ability of tumor cell-derived exosomes may be related to the expression of integrin (Qiao et al., 2020). Because of this characteristic, tumor-derived exosomes are natural vectors that have great potential for targeted delivery of antitumor drugs. Studies have constructed nano aspirin exosome drug delivery systems that can effectively deliver aspirin to the tumor site *in vivo*; this induces apoptosis and autophagy of colorectal cancer cells, thus producing a good tumor treatment effect (Tran et al., 2019). In other study, exosomes were isolated from A33-positive LIM1215 cells and loaded with doxorubicin (DOX) to target A33-positive colorectal cancer cells. The results revealed that DOX-loaded exosomes have good tumor-targeting ability; they could also inhibit colorectal cancer growth (Li et al., 2018). In addition, researchers have loaded DOX into exosomes secreted by MSCs, which effectively inhibited colorectal tumor growth (Bagheri et al., 2020). Furthermore, there is evidence shown that exosomes coated with oxaliplatin and PGM5-AS1 can reverse drug resistance in colorectal cancer cells and inhibit their growth (Hui et al., 2021).

In addition to being used to package drugs, exosomes can also be used to deliver biological molecules for RNA-based therapeutic strategies. Hepatocyte growth factor (HGF) can promote the growth of tumor cells and blood vessels. HGF siRNA can be transported to cancer cells using exosomes, which can down-regulate expression of HGF, thereby inhibiting the proliferation and migration of gastric cancer cells (Zhang H. et al., 2018). Furthermore, exosomes can be used as nanoparticles to deliver anti-miR-214 and down-regulate the expression of miR-214 in gastric cancer cells, which can reduce cisplatin chemotherapy resistance in gastric cancer and inhibit tumor growth (Wang et al., 2018). Other research has shown that delivering c-Met siRNA via exosomes can promote cell apoptosis, inhibit tumor growth *in vivo*, and reduce gastric cancer cisplatin resistance (Zhang et al., 2020). It has also been reported that exosomes can be used to simultaneously deliver 5-FU and miR-21 inhibitors to colorectal cancer cells; researchers found that the constructed exosomes had significant anti-tumor effects both *in vivo* and *in vitro* (Liang et al., 2020). Furthermore, exosomes loaded with miR-128-3p from normal intestinal FHC cells have been found to be able to inhibit the expression of MRP5, thereby improving the colorectal cancer cells' sensitivity to oxaliplatin (both *in vivo* and *in vitro*) (Liu et al., 2019).

Tumor-derived exosomes have been shown to be able to target tumors, and exosomes can be used to directly package anticancer drugs and deliver them specifically to gastric and colorectal tumors to inhibit tumor development. They can also use regulatory mechanisms to inhibit the growth of cancer cells or reverse drug resistance by deliver biomolecules such as siRNA.

DISCUSSION

Gastric and colorectal cancer are common and significant cancers worldwide that cause high morbidity and mortality rate (Athauda et al., 2019). Therefore, it is urgent to develop new diagnostic and therapeutic methods. Increasing numbers of studies indicate that exosomes are involved in various stages of cancer progression and can influence its overall course (Bebelman et al., 2018). For example, exosomes regulate tumor progression due to their ability to communicate between cells and deliver various substances. The contents related to exosomes and cancer regulation introduced in this review mainly include ncRNAs (miRNA, lncRNA, circRNA, piRNA) and proteins, and the role of exosomes includes regulating tumor microenvironments and affecting tumor proliferation, metastasis, and drug resistance (Mashouri et al., 2019). Exosomes secreted by different cells also have different functions and characteristics. In the case of gastric and colorectal cancer cells, they have been shown to promote the proliferation and invasion of their own cancer cells through exosomes and to make the surrounding environment more suitable for tumor growth. Moreover, exosomes secreted by tumor cells can be better ingested by the same type of tumor cells, which indicates that they can be used to carry treatment drugs. In addition, other cells in the tumor microenvironment, such as CAFs, TAMs, and MSCs, have a bidirectional molecular transfer process with tumor cells. In some cases, these cells can promote tumor growth, while in other cases they can deliver substances that inhibit tumor growth. This suggests that the tumor-inhibiting effects can be produced by regulating the tumor microenvironment.

Depending on exosome specificity, exosomes can selectively deliver drugs to specific tumor cells with the advantages of high efficiency and low toxicity (Wu et al., 2021). However, only a few studies have examined the application of exosomal delivery drugs for gastric cancer and colorectal cancer. Moreover, exosomes are rich in miRNAs, which can alter the fate of tumor cells by influencing the expression of related miRNAs in tumor cells (Sun Z. et al., 2018). Studies have found new types of RNA, such

as piRNA and tsRNA, in addition to miRNA, lncRNA, and cicrRNA. There have only been a few investigations into the existence and function of these types of ncRNA in gastric cancer and colorectal cancer exosomes. Therefore, although the treatment of exosomes has shown great application prospects in gastric and colorectal cancers, many challenges remain before we can use exosomes in the clinical treatment of cancer. For example, information is still needed regarding the detailed mechanisms of exosomes in cancer cells, the isolation of a large number of exosomes, their precise detection, and the drug loading efficiency and preservation methods of exosomes. In an attempt to improve the safety and quality of exosome engineering in the clinical application of treatments for both gastric and colorectal cancer, it is imperative that further studies be carried out to determine how to improve drug delivery methods and the targeting effect of exosomes.

CONCLUSION

Exosomes play an important role in the occurrence and development of gastric and colorectal cancer, and substances such as miRNA that are contained in exosomes can affect tumor progression. Due to their biological characteristics, exosomes can be used for targeted transport of tumor drugs. Therefore, it is reasonable to presume that exosomes can be modified for use as biomarkers, vaccines, and drug carriers to develop more effective clinical diagnosis and treatment strategies for cancer.

AUTHOR CONTRIBUTIONS

QL and DW wrote the manuscript. QL, DD, YF, RH, DL, CL, and YG collected the references and prepared figures. All authors reviewed the manuscript.

FUNDING

This work was supported by the Jilin Health Commission Program under Grant 2020J05S, the Fundamental Research Funds for the Central Universities under Grant 2019JCKT-70, the Jilin Education Department Program under Grant JJKH20200950KJ, and the Jilin Scientific and Technological Development Program under Grant 20190103071JH, 202002006JC and 20210101010JC.

REFERENCES

Athauda, A., Segelov, E., Ali, Z., and Chau, I. (2019). Integrative Molecular Analysis of Colorectal Cancer and Gastric Cancer: What Have We Learnt? *Cancer Treat. Rev.* 73, 31–40. doi:10.1016/j.ctrv.2018.12.004

Bagheri, E., Abnous, K., Farzad, S. A., Taghdisi, S. M., Ramezani, M., and Alibolandi, M. (2020). Targeted Doxorubicin-Loaded Mesenchymal Stem Cells-Derived Exosomes as a Versatile Platform for Fighting against Colorectal Cancer. *Life Sci.* 261, 118369. doi:10.1016/j.lfs.2020.118369

Bebelman, M. P., Smit, M. J., Pegtel, D. M., and Baglio, S. R. (2018). Biogenesis and Function of Extracellular Vesicles in Cancer. *Pharmacol. Ther.* 188, 1–11. doi:10.1016/j.pharmthera.2018.02.013

Chen, X., Liu, Y., Zhang, Q., Liu, B., Cheng, Y., Zhang, Y., et al. (2021a). Exosomal miR-590-3p Derived from Cancer-Associated Fibroblasts Confers Radioresistance in Colorectal Cancer. *Mol. Ther. Nucleic Acids* 24, 113–126. doi:10.1016/j.omtn.2020.11.003

Chen, Z., Xie, Y., Chen, W., Li, T., Chen, X., and Liu, B. (2021b). microRNA-6785-5p-loaded Human Umbilical Cord Mesenchymal Stem Cells-Derived Exosomes Suppress Angiogenesis and Metastasis in Gastric Cancer via INHBA. *Life Sci.* 284, 119222. doi:10.1016/j.lfs.2021.119222

Cianciaruso, C., Beltraminelli, T., Duval, F., Nassiri, S., Hamelin, R., Mozes, A., et al. (2019). Molecular Profiling and Functional Analysis of Macrophage-Derived Tumor Extracellular Vesicles. *Cell Rep* 27 (10), 3062–e11. doi:10.1016/j.celrep.2019.05.008

Dekker, E., Tanis, P. J., Vleugels, J. L. A., Kasi, P. M., and Wallace, M. B. (2019). Colorectal Cancer. *Lancet* 394 (10207), 1467–1480. doi:10.1016/S0140-6736(19)32319-0

Ganig, N., Baenke, F., Thepkaysone, M. L., Lin, K., Rao, V. S., Wong, F. C., et al. (2021). Proteomic Analyses of Fibroblast- and Serum-Derived Exosomes Identify QSOX1 as a Marker for Non-invasive Detection of Colorectal Cancer. *Cancers (Basel)* 13 (6), 1351. doi:10.3390/cancers13061351

Gao, T., Liu, X., He, B., Nie, Z., Zhu, C., Zhang, P., et al. (2018). Exosomal lncRNA 91H Is Associated with Poor Development in Colorectal Cancer by Modifying HNRNPK Expression. *Cancer Cell Int* 18, 11. doi:10.1186/s12935-018-0506-2

Ge, L., Zhang, N., Li, D., Wu, Y., Wang, H., and Wang, J. (2020). Circulating Exosomal Small RNAs Are Promising Non-invasive Diagnostic Biomarkers for Gastric Cancer. *J. Cell Mol Med* 24 (24), 14502–14513. doi:10.1111/jcmm.16077

Hoshino, A., Kim, H. S., Bojmar, L., Gyan, K. E., Cioffi, M., Hernandez, J., et al. (2020). Extracellular Vesicle and Particle Biomarkers Define Multiple Human Cancers. *Cell* 182 (4), 1044–e18. doi:10.1016/j.cell.2020.07.009

Hui, B., Lu, C., Wang, J., Xu, Y., Yang, Y., Ji, H., et al. (2021). Engineered Exosomes for Co-delivery of PGM5-AS1 and Oxaliplatin to Reverse Drug Resistance in colon Cancer. *J. Cell Physiol.* doi:10.1002/jcp.30566

Jänne, P. A., and Mayer, R. J. (2000). Chemoprevention of Colorectal Cancer. *N. Engl. J. Med.* 342 (26), 1960–1968. doi:10.1056/NEJM200006293422606

Jiang, K., Chen, H., Fang, Y., Chen, L., Zhong, C., Bu, T., et al. (2021). Exosomal ANGPTL1 Attenuates Colorectal Cancer Liver Metastasis by Regulating Kupffer Cell Secretion Pattern and Impeding MMP9 Induced Vascular Leakiness. *J. Exp. Clin. Cancer Res.* 40 (1), 21. doi:10.1186/s13046-020-01816-3

Keehn, R. J., and Higgins, G. A., Jr. (1981). Chemotherapy for Gastric Cancer. *Lancet* 1 (8215), 323. doi:10.1016/s0140-6736(81)91928-0

Lan, J., Sun, L., Xu, F., Liu, L., Hu, F., Song, D., et al. (2019). M2 Macrophage-Derived Exosomes Promote Cell Migration and Invasion in Colon Cancer. *Cancer Res.* 79 (1), 146–158. doi:10.1158/0008-5472.CAN-18-0014

Li, H., and Li, F. (2018). Exosomes from BM-MSCs Increase the Population of CSCs via Transfer of miR-142-3p. *Br. J. Cancer* 119 (6), 744–755. doi:10.1038/s41416-018-0254-z

Li, Y., Gao, Y., Gong, C., Wang, Z., Xia, Q., Gu, F., et al. (2018). A33 Antibody-Functionalized Exosomes for Targeted Delivery of Doxorubicin against Colorectal Cancer. *Nanomedicine* 14 (7), 1973–1985. doi:10.1016/j.nano.2018.05.020

Li, Z., Suo, B., Long, G., Gao, Y., Song, J., Zhang, M., et al. (2020). Exosomal miRNA-16-5p Derived from M1 Macrophages Enhances T Cell-dependent Immune Response by Regulating PD-L1 in Gastric Cancer. *Front Cell Dev Biol* 8, 572689. doi:10.3389/fcell.2020.572689

Liang, G., Zhu, Y., Ali, D. J., Tian, T., Xu, H., Si, K., et al. (2020). Engineered Exosomes for Targeted Co-delivery of miR-21 Inhibitor and Chemotherapeutics to Reverse Drug Resistance in colon Cancer. *J. Nanobiotechnology* 18 (1), 10. doi:10.1186/s12951-019-0563-2

Lin, H., Zhang, L., Zhang, C., and Liu, P. (2020). Exosomal MiR-500a-3p Promotes Cisplatin Resistance and Stemness via Negatively Regulating FBXW7 in Gastric Cancer. *J. Cell Mol Med* 24 (16), 8930–8941. doi:10.1111/jcmm.15524

Liu, T., Zhang, X., Du, L., Wang, Y., Liu, X., Tian, H., et al. (2019). Exosome-transmitted miR-128-3p Increase Chemosensitivity of Oxaliplatin-Resistant Colorectal Cancer. *Mol. Cancer* 18 (1), 43. doi:10.1186/s12943-019-0981-7

Maconi, G., Manes, G., and Porro, G. B. (2008). Role of Symptoms in Diagnosis and Outcome of Gastric Cancer. *World J. Gastroenterol.* 14 (8), 1149–1155. doi:10.3748/wjg.14.1149

Mao, J., Liang, Z., Zhang, B., Yang, H., Li, X., Fu, H., et al. (2017). UBR2 Enriched in P53 Deficient Mouse Bone Marrow Mesenchymal Stem Cell-Exosome Promoted Gastric Cancer Progression via Wnt/β-Catenin Pathway. *Stem Cells* 35 (11), 2267–2279. doi:10.1002/stem.2702

Mashouri, L., Yousefi, H., Aref, A. R., Ahadi, A. M., Molaei, F., and Alahari, S. K. (2019). Exosomes: Composition, Biogenesis, and Mechanisms in Cancer Metastasis and Drug Resistance. *Mol. Cancer* 18 (1), 75. doi:10.1186/s12943-019-0991-5

Nabariya, D. K., Pallu, R., and Yenuganti, V. R. (2020). Exosomes: The Protagonists in the Tale of Colorectal Cancer? *Biochim. Biophys. Acta Rev. Cancer* 1874 (2), 188426. doi:10.1016/j.bbcan.2020.188426

Poggi, A., and Giuliani, M. (2016). Mesenchymal Stromal Cells Can Regulate the Immune Response in the Tumor Microenvironment. *Vaccines (Basel)* 4 (4). doi:10.3390/vaccines4040041

Qiao, L., Hu, S., Huang, K., Su, T., Li, Z., Vandergriff, A., et al. (2020). Tumor Cell-Derived Exosomes home to Their Cells of Origin and Can Be Used as Trojan Horses to Deliver Cancer Drugs. *Theranostics* 10 (8), 3474–3487. doi:10.7150/thno.39434

Quail, D. F., and Joyce, J. A. (2013). Microenvironmental Regulation of Tumor Progression and Metastasis. *Nat. Med.* 19 (11), 1423–1437. doi:10.1038/nm.3394

Ren, J., Ding, L., Zhang, D., Shi, G., Xu, Q., Shen, S., et al. (2018). Carcinoma-associated Fibroblasts Promote the Stemness and Chemoresistance of Colorectal Cancer by Transferring Exosomal lncRNA H19. *Theranostics* 8 (14), 3932–3948. doi:10.7150/thno.25541

Shi, L., Wang, Z., Geng, X., Zhang, Y., and Xue, Z. (2020). Exosomal miRNA-34 from Cancer-Associated Fibroblasts Inhibits Growth and Invasion of Gastric Cancer Cells *In Vitro* and *In Vivo*. *Aging (Albany NY)* 12 (9), 8549–8564. doi:10.18632/aging.103157

Smyth, E. C., Nilsson, M., Grabsch, H. I., van Grieken, N. C., and Lordick, F. (2020). Gastric Cancer. *Lancet* 396 (10251), 635–648. doi:10.1016/S0140-6736(20)31288-5

Sun, W., Luo, J. D., Jiang, H., and Duan, D. D. (2018a). Tumor Exosomes: a Double-Edged Sword in Cancer Therapy. *Acta Pharmacol. Sin* 39 (4), 534–541. doi:10.1038/aps.2018.17

Sun, Z., Shi, K., Yang, S., Liu, J., Zhou, Q., Wang, G., et al. (2018b). Effect of Exosomal miRNA on Cancer Biology and Clinical Applications. *Mol. Cancer* 17 (1), 147. doi:10.1186/s12943-018-0897-7

Théry, C., Zitvogel, L., and Amigorena, S. (2002). Exosomes: Composition, Biogenesis and Function. *Nat. Rev. Immunol.* 2 (8), 569–579. doi:10.1038/nri855

Tran, P. H. L., Wang, T., Yin, W., Tran, T. T. D., Nguyen, T. N. G., Lee, B. J., et al. (2019). Aspirin-loaded Nanoexosomes as Cancer Therapeutics. *Int. J. Pharm.* 572, 118786. doi:10.1016/j.ijpharm.2019.118786

Uemura, N., Okamoto, S., Yamamoto, S., Matsumura, N., Yamaguchi, S., Yamakido, M., et al. (2001). *Helicobacter pylori* Infection and the Development of Gastric Cancer. *N. Engl. J. Med.* 345 (11), 784–789. doi:10.1056/NEJMoa001999

Wang, M., Zhao, C., Shi, H., Zhang, B., Zhang, L., Zhang, X., et al. (2014). Deregulated microRNAs in Gastric Cancer Tissue-Derived Mesenchymal Stem Cells: Novel Biomarkers and a Mechanism for Gastric Cancer. *Br. J. Cancer* 110 (5), 1199–1210. doi:10.1038/bjc.2014.14

Wang, X., Zhang, H., Bai, M., Ning, T., Ge, S., Deng, T., et al. (2018). Exosomes Serve as Nanoparticles to Deliver Anti-miR-214 to Reverse Chemoresistance to Cisplatin in Gastric Cancer. *Mol. Ther.* 26 (3), 774–783. doi:10.1016/j.ymthe.2018.01.001

Wei, S., Peng, L., Yang, J., Sang, H., Jin, D., Li, X., et al. (2020). Exosomal Transfer of miR-15b-3p Enhances Tumorigenesis and Malignant Transformation through the DYNLT1/Caspase-3/Caspase-9 Signaling Pathway in Gastric Cancer. *J. Exp. Clin. Cancer Res.* 39 (1), 32. doi:10.1186/s13046-019-1511-6

Wu, S., Li, T., Liu, W., and Huang, Y. (2021). Ferroptosis and Cancer: Complex Relationship and Potential Application of Exosomes. *Front. Cell Dev Biol* 9, 733751. doi:10.3389/fcell.2021.733751

Xie, M., Yu, T., Jing, X., Ma, L., Fan, Y., Yang, F., et al. (2020a). Exosomal circSHKBP1 Promotes Gastric Cancer Progression via Regulating the miR-582-3p/HUR/VEGF axis and Suppressing HSP90 Degradation. *Mol. Cancer* 19 (1), 112. doi:10.1186/s12943-020-01208-3

Xie, Y., Li, J., Li, P., Li, N., Zhang, Y., Binang, H., et al. (2020b). RNA-seq Profiling of Serum Exosomal Circular RNAs Reveals Circ-PNN as a Potential Biomarker for Human Colorectal Cancer. *Front. Oncol.* 10, 982. doi:10.3389/fonc.2020.00982

Xing, H., Liang, C., Xu, X., Sun, H., Ma, X., and Jiang, Z. (2020). Mesenchymal Stroma/stem-like Cells of GARP Knockdown Inhibits Cell Proliferation and Invasion of Mouse colon Cancer Cells (MC38) through Exosomes. *J. Cell Mol Med* 24 (23), 13984–13990. doi:10.1111/jcmm.16008

Xu, G., Zhang, B., Ye, J., Cao, S., Shi, J., Zhao, Y., et al. (2019). Exosomal miRNA-139 in Cancer-Associated Fibroblasts Inhibits Gastric Cancer Progression by Repressing MMP11 Expression. *Int. J. Biol. Sci.* 15 (11), 2320–2329. doi:10.7150/ijbs.33750

Yang, B., Chen, Y., and Shi, J. (2019). Exosome Biochemistry and Advanced Nanotechnology for Next-Generation Theranostic Platforms. *Adv. Mater.* 31 (2), e1802896. doi:10.1002/adma.201802896

Zeng, Z., Li, Y., Pan, Y., Lan, X., Song, F., Sun, J., et al. (2018). Cancer-derived Exosomal miR-25-3p Promotes Pre-metastatic Niche Formation by Inducing Vascular Permeability and Angiogenesis. *Nat. Commun.* 9 (1), 5395. doi:10.1038/s41467-018-07810-w

Zhang, H., Deng, T., Liu, R., Bai, M., Zhou, L., Wang, X., et al. (2017). Exosome-delivered EGFR Regulates Liver Microenvironment to Promote Gastric Cancer Liver Metastasis. *Nat. Commun.* 8, 15016. doi:10.1038/ncomms15016

Zhang, H., Wang, Y., Bai, M., Wang, J., Zhu, K., Liu, R., et al. (2018a). Exosomes Serve as Nanoparticles to Suppress Tumor Growth and Angiogenesis in Gastric Cancer by Delivering Hepatocyte Growth Factor siRNA. *Cancer Sci.* 109 (3), 629–641. doi:10.1111/cas.13488

Zhang, Q., Liu, R. X., Chan, K. W., Hu, J., Zhang, J., Wei, L., et al. (2019). Exosomal Transfer of P-STAT3 Promotes Acquired 5-FU Resistance in Colorectal Cancer Cells. *J. Exp. Clin. Cancer Res.* 38 (1), 320. doi:10.1186/s13046-019-1314-9

Zhang, Q., Zhang, H., Ning, T., Liu, D., Deng, T., Liu, R., et al. (2020). Exosome-Delivered C-Met siRNA Could Reverse Chemoresistance to Cisplatin in Gastric Cancer. *Int. J. Nanomedicine* 15, 2323–2335. doi:10.2147/IJN.S231214

Zhang, Y., Yu, G., Chu, H., Wang, X., Xiong, L., Cai, G., et al. (2018b). Macrophage-Associated PGK1 Phosphorylation Promotes Aerobic Glycolysis and Tumorigenesis. *Mol. Cell* 71 (2), 201–e7. doi:10.1016/j.molcel.2018.06.023

Zhao, R., Zhang, Y., Zhang, X., Yang, Y., Zheng, X., Li, X., et al. (2018). Exosomal Long Noncoding RNA HOTTIP as Potential Novel Diagnostic and Prognostic Biomarker Test for Gastric Cancer. *Mol. Cancer* 17 (1), 68. doi:10.1186/s12943-018-0817-x

Zheng, P., Chen, L., Yuan, X., Luo, Q., Liu, Y., Xie, G., et al. (2017). Exosomal Transfer of Tumor-Associated Macrophage-Derived miR-21 Confers Cisplatin Resistance in Gastric Cancer Cells. *J. Exp. Clin. Cancer Res.* 36 (1), 53. doi:10.1186/s13046-017-0528-y

Zheng, P., Luo, Q., Wang, W., Li, J., Wang, T., Wang, P., et al. (2018). Tumor-associated Macrophages-Derived Exosomes Promote the Migration of Gastric Cancer Cells by Transfer of Functional Apolipoprotein E. *Cell Death Dis* 9 (4), 434. doi:10.1038/s41419-018-0465-5

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Li, Wang, Ding, Feng, Hou, Liu, Lin and Gao. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Oncology

ORIGINAL RESEARCH
published: 03 February 2022
doi: 10.3389/fonc.2022.842406

Ailanthone Inhibits Proliferation, Migration and Invasion of Osteosarcoma Cells by Downregulating the Serine Biosynthetic Pathway

Yawen Zhang[1†], Runze Gong[1†], Yong Liu[2], Xipeng Sun[3], Jinrong Liang[1], Yan Zhou[1], Yaling Wang[4], Wenxi Yu[1], Yonggang Wang[1], Lina Tang[1], Aina He[1], Zan Shen[1], Yang Yao[1], Haiyan Hu[1], Xin Liu[5,6*] and Jianjun Zhang[1*]*

[1] Department of Oncology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China, [2] School of Artificial Intelligence, Guangxi University for Nationalities, Nanning, China, [3] Department of Pharmacy, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China, [4] Department of Oncology, The Eighth People's Hospital of Shanghai, Shanghai, China, [5] Department of Medical Oncology, Fudan University Shanghai Cancer Center, Shanghai, China, [6] Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, China

OPEN ACCESS

Edited by:
Yongye Huang,
Northeastern University, China

Reviewed by:
Hengyuan Li,
Zhejiang University, China
Nouf Laqtom,
Stanford University, United States
Jinxia Hu,
Binzhou Medical University, China

***Correspondence:**
Haiyan Hu
xuri1104@163.com
Xin Liu
jeanettexin@hotmail.com
Jianjun Zhang
robustzhang168@aliyun.com

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Oncology

Received: 23 December 2021
Accepted: 17 January 2022
Published: 03 February 2022

Citation:
Zhang Y, Gong R, Liu Y, Sun X, Liang J, Zhou Y, Wang Y, Yu W, Wang Y, Tang L, He A, Shen Z, Yao Y, Hu H, Liu X and Zhang J (2022) Ailanthone Inhibits Proliferation, Migration and Invasion of Osteosarcoma Cells by Downregulating the Serine Biosynthetic Pathway. Front. Oncol. 12:842406. doi: 10.3389/fonc.2022.842406

Osteosarcoma (OS) is the most common primary bone sarcoma, chemoresistance becomes an obstacle to its treatment. Metabolic reprogramming is a hallmark of malignancy, targeting the metabolic pathways might provide a reasonable therapeutic strategy for OS. Here we demonstrated that Ailanthone (AIL), a major component of the Chinese medicine Ailanthus altissima, significantly suppressed OS cell growth *in vitro* and *in vivo*. Furthermore, AIL dose-dependently inhibited cell migration and invasion, induced cell cycle arrest and apoptosis in OS cells. Combined transcriptomics, proteomics and metabolomics analyses revealed that AIL induced widespread changes in metabolic programs in OS cells, while the serine biosynthetic pathway (SSP) was the most significantly altered pathway. qRT-PCR and Western blot assay confirmed that the transcript and protein levels of the SSP genes (*PHGDH*, *PSAT1* and *PSPH*) were downregulated dose-dependently by AIL. In addition, we found out that many downstream pathways of the SSP including the one-carbon pool by folate, purine metabolism, pyrimidine metabolism, DNA replication and sphingolipid metabolism were downregulated after AIL treatment. In the revere test, *PHGDH* overexpression but not exogenous serine supplementation clearly attenuated the effects of AIL on OS cells. Taken together, AIL exerts antitumor effects on OS through mediating metabolic reprogramming, at least in part, by suppressing the SSP. Our findings suggest that AIL could emerge as a potential therapeutic strategy in OS.

Keywords: ailanthone, osteosarcoma, cancer metabolism, serine biosynthesis, PHGDH, multiomics analysis

INTRODUCTION

Osteosarcoma (OS) is the most common primary bone sarcoma, affecting mainly adolescents and young adults (1). The survival of patients with OS has improved considerably with the advent of multi-drug chemotherapy regimen including high-dose methotrexate, doxorubicin, cisplatin and ifosfamide since the late 1970s (2). However, frequent acquisition of drug-resistance becomes an obstacle to its treatment, patients who do not respond to chemotherapy have an overall survival of only 20-25% at 5 years (3, 4). During the past 30 years, OS therapy and prognosis had only modest improvements (5). New alternative anticancer agents for OS are in urgent need.

Metabolic reprogramming is a hallmark of malignancy (6). This metabolic alteration allows tumors to achieve excessive cell growth, proliferation, metastasis potential, as well as drug-resistance (7). Ongoing studies have revealed that the origin and development of OS are associated with alterations in metabolic pathways. For instance, OS cells have higher mitochondrial biogenesis compared to normal osteoblasts (8). Metabolic shift from oxidative phosphorylation to glycolysis promotes proliferation in osteosarcoma (9) while a higher glycolysis rate of OS cells confers to their cisplatin-resistant property (10). Recent studies reported that activation of the serine biosynthetic pathway (SSP) drives oncogenesis and contributes to tumor growth (11–13). The upregulation of the first rate-limiting enzyme of the SSP, 3-phosphoglycerate dehydrogenase (PHGDH), has been identified in a large subset of cancers (13–20). OS cell lines including Saos-2, U2-OS, MG63, MNNG and NOS1 were found to have significantly higher PHGDH protein expression than that of mesenchymal cells. Clinically, higher PHGDH protein expression was correlated with poorer outcomes (21). Given the important role of metabolic reprogramming in OS, targeting the metabolic pathways might provide a new therapeutic strategy for this disease.

In this study, we identified that Ailanthone (AIL), a quassinoid extracted from the traditional Chinese medicine plant Ailanthus altissima (22), exhibited significant antitumor activity in OS *in vitro* and *in vivo*. Mechanistically, combined transcriptomics, proteomics and metabolomics analysis revealed that AIL induced widespread changes in metabolic programs in OS cells. In particular, the SSP was the most significantly affected pathway by AIL treatment. Moreover, many downstream pathways of the SSP were downregulated. In the reverse test, *PHGDH* overexpression clearly reduced the inhibitory effects of AIL on OS cells, revealing a mechanistic link between AIL and the SSP. Overall, our findings indicate that AIL suppresses OS cell growth through mediating metabolic reprogramming, suggesting that AIL could be used as a promising therapeutic candidate in OS treatment.

MATERIALS AND METHODS

Cell Culture and Proliferation Assay

Human Saos-2 and U-2OS OS cells were purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The cell lines were routinely cultured in DMEM (#8119283, Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, #10270-106, Gibco), 100 μg/mL streptomycin, and 100 U/mL penicillin at 37°C in an incubator with a constant airflow of 5% CO_2 and 95% O_2. As for reverse test, the above-mentioned medium was supplemented with extra 400 μM serine (#GA10157, GlpBio Technology, CA, USA) in the serine supplementation group. AIL (#GC31742, molecular weight, 376.4, purity > 98.00%, GlpBio Technology) was dissolved to 10mM in dimethyl sulfoxide (DMSO, #D4540, Sigma-Aldrich, St. Louis, MO, USA) and stored at -80°C. The final concentrations were 0.039-10 μM. Cell proliferation assay was performed using Cell Counting Kit-8 (CCK-8, #96992, Dojindo Molecular Technologies, Dojindo, Japan). The absorbance value was measured at a wavelength of 450 nm, with the reference wavelength set at 630 nm. Data were collected from three independent experiments, and the AIL-induced cell growth inhibition rate was calculated by comparison to untreated control cells.

Wound Healing Assay

The migration ability of OS cells was detected using a wound healing assay as outlined previously (23). Briefly, OS cells in a 6-well plate were carefully scratched using 10 μL sterile pipette tips. Subsequently, the cells were washed twice and were cultured in DMEM with different concentrations (0, 0.01, 0.05 and 0.1 μM) of AIL for 24 h. Images were then taken to determine the width of the wound using a microscope (10× objective) (1×71, Olympus).

Transwell Assay

Transwell assays were performed using Boyden chambers (Corning Inc., Corning, NY, USA) as previously described (24). Briefly, OS cells were treated with AIL at various concentrations for 24h. Next, 1×10^5 cells viable cells (confirmed by trypan blue exclusion) from each well were seeded in serum-free medium onto the Matrigel-coated upper chamber, while complete medium was added in the lower compartment as a chemoattractant. After incubation of 24 h, the cells that migrated through the filter were fixed and stained (23). The invading cells were evaluated in five random fields under the microscope, and images were obtained.

Apoptosis Analysis

OS cells were seeded in 6-cm plates and incubated with different concentrations of AIL. After 24 h, all the cells including the floating and adherent ones were harvested and stained with Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) apoptosis detection kit (#556547, BD Biosciences Pharmingen, San Diego, CA, USA) according to the manufacturer's protocols. The resulting fluorescence was assessed by fluorescence-activated cell sorting scan (FACS) flow cytometry (Becton Dickinson, Mountain View, CA, USA) as reported previously (25).

Cell Cycle Analysis

After being treated with different concentrations of AIL for 24 h, the OS cells were harvested and fixed with 70% ethanol overnight at -20°C and stained with PI. The DNA content was detected

using a FACS flow cytometry (Becton Dickinson) according to the manufacturer's protocols. The cell cycle distribution was determined using FlowJo software 8.7.1 (Tree Star, Inc., Ashland, OR, USA).

TMT-Based Quantitative Proteomics

Tandem mass tag (TMT)-based quantitative proteomic analysis was carried out on Saos-2 cells treated with IC50 (0.20 μM) of AIL or DMSO for 24 h (3 biological replicates each group). A schematic description of the experimental design and data process strategy is presented in **Figure 5A**. SDT (4%SDS, 100mM Tris-HCl, 1mM DTT, pH7.6) buffer was used for cell lysis and protein extraction. Quantitative proteomic analysis was performed at Shanghai Applied Protein Technology Co., Ltd as described previously (26). Briefly, 100 μg peptide of each sample was labeled using TMT-6-plex Isobaric Label Reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Then, the labeled peptides were separated into 10 fractions using the High pH Reversed-Phase Peptide Fractionation Kit (Thermo Fisher Scientific). Each fraction was separated with EASY-nLC 1000 Liquid Chromatograph (Thermo Fisher Scientific) and then subjected to LC-MS/MS analysis on a Q Exactive HF-X mass spectrometer (Thermo Fisher Scientific) for 60 min. The MS raw data for each sample were searched using the MASCOT engine (version 2.2, Matrix Science, London, UK) embedded into Proteome Discoverer 1.4 software (Thermo Fisher Scientific) against the SwissProt human proteome database (20395 items, released on 2021.01.06) concatenated with reverse decoy database for identification and quantitation analysis. All identified proteins were determined using a false discovery rate (FDR) threshold of ≤ 0.01. The methods for protein quantification were as followed: first, the ratio of label signals was output and calculated as a ratio to the reference sample; secondly, the necessary normalization on protein median was performed to eliminate experimental error utilizing Correcting Experimental Bias function with built-in settings in Proteome Discoverer 1.4 software (Thermo Fisher Scientific); finally, the protein is quantified based on the median of the relative intensity of the unique peptide of each protein.

RNA-Seq Analysis

Cell culture and AIL treatment were the same as those in quantitative proteomic analysis. RNA samples were prepared using TRIzol (#15596026, Invitrogen) and subjected to RNA-seq analysis by Genome Center, WuXi App Tec as reported previously (27). Generally, 1 μg of qualified RNA (RIN > 8.0) was used as input for library construction following the Illumina TruSeq RNA Sample Preparation protocol. Then, RNA libraries were sequenced on an Illumina NovaSeq 6000 platform, PE 2×150bp. The average data yield for each sample was 20M PE reads with % of Q30 bases > 90. Subsequently, RNA-seq reads of each sample were aligned to the human genome (build 38) using short reads aligner STAR (version 2.5.1b). Gene annotation and quantification were then performed using RSEM24 with GENCODE annotation (release 24: http://www.gencodegenes.org).

Untargeted Metabolomic Relative Quantitative Analysis

Untargeted metabolomics profiling of Saos-2 cells treated with AIL or DMSO was performed using ultra-high-performance liquid chromatography (1290 Infinity LC, Agilent Technologies, USA) coupled with a quadrupole time-of-flight system (AB Sciex TripleTOF 6600, AB SCIEX, USA) at Shanghai Applied Protein Technology Co., Ltd. Briefly, the cell samples were collected in 15-mL Vacutainer tubes and then centrifuged for 15 minutes. Aliquots of the samples were stored at −80°C for use. Then the samples were thawed at 4°C, and 100-μL aliquots were mixed with 400 μL of cold methanol/acetonitrile (1:1, v/v) to remove the protein. The mixture was centrifuged for 20 minutes. The supernatants were dried in a vacuum centrifuge. Detailed experimental procedures for mass spectrometry and data process methods are described previously (28). After normalization to the total peak intensity, the data were exported to the SIMCA-P software for multivariate data analysis to screen for differential metabolites.

qRT-PCR Assay

The mRNA levels of *PHGDH*, phosphoserine aminotransferase 1 (*PSAT1*) and phosphoserine phosphatase (*PSPH*) were detected using a PrimeScript RNA RT-PCR Kit (#Q711-03, Vazyme Biotech co., ltd, Nanjing, China) according to the manufacturer's protocol. The primer pairs are shown in **Table 1**. The relative mRNA levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Western Blot Assay

Expression of proteins in OS cells was quantified using a BCA Protein Assay Reagent Kit (#23228, Thermo Fisher Scientific) as described previously (23). The rabbit polyclonal cleaved-Caspase-3 (#9664), BCL-2 (#3498), BAX (#5023), cyclinD1 (#55506) and CDK4 (#12790) antibodies were purchased from Cell Signaling Technology (Boston, USA). The PHGDH (#14719-1-AP), PSAT1 (#10501-1-AP) and PSPH (#14513-1-AP) antibodies were purchased from Proteintech Group, Inc. (Chicago, IL, USA), and the band density was quantified using ImageJ software 1.8.0 (National Institutes of Health, Bethesda, MD, USA).

TABLE 1 | The qRT-PCR primers.

	Forward	Reverse
PHGDH	5′-ATCTGCGGAAAGTGCTCATCA-3′	5′-GTGGCAGAGCGAACAATAAGG-3′
PSAT1	5′-CGCAGAAGAAGCCAAGAAGTT-3′	5′-TGGCTTGGACAGGAAGTTTGA-3′
PSPH	5′-AAAATCTGTGGCGTTGAGGAC-3′	5′-TGTGGGGGTTGCTCTGCTAT-3′
GAPDH	5′-AACGGATTTGGTCGTATTGGG-3′	5′-CCTGGAAGATGGTGATGGGAT-3′

Construction of the Plasmids and Transfection

The *PHGDH* overexpression pcDNA3.1 plasmid was constructed by Genechem Co., Ltd. (Shanghai, China). The plasmid was transfected into Saos-2 and U-2OS cells using Lipofectamine 3000 (#L3000015, Invitrogen) following the manufacturer's protocol.

Mice Xenograft Experiment

The animal study was performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals approved by the Scientific Investigation Board of Shanghai Jiao Tong University Affiliated Sixth People's Hospital. 5×10^6 Saos-2 cells were suspended in 75 μL serum-free DMEM and combined with 75 μL of Matrigel (#356235, Corning, NY, USA), and then the mixture was injected into the right hind thigh of 6-8 weeks NOD-*Prkdc*^{scid} *Il2rg*^{em1}*/Smoc* (NSG) mice (Model organisms center, Shanghai, China). Cell line-derived xenografts were allowed to grow to an average volume of 100 mm^3, then mice were intraperitoneally injected with AIL (2mg kg^{-1}) or DMSO (as controls) every 3 days. Length and width of resulting tumors as well as weight of mice were measured every 5 days, and tumor volumes were calculated using the formula (length×width^2)/2. Mice were sacrificed and tumors were harvested 30 days after treatment initiation.

Bioinformatics Analysis and Statistics

For proteomics analysis, Perseus software (v1.6.5.0) was used. Differentially ex-pressed proteins (DEPs) were filtered at $P < 0.05$ and with a fold change of ≥ 1.2 or ≤ 0.83. Gene Ontology (GO) annotation for DEPs was performed using the DAVID Bioinformatics Resources 6.8 (https://david.ncifcrf.gov/). Moreover, proteome and transcriptome data were subjected to Gene Set Enrichment Analysis (GSEA) separately based on the whole gene set with the rank list of all the available expression values. C2.cp.kegg from the Molecular Signatures Database (MSigDB, v7.4) were selected as gene sets for the KEGG pathway enrichment analysis. Significantly enriched pathways were selected at a level of 0.05 norminal *P*-value and 0.25 FDR *q*-value. Subsequently, enriched pathways from proteome and transcriptome data were combined to determine the pathway that was most significantly affected by AIL treatment.

For metabolomics data, differentially expressed metabolites were determined using an unpaired Student's t-test. The variable importance in the projection (VIP) value >1 and $P < 0.05$ was considered as statistically significant. The above-obtained metabolites were blasted against the online KEGG database to retrieve their Compounds and were subsequently subjected to enrichment analysis. Only pathways with *P*-values under a threshold of 0.05 were considered as significantly changed pathways.

The non-omics data analysis was performed using GraphPad Prism 7 (GraphPad Software Inc., CA, USA). *P* values were two-tailed, and $P < 0.05$ was considered statistically significant. Continuous variables are presented as the mean ± SD.

RESULTS

AIL Inhibited Proliferation, Migration and Invasion of OS Cells *In Vitro*

The proliferation assay revealed that AIL significantly inhibited the proliferation of Saos-2 and U-2OS cells in a dose and time-dependent manner (**Figure 1A**). The IC50 at 24 h was determined to be 0.20 and 0.13 μM for the Saos-2 and U-2OS cells, respectively. Wound healing assays and Transwell assays were performed to determine the migratory and invasive abilities of OS cells. As shown in **Figures 1B, D**, compared with the migration rates of the control group, the migration rates of the Saos-2 and U-2OS cells treated with AIL were decreased dose-dependently. In the Transwell assay, AIL dose-dependently suppressed OS cell invasion (**Figures 1C, D**).

AIL Suppressed Subcutaneously Xenografted OS Tumor Growth

To evaluated the efficacy of AIL *in vivo*, we treated Saos-2 xenografts in nude mice with AIL every 3 days. As a result, AIL significantly inhibited the increase of tumor volume in Saos-2 xenografts (**Figure 2A**). Mice were sacrificed after treatment, the mean tumor volume in AIL-treated mice was significantly lower than that in DMSO-treated mice (**Figures 2B, C**). In addition, there was no significant difference in mice weight between the two groups (**Figure 2D**), indicating that the dose of AIL was well tolerated.

AIL Induced Apoptosis and Cell Cycle Arrest in OS Cells

Since AIL showed a significant inhibitory effect on OS cell growth both *in vitro* and *in vivo*, we next explored the mechanism of action. As a result, AIL induced apoptosis in Saos-2 and U-2OS cells dose-dependently, which was supported by the downregulation of BCL-2 protein and upregulation of cleaved-Caspase-3 and BAX protein by Western blot assay (**Figure 3**). Cell cycle analysis showed that the S phase was significantly decreased, while the G1 phase was significantly increased in Saos-2 and U-2OS cells after AIL treatment (**Figures 4A, B**). G1-S transition related protein cyclinD1 and CDK4 were downregulated dose-dependently (**Figures 4C, D**). The results indicated that AIL blocked G1-S transition in OS cells in a dose-dependent manner.

Treatment With AIL Led to Widespread Changes in Metabolic Programs

To investigate the possible network related to the inhibitory effect of AIL on OS cells, we performed a comparative proteomic analysis in Saos-2 cells treated with AIL or DMSO. In total, 6860 unique proteins (5684 proteins identified with at least two peptide fragments) were identified with high confidence (FDR ≤ 0.01). Among them, 6831 proteins were quantifiable (5674 proteins were quantifiable with at least two peptide fragments). 899 DEPs (342 upregulated and 557 downregulated, **Supplementary Table 1**) were filtered at $P < 0.05$ and fold change ≥ 1.2 or ≤ 0.83. The classification of DEPs by GO database annotation showed that

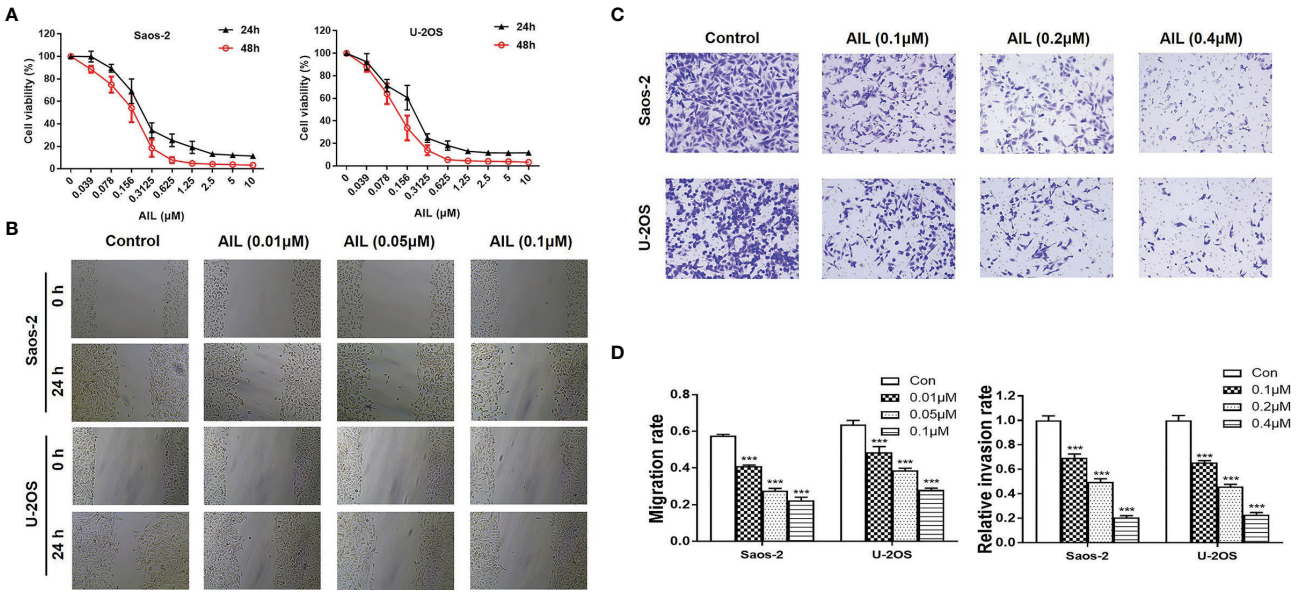


FIGURE 1 | AIL inhibited proliferation, migration and invasion of OS cells. **(A)** After exposure to various concentrations (0.039-10 μM) of AIL for 24 h and 48h, the viability of Saos-2 and U-2OS cells was assessed with a CCK-8 assay. The cell viability decreased dose- and time-dependently. **(B)** After incubation with various concentrations of AIL, the wound healing assay indicated that AIL impaired wound healing dose-dependently. **(C)** Transwell assays showed that the invasion rates of OS cells decreased dose-dependently in the AIL groups. **(D)** Comparisons of the migration rates and relative invasion rates between the AIL and control groups are shown (***$P < 0.001$ compared with the control).

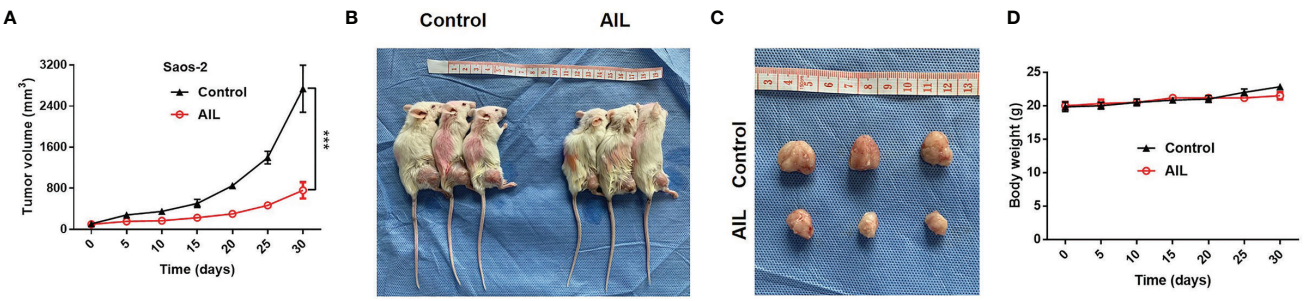


FIGURE 2 | AIL inhibited subcutaneously xenografted tumor growth. **(A)** Saos-2 tumor-bearing mice were treated with AIL or DMSO intraperitoneally. The tumor volumes were measured every 5 days, growth curves for tumor proliferation were drawn accordingly (***$P < 0.001$). **(B, C)** Mice were sacrificed and tumors were harvested (30 days after treatment), the mean tumor volume in AIL-treated mice and DMSO-treated mice was 2739 ± 796 mm^3 and 760 ± 282 mm^3, respectively (***$P < 0.001$). **(D)** There was no significant difference in mice weight between the two groups.

617 (68.6%) proteins are involved in the metabolic process (**Figure 5B**), suggesting that AIL treatment leads to widespread changes in metabolic programs in OS cells. To identify the specific signaling pathways in detail, GSEA was performed. As a result, 28 KEGG pathways were significantly enriched (**Figure 5C**). Furthermore, we performed RNA-Seq analysis to interrogate changes at transcript level in OS cells exposed to AIL. GSEA analysis revealed that 30 KEGG pathways were significantly enriched (**Figure 5D**). Then we combined the significantly enriched pathways from proteome and transcriptome data, we found out that 7 KEGG pathways were common (**Figure 5E**). Among the 7 common pathways, the serine biosynthetic or metabolic pathway was the pathway most significantly changed (**Figures 5C–G**). Notably, the GSEA Blue-Pink O'gram (**Figure 5H**) revealed that PHGDH, PSPH and PSAT1, the three rate-limiting enzymes in SSP, are the top three downregulated proteins. These bioinformatic results suggested that the SSP is a key target of AIL in OS cells. Serine is a precursor to folate, which is the principal donor of one-carbon units (29, 30), it serves as a major source of metabolic intermediates that are required for nucleotide synthesis. Serine is also the precursor to sphingolipids (29, 31–33). Interestingly, GSEA results indicated that the

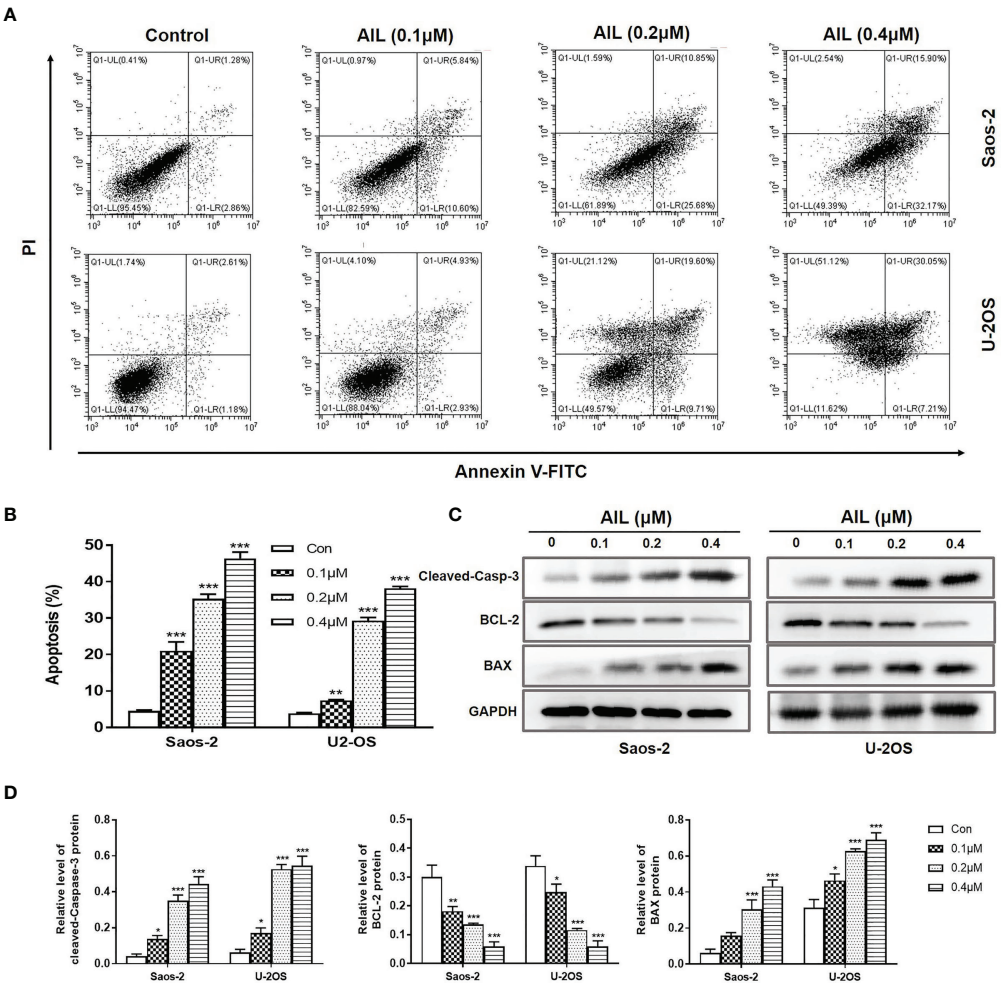


FIGURE 3 | AIL induced apoptosis in OS cells. **(A)** Saos-2 and U-2OS cells were, respectively, treated with AIL at different concentrations (0, 0.1, 0.2 or 0.4 μ M) for 24 h. Then cells were stained with Annexin V-FITC and PI and then subjected to flow cytometry analysis of cell apoptosis. **(B)** Data of the percentage of apoptosis at various concentrations were pooled from **(A)**. **(C)** Apoptosis protein analysis. The protein levels of cleaved-Caspase-3, BCL-2 and BAX was determined by Western blot assay. **(D)** The protein levels were evaluated by ratio values quantified from protein bands of each marker versus GAPDH (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the control).

one-carbon pool by folate, DNA replication and sphingolipid metabolism pathways were among the 7 common pathways downregulated by AIL treatment (**Figures 5E, I–K**). These data further support the connection between the effects of AIL and the SSP.

Treatment With AIL Led to Reduced SSP Expression

To validate the changes of SSP expression induced by AIL, we measured the expression of mRNA and protein of the SSP in Saos-2 and U-2OS cells using qRT-PCR and Western blot assay, respectively. In line with the RNA-Seq-based gene expression, all of the *PHGDH*, *PSAT1* and *PSPH* mRNA was downregulated significantly in both of the two cell lines after AIL treatment (**Figure 6A**). Similarly, protein expression of each enzyme in the SSP was confirmed to be downregulated by AIL dose-dependently (**Figures 6B, C**).

Treatment With AIL Led to Reduced Intermediate Metabolites of the SSP and Its Downstream Pathways

Since the above-mentioned data suggested that the effects of AIL are associated with changes in metabolic programs, untargeted metabolomics with high resolution, high throughput, and highly sensitive technology was performed to execute a large-scale detection of the metabolite features after AIL treatment (**Figure 7A**). As shown in **Figure 7B**, 21 KEGG pathways were significantly enriched, out of which, 13 were involved in metabolism and **Supplementary Table 2**. To be noted, glycine serine and threonine metabolism was one of the pathways

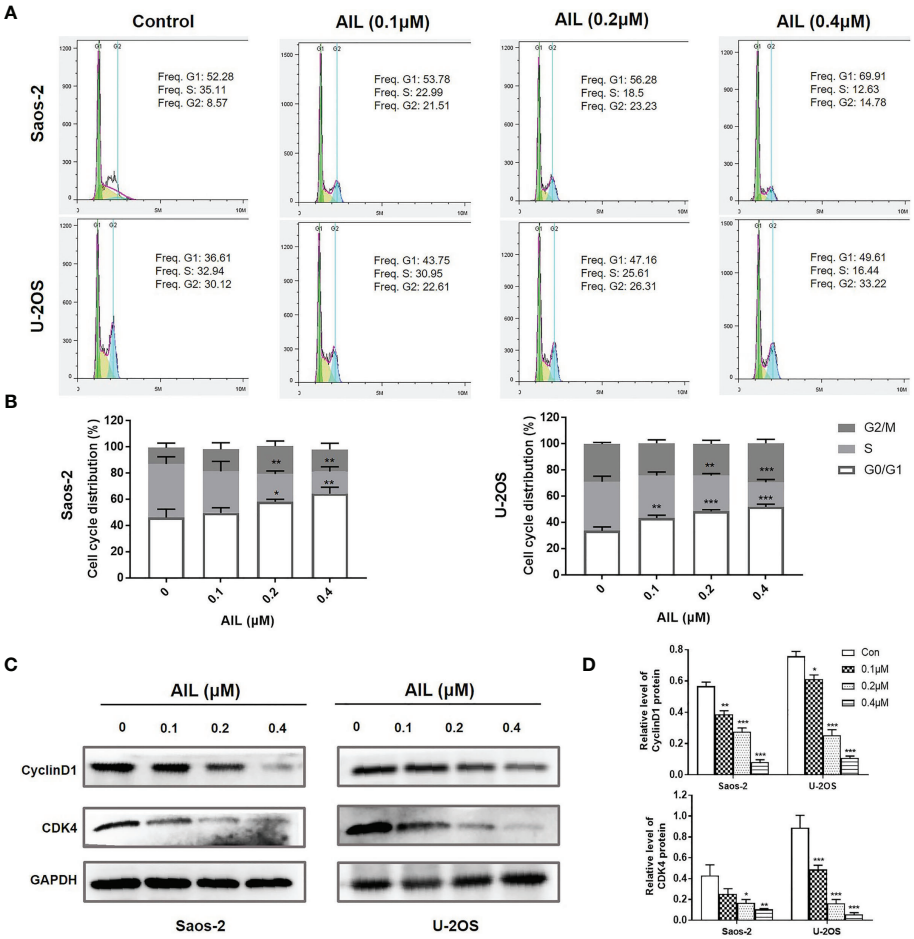


FIGURE 4 | AIL blocked G1-S cell cycle transition in OS cells. **(A)** Cell cycle profiles were measured by flow cytometry following treatment of the cells with various concentrations of AIL (0, 0.1, 0.2 or 0.4 μ M) for 24 h. **(B)** The S phase was significantly decreased, while the G1 phase was significantly increased in Saos-2 and U-2OS cells after AIL treatment (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the control). **(C)** The expression of cell cycle-related protein CyclinD1 and CDK4 was determined by Western blot assay. **(D)** The protein levels were evaluated by ratio values quantified from protein bands of each marker versus GAPDH (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the control).

that are changed the most. And then, we asked about the change of intermediate metabolites of the SSP. As expected, the relative levels of 3-phosphohydroxypyruvate, 3-phosphoserine and serine in OS cells were reduced significantly after AIL treatment (**Figures 7A, C**). Serine is important in metabolism in that it participates in the biosynthesis of purines, pyrimidines and sphingolipids. Interestingly, the intermediate metabolites of the downstream pathways of the SSP including the purine metabolism, pyrimidine metabolism and sphingolipid metabolism were also downregulated significantly (**Figure 7C**). These results are in line with the data at mRNA and protein levels. Taken together, we proposed that AIL inhibited proliferation, migration and invasion of OS cells by downregulating the SSP, further downregulating the pathways of one-carbon pool by folate, purine metabolism, pyrimidine metabolism, DNA replication and sphingolipid metabolism (**Figure 7D**).

Overexpression of *PHGDH* but Not Exogenous Serine Supplementation Reversed the Effects of AIL on OS Cells

To further determine the functional importance of the SSP in AIL effects, the *PHGDH* in OS cells were overexpressed by the treatment with IC50 of AIL (**Figures 8A, B**). Besides, we supplemented exogenous serine in the media to explore if downregulation of serine biosynthesis by AIL can be compensated for with exogenous serine. As expected, the viability of Saos-2 and U-2OS cells significantly increased in the *PHGDH* overexpression group compared with that in the negative control group. However, the inhibitory effect of AIL on OS cell proliferation can't be reversed by exogenous serine supplementation. Moreover, exogenous serine supplementation didn't affect the reverse effect of *PHGDH* overexpression (**Figure 8C**). Similarly, the wound healing assay and Transwell assay revealed that overexpression of *PHGDH* but not exogenous serine supplementation attenuated the

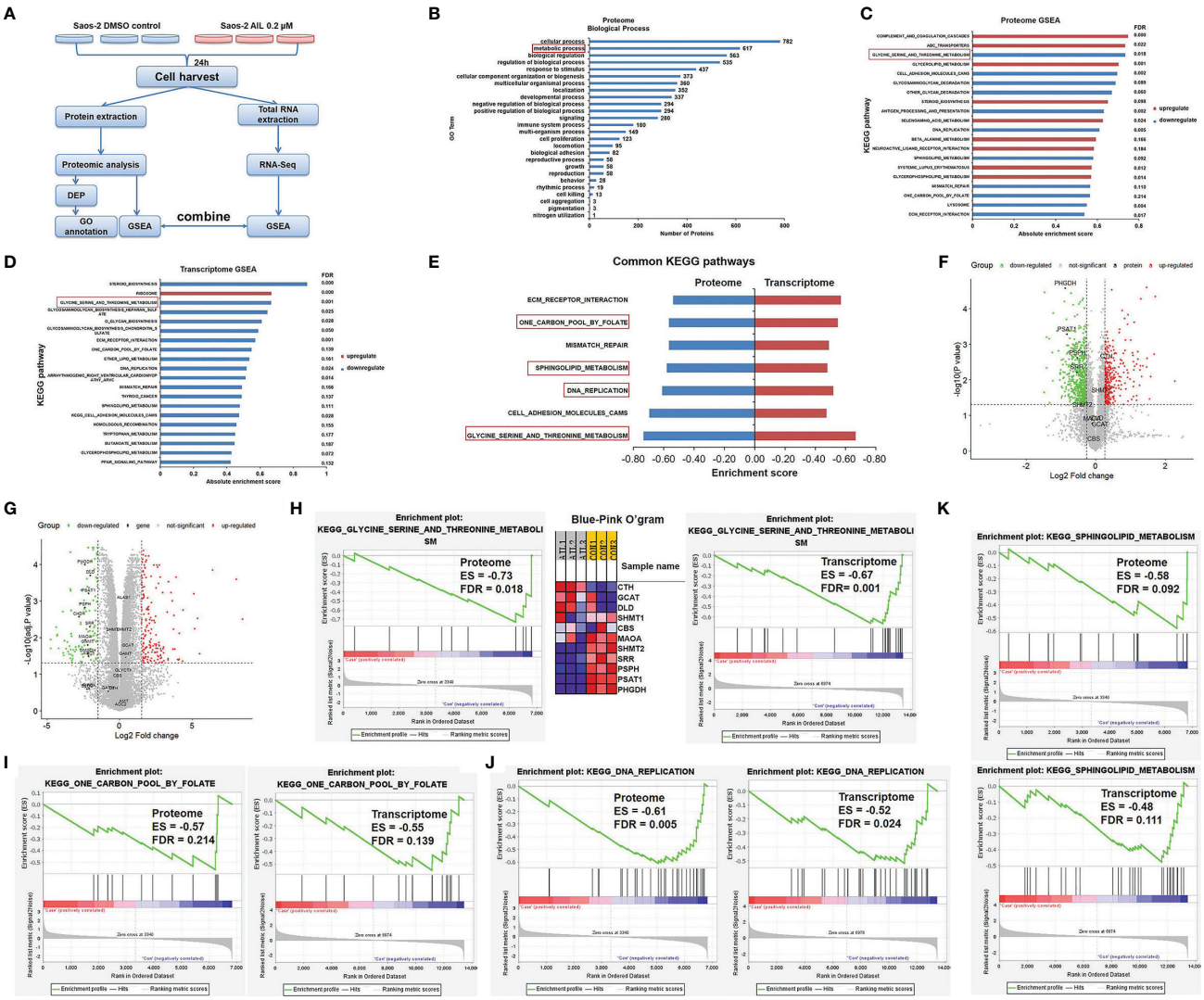


FIGURE 5 | Proteomics and transcriptomics analysis of AIL treated Saos-2 cells identified the SSP as a key target. **(A)** Schematic overview of proteomics and transcriptomics analysis of Saos-2 cells treated with AIL or DMSO. **(B)** Gene ontology (GO) annotation showed that 617 (68.6%) out of the 899 differentially expressed proteins are involved in metabolic process. **(C)** GSEA of proteome data, showing the top 20 significant altered KEGG pathways after AIL treatment. **(D)** GSEA of transcriptome data, showing the top 20 significant altered KEGG pathways after AIL treatment. **(E)** Details of the 7 common KEGG pathways from proteome and transcriptome data. **(F)** Volcano plot of the global profile of proteins after AIL treatment, marked black proteins are involved in the serine biosynthetic or metabolic pathway. **(G)** Volcano plot of the global profile of mRNA after AIL treatment, marked black genes are involved in the serine biosynthetic or metabolic pathway. **(H)** Enrichment plot and Blue-Pink O'gram of the glycine, serine and threonine metabolism pathway from proteome and transcriptome data. **(I)** Enrichment plots of the KEGG one-carbon pool by folate pathway from proteome and transcriptome data. **(J)** Enrichment plots of the KEGG DNA replication pathway from proteome and transcriptome data. **(K)** Enrichment plots of the KEGG sphingolipid metabolism pathway from proteome and transcriptome data.

suppression of AIL on the migration and invasion of OS cells (**Figures 8D–F**). These data demonstrated that AIL suppresses OS cells through downregulating the serine *de novo* biosynthesis but not exogenous serine importation.

DISCUSSION

In the past decade, an increasing number of studies have focused on AIL due to its antitumor activity in a variety of malignances, including leukemia, lung, breast, gastric, liver and prostate cancer (22, 34–38). However, the effects of AIL on OS remain largely unexplored. In this study, we found that AIL dose-dependently inhibited cell proliferation, migration and invasion while inducing G1-S cell cycle arrest and apoptosis in Saos-2 and U-2OS cells. Our results were similar to that of researches in gastric and liver cancer (37, 38).

The antitumor mechanism of AIL differs a lot in different cancer types (39). To explore the underlying networks that are regulated by AIL in OS cells, we performed combined transcriptomics,

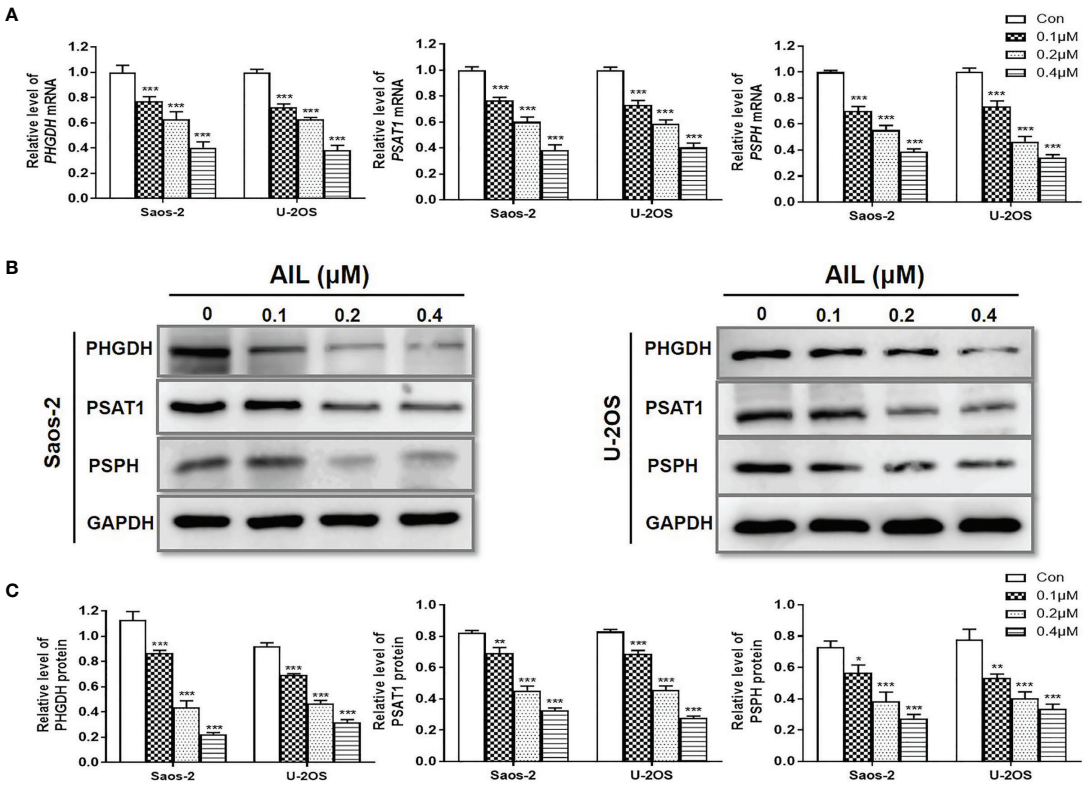


FIGURE 6 | Treatment with AIL led to reduced SSP expression. **(A)** qRT-PCR revealed that the *PHGDH*, *PSAT1* and *PSPH* mRNA levels were downregulated by AIL dose-dependently in Saos-2 and U-2OS cells (**P* < 0.05, ***P* < 0.01, ****P* < 0.001 compared with the control). **(B)** The expression of PHGDH, PSAT1 and PSPH protein was downregulated by AIL dose-dependently in Saos-2 and U-2OS cells. **(C)** Data of relative protein level were pooled from three independent experiments (**P* < 0.05, ***P* < 0.01, ****P* < 0.001 compared with the control).

proteomics and metabolomics analysis. The results revealed that AIL induced widespread changes in metabolic programs in OS cells. In particular, AIL treatment led to significantly reduced SSP at transcript, protein, as well as metabolite levels. Then we selected the SSP for further validation, not only because the SSP was the pathway most significantly affected by AIL, but also because the SPP was confirmed to be abnormally activated in OS and serine is a central hub of cancer metabolism (21). The SSP represents a crucial turning point in glucose conversion (40). It converts the glycolytic intermediate, 3-phosphoglycerate, to serine *via* three sequential enzymes PHGDH, PSAT1 and PSPH. Serine is then converted to glycine, concomitantly charging the folate pool with one-carbon units (29, 30, 41–43). Both glycine and folate one-carbon units are used to make nucleotides. Serine is also required for the synthesis of sphingolipids, a major component of cellular membranes (31–33). Moreover, serine biosynthesis affects cellular antioxidative capacity, thus maintaining tumor homeostasis (11, 20). Hyperactive SPP frequently enables cancer cells to survive and proliferate irrespective of the availability of exogenous serine (44, 45).

In the subsequent experiments, the transcript and protein levels of the SSP genes (*PHGDH*, *PSAT1* and *PSPH*) were confirmed to be downregulated dose-dependently by AIL in OS cells using qRT-PCR or Western blot assay. To validate the key role of the SSP, the reverse test was performed. As expected, after overexpressing *PHGDH*, the suppression of AIL on OS cell proliferation, migration, and invasion was clearly reduced. We reason that inhibition of *de novo* serine biosynthesis causes failure to fuel the subsequent DNA synthesis and cellular membrane production, which are essential to cancer proliferation. This proposed mechanism was further supported by the downregulation of several downstream pathways of the SSP and the cell cycle arrest in OS cells after AIL treatment. Similarly, Ni et al. reported that AIL inhibited non-small cell lung cancer cell growth through repressing DNA synthesis (35). Proliferating cells can import serine exogenously or through *de novo* serine biosynthesis *via* the SSP. However, we found that exogenous serine supplementation didn't reverse the effects of AIL on OS cells. We then asked why *PHGDH* inhibition cannot be compensated for with exogenous serine, one possible explanation is the relatively low-baseline mRNA expression of the serine transporter *SLC1A5* in OS cells, as shown in data from the Cancer Cell Line Encyclopedia (46). Indeed, the detailed mechanistic understanding of why some cancer cells are addicted to serine synthesis despite the availability of extracellular serine for import remains unclear (47).

The importance of the metabolic pathways is underlined in OS by the fact that the inhibitor of dihydrofolate reductase

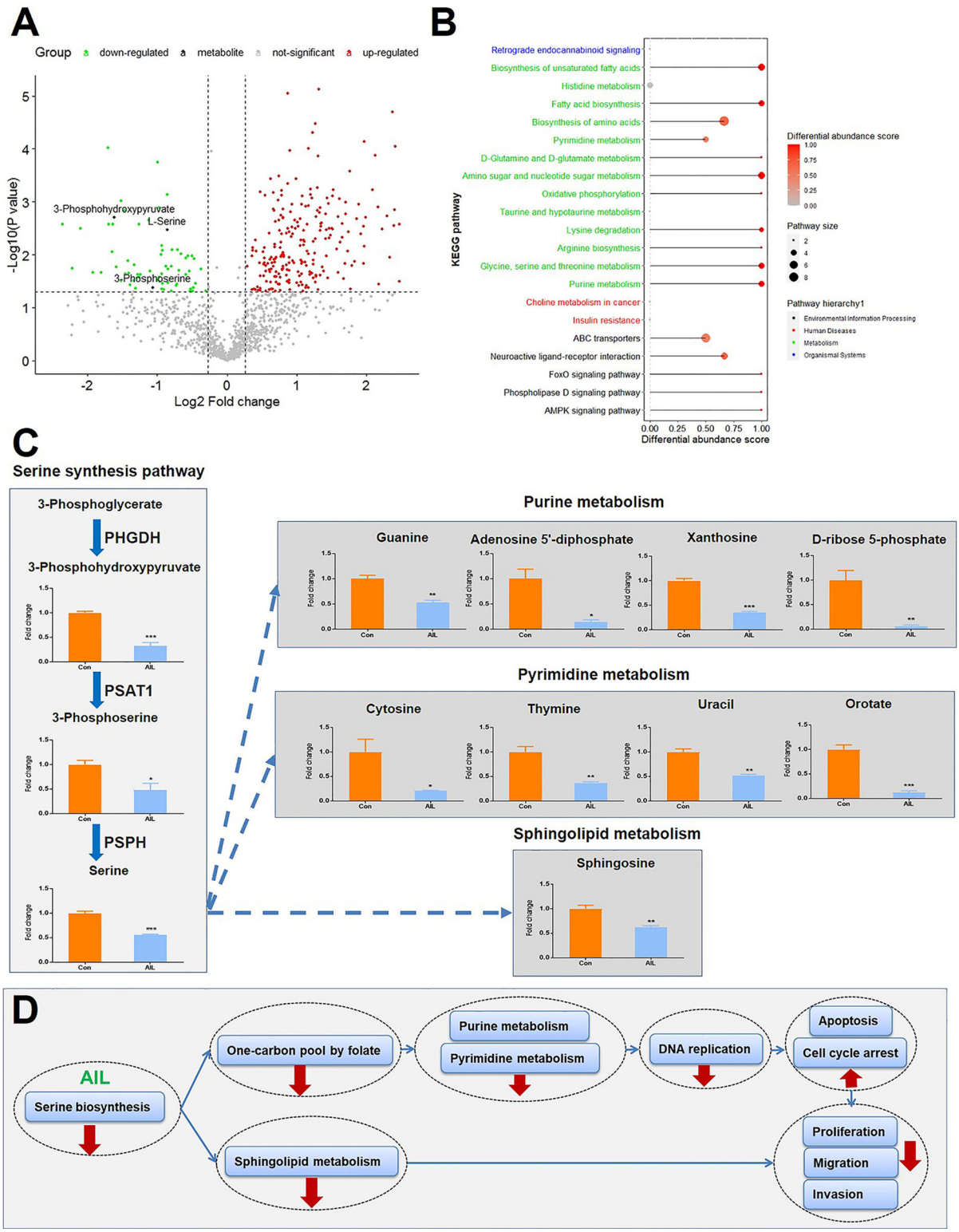


FIGURE 7 | Treatment with AIL led to reduced intermediate metabolites of the SSP and its downstream pathways. **(A)** Volcano plot of the global profile of metabolites after AIL treatment, marked black metabolites are involved in the serine biosynthetic or metabolic pathway. **(B)** KEGG enrichment of differentially expressed metabolites, showing 21 significantly enriched pathways after AIL treatment. **(C)** Fold-changes in the intermediate metabolites of the SSP and its downstream pathways (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the control). **(D)** Proposed schematic overview of the antitumor mechanism of AIL in OS.

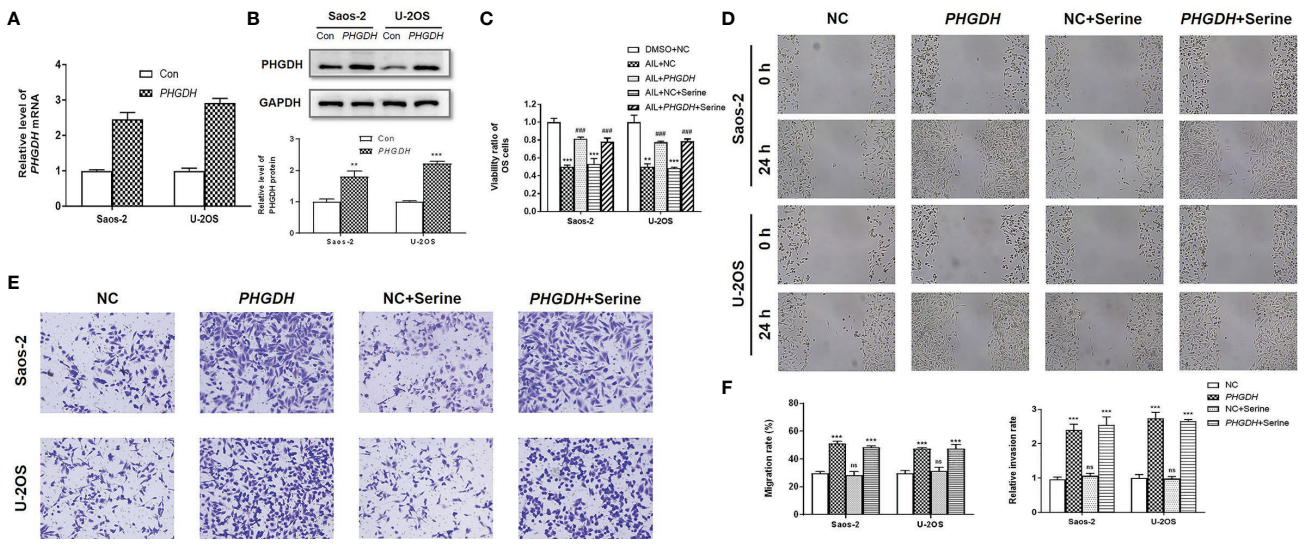


FIGURE 8 | Overexpression of *PHGDH* but not exogenous serine supplementation reversed the effects of AIL on OS cells. **(A, B)** Overexpression efficiency of *PHGDH* was verified by qRT-PCR and Western blot assay. **(C)** OS cells were assigned to five groups: negative control (NC) treated with DMSO (DMSO + NC), NC treated with AIL (AIL + NC), *PHGDH* overexpression treated with AIL (AIL + *PHGDH*), NC treated with AIL and serine (AIL + NC + Serine), *PHGDH* overexpression treated with AIL and serine (AIL + *PHGDH* + Serine). The viability ratio of Saos-2 and U-2OS cells in the AIL + *PHGDH* group and AIL + *PHGDH* + Serine group but not in the AIL + NC + Serine group was significantly higher than that in the AIL + NC group (**$P < 0.01$, ***$P < 0.001$ compared with the DMSO + NC group, $^{###}P < 0.001$ compared with the AIL + NC group). **(D)** The wound healing assay revealed that overexpression of *PHGDH* but not exogenous serine supplementation reversed the suppressive effect of AIL on the migration of OS cells. **(E)** The Transwell assay demonstrated that overexpression of *PHGDH* but not exogenous serine supplementation reversed the suppressive effect of AIL on OS cell invasion. **(F)** Comparisons of the migration rates and relative invasion rates between the NC group and the other groups are shown (***$P < 0.001$, $^{ns}P > 0.05$).

methotrexate is one of the most effective chemotherapeutic agents for OS (48). Given the feasibility of regimens targeting the folate pathway in OS, inhibition of the upstream SSP deserves to be explored as a possible replacement for the toxic high-dose methotrexate, especially for patients who do not benefit from methotrexate.

In summary, our study demonstrated that AIL significantly inhibited OS cell proliferation, migration and invasion through targeting metabolic pathways, at least in part, by suppressing the SSP. AIL is worthy to be further explored as a candidate for the treatment of OS.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

The animal study was reviewed and approved by the Scientific Investigation Board of Shanghai Jiao Tong University Affiliated Sixth People's Hospital.

AUTHOR CONTRIBUTIONS

JZ, XL and HH designed the research. YZha, RG and XS performed the *in vitro* research. YZha and JL completed the animal study. YL and YZho contributed to bioinformatic analysis. YgW, LT and AH performed the non-omics data analysis. WY and YlW prepared the figures. ZS and YY polished the English translation. XL and JZ wrote the paper. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by grants from the National Natural Science Foundation of China (82072967), Shanghai Senior Integrative Chinese and Western Medicine Talents Program (ZY(2018-2020)-RCPY-2017) and Shanghai Pujiang Program (21PJD051).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.842406/full#supplementary-material

REFERENCES

1. Zhou Y, Yang D, Yang Q, Lv X, Huang W, Zhou Z, et al. Single-Cell RNA Landscape of Intratumoral Heterogeneity and Immunosuppressive Microenvironment in Advanced Osteosarcoma. *Nat Commun* (2020) 11 (1):6322. doi: 10.1038/s41467-020-20059-6
2. Bielack SS, Smeland S, Whelan JS, Marina N, Jovic G, Hook JM, et al. Methotrexate, Doxorubicin, and Cisplatin (MAP) Plus Maintenance Pegylated Interferon Alfa-2b Versus MAP Alone in Patients With Resectable High-Grade Osteosarcoma and Good Histologic Response to Preoperative MAP: First Results of the EURAMOS-1 Good Response Randomized Controlled Trial. *J Clin Oncol* (2015) 33(20):2279–87. doi: 10.1200/JCO.2014.60.0734
3. Hawkins DS, Arndt CA. Pattern of Disease Recurrence and Prognostic Factors in Patients With Osteosarcoma Treated With Contemporary Chemotherapy. *Cancer* (2003) 98(11):2447–56. doi: 10.1002/cncr.11799
4. Marina NM, Smeland S, Bielack SS, Bernstein M, Jovic G, Krailo MD, et al. Comparison of MAPIE Versus MAP in Patients With a Poor Response to Preoperative Chemotherapy for Newly Diagnosed High-Grade Osteosarcoma (EURAMOS-1): An Open-Label, International, Randomised Controlled Trial. *Lancet Oncol* (2016) 17(10):1396–408. doi: 10.1016/S1470-2045(16)30214-5
5. Isakoff MS, Bielack SS, Meltzer P, Gorlick R. Osteosarcoma: Current Treatment and a Collaborative Pathway to Success. *J Clin Oncol* (2015) 33 (27):3029–35. doi: 10.1200/JCO.2014.59.4895
6. Faubert B, Solmonson A, DeBerardinis RJ. Metabolic Reprogramming and Cancer Progression. *Science* (2020) 368(6487):eaaw5473. doi: 10.1126/science.aaw5473
7. Song HT, Qin Y, Yao GD, Tian ZN, Fu SB, Geng JS. Astrocyte Elevated Gene-1 Mediates Glycolysis and Tumorigenesis in Colorectal Carcinoma Cells *via* AMPK Signaling. *Mediators Inflamm* (2014) 2014:287381. doi: 10.1155/2014/287381
8. Chen J, Xu X, Fan M. Inhibition of Mitochondrial Translation Selectively Targets Osteosarcoma. *Biochem Biophys Res Commun* (2019) 515(1):9–15. doi: 10.1016/j.bbrc.2019.05.070
9. Zhu R, Li X, Ma Y. miR-23b-3p Suppressing PGC1alpha Promotes Proliferation Through Reprogramming Metabolism in Osteosarcoma. *Cell Death Dis* (2019) 10(6):381. doi: 10.1038/s41419-019-1614-1
10. Wen JF, Jiang YQ, Li C, Dai XK, Wu T, Yin WZ. LncRNA-SARCC Sensitizes Osteosarcoma to Cisplatin Through the miR-143-Mediated Glycolysis Inhibition by Targeting Hexokinase 2. *Cancer Biomark* (2020) 28(2):231–46. doi: 10.3233/CBM-191181
11. Amelio I, Cutruzzola F, Antonov A, Agostini M, Melino G. Serine and Glycine Metabolism in Cancer. *Trends Biochem Sci* (2014) 39(4):191–8. doi: 10.1016/j.tibs.2014.02.004
12. Li AM, Ye J. Reprogramming of Serine, Glycine and One-Carbon Metabolism in Cancer. *Biochim Biophys Acta Mol Basis Dis* (2020) 1866(10):165841. doi: 10.1016/j.bbadis.2020.165841
13. Locasale JW, Grassian AR, Melman T, Lyssiotis CA, Mattaini KR, Bass AJ, et al. Phosphoglycerate Dehydrogenase Diverts Glycolytic Flux and Contributes to Oncogenesis. *Nat Genet* (2011) 43(9):869–74. doi: 10.1038/ng.890
14. Mullarky E, Mattaini KR, Vander Heiden MG, Cantley LC, Locasale JW. PHGDH Amplification and Altered Glucose Metabolism in Human Melanoma. *Pigment Cell Melanoma Res* (2011) 24(6):1112–5. doi: 10.1111/j.1755-148X.2011.00919.x
15. Svoboda LK, Teh SSK, Sud S, Kerk S, Zebolsky A, Treichel S, et al. Menin Regulates the Serine Biosynthetic Pathway in Ewing Sarcoma. *J Pathol* (2018) 245(3):324–36. doi: 10.1002/path.5085
16. Jia XQ, Zhang S, Zhu HJ, Wang W, Zhu JH, Wang XD, et al. Increased Expression of PHGDH and Prognostic Significance in Colorectal Cancer. *Transl Oncol* (2016) 9(3):191–6. doi: 10.1016/j.tranon.2016.03.006
17. Song Z, Feng C, Lu Y, Lin Y, Dong C. PHGDH Is an Independent Prognosis Marker and Contributes Cell Proliferation, Migration and Invasion in Human Pancreatic Cancer. *Gene* (2018) 642:43–50. doi: 10.1016/j.gene.2017.11.014
18. Zhu J, Ma J, Wang X, Ma T, Zhang S, Wang W, et al. High Expression of PHGDH Predicts Poor Prognosis in Non-Small Cell Lung Cancer. *Transl Oncol* (2016) 9(6):592–9. doi: 10.1016/j.tranon.2016.08.003
19. Rinaldi G, Pranzini E, Van Elsen J, Broekaert D, Funk CM, Planque M, et al. *In Vivo* Evidence for Serine Biosynthesis-Defined Sensitivity of Lung Metastasis, But Not of Primary Breast Tumors, to Mtorc1 Inhibition. *Mol Cell* (2021) 81 (2):386–97.e7. doi: 10.1016/j.molcel.2020.11.027
20. Samanta D, Park Y, Andrabi SA, Shelton LM, Gilkes DM, Semenza GL. PHGDH Expression Is Required for Mitochondrial Redox Homeostasis, Breast Cancer Stem Cell Maintenance, and Lung Metastasis. *Cancer Res* (2016) 76(15):4430–42. doi: 10.1158/0008-5472.CAN-16-0530
21. Rathore R, Caldwell KE, Schutt C, Brashears CB, Prudner BC, Ehrhardt WR, et al. Metabolic Compensation Activates Pro-Survival Mtorc1 Signaling Upon 3-Phosphoglycerate Dehydrogenase Inhibition in Osteosarcoma. *Cell Rep* (2021) 34(4):108678. doi: 10.1016/j.celrep.2020.108678
22. He Y, Peng S, Wang J, Chen H, Cong X, Chen A, et al. Ailanthone Targets P23 to Overcome MDV3100 Resistance in Castration-Resistant Prostate Cancer. *Nat Commun* (2016) 7:13122. doi: 10.1038/ncomms13122
23. Zhang JJ, Zhou XH, Zhou Y, Wang YG, Qian BZ, He AN, et al. Bufalin Suppresses the Migration and Invasion of Prostate Cancer Cells Through HOTAIR, the Sponge of miR-520b. *Acta Pharmacol Sin* (2019) 40(9):1228–36. doi: 10.1038/s41401-019-0234-8
24. Li Y, Zhang J, Ma D, Zhang L, Si M, Yin H, et al. Curcumin Inhibits Proliferation and Invasion of Osteosarcoma Cells Through Inactivation of Notch-1 Signaling. *FEBS J* (2012) 279(12):2247–59. doi: 10.1111/j.1742-4658.2012.08607.x
25. Zhang J, Sha J, Zhou Y, Han K, Wang Y, Su Y, et al. Bufalin Inhibits Proliferation and Induces Apoptosis in Osteosarcoma Cells by Downregulating MicroRNA-221. *Evid Based Complement Alternat Med* (2016) 2016:7319464. doi: 10.1155/2016/7319464
26. Zheng Q, Li G, Wang S, Zhou Y, Liu K, Gao Y, et al. Trisomy 21-Induced Dysregulation of Microglial Homeostasis in Alzheimer's Brains Is Mediated by USP25. *Sci Adv* (2021) 7(1):eabe1340. doi: 10.1126/sciadv.abe1340
27. Yu P, Wilhelm K, Dubrac A, Tung JK, Alves TC, Fang JS, et al. FGF-Dependent Metabolic Control of Vascular Development. *Nature* (2017) 545 (7653):224–8. doi: 10.1038/nature22322
28. Lu L, Li H, Wu X, Rao J, Zhou J, Fan S, et al. HJC0152 Suppresses Human Non-Small-Cell Lung Cancer by Inhibiting STAT3 and Modulating Metabolism. *Cell Prolif* (2020) 53(3):e12777. doi: 10.1111/cpr.12777
29. de Koning TJ, Snell K, Duran M, Berger R, Poll-The BT, Surtees R. L-Serine in Disease and Development. *Biochem J* (2003) 371(Pt 3):653–61. doi: 10.1042/BJ20021785
30. Li AM, Ducker GS, Li Y, Seoane JA, Xiao Y, Melemenidis S, et al. Metabolic Profiling Reveals a Dependency of Human Metastatic Breast Cancer on Mitochondrial Serine and One-Carbon Unit Metabolism. *Mol Cancer Res* (2020) 18(4):599–611. doi: 10.1158/1541-7786.MCR-19-0606
31. Hwang S, Gustafsson HT, O'Sullivan C, Bisceglia G, Huang X, Klose C, et al. Serine-Dependent Sphingolipid Synthesis Is a Metabolic Liability of Aneuploid Cells. *Cell Rep* (2017) 21(13):3807–18. doi: 10.1016/j.celrep.2017.11.103
32. Muthusamy T, Cordes T, Handzlik MK, You L, Lim EW, Gengatharan J, et al. Serine Restriction Alters Sphingolipid Diversity to Constrain Tumour Growth. *Nature* (2020) 586(7831):790–5. doi: 10.1038/s41586-020-2609-x
33. Futerman AH, Riezman H. The Ins and Outs of Sphingolipid Synthesis. *Trends Cell Biol* (2005) 15(6):312–8. doi: 10.1016/j.tcb.2005.04.006
34. Zhang Y, Zhang C, Min D. Ailanthone Up-Regulates miR-449a to Restrain Acute Myeloid Leukemia Cells Growth, Migration and Invasion. *Exp Mol Pathol* (2019) 108:114–20. doi: 10.1016/j.yexmp.2019.04.011
35. Ni Z, Yao C, Zhu X, Gong C, Xu Z, Wang L, et al. Ailanthone Inhibits Non-Small Cell Lung Cancer Cell Growth Through Repressing DNA Replication *via* Downregulating RPA1. *Br J Cancer* (2017) 117(11):1621–30. doi: 10.1038/bjc.2017.319
36. Gao W, Ge S, Sun J. Ailanthone Exerts Anticancer Effect by Up-Regulating miR-148a Expression in MDA-MB-231 Breast Cancer Cells and Inhibiting Proliferation, Migration and Invasion. *BioMed Pharmacother* (2019) 109:1062–9. doi: 10.1016/j.biopha.2018.10.114
37. Chen Y, Zhu L, Yang X, Wei C, Chen C, He Y, et al. Ailanthone Induces G2/M Cell Cycle Arrest and Apoptosis of SGC7901 Human Gastric Cancer Cells. *Mol Med Rep* (2017) 16(5):6821–7. doi: 10.3892/mmr.2017.7491
38. Zhuo Z, Hu J, Yang X, Chen M, Lei X, Deng L, et al. Ailanthone Inhibits Huh7 Cancer Cell Growth *via* Cell Cycle Arrest and Apoptosis *In Vitro* and *In Vivo*. *Sci Rep* (2015) 5:16185. doi: 10.1038/srep16185
39. Ding H, Yu X, Hang C, Gao K, Lao X, Jia Y, et al. Ailanthone: A Novel Potential Drug for Treating Human Cancer. *Oncol Lett* (2020) 20(2):1489–503. doi: 10.3892/ol.2020.11710

40. Kalhan SC, Hanson RW. Resurgence of Serine: An Often Neglected But Indispensable Amino Acid. *J Biol Chem* (2012) 287(24):19786–91. doi: 10.1074/jbc.R112.357194
41. Tibbetts AS, Appling DR. Compartmentalization of Mammalian Folate-Mediated One-Carbon Metabolism. *Annu Rev Nutr* (2010) 30:57–81. doi: 10.1146/annurev.nutr.012809.104810
42. Labuschagne CF, van den Broek NJ, Mackay GM, Vousden KH, Maddocks OD. Serine, But Not Glycine, Supports One-Carbon Metabolism and Proliferation of Cancer Cells. *Cell Rep* (2014) 7(4):1248–58. doi: 10.1016/j.celrep.2014.04.045
43. Garcia-Canaveras JC, Lancho O, Ducker GS, Ghergurovich JM, Xu X, da Silva-Diz V, et al. SHMT Inhibition Is Effective and Synergizes With Methotrexate in T-Cell Acute Lymphoblastic Leukemia. *Leukemia* (2021) 35(2):377–88. doi: 10.1038/s41375-020-0845-6
44. Possemato R, Marks KM, Shaul YD, Pacold ME, Kim D, Birsoy K, et al. Functional Genomics Reveal That the Serine Synthesis Pathway Is Essential in Breast Cancer. *Nature* (2011) 476(7360):346–50. doi: 10.1038/nature10350
45. Sun L, Song L, Wan Q, Wu G, Li X, Wang Y, et al. Cmyc-Mediated Activation of Serine Biosynthesis Pathway Is Critical for Cancer Progression Under Nutrient Deprivation Conditions. *Cell Res* (2015) 25(4):429–44. doi: 10.1038/cr.2015.33
46. Barretina J, Caponigro G, Stransky N, Venkatesan K, Margolin AA, Kim S, et al. The Cancer Cell Line Encyclopedia Enables Predictive Modelling of Anticancer Drug Sensitivity. *Nature* (2012) 483(7391):603–7. doi: 10.1038/nature11003
47. Mullarky E, Lucki NC, Beheshti Zavareh R, Anglin JL, Gomes AP, Nicolay BN, et al. Identification of a Small Molecule Inhibitor of 3-Phosphoglycerate Dehydrogenase to Target Serine Biosynthesis in Cancers. *Proc Natl Acad Sci USA* (2016) 113(7):1778–83. doi: 10.1073/pnas.1521548113
48. van Dalen EC, van As JW, de Camargo B. Methotrexate for High-Grade Osteosarcoma in Children and Young Adults. *Cochrane Database Syst Rev* (2011) (5):CD006325. doi: 10.1002/14651858.CD006325.pub3

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Zhang, Gong, Liu, Sun, Liang, Zhou, Wang, Yu, Wang, Tang, He, Shen, Yao, Hu, Liu and Zhang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers | Frontiers in Pharmacology

REVIEW
published: 03 May 2022
doi: 10.3389/fphar.2022.903699

Research Progress of RNA Methylation Modification in Colorectal Cancer

Weizheng Liang[1], Hongyang Yi[2], Chenyu Mao[3], Qingxue Meng[1], Xueliang Wu[4], Shanliang Li[5] and Jun Xue[4]**

[1]Central Laboratory, The First Affiliated Hospital of Hebei North University, Zhangjiakou, China, [2]The Third People's Hospital of Shenzhen, Shenzhen, China, [3]School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA, United States, [4]Department of General Surgery, The First Affiliated Hospital of Hebei North University, Zhangjiakou, China, [5]Department of Pharmacology, Guangxi University of Chinese Medicine, Nanning, China

Accumulating evidence indicates that RNA methylation, as the most common modification of mRNA, is of great significance in tumor progression and metastasis. Colorectal cancer is a common malignant tumor of the digestive system that seriously affects the health of middle-aged and elderly people. Although there have been many studies on the biological mechanism of the occurrence and development of colorectal cancer, there are still major deficiencies in the diagnosis and prognosis of colorectal cancer. With the deep study of RNA methylation, it was found that RNA modification is highly related to colorectal cancer tumorigenesis, development and prognosis. Here, we will highlight various RNA chemical modifications including N6-methyladenosine, 5-methylcytosine, N1-methyladenosine, 7-methylguanine, pseudouridine and their modification enzymes followed by summarizing their functions in colorectal cancer.

Keywords: colorectal cancer, RNA modification, RNA methylation, N6-methyladenosine, 5-methylcytosine, N1-methyladenosine, 7-methylguanine, circular RNA

OPEN ACCESS

Edited by:
Yongye Huang,
Northeastern University, China

Reviewed by:
Jiangbo Wei,
The University of Chicago,
United States
Xiaolong Cui,
The University of Chicago,
United States
Longtao Huangfu,
Peking University, China

****Correspondence:***
Weizheng Liang
jmbb1203@126.com
Jun Xue
xuejun@hebeinu.edu.cn

Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

Received: *24 March 2022*
Accepted: *19 April 2022*
Published: *03 May 2022*

Citation:
Liang W, Yi H, Mao C, Meng Q, Wu X, Li S and Xue J (2022) Research Progress of RNA Methylation Modification in Colorectal Cancer. Front. Pharmacol. 13:903699. doi: 10.3389/fphar.2022.903699

INTRODUCTION

With the fourth modality rate among all diseases, colorectal cancer (CRC) is one of the most commonly seen tumors of the gastrointestinal tract, and its incidence is the highest in developed countries (Brody, 2015; Bray et al., 2018; Siegel et al., 2020). At present, colorectal cancer is gradually tending to be younger (Akimoto et al., 2021). The pathogenesis of colorectal cancer is complex and diverse, which may be led by an unhealthy diet, obesity, lack of exercise, and microbial infection. Long-term exposure to these risk factors affects the intestinal microbiota and host immunity, resulting in genetic and epigenetic alterations in colorectal epithelial cells, ultimately predisposing them to colorectal cancer (Brody, 2015; Akimoto et al., 2021). The progression of colorectal tumors from adenoma to colorectal cancer is a multi-step pathological process of tumorigenesis that takes about 10 years. Abnormal mutations, accumulation of proto-oncogenes and tumor suppressor genes play a huge part in this process which includes heritable changes in the genome with changes in the basic nucleotides of DNA and stable inheritance of unchanged nucleotide sequences that cause changes in gene expression and function, that is, epigenetics (Sjöblom et al., 2006; Markowitz and Bertagnolli, 2009).

Epigenetic modifications are dynamically reversible and heritable, including DNA methylation, histone modifications, chromatin remodeling, microRNAs (miRNAs) and noncoding RNAs (ncRNAs) which are of great importance in the progression and metastasis

of colorectal cancer (Huang et al., 2021a). Recently, major breakthroughs have been made in the research on DNA omics and proteomics in the occurrence and development of tumors, and the epigenetic modification at the RNA level has also attracted the attention of many researchers (Skvortsova et al., 2019; Sivanand and Vander Heiden, 2020; Mobet et al., 2022; Wishart, 2022).

RNA modifications play a key regulatory role in gene expression (Helm and Motorin, 2017; Wu et al., 2021). In recent years, over 170 RNA chemical modifications have been discovered, involving both coding and noncoding RNAs (Boccaletto et al., 2022). In eukaryotic, the most abundant modification found is N6-methyladenosine (m6A), which is considered as eukaryotic characteristic internal modification since its discovery in the 1870s (Gilbert et al., 2016; Davalos et al., 2018). RNA is not only an intermediate or effector molecule in protein synthesis but also has direct functional effects on gene expression through a variety of other noncoding RNAs. Dynamic modification of RNA enables cells to respond rapidly to changes in the external environment, and the ability to adapt to changing microenvironments (such as stimuli and stress) is critical for tumor cell survival. Recent research has demonstrated that RNA modification has become a main emerging regulator in cancer by regulating various RNA metabolic processes (Cui et al., 2017; Barbieri and Kouzarides, 2020; Miano et al., 2021). More and more evidence has shown that the abnormal expression of various m6A regulatory proteins plays a role in promoting or suppressing tumors in human tumors (Nombela et al., 2021). Abnormal changes in RNA modification are often functionally related to cell proliferation, differentiation, stress adaptation, invasion and resistance to chemotherapy. Therefore, targeting abnormal RNA modifications in cancer cells is expected to be an effective way to treat tumors.

In this review, we will summarize the biological characteristics of various RNA modifications, including m6A, m5C, N1-methyladenosine, 7-methylguanine, pseudouridine and their roles in the occurrence and development of colorectal tumors by providing a basis for further search for biomarkers and therapeutic targets.

m6A Modification and Colorectal Cancer

m6A methylation modification is a common RNA methylation modification on the 6th N of adenine. It is a dynamically reversible post-transcriptional modification that is most abundant in mRNA and noncoding RNA (**Figure 1**). The modification of m6A occurs mainly in the RRACH sequence motif (where R = A or G, H = A, C, or U) and is significantly enriched in the mRNA 3′UTR as well as CDS regions (Dominissini et al., 2012; Meyer et al., 2012). m6A is involved in multiple processes of RNA metabolism, including post-transcriptional splicing, translation efficiency and stability maintenance, and is essential in normal physiological processes such as growth and development, learning and memory, and is also involved in regulating the response to body heat shock (Zhou et al., 2015; Shi et al., 2018; Wang et al., 2018). It is also crucial for tumor development and tumor immune drug resistance (Han et al., 2019). m6A modification is regulated by methyltransferases, demethylases, and methyl-recognition proteins as a post-translational RNA modification. Among them, methyltransferase catalyzes the modification of adenylate by m6A in RNA, which is composed of various proteins such as METTL3 (methyltransferase like 3), METTL14 (methyltransferase like 14), WTAP (Wilms tumor 1 associating protein) (Frye et al., 2018; Shi et al., 2019) and KIAA1429 (Liu et al., 2014). The core proteins of demethylase include FTO (fat mass and obesity-associated protein) and ALKBH5 (Alkb homolog 5), which can demethylate bases that undergo a modification of m6A, which is also the cause of dynamic reversibility. Methylation recognition proteins can recognize and bind to m6A-modified bases, and regulate biological processes such as RNA degradation and stabilization, nuclear export, and translation efficiency. Given their functional characteristics, these proteins are called "writer," "eraser," and "reader" (Zaccara et al., 2019).

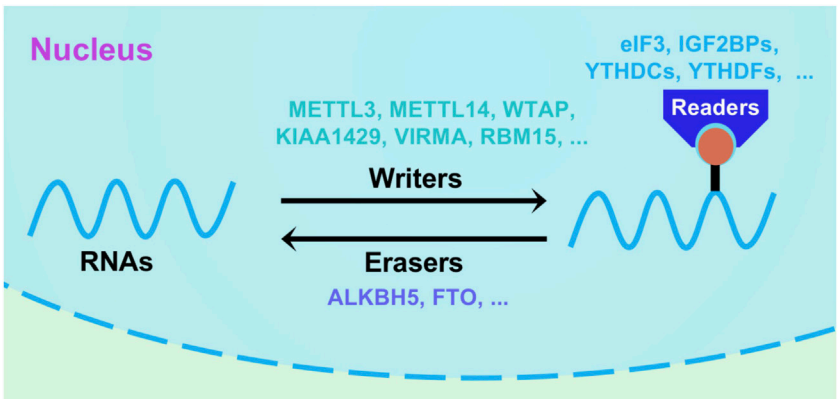


FIGURE 1 | Schematic diagram of RNA m6A methylation mechanism. m6A modification is a dynamic and reversible process, which could be regulated by writers (WTAP, METTL3/14, KIAA1429, VIRMA and RBM15), erasers (FTO and ALKBH5) and readers (eIF3, YTHDCs, YTDFs and IGF2BPs).

In recent years, m6A modification has gradually become the hot field in studying colorectal tumorigenesis, development and metastasis. m6A modifications have widely altered the expression of certain colorectal tumor-associated genes. Abnormally expressed writer protein, eraser protein, and reader protein, by regulating the level of m6A in different RNAs, affects the function of downstream pathways, such as the classical pathway related to colorectal tumorigenesis and apoptosis, the Wnt pathway and the Hippo pathway, and plays pro- or anti-cancer effects (Chen et al., 2020a; Xu et al., 2020a; Chen et al., 2020b).

It was found that METTL3 expression was increased in tumor tissues of colorectal cancer patients, and the higher its expression, the worse the prognosis of patients (Zhou et al., 2021a; Chen et al., 2021). METTL3 regulates the expression of various oncogenes and tumor suppressor genes at the post-transcriptional level to enhance the progression of colorectal cancer. Overexpression of METTL3 represses SOCS2 and promotes LGR5 promoter activity to maintain cell proliferation in colon cancer cells (Xu et al., 2020b). Knockdown of METTL3 can inhibit the development of colorectal cancer significantly. Research have suggested that METTL3 targets the YPEL5 m6A modification site to inhibit its expression and promote the progression of colorectal cancer (Zhou et al., 2021a). On the other hand, METTL3 stabilizes its expression level by methylating the m6A site of the CCNE1 3′UTR mRNA to increase the expression of the cyclin E1 to promote colorectal cancer cell proliferation (Zhu et al., 2020). Furthermore, a group found that the m6A methyltransferase METTL3 promoted the malignant proliferation of colorectal cancer cells by directly or indirectly upregulating MYC expression (Xiang et al., 2020). In an indirect mechanism, it was found that METTL3 may upregulate MYC expression through the WNT, TGF-β and other signaling pathways. The expression of MYC was also found to be recognized by IGF2BP1 and its stability was enhanced dependent on m6A-IGF2BP1. In addition, METTL3 increases the expression of PTTG3P through an m6A/IGF2BP2-dependent mechanism and regulates the PTTG3P/YAP1 axis to induce colon cancer cell proliferation and promote colon cancer progression (Zheng et al., 2021). METTL3 also regulates the progression of colorectal cancer by affecting the metabolism of tumor cells. Shen et al. found that the m6A methyltransferase METTL3 regulates the expression levels of HK2 and SLC2A1 by regulating m6A expression and stabilizing m6A under the action of the methyl-binding protein IGF2BP, which further promotes the activation of the glycolytic pathway, inducing malignant proliferation of colorectal cancer cells (Shen et al., 2020). Another study has confirmed that METTL3 carries out m6A modification on GLUT1 mRNA to improve its mRNA stability and translation level, and promote glucose metabolism and colorectal cancer occurrence (Chen et al., 2021). Through the regulation of the m6A-GLUT1-mTORC1 axis, METTL3 is actively involved in the proliferation of colorectal cancer, and simultaneous inhibition of mTORC1 and METTL3 has an additive effect on the treatment of colorectal cancer. In addition to the effects on cell proliferation, METTL3 also has relations with the progression and metastasis of colorectal cancer. Peng et al. (2019) demonstrated that the overexpressed m6A methyltransferase METTL3 in colorectal cancer can increase miRNA-1246 expression by upregulating the methylation level of pri-miR-1246, and further lead to tumor metastasis by downregulating the expression of the metastasis-related suppressor gene SPRED2. In addition, it was found that SPRED2 regulates colorectal cancer metastasis through the MAPK signaling pathway. Research has suggested that MAPK can regulate the epithelial-mesenchymal transition (EMT) process, thus impacting tumor metastasis (Huang et al., 2021b). Li et al. (2019) shown that METTL3 regulates the progression and migration of colorectal cancer by increasing the methylation level of SOX2 and enhancing the stability of methylated SOX2 through an m6A-IGF2BP2-dependent mechanism. It was also found that the tumor-suppressing METTL3 had low expression level in colorectal tumor, and it lost its inhibitory effect on p38/ERK in the MAPK signaling pathway and promoted colorectal cancer migration (Deng et al., 2019). In sum, METTL3 modulates the expression and function of various target molecules in a m6A-modified manner to affect the proliferation, apoptosis, metabolism, invasion and metastasis of colorectal cancer cells. Therefore, specific targeting of METTL3 has important application prospects in the treatment of colorectal cancer.

In contrast, another m6A writer protein, METTL14, expressed at a low level in colorectal cancer. The higher the expression of METTL14, the longer the overall survival of the patients, and overexpression of METTL14 can suppress the metastasis and progression of colorectal cancer (Chen et al., 2020b). METTL14 knockout was shown to reduce the level of m6A of its downstream target SOX4, resulting in reduced recognition of the modified SOX4 mRNA by YTHDF2, thus increasing SOX4 gene expression and promoting the SOX4-mediated EMT process and activity of the PI3K/AKT signaling pathway, leading to malignant progression of colorectal cancer (Chen et al., 2020a). This was also confirmed in another study in which the researcher found that the suppression of METTL14 can enhance the progression and metastasis of colorectal cancer (Wang et al., 2021). Tumor-associated macrophages (TAM) can inhibit the antitumor activity of T cells, but the mechanism is not clear. A recent study found that in colorectal cancer patients' tumor tissue, the expression of METTL14 was negatively correlated with $CD8^+$ T cell infiltration (Dong et al., 2021). Reduced expression of METTL14 in TAM subset C1q + cells can promote colon cancer cell growth and impede $CD8^+$ T cell infiltration. It was further found that the reduction of Ebi3 expression level in C1q + cells can restore the anti-tumor killing ability of $CD8^+$ T cells, while the loss of METTL14 expression can reduce the m6A modification level of Ebi3 mRNA in C1q + cells and promote the increase of its transcription level. Therefore, increasing the expression of METTL14 in TAM subset C1q + cells can promote $CD8^+$ T cell infiltration and antitumor effects (Dong et al., 2021).

The third writer protein WTAP has been shown to have tumor-promoting action in many malignancies, including liver

cancer, gastric cancer and osteosarcoma (Chen et al., 2019a; Li et al., 2020a; Chen et al., 2020c; Zhou et al., 2021b; Feng et al., 2021). In colorectal cancer, studies have pointed out WTAP is also oncogenic and can promote the progression of colorectal cancer through the WTAP/WT1/TBL1 axis in the canonical Wnt signaling pathway (Zhang et al., 2016a). Another group also suggested the higher expression of WTAP protein in colorectal cancer (Dong et al., 2022). In sum, both the dual role of METTL3 in colorectal tumors and the pro-oncogenic effect of METTL14 and WTAP in other tumors suggest that the m6A modification sites on different downstream target genes are different, which affect the activation status of various signaling pathways they are involved in, and thus play specific roles in tumors. Therefore, the next step in the investigation of m6A modification in the progression of colorectal tumor disease could be how to preserve the oncogenic role of the regulator by using inhibitors or developing new drugs to block its oncogenic signaling in specific signaling pathways.

However, unlike m6A methyltransferase, eraser and reader proteins have been less studied in colorectal cancer. It has been found that FTO is regulated by miR-1266 in the nucleus and initiates cell signaling molecules STAT3, cyclin D1 and MMPs to promote the development of colorectal cancer (Shen et al., 2018; Roslan et al., 2019; Ge et al., 2021). In addition, one group recently found that FTO is regulated by miR-96, which affects the methylation level of MYC and increase the expression of MYC, thus participating in the pro-proliferative and anti-apoptotic effects of miR-96 in colorectal cancer (Yue et al., 2020). While in the cytoplasm, FTO can dynamically regulate m6A modifications to contribute to the stemness of colorectal cancer cells and influence tumor drug resistance (Relier et al., 2021). Preliminary progress has also been made in the mechanism study of another demethylase, ALKBH5, in mediating colorectal cancer metastasis and immunotherapy resistance. The expression of ALKBH5 is downregulated in colon cancer and is associated with tumor metastasis, and it is pointed out that this molecule can be used as an independent predictor of patient prognosis (Yang et al., 2020). This study further confirmed the tumor suppressor effect of ALKBH5 both *in vitro* and *in vivo* (Yang et al., 2020). One study found that ALKBH5 can affect the tumor microenvironment of colorectal cancer, thus mediating the resistance of colorectal cancer patients to the anti-PD-1 therapy response (Li et al., 2020b). Meanwhile, ALKBH5 inhibitors can significantly improve immunotherapy efficacy, providing new possibilities for targeted therapy of colorectal cancer, melanoma, and other malignant tumors. Together, these demonstrated that ALKBH5 can not only be used as a breakthrough target for the clinical treatment of colorectal cancer in the future, but also has the potential to become a new biomarker for the patient population. Furthermore, there is still a gap in the study of the functional mechanisms of other members of the Alkb subfamily in colorectal tumors. The search for new molecules related to m6A modification and the study of their mechanisms is still a promising direction worth studying in the future.

Reader protein reads m6A modification sites of key molecules of colorectal tumor signaling pathways, affecting the expression level and RNA stability of these genes. In addition, the reader protein also forms complexes with noncoding RNAs related to colorectal tumors to improve its stability. Transcriptional and translational levels of YTHDF1 are significantly elevated in colorectal cancer patients. One study found YTHDF1 could enhance tumor cell stemness to promote tumorigenesis by inhibiting the canonical Wnt/β-catenin pathway (Bai et al., 2019). Another member of the YTHDF family, YTHDF3, affects colorectal cancer progression by regulating the negative feedback axis of the lncRNA GAS5-YAP-YTHDF3 in the Hippo pathway (Ni et al., 2019). Downregulation of the YTHDC2 gene can significantly inhibit the translation of tumor metastasis-related genes, such as hypoxia-inducible factor-1alpha (HIF-1α), and the high expression of this molecule is positively correlated with tumor stage and colon cancer metastasis (Tanabe et al., 2016).

circRNA is an RNA molecule with a novel structure. In recent years, modification of m6A has also been found in some circRNAs (**Figure 2**), with functions in the tumor occurrence and progression being reported (Zhao et al., 2019a; Li et al., 2021; Rao et al., 2021). In colorectal cancer, a study reported that circNSUN2 modified with m6A was frequently upregulated in tumor tissue and serum samples from patients with liver metastases of colorectal cancer (Chen et al., 2019b). The results show that after being recognized by YTHDC1, m6A-modified circNSUN2 will be exported to the cytoplasm, contributing to the formation of the ternary complex of circNSUN2-insulin-like growth factor 2 mRNA binding protein. circNSUN2 enhances the stability of HMGA2 mRNA, thereby promoting colorectal cancer invasion and liver metastasis (Chen et al., 2019b). circNSUN2 may become a potential therapeutic target for liver metastases of colorectal cancer.

m5C Modification and Colorectal Cancer

m5C modifications are widely found in mRNA, rRNA, tRNA, and ncRNA, with the greatest abundance in tRNA and rRNA in eukaryotes (**Figure 3**). Enzymes responsible for RNA m5C modification include NSUN1 to NSUN7 of the NSUN family and DNA Methyltransferase-2 (DNMT2) (Bohnsack et al., 2019; Kuznetsova et al., 2019). NSUN1 and NSUN5 are involved in regulating 28S rRNA m5C modification, while NSUN3 and NSUN4 regulate mitochondrial tRNA and rRNA m5C modification, respectively. NSUN2 and DNMT2 regulate tRNA m5C modification in the cytoplasm, while NSUN7 targets eRNAs. The m5C regulator also functions in some digestive tumors. Studies have shown that the copy number of the NSUN2 gene is significantly increased in colorectal cancer, and the RNA methyltransferase NSUN2 is associated with the oncogene MYC. When the MYC protein is activated, the expression of NSUN2 is also upregulated (Alboushi et al., 2021). To date, no specific m5C methyltransferase inhibitor has been developed. However, studies have shown that azacytidine, as a novel antitumor drug, can reduce the

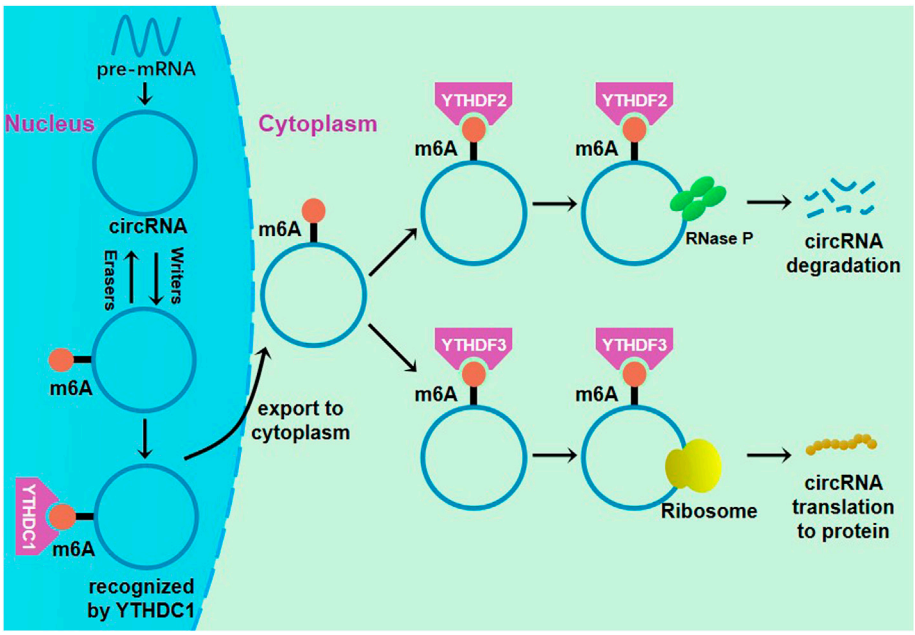


FIGURE 2 | Regulation of m6A methylation in circRNA. YTHDC1 could promote circRNA nuclear export through binding to m6A methylation sites. The complex of eIF4G2, eIF4, eIF4B and YTHDF3 could initiate the translation of m6A-modified circRNA, and the circRNA could be cleaved by YTHDF2-HRSP12-RNase P/MRP complex.

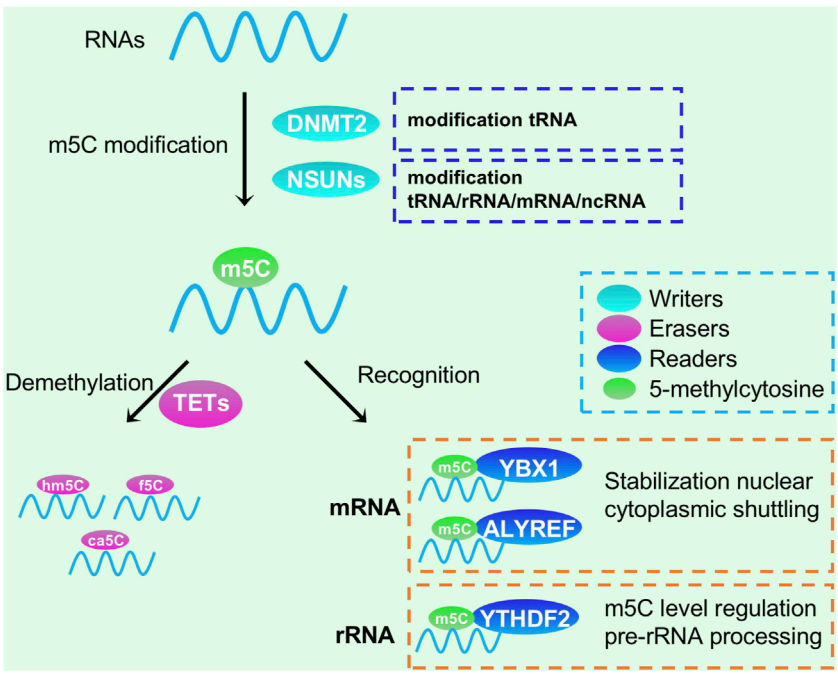


FIGURE 3 | Functions of RNA m5C modification in RNA processing and metabolism. DNMT2 and NSUNs could methylate tRNA, rRNA, mRNA and ncRNA; YTHDF1, YBX1 and ALYREF could recognize m5C modification, while TETs could demethelate them.

proliferation ability of cancer cells by inhibiting DNMT2-mediated m5C modification, suggesting that reducing m5C modification of tRNA may be an effective cancer treatment strategy (Esteller and Pandolfi, 2017).

m1A Modification and Colorectal Cancer

m1A modification means the attachment of a methyl group to the N1 position of RNA adenosine. The modification of m1A can significantly alter the structure of the RNA and the strength

of protein-RNA interactions, thus affecting the protein translation process (Wiener and Schwartz, 2021). TRM10 and the TRM6-TRM61 complex mediate m1A modification of tRNA (Xiong et al., 2018; Zhang and Jia, 2018). The presence of m1A modification was also detected in 28S rRNA (Sloan et al., 2017). The YTH protein family can bind to m1A with low affinity, so it is considered as a potential m1A reader (Dai et al., 2018). ALKBH1 and ALKBH3, as demethylases, are involved in the regulation of m1A modification. Abnormal expression of m1A-related regulatory genes in digestive tumors is closely related to patient prognosis. Overexpression of ALKBH1 in colorectal cancer is one of the causative factors of a lower survival rate, as well as low expression of ALKBH3 (Zhao et al., 2019b).

m7G Modification and Colorectal Cancer

The epigenetic modification m7G was originally found to exist within eukaryotic mRNA, tRNA, and rRNA. The most typical enzyme characterizing m7G methylation modification is METTL1. In tRNA, METTL1-WDR4 complex-mediated m7G modification maintains its structural integrity (Lin et al., 2018). The m7G in rRNA is mediated by the Williams-Beuren syndrome chromosome region 22 (WBSCR22), but its role is not fully understood. The m7G modification on rRNA may be involved in ribosome maturation, but has little effect on translation (Haag et al., 2015). The m7G modification within mRNA is enriched at the 5′UTR and is dynamically regulated with stress changes, and its role is to promote the translation process (Malbec et al., 2019). As an important regulator of m7G, METTL1 functions as a tumor suppressor in colorectal cancer (Liu et al., 2020). In addition, overexpression of METTL1 also enhance the chemosensitivity of colorectal cancer cells to cisplatin by regulating the miR-149-3p/S100A4/p53 axis (Liu et al., 2019). These results suggest that maintaining high levels of functional tRNA may be crucial for METTL1 executing function in cancer cells. Although the effect of METTL1 on tRNA may be cancer-promoting, there is no direct evidence that m7G modification plays a role in cancer cells. Another study demonstrated that WBSCR22 expression in colorectal cancer tissues is remarkably increased and upregulated WBSCR22 predicts a poor prognosis in patients (Yan et al., 2017). Knockdown of WBSCR22 can significantly improve the sensitivity of colorectal cancer cells to oxaliplatin, while overexpression of WBSCR22 increases cell resistance. Knockdown of WBSCR22 can increase oxaliplatin-induced intracellular ROS production and ROS-induced accumulation of 8-oxoguanine oxidative damage, which makes cancer cells more susceptible to oxaliplatin treatment (Yan et al., 2017).

Pseudouridine (ψ) Modification and Colorectal Cancer

Pseudouridine is another abundant RNA modification in mRNA, tRNA, rRNA, snRNA, and lncRNA. In human cell lines, the proportion of pseudouridine (ψ) modifications in mRNA and lncRNA is about 30%–84% (Zhang et al., 2019). Pseudouridine is important in regulating the response to environmental stress. The presence of ψ can increase the rigidity of the RNA backbone, affecting its thermodynamic stability and spatial conformation, thus making the structure and function of the RNA more stable. ψ modification is involved in maintaining structural stability in tRNA and in the assembly of ribosomes in rRNA. One of the most studied diseases associated with defective pseudouridine modification is dyskeratosis congenita (DC) caused by inactivating mutations in pseudouridine synthase 1 (DKC1). Deficiency of DKC1 activity results in impaired telomerase activity and impaired mRNA translation, resulting in reduced cell replicative potential and premature senescence. Abnormal mutations in DKC1 are associated with various tumors such as colorectal cancer and hepatocellular carcinoma (McMahon,

TABLE 1 | The main factors of RNA modification.

Genes	Modification type	Target genes	Functions in cancer	References
Writer				
METTL3	m6A	MYC, EGFR, SP1, SP2, SOX2	Oncogene	Lin et al., (2016); Barbieri et al., (2017); Cui et al., (2017); Vu et al. (2017);
METTL14	m6A			Weng et al. (2018)
NSUNs	m5C	rRNA, NMR, HDGF	Oncogene	Saijo et al. (2001); Bantis et al. (2004); Li et al. (2018); Chen et al. (2019c)
METTL1	m7G	Pri-let7	Oncogene	Pandolfini et al. (2019)
PUS10	Pseudouridine	Unknown	Tumour suppressor	Jana et al. (2017)
DKC1	Pseudouridine	rRNA	Tumour suppressor	Ruggero et al. (2003); Montanaro et al. (2010)
		TERC	Oncogene	Penzo et al. (2015)
Eraser				
FTO	m6A	PDCD1, CXCR4, SOX10, ASB2, RARA	Oncogene	Iles et al. (2013); Li et al. (2017); Yang et al. (2019)
ALKBH5	m6A	FOXM1, NANOG	Oncogene	Zhang et al., (2016b); Zhang et al. (2017)
ALKBH3	m1A	tRNA, CSF1	Oncogene	Konishi et al. (2005); Chen et al. (2019d); Woo and Chambers, (2019)
Reader				
YTHDC2	m6A	HIF1A	Oncogene	Tanabe et al. (2016)
YTHDF1	m6A	FZD9, WNT6	Oncogene	Bai et al. (2019)
YTHDF2	m6A	TNFRSF1B	Oncogene	Paris et al. (2019)
IGF2BP1	m6A	SRF	Oncogene	Müller, (2019)
IGF2BP2	m6A	SOX2, MYC	Oncogene	Li et al. (2019); Wang et al. (2019)

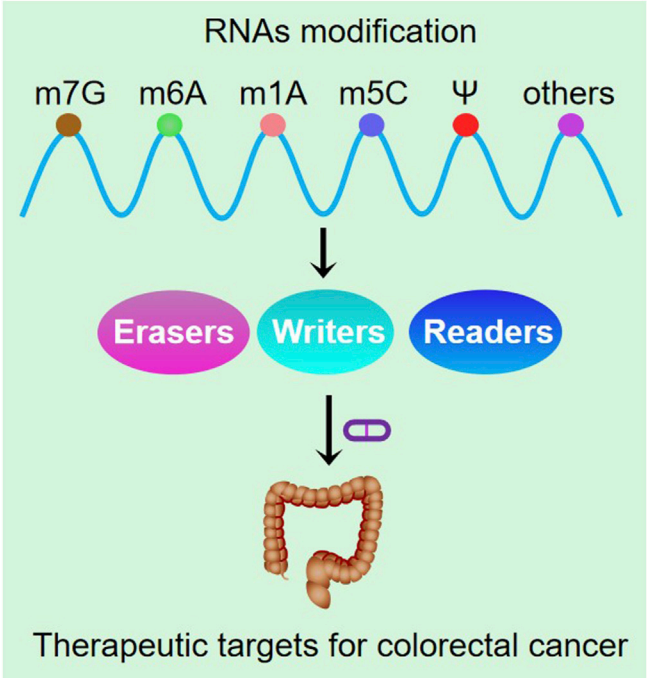


FIGURE 4 | Schematic diagram of RNA methylation application in the clinic.

2019). In colorectal cancer, reduced ψ modification on rRNA alters translation in cancer cells by affecting its interaction with the ribosomal P site (Babaian et al., 2020).

CONCLUSIONS AND PERSPECTIVES

RNA modification plays an important role as a key post-transcriptional regulator in gene expression, and researchers gradually realize that the functional network of its interaction involves many fields such as metabolism, epigenetics, chromatin remodeling, as well as the immune system. Although research on the epitranscriptome has made great progress, most studies have only focused on the biological functions of m6A modifications in mRNA, and the epitranscriptome includes more than 170 chemical modifications that modify coding and noncoding RNAs. Therefore, the association of hundreds of other RNA modifications with coding and noncoding RNAs remains to be explored. To explore the specific biological functions of various RNA modifications, it is first necessary to develop systematic methods and tools for rapid and quantitative detection of RNA modifications. Most existing sequencing methods are based on second generation sequencing technology, which cannot accurately identify chemical modifications on RNA. When researchers detect specific RNA modifications, they often need to detect RNA modifications using specific antibody-based immunoprecipitation techniques or indirect methods such as chemical labeling and the unique base modification properties of RNA pairings. Although these methods have good feasibility, its reproducibility is still low due to the reasons such as technology and incomplete algorithms. Therefore, future detection technologies and methods for various RNA modifications still need further research and exploration.

To date, treatments for colorectal cancer are still very limited. The rapid development of experimental technologies based on high-throughput sequencing and proteomics provides more possibilities for a deep study of the mechanism of RNA modification in colorectal cancer. In colorectal cancer, the complex of methyltransferase modified by m6A methylation, including METTL3, which plays a major catalytic role, has been thoroughly studied and is involved in the regulation of multiple classical signaling pathways in tumor development and apoptosis. However, there are still few studies on other writer molecules, and the roles of WTAP and METTL16, which can act independently, in colorectal tumors are still unclear. Similarly, eraser and reader proteins have been shown to contribute to the occurrence, development, metastasis and drug resistance of colorectal cancers by regulating classical signaling pathways, such as the Wnt pathway and the PI3K/AKT pathway, as well as changing the m6A modification level of noncoding RNAs, but there is still wide room for improvement in the transformation from basic to clinical. Although m6A methylation modification is abundant at the mRNA level, tumor-associated noncoding RNAs (miRNAs, lncRNAs and circRNAs) are also regulated by m6A methylation and act in the disease progression of colorectal cancer. circRNAs have recently been found to encode polypeptides. Whether m6A modification plays a key role and the specific mechanism has attracted the attention of researchers. Therefore, it is also essential to pay attention to tumor-associated noncoding RNAs. In addition, few eraser proteins have been found and only two more abundantly studied molecules, FTO and ALKBH5. ALKBH3 has been confirmed to have the function of demethylase, but there are still many areas that we have not studied in the Alk homolog family. It is still a research direction to explore whether more members of this family can function as demethylases. There are many types of reader proteins, and it is still unknown that reader proteins specifically read m6A modifications on different RNAs and play different downstream functions.

Various RNA modifications including m6A, m5C and ψ function in the regulation of stem cell function and cell survival under stress. The regulation of these RNA modifications may be of great value in reducing tumor cell chemotherapy resistance and tumor recurrence. The identification of precise epitranscriptomic biomarkers in a specific cell type or tumor microenvironment and the identification of aberrantly modified oncogenic or tumor suppressor effects are of great significance for finding precise molecular targets and developing highly selective and effective therapeutic approaches (**Table 1**). In the future, precision medicine based on epitranscriptomic signatures may be used for the diagnosis and treatment of colorectal cancer (**Figure 4**).

AUTHOR CONTRIBUTIONS

WL and JX contributed directly to this review. WL designed and wrote the preliminary version of the manuscript. All authors were involved in the manuscript writing, including discussion of content and writing and editing of the manuscript. All authors approved the final text.

FUNDING

This study was supportive by Hebei Funding Project for High-level Talents (A202101062); Training Project for Excellent Clinical Medical Talents of Hebei Provincial Department of Finance (361009); Hebei Provincial Health Commission Medical Science Research Key Project Program (20210954).

REFERENCES

Akimoto, N., Ugai, T., Zhong, R., Hamada, T., Fujiyoshi, K., Giannakis, M., et al. (2021). Rising Incidence of Early-Onset Colorectal Cancer - a Call to Action. *Nat. Rev. Clin. Oncol.* 18 (4), 230–243. doi:10.1038/s41571-020-00445-1

Alboushi, L., Hackett, A. P., Naeli, P., Bakhti, M., and Jafarnejad, S. M. (2021). Multifaceted Control of mRNA Translation Machinery in Cancer. *Cell Signal* 84, 110037. doi:10.1016/j.cellsig.2021.110037

Babaian, A., Rothe, K., Girodat, D., Minia, I., Djondovic, S., Milek, M., et al. (2020). Loss of $M^1acp^3\Psi$ Ribosomal RNA Modification Is a Major Feature of Cancer. *Cell Rep* 31 (5), 107611. doi:10.1016/j.celrep.2020.107611

Bai, Y., Yang, C., Wu, R., Huang, L., Song, S., Li, W., et al. (2019). YTHDF1 Regulates Tumorigenicity and Cancer Stem Cell-like Activity in Human Colorectal Carcinoma. *Front. Oncol.* 9, 332. doi:10.3389/fonc.2019.00332

Bantis, A., Giannopoulos, A., Gonidi, M., Liossi, A., Aggelonidou, E., Petrakakou, E., et al. (2004). Expression of P120, Ki-67 and PCNA as Proliferation Biomarkers in Imprint Smears of Prostate Carcinoma and Their Prognostic Value. *Cytopathology* 15 (1), 25–31. doi:10.1046/j.0956-5507.2003.00090.x

Barbieri, I., and Kouzarides, T. (2020). Role of RNA Modifications in Cancer. *Nat. Rev. Cancer* 20 (6), 303–322. doi:10.1038/s41568-020-0253-2

Barbieri, I., Tzelepis, K., Pandolfini, L., Shi, J., Millán-Zambrano, G., Robson, S. C., et al. (2017). Promoter-bound METTL3 Maintains Myeloid Leukaemia by m^6A-dependent Translation Control. *Nature* 552 (7683), 126–131. doi:10.1038/nature24678

Boccaletto, P., Stefaniak, F., Ray, A., Cappannini, A., Mukherjee, S., Purta, E., et al. (2022). MODOMICS: a Database of RNA Modification Pathways. 2021 Update. *Nucleic Acids Res.* 50 (D1), D231–d235. doi:10.1093/nar/gkab1083

Bohnsack, K. E., Höbartner, C., and Bohnsack, M. T. (2019). Eukaryotic 5-methylcytosine (m^5C) RNA Methyltransferases: Mechanisms, Cellular Functions, and Links to Disease. *Genes (Basel)* 10 (2), 20102. doi:10.3390/genes10020102

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., and Jemal, A. (2018). Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.* 68 (6), 394–424. doi:10.3322/caac.21492

Brody, H. (2015). Colorectal Cancer. *Nature* 521 (7551), S1. doi:10.1038/521S1a

Chen, H., Gao, S., Liu, W., Wong, C. C., Wu, J., Wu, J., et al. (2021). RNA N^6-Methyladenosine Methyltransferase METTL3 Facilitates Colorectal Cancer by Activating the m^6A-GLUT1-mTORC1 Axis and Is a Therapeutic Target. *Gastroenterology* 160 (4), 1284–e16. e16. doi:10.1053/j.gastro.2020.11.013

Chen, R. X., Chen, X., Xia, L. P., Zhang, J. X., Pan, Z. Z., Ma, X. D., et al. (2019). N^6-methyladenosine Modification of circNSUN2 Facilitates Cytoplasmic export and Stabilizes HMGA2 to Promote Colorectal Liver Metastasis. *Nat. Commun.* 10 (1), 4695. doi:10.1038/s41467-019-12651-2

Chen, S., Li, Y., Zhi, S., Ding, Z., Wang, W., Peng, Y., et al. (2020). WTAP Promotes Osteosarcoma Tumorigenesis by Repressing HMBOX1 Expression in an m^6A-dependent Manner. *Cell Death Dis* 11 (8), 659. doi:10.1038/s41419-020-02847-6

Chen, X., Li, A., Sun, B. F., Yang, Y., Han, Y. N., Yuan, X., et al. (2019). 5-methylcytosine Promotes Pathogenesis of Bladder Cancer through Stabilizing mRNAs. *Nat. Cel Biol* 21 (8), 978–990. doi:10.1038/s41556-019-0361-y

Chen, X., Xu, M., Xu, X., Zeng, K., Liu, X., Pan, B., et al. (2020). METTL14-mediated N6-Methyladenosine Modification of SOX4 mRNA Inhibits Tumor Metastasis in Colorectal Cancer. *Mol. Cancer* 19 (1), 106. doi:10.1186/s12943-020-01220-7

Chen, X., Xu, M., Xu, X., Zeng, K., Liu, X., Sun, L., et al. (2020). METTL14 Suppresses CRC Progression via Regulating N6-methyladenosine-dependent Primary miR-375 Processing. *Mol. Ther.* 28 (2), 599–612. doi:10.1016/j.ymthe.2019.11.016

Chen, Y., Peng, C., Chen, J., Chen, D., Yang, B., He, B., et al. (2019). WTAP Facilitates Progression of Hepatocellular Carcinoma via m6A-HuR-dependent Epigenetic Silencing of ETS1. *Mol. Cancer* 18 (1), 127. doi:10.1186/s12943-019-1053-8

Chen, Z., Qi, M., Shen, B., Luo, G., Wu, Y., Li, J., et al. (2019). Transfer RNA Demethylase ALKBH3 Promotes Cancer Progression via Induction of tRNA-Derived Small RNAs. *Nucleic Acids Res.* 47 (5), 2533–2545. doi:10.1093/nar/gky1250

Cui, Q., Shi, H., Ye, P., Li, L., Qu, Q., Sun, G., et al. (2017). m6A RNA Methylation Regulates the Self-Renewal and Tumorigenesis of Glioblastoma Stem CellsA RNA Methylation Regulates the Self-Renewal and Tumorigenesis of Glioblastoma Stem Cells. *Cel Rep* 18 (11), 2622–2634. doi:10.1016/j.celrep.2017.02.059

Dai, X., Wang, T., Gonzalez, G., and Wang, Y. (2018). Identification of YTH Domain-Containing Proteins as the Readers for N1-Methyladenosine in RNA. *Anal. Chem.* 90 (11), 6380–6384. doi:10.1021/acs.analchem.8b01703

Davalos, V., Blanco, S., and Esteller, M. (2018). SnapShot: Messenger RNA Modifications. *Cell* 174 (2), 498–e1. doi:10.1016/j.cell.2018.06.046

Deng, R., Cheng, Y., Ye, S., Zhang, J., Huang, R., Li, P., et al. (2019). m6A Methyltransferase METTL3 Suppresses Colorectal Cancer Proliferation and Migration through P38/ERK pathwaysA Methyltransferase METTL3 Suppresses Colorectal Cancer Proliferation and Migration through P38/ERK Pathways. *Onco Targets Ther.* 12, 4391–4402. doi:10.2147/OTT.S201052

Dominissini, D., Moshitch-Moshkovitz, S., Schwartz, S., Salmon-Divon, M., Ungar, L., Osenberg, S., et al. (2012). Topology of the Human and Mouse m6A RNA Methylomes Revealed by m6A-Seq. *Nature* 485 (7397), 201–206. doi:10.1038/nature11112

Dong, L., Chen, C., Zhang, Y., Guo, P., Wang, Z., Li, J., et al. (2021). The Loss of RNA N6-Adenosine Methyltransferase Mettl14 in Tumor-Associated Macrophages Promotes CD8+ T Cell Dysfunction and Tumor Growth. *Cancer Cell* 39 (7), 945–e10. e10. doi:10.1016/j.ccell.2021.04.016

Dong, X. F., Wang, Y., Tang, C. H., Huang, B. F., Du, Z., Wang, Q., et al. (2022). Upregulated WTAP Expression in Colorectal Cancer Correlates with Tumor Site and Differentiation. *PLoS One* 17 (2), e0263749. doi:10.1371/journal.pone.0263749

Esteller, M., and Pandolfi, P. P. (2017). The Epitranscriptome of Noncoding RNAs in Cancer. *Cancer Discov.* 7 (4), 359–368. doi:10.1158/2159-8290.CD-16-1292

Feng, Z. Y., Wang, T., Su, X., and Guo, S. (2021). Identification of the m6A RNA Methylation Regulators WTAP as a Novel Prognostic Biomarker and Genomic Alterations in Cutaneous Melanoma. *Front. Mol. Biosci.* 8, 665222. doi:10.3389/fmolb.2021.665222

Frye, M., Harada, B. T., Behm, M., and He, C. (2018). RNA Modifications Modulate Gene Expression during Development. *Science* 361 (6409), 1346–1349. doi:10.1126/science.aau1646

Ge, Y., Liu, T., Wang, C., Zhang, Y., Xu, S., Ren, Y., et al. (2021). N6-methyladenosine RNA Modification and its Interaction with Regulatory Non-coding RNAs in Colorectal Cancer. *RNA Biol.* 18 (Suppl. 2), 551–561. doi:10.1080/15476286.2021.1974749

Gilbert, W. V., Bell, T. A., and Schaening, C. (2016). Messenger RNA Modifications: Form, Distribution, and Function. *Science* 352 (6292), 1408–1412. doi:10.1126/science.aad8711

Haag, S., Kretschmer, J., and Bohnsack, M. T. (2015). WBSCR22/Merm1 Is Required for Late Nuclear Pre-ribosomal RNA Processing and Mediates N7-Methylation of G1639 in Human 18S rRNA. *Rna* 21 (2), 180–187. doi:10.1261/rna.047910.114

Han, D., Liu, J., Chen, C., Dong, L., Liu, Y., Chang, R., et al. (2019). Anti-tumour Immunity Controlled through mRNA m^6A Methylation and YTHDF1 in Dendritic Cells. *Nature* 566 (7743), 270–274. doi:10.1038/s41586-019-0916-x

Helm, M., and Motorin, Y. (2017). Detecting RNA Modifications in the Epitranscriptome: Predict and Validate. *Nat. Rev. Genet.* 18 (5), 275–291. doi:10.1038/nrg.2016.169

Huang, Y., Yuan, K., Tang, M., Yue, J., Bao, L., Wu, S., et al. (2021). Melatonin Inhibiting the Survival of Human Gastric Cancer Cells under ER Stress Involving Autophagy and Ras-Raf-MAPK Signalling. *J. Cel Mol Med* 25 (3), 1480–1492. doi:10.1111/jcmm.16237

Huang, Y., Zhou, Z., Zhang, J., Hao, Z., He, Y., Wu, Z., et al. (2021). lncRNA MALAT1 Participates in Metformin Inhibiting the Proliferation of Breast Cancer Cell. *J. Cel Mol Med* 25 (15), 7135–7145. doi:10.1111/jcmm.16742

Iles, M. M., Law, M. H., Stacey, S. N., Han, J., Fang, S., Pfeiffer, R., et al. (2013). A Variant in FTO Shows Association with Melanoma Risk Not Due to BMI. *Nat. Genet.* 45 (4), 428432e1–432e1. doi:10.1038/ng.2571

Jana, S., Hsieh, A. C., and Gupta, R. (2017). Reciprocal Amplification of Caspase-3 Activity by Nuclear export of a Putative Human RNA-Modifying Protein, PUS10 during TRAIL-Induced Apoptosis. *Cel Death Dis* 8 (10), e3093. doi:10.1038/cddis.2017.476

Konishi, N., Nakamura, M., Ishida, E., Shimada, K., Mitsui, E., Yoshikawa, R., et al. (2005). High Expression of a New Marker PCA-1 in Human Prostate Carcinoma. *Clin. Cancer Res.* 11 (14), 5090–5097. doi:10.1158/1078-0432.CCR-05-0195

Kuznetsova, S. A., Petrukov, K. S., Pletnev, F. I., Sergiev, P. V., and Dontsova, O. A. (2019). RNA (C5-Cytosine) Methyltransferases. *Biochemistry (Mosc)* 84 (8), 851–869. doi:10.1134/S0006297919080029

Li, B., Zhu, L., Lu, C., Wang, C., Wang, H., Jin, H., et al. (2021). CircNDUFB2 Inhibits Non-small Cell Lung Cancer Progression via Destabilizing IGF2BPs and Activating Anti-tumor Immunity. *Nat. Commun.* 12 (1), 295. doi:10.1038/s41467-020-20527-z

Li, H., Su, Q., Li, B., Lan, L., Wang, C., Li, W., et al. (2020). High Expression of WTAP Leads to Poor Prognosis of Gastric Cancer by Influencing Tumour-Associated T Lymphocyte Infiltration. *J. Cel Mol Med* 24 (8), 4452–4465. doi:10.1111/jcmm.15104

Li, N., Kang, Y., Wang, L., Huff, S., Tang, R., Hui, H., et al. (2020). ALKBH5 Regulates Anti-PD-1 Therapy Response by Modulating Lactate and Suppressive Immune Cell Accumulation in Tumor Microenvironment. *Proc. Natl. Acad. Sci. U S A.* 117 (33), 20159–20170. doi:10.1073/pnas.1918986117

Li, T., Hu, P. S., Zuo, Z., Lin, J. F., Li, X., Wu, Q. N., et al. (2019). METTL3 Facilitates Tumor Progression via an m^6A-igf2bp2-dependent Mechanism in Colorectal Carcinoma. *Mol. Cancer* 18 (1), 112. doi:10.1186/s12943-019-1038-7

Li, Y., Li, J., Luo, M., Zhou, C., Shi, X., Yang, W., et al. (2018). Novel Long Noncoding RNA NMR Promotes Tumor Progression via NSUN2 and BPTF in Esophageal Squamous Cell Carcinoma. *Cancer Lett.* 430, 57–66. doi:10.1016/j.canlet.2018.05.013

Li, Z., Weng, H., Su, R., Weng, X., Zuo, Z., Li, C., et al. (2017). FTO Plays an Oncogenic Role in Acute Myeloid Leukemia as a N^6-Methyladenosine RNA Demethylase. *Cancer Cell* 31 (1), 127–141. doi:10.1016/j.ccell.2016.11.017

Lin, S., Choe, J., Du, P., Triboulet, R., and Gregory, R. I. (2016). The M(6)A Methyltransferase METTL3 Promotes Translation in Human Cancer Cells. *Mol. Cel* 62 (3), 335–345. doi:10.1016/j.molcel.2016.03.021

Lin, S., Liu, Q., Lelyveld, V. S., Choe, J., Szostak, J. W., and Gregory, R. I. (2018). Mettl1/Wdr4-Mediated m^7G tRNA Methylome Is Required for Normal mRNA Translation and Embryonic Stem Cell Self-Renewal and Differentiation. *Mol. Cel* 71 (2), 244–e5. e5. doi:10.1016/j.molcel.2018.06.001

Liu, J., Yue, Y., Han, D., Wang, X., Fu, Y., Zhang, L., et al. (2014). A METTL3-METTL14 Complex Mediates Mammalian Nuclear RNA N6-Adenosine Methylation. *Nat. Chem. Biol.* 10 (2), 93–95. doi:10.1038/nchembio.1432

Liu, Y., Yang, C., Zhao, Y., Chi, Q., Wang, Z., and Sun, B. (2019). Overexpressed Methyltransferase-like 1 (METTL1) Increased Chemosensitivity of colon Cancer Cells to Cisplatin by Regulating miR-149-3p/S100A4/p53 axis. *Aging (Albany NY)* 11 (24), 12328–12344. doi:10.18632/aging.102575

Liu, Y., Zhang, Y., Chi, Q., Wang, Z., and Sun, B. (2020). Methyltransferase-like 1 (METTL1) served as a tumor suppressor in colon cancer by activating 7-methyguanosine (m7G) regulated let-7e miRNA/HMGA2 axis. *Life Sci.* 249, 117480. doi:10.1016/j.lfs.2020.117480

Malbec, L., Zhang, T., Chen, Y. S., Zhang, Y., Sun, B. F., Shi, B. Y., et al. (2019). Dynamic Methylome of Internal mRNA N^7-Methylguanosine and its Regulatory Role in Translation. *Cell Res* 29 (11), 927–941. doi:10.1038/s41422-019-0230-z

Markowitz, S. D., and Bertagnolli, M. M. (2009). Molecular Origins of Cancer: Molecular Basis of Colorectal Cancer. *N. Engl. J. Med.* 361 (25), 2449–2460. doi:10.1056/NEJMra0804588

McMahon, M. (2019). A Single H/ACA Small Nucleolar RNA Mediates Tumor Suppression Downstream of Oncogenic RAS. *Elife* 8, 48847. doi:10.7554/elife.48847

Meyer, K. D., Saletore, Y., Zumbo, P., Elemento, O., Mason, C. E., and Jaffrey, S. R. (2012). Comprehensive Analysis of mRNA Methylation Reveals Enrichment in 3' UTRs and Near Stop Codons. *Cell* 149 (7), 1635–1646. doi:10.1016/j.cell.2012.05.003

Miano, V., Codino, A., Pandolfini, L., and Barbieri, I. (2021). The Non-coding Epitranscriptome in Cancer. *Brief. Funct. Genomics* 20 (2), 94–105. doi:10.1093/bfgp/elab003

Mobet, Y., Liu, X., Liu, T., Yu, J., and Yi, P. (2022). Interplay between m^6A RNA Methylation and Regulation of Metabolism in Cancer. *Front Cel Dev Biol* 10, 813581. doi:10.3389/fcell.2022.813581

Montanaro, L., Calienni, M., Bertoni, S., Rocchi, L., Sansone, P., Storci, G., et al. (2010). Novel Dyskerin-Mediated Mechanism of P53 Inactivation through Defective mRNA Translation. *Cancer Res.* 70 (11), 4767–4777. doi:10.1158/0008-5472.CAN-09-4024

Müller, S., (2019). IGF2BP1 Promotes SRF-dependent Transcription in Cancer in a m6A- and miRNA-dependent Manner. *Nucleic Acids Res.* 47 (1), 375–390. doi:10.1093/nar/gky1012

Ni, W., Yao, S., Zhou, Y., Liu, Y., Huang, P., Zhou, A., et al. (2019). Long Noncoding RNA GAS5 Inhibits Progression of Colorectal Cancer by Interacting with and Triggering YAP Phosphorylation and Degradation and Is Negatively Regulated by the m^6A Reader YTHDF3. *Mol. Cancer* 18 (1), 143. doi:10.1186/s12943-019-1079-y

Nombela, P., Miguel-López, B., and Blanco, S. (2021). The Role of m^6A, m5C and Ψ RNA Modifications in Cancer: Novel Therapeutic Opportunities. *Mol. Cancer* 20 (1), 18. doi:10.1186/s12943-020-01263-w

Pandolfini, L., Barbieri, I., Bannister, A. J., Hendrick, A., Andrews, B., Webster, N., et al. (2019). METTL1 Promotes Let-7 MicroRNA Processing via m7G Methylation. *Mol. Cel* 74 (6), 1278–e9. e9. doi:10.1016/j.molcel.2019.03.040

Paris, J., Morgan, M., Campos, J., Spencer, G. J., Shmakova, A., Ivanova, I., et al. (2019). Targeting the RNA m^6A Reader YTHDF2 Selectively Compromises Cancer Stem Cells in Acute Myeloid Leukemia. *Cell Stem Cell* 25 (1), 137–e6. e6. doi:10.1016/j.stem.2019.03.021

Peng, W., Li, J., Chen, R., Gu, Q., Yang, P., Qian, W., et al. (2019). Upregulated METTL3 Promotes Metastasis of Colorectal Cancer via miR-1246/SPRED2/MAPK Signaling Pathway. *J. Exp. Clin. Cancer Res.* 38 (1), 393. doi:10.1186/s13046-019-1408-4

Penzo, M., Ludovini, V., Treré, D., Siggillino, A., Vannucci, J., Bellezza, G., et al. (2015). Dyskerin and TERC Expression May Condition Survival in Lung Cancer Patients. *Oncotarget* 6 (25), 21755–21760. doi:10.18632/oncotarget.4580

Rao, X., Lai, L., Li, X., Wang, L., Li, A., and Yang, Q. (2021). N^6 -methyladenosine Modification of Circular RNA Circ-ARL3 Facilitates Hepatitis B Virus-Associated Hepatocellular Carcinoma via Sponging miR-1305. *IUBMB Life* 73 (2), 408–417. doi:10.1002/iub.2438

Relier, S., Ripoll, J., Guillorit, H., Amalric, A., Achour, C., Boissière, F., et al. (2021). FTO-mediated Cytoplasmic m^6A_m Demethylation Adjusts Stem-like Properties in Colorectal Cancer Cell. *Nat. Commun.* 12 (1), 1716. doi:10.1038/s41467-021-21758-4

Roslan, N. H., Makpol, S., and Mohd Yusof, Y. A. (2019). A Review on Dietary Intervention in Obesity Associated Colon Cancer. *Asian Pac. J. Cancer Prev.* 20 (5), 1309–1319. doi:10.31557/APJCP.2019.20.5.1309

Ruggero, D., Grisendi, S., Piazza, F., Rego, E., Mari, F., Rao, P. H., et al. (2003). Dyskeratosis Congenita and Cancer in Mice Deficient in Ribosomal RNA Modification. *Science* 299 (5604), 259–262. doi:10.1126/science.1079447

Saijo, Y., Sato, G., Usui, K., Sato, M., Sagawa, M., Kondo, T., et al. (2001). Expression of Nucleolar Protein P120 Predicts Poor Prognosis in Patients with Stage I Lung Adenocarcinoma. *Ann. Oncol.* 12 (8), 1121–1125. doi:10.1023/a:1011617707999

Shen, C., Xuan, B., Yan, T., Ma, Y., Xu, P., Tian, X., et al. (2020). m6A-dependent Glycolysis Enhances Colorectal Cancer progressionA-dependent Glycolysis Enhances Colorectal Cancer Progression. *Mol. Cancer* 19 (1), 72. doi:10.1186/s12943-020-01190-w

Shen, X. P., Ling, X., Lu, H., Zhou, C. X., Zhang, J. K., and Yu, Q. (2018). Low Expression of microRNA-1266 Promotes Colorectal Cancer Progression via Targeting FTO. *Eur. Rev. Med. Pharmacol. Sci.* 22 (23), 8220–8226. doi:10.26355/eurrev_201812_16516

Shi, H., Wei, J., and He, C. (2019). Where, When, and How: Context-dependent Functions of RNA Methylation Writers, Readers, and Erasers. *Mol. Cel* 74 (4), 640–650. doi:10.1016/j.molcel.2019.04.025

Shi, H., Zhang, X., Weng, Y. L., Lu, Z., Liu, Y., Lu, Z., et al. (2018). m6A Facilitates Hippocampus-dependent Learning and Memory through YTHDF1A Facilitates Hippocampus-dependent Learning and Memory through YTHDF1. *Nature* 563 (7730), 249–253. doi:10.1038/s41586-018-0666-1

Siegel, R. L., Miller, K. D., Goding Sauer, A., Fedewa, S. A., Butterly, L. F., Anderson, J. C., et al. (2020). Colorectal Cancer Statistics, 2020. *CA Cancer J. Clin.* 70 (3), 145–164. doi:10.3322/caac.21601

Sivanand, S., and Vander Heiden, M. G. (2020). Emerging Roles for Branched-Chain Amino Acid Metabolism in Cancer. *Cancer Cell* 37 (2), 147–156. doi:10.1016/j.ccell.2019.12.011

Sjöblom, T., Jone, S., Wood, L. D., Parsons, D. W., Lin, J., Barber, T. D., et al. (2006). The Consensus Coding Sequences of Human Breast and Colorectal Cancers. *Science* 314 (5797), 268–274. doi:10.1126/science.1133427

Skvortsova, K., Stirzaker, C., and Taberlay, P. (2019). The DNA Methylation Landscape in Cancer. *Essays Biochem.* 63 (6), 797–811. doi:10.1042/EBC20190037

Sloan, K. E., Warda, A. S., Sharma, S., Entian, K. D., Lafontaine, D. L. J., and Bohnsack, M. T. (2017). Tuning the Ribosome: The Influence of rRNA Modification on Eukaryotic Ribosome Biogenesis and Function. *RNA Biol.* 14 (9), 1138–1152. doi:10.1080/15476286.2016.1259781

Tanabe, A., Tanikawa, K., Tsunetomi, M., Takai, K., Ikeda, H., Konno, J., et al. (2016). RNA Helicase YTHDC2 Promotes Cancer Metastasis via the Enhancement of the Efficiency by Which HIF-1α mRNA Is Translated. *Cancer Lett.* 376 (1), 34–42. doi:10.1016/j.canlet.2016.02.022

Vu, L. P., Pickering, B. F., Cheng, Y., Zaccara, S., Nguyen, D., Minuesa, G., et al. (2017). The N^6-Methyladenosine (m^6A)-Forming Enzyme METTL3 Controls Myeloid Differentiation of normal Hematopoietic and Leukemia Cells. *Nat. Med.* 23 (11), 1369–1376. doi:10.1038/nm.4416

Wang, S., Gan, M., Chen, C., Zhang, Y., Kong, J., Zhang, H., et al. (2021). Methyl CpG Binding Protein 2 Promotes Colorectal Cancer Metastasis by Regulating N^6 -methyladenosine Methylation through Methyltransferase-like 14. *Cancer Sci.* 112 (8), 3243–3254. doi:10.1111/cas.15011

Wang, Y., Li, Y., Yue, M., Wang, J., Kumar, S., Wechsler-Reya, R. J., et al. (2018). N6-methyladenosine RNA Modification Regulates Embryonic Neural Stem Cell Self-Renewal through Histone Modifications. *Nat. Neurosci.* 21 (2), 195–206. doi:10.1038/s41593-017-0057-1

Wang, Y., Lu, J. H., Wu, Q. N., Jin, Y., Wang, D. S., Chen, Y. X., et al. (2019). LncRNA LINRIS Stabilizes IGF2BP2 and Promotes the Aerobic Glycolysis in Colorectal Cancer. *Mol. Cancer* 18 (1), 174. doi:10.1186/s12943-019-1105-0

Weng, H., Huang, H., Wu, H., Qin, X., Zhao, B. S., Dong, L., et al. (2018). $METTL_{14}$ Inhibits Hematopoietic Stem/Progenitor Differentiation and Promotes Leukemogenesis via mRNA m^6A Modification. *Cell Stem Cell* 22 (2), 191–e9. e9. doi:10.1016/j.stem.2017.11.016

Wiener, D., and Schwartz, S. (2021). The Epitranscriptome beyond m6A. *Nat. Rev. Genet.* 22 (2), 119–131. doi:10.1038/s41576-020-00295-8

Wishart, D. (2022). Metabolomics and the Multi-Omics View of Cancer. *Metabolites* 12 (2), 154. doi:10.3390/metabo12020154

Woo, H. H., and Chambers, S. K. (2019). Human ALKBH3-Induced m1A Demethylation Increases the CSF-1 mRNA Stability in Breast and Ovarian Cancer Cells. *Biochim. Biophys. Acta Gene Regul. Mech.* 1862 (1), 35–46. doi:10.1016/j.bbagrm.2018.10.008

Wu, S., Li, T., Liu, W., and Huang, Y. (2021). Ferroptosis and Cancer: Complex Relationship and Potential Application of Exosomes. *Front. Cel Dev Biol* 9, 733751. doi:10.3389/fcell.2021.733751

Xiang, S., Liang, X., Yin, S., Liu, J., and Xiang, Z. (2020). N6-methyladenosine Methyltransferase METTL3 Promotes Colorectal Cancer Cell Proliferation through Enhancing MYC Expression. *Am. J. Transl Res.* 12 (5), 1789–1806.

Xiong, X., Li, X., and Yi, C. (2018). N1-methyladenosine Methylome in Messenger RNA and Non-coding RNA. *Curr. Opin. Chem. Biol.* 45, 179–186. doi:10.1016/j.cbpa.2018.06.017

Xu, D., Shao, J., Song, H., and Wang, J. (2020). The YTH Domain Family of N6-Methyladenosine "Readers" in the Diagnosis and Prognosis of Colonic Adenocarcinoma. *Biomed. Res. Int.* 2020, 9502560. doi:10.1155/2020/9502560

Xu, J., Chen, Q., Tian, K., Liang, R., Chen, T., Gong, A., et al. (2020). m6A Methyltransferase METTL3 Maintains colon Cancer Tumorigenicity by Suppressing SOCS2 to Promote Cell Proliferation. *Oncol. Rep.* 44 (3), 973–986. doi:10.3892/or.2020.7665

Yan, D., Tu, L., Yuan, H., Fang, J., Cheng, L., Zheng, X., et al. (2017). WBSCR22 Confers Oxaliplatin Resistance in Human Colorectal Cancer. *Sci. Rep.* 7 (1), 15443. doi:10.1038/s41598-017-15749-z

Yang, P., Wang, Q., Liu, A., Zhu, J., and Feng, J. (2020). ALKBH5 Holds Prognostic Values and Inhibits the Metastasis of Colon Cancer. *Pathol. Oncol. Res.* 26 (3), 1615–1623. doi:10.1007/s12253-019-00737-7

Yang, S., Wei, J., Cui, Y. H., Park, G., Shah, P., Deng, Y., et al. (2019). m6A mRNA Demethylase FTO Regulates Melanoma Tumorigenicity and Response to Anti-PD-1 blockadeA mRNA Demethylase FTO Regulates Melanoma Tumorigenicity and Response to Anti-PD-1 Blockade. *Nat. Commun.* 10 (1), 2782. doi:10.1038/s41467-019-10669-0

Yue, C., Chen, J., Li, Z., Li, L., Chen, J., and Guo, Y. (2020). microRNA-96 Promotes Occurrence and Progression of Colorectal Cancer via Regulation of the AMPKα2-FTO-m6A/MYC axis. *J. Exp. Clin. Cancer Res.* 39 (1), 240. doi:10.1186/s13046-020-01731-7

Zaccara, S., Ries, R. J., and Jaffrey, S. R. (2019). Reading, Writing and Erasing mRNA Methylation. *Nat. Rev. Mol. Cel Biol* 20 (10), 608–624. doi:10.1038/s41580-019-0168-5

Zhang, C., and Jia, G. (2018). Reversible RNA Modification N1-Methyladenosine (m1A) in mRNA and tRNA. *Genomics Proteomics Bioinformatics* 16 (3), 155–161. doi:10.1016/j.gpb.2018.03.003

Zhang, C., Samanta, D., Lu, H., Bullen, J. W., Zhang, H., Chen, I., et al. (2016). Hypoxia Induces the Breast Cancer Stem Cell Phenotype by HIF-dependent and ALKBH5-Mediated m^6A-Demethylation of NANOG mRNA. *Proc. Natl. Acad. Sci. U S A.* 113 (14), E2047–E2056. doi:10.1073/pnas.1602883113

Zhang, J., Tsoi, H., Li, X., Wang, H., Gao, J., Wang, K., et al. (2016). Carbonic Anhydrase IV Inhibits colon Cancer Development by Inhibiting the Wnt Signalling Pathway through Targeting the WTAP-WT1-TBL1 axis. *Gut* 65 (9), 1482–1493. doi:10.1136/gutjnl-2014-308614

Zhang, S., Zhao, B. S., Zhou, A., Lin, K., Zheng, S., Lu, Z., et al. (2017). m6A Demethylase ALKBH5 Maintains Tumorigenicity of Glioblastoma Stem-like Cells by Sustaining FOXM1 Expression and Cell Proliferation ProgramA Demethylase ALKBH5 Maintains Tumorigenicity of Glioblastoma Stem-like Cells by Sustaining FOXM1 Expression and Cell Proliferation Program. *Cancer Cell* 31 (4), 591–e6. doi:10.1016/j.ccell.2017.02.013

Zhang, W., Eckwahl, M. J., Zhou, K. I., and Pan, T. (2019). Sensitive and Quantitative Probing of Pseudouridine Modification in mRNA and Long Noncoding RNA. *Rna* 25 (9), 1218–1225. doi:10.1261/rna.072124.119

Zhao, J., Lee, E. E., Kim, J., Yang, R., Chamseddin, B., Ni, C., et al. (2019). Transforming Activity of an Oncoprotein-Encoding Circular RNA from Human Papillomavirus. *Nat. Commun.* 10 (1), 2300. doi:10.1038/s41467-019-10246-5

Zhao, Y., Zhao, Q., Kaboli, P. J., Shen, J., Li, M., Wu, X., et al. (2019). m1A Regulated Genes Modulate PI3K/AKT/mTOR and ErbB Pathways in Gastrointestinal Cancer. *Transl Oncol.* 12 (10), 1323–1333. doi:10.1016/j.tranon.2019.06.007

Zheng, Y., Wang, Y., Liu, Y., Xie, L., Ge, J., Yu, G., et al. (2021). N6-Methyladenosine Modification of PTTG3P Contributes to Colorectal Cancer Proliferation via YAP1. *Front. Oncol.* 11, 669731. doi:10.3389/fonc.2021.669731

Zhou, D., Tang, W., Xu, Y., Xu, Y., Xu, B., Fu, S., et al. (2021). $METTL_3/YTHDF_2$ m6A axis Accelerates Colorectal Carcinogenesis through Epigenetically Suppressing YPEL5. *Mol. Oncol.* 15 (8), 2172–2184. doi:10.1002/1878-0261.12898

Zhou, J., Wan, J., Gao, X., Zhang, X., Jaffrey, S. R., and Qian, S. B. (2015). Dynamic M(6)A mRNA Methylation Directs Translational Control of Heat Shock Response. *Nature* 526 (7574), 591–594. doi:10.1038/nature15377

Zhou, X., Chang, Y., Zhu, L., Shen, C., Qian, J., and Chang, R. (2021). LINC00839/miR-144-3p/WTAP (WT1 Associated Protein) axis Is Involved in Regulating Hepatocellular Carcinoma Progression. *Bioengineered* 12 (2), 10849–10861. doi:10.1080/21655979.2021.1990578

Zhu, W., Si, Y., Xu, J., Lin, Y., Wang, J. Z., Cao, M., et al. (2020). Methyltransferase like 3 Promotes Colorectal Cancer Proliferation by Stabilizing CCNE1 mRNA in an m6A-dependent Manner. *J. Cel Mol Med* 24 (6), 3521–3533. doi:10.1111/jcmm.15042

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Liang, Yi, Mao, Meng, Wu, Li and Xue. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers | Frontiers in Pharmacology

ORIGINAL RESEARCH
published: 23 May 2022
doi: 10.3389/fphar.2022.921517

Puerarin: A Potential Therapeutic for Colon Adenocarcinoma (COAD) Patients Suffering From SARS-CoV-2 Infection

Weizheng Liang[1,2*†], *Xiushen Li*[3,4,5†], *Yue Yao*[6†], *Qingxue Meng*[1], *Xueliang Wu*[2], *Hao Wang*[3,4,5*] *and Jun Xue*[1,2*]

[1]Central Laboratory, The First Affiliated Hospital of Hebei North University, Zhangjiakou, China, [2]Department of General Surgery, The First Affiliated Hospital of Hebei North University, Zhangjiakou, China, [3]Department of Obstetrics and Gynecology, Shenzhen University General Hospital, Shenzhen, China, [4]Guangdong Key Laboratory for Biomedical Measurements and Ultrasound Imaging, School of Biomedical Engineering, Shenzhen University Health Science Center, Shenzhen, China, [5]Shenzhen Key Laboratory, Shenzhen University General Hospital, Shenzhen, China, [6]Department of Internal Medicine of Traditional Chinese Medicine, The First Clinical Medical School, Guangzhou University of Chinese Medicine, Guangzhou, China

OPEN ACCESS

Edited by:
Yongye Huang,
Northeastern University, China

Reviewed by:
Bowang Chen,
Chinese Academy of Medical Sciences and Peking Union Medical College, China
Yuting Chen,
Genesis Healthcare, Japan

***Correspondence:**
Weizheng Liang
jmbb1203@126.com
Hao Wang
haowang0806@gmail.com
Jun Xue
xuejun@hebeinu.edu.cn

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

Received: 16 April 2022
Accepted: 06 May 2022
Published: 23 May 2022

Citation:
Liang W, Li X, Yao Y, Meng Q, Wu X, Wang H and Xue J (2022) Puerarin: A Potential Therapeutic for Colon Adenocarcinoma (COAD) Patients Suffering From SARS-CoV-2 Infection. Front. Pharmacol. 13:921517. doi: 10.3389/fphar.2022.921517

Patients with colonic adenocarcinoma (COAD) are at relatively high risk of SARS-CoV-2 infection. However, there is a lack of medical strategies to treat COVID-19/COAD comorbidity. Puerarin, a natural product, is a known antiviral, antitumor, and immunomodulatory effect. Therefore, we hypothesised that puerarin could be used to treat COVID-19/COAD patients. Based on network pharmacology and bioinformatics analysis, the potential targets and pharmacological mechanisms of puerarin in COVID-19/COAD were identified. By intersecting therapeutic target genes for puerarin, COVID-19-related genes and COAD-related genes, 42 target genes of puerarin that could potentially treat COVID-19/COAD comorbidity were obtained. By using the 42 potential target genes to construct the protein-protein interaction (PPI) network, we obtained five core target genes, namely RELA, BCL2, JUN, FOS, and MAPK1. The results of bioinformatics analysis revealed that puerarin could be able to treat COVID-19/COAD comorbidity through apoptosis, antiviral, antioxidant, NF-κB signaling pathway, MAPK signaling pathway, IL-17 signaling pathway, TNF signaling pathway, and HIF-1 signaling pathway etc. This study found that puerarin has the potential to treat COVID-19/COAD patients and that the therapeutic target genes obtained in the study may provide clues for the treatment of COVID19/COAD comorbidity.

Keywords: puerarin, COVID-19, COAD, comorbidity, network pharmacology, bioinformatics

Abbreviations: COAD, Colon Adenocarcinoma; PPI, Protein-Protein Interaction; TCM, Traditional Chinese Medicine; GEO, Gene Expression Omnibus; DEGs, Differentially Expressed Genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

INTRODUCTION

Since the emergence of the SARS-CoV-2 virus, COVID-19 has spread rapidly worldwide. The latest data from the World Health Organization show that the cumulative number of confirmed cases and deaths worldwide has been more than 500 million and six million (updated on 05 May 2022). With a global daily average of about 500,000 patients with COVID-19, the situation remains critical for epidemic prevention. Similar to SARS-CoV, SARS-CoV-2 was a highly pathogenic coronavirus, which were mainly transmitted through aerosols or droplets, leading to the development of respiratory diseases and even death (Xu et al., 2020). Due to the small size of droplets and aerosols carrying the SARS-CoV-2 virus, it is difficult to achieve the expected preventive effect (Jayaweera et al., 2020). The common clinical symptoms in most SARS-CoV-2 infected patients were cough and fever, with partial patients also presenting with sore throat, diarrhea, and loss of taste (Giacomelli et al., 2020; Li et al., 2020). However, a few of patients with COVID-19 probably developed acute respiratory failure or acute respiratory distress syndrome, which might even lead to death (Chen et al., 2020). The emergence of the SARS-CoV-2 virus has caused enormous economic and health losses to people around the world. Through the continuous efforts of scientists from around the world, several vaccines and drugs have been used for COVID-19 (Liu et al., 2020; Lazarus et al., 2021). However, there was no significant change in the number of additional COVID-19 patients per day.

Both malignant tumor and COVID-19 are major public health problems worldwide. Colorectal cancer, a common gastrointestinal malignancy, had the third-highest incidence and mortality rate of all cancers in the United States, for both men and women (Siegel et al., 2022). With the rapid development of modern medicine, significant breakthroughs have been made in basic research, early diagnosis, chemotherapy, and targeted therapy for colorectal cancer, yet the survival rate of colorectal cancer patients has not been significantly improved (Durinikova et al., 2021). Colon adenocarcinoma (COAD) is a common malignant tumor among all types of colon cancer, with a high mortality rate and high recurrence rate (Liu, 2021). Due to the lack of specific early diagnostic symptoms and markers, most patients were already at an advanced stage at the time of diagnosis, and the high metastatic rate characteristic of advanced COAD was a major factor in the high mortality rate of COAD patients (Brown and Solomon, 2018). Therefore, exploring the mechanism of COAD pathogenesis, progression, and metastasis and finding effective biomarkers and therapeutic targets can greatly help to improve the survival rate of COAD patients. The latest study found that the vast majority of patients with malignancies are chronically immunosuppressed and therefore more susceptible to SARS-CoV-2 infection than healthy individuals, often resulting in a poorer prognosis (Kamboj and Sepkowitz, 2009; Liang et al., 2020). If patients with COAD are infected with the SARS-CoV-2 virus, it may lead to a worse prognosis. Therefore, it is necessary to find drugs that can be used to treat COVID-19/COAD patients.

Traditional Chinese medicine (TCM), derived from more than 2,000 years of clinical experience, has been a crucial role in the treatment of a variety of epidemics and has made significant contributions to the health of the Chinese people. With the outbreak of COVID-19, combinations of TCM treatment protocols have been widely used in China, achieving better clinical outcomes. In China, more than four-fifths of COVID-19 patients have received TCM treatment (Ren et al., 2020). Pueraria Lobata, one of the commonly used TCMs, has be reportedly used for anti-inflammatory, immunomodulatory, anti-cancer and so on (Wang et al., 2020). Puerarin, as the main active ingredient of Pueraria Lobata, has similar pharmacological effects to Pueraria Lobata. As a potent inhibitor of influenza virus neuraminidase, puerarin could reduce influenza virus titers, decrease respiratory inflammatory responses and diminish mortality (Wang et al., 2021). Puerarin nanosuspension showed good anticancer activity and low toxicity and has the potential to become a therapeutic medicine for patients with COAD (Wang et al., 2013). On this basis, we hypothesized that puerarin might be used for COVID-19/COAD patients. Therefore, network pharmacology and other bioinformatic methods were employed to explore the therapeutic targets and pharmacological mechanisms of puerarin in COVID-19/COAD and to elucidate the potential therapeutic value of puerarin.

MATERIALS AND METHODS

Flowchart of the Study

Figure 1 depicts the entire flow of this study. Firstly, targets associated with COVID-19 were identified though the Gene Expression Omnibus (GEO) dataset and multiple disease-target related databases; then targets associated with COAD were identified by using the TCGA-COAD dataset, the GTEx-colon data dataset, and multiple disease-target related databases; next, multiple drug-target related databases were used to find the therapeutic targets of puerarin. The COVID-19-related targets, COAD-related targets, and therapeutic targets of Puerarin were intersected. Finally, bioinformatics analysis was used to explore the pharmacological mechanisms of puerarin in COVID-19/COAD comorbidity.

Identification of Targets Associated With COVID-19 or COAD

Transcriptome sequencing data from the COVDID-19-related datasets (GSE157103, GSE152075, GSE171110) were downloaded though the GEO database (https://www.ncbi.nlm.nih.gov/geo/). The differentially expressed genes (DEGs) associated with COVDID-19 were filtered according to the filtering criteria ($p < 0.05$ and absolute value of log fold change >1) by using the "limma" package in R. In addition, we also searched seven authoritative databases containing CTD (http://ctdbase.org/), OMIM (https://omim.org/), DisGeNET (https://www.disgenet.org/), GeneCards (https://www.genecards.org), NCBI (https://www.ncbi.nlm.nih.gov/), TTD (http://db.idrblab.net/), pharmaGKB (https://www.pharmgkb.org/) to further explore genes associated with COVID-19.

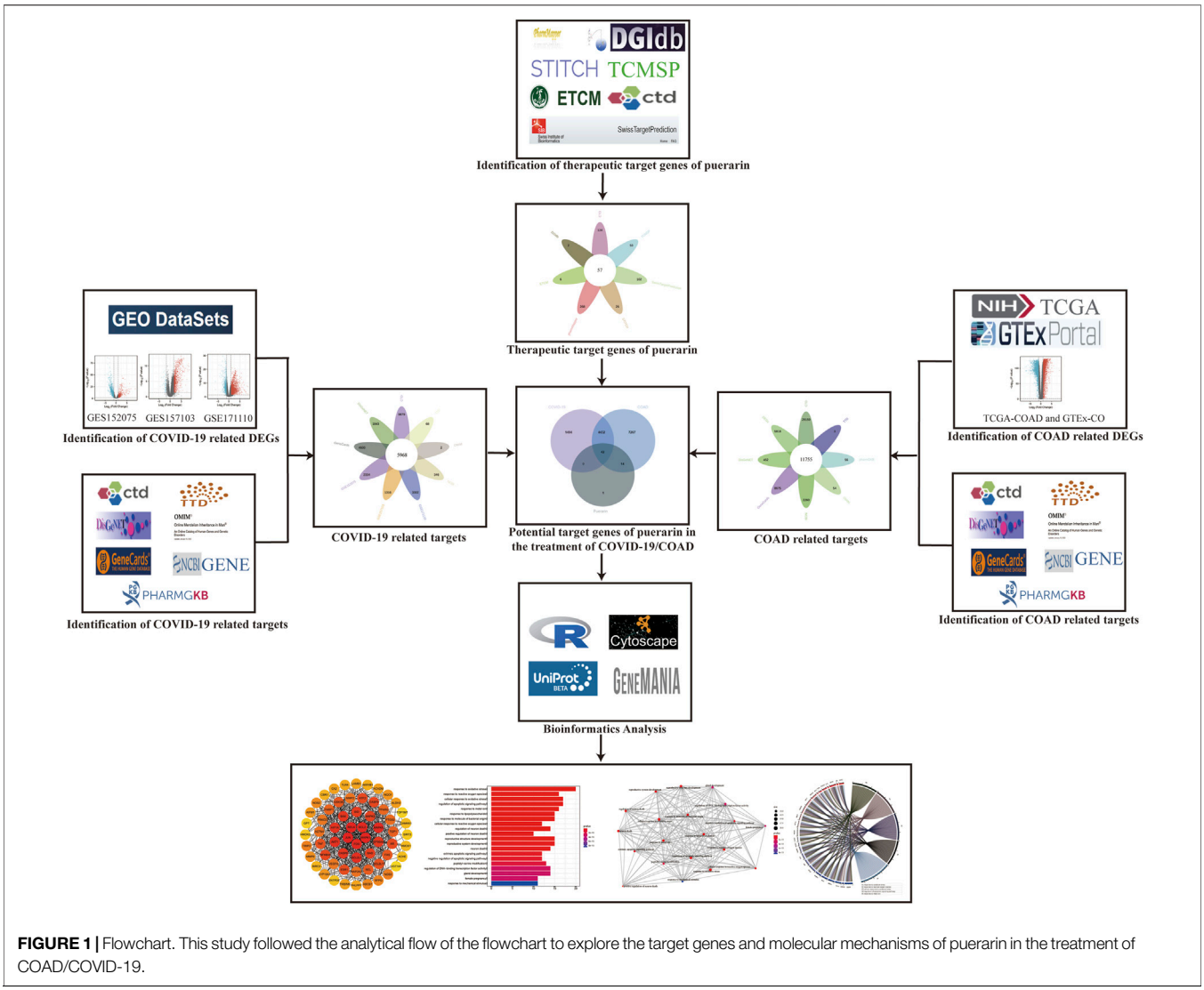


FIGURE 1 | Flowchart. This study followed the analytical flow of the flowchart to explore the target genes and molecular mechanisms of puerarin in the treatment of COAD/COVID-19.

Gene names obtained from the different databases were harmonized. We considered the genes that appeared at least twice as COVID-19-related genes.

Transcriptome sequencing data from the TCGA-COAD and GTEx-CO datasets were obtained via the UCSC Xena (http://xena.ucsc.edu/) database. DEGs associated with COAD ($p < 0.05$ and absolute value of log fold change >1) were screened by the R language "limma" package. In addition, CTD, OMIM, DisGeNET, GeneCards, NCBI, TTD, and pharmaGKB databases were used to obtain COAD-associated genes. We considered the genes that appeared at least twice as COAD-related genes.

Identification of Puerarin Therapeutic Targets

To fully explore the therapeutic targets of puerarin, we searched seven authoritative databases related to drug targets: 1) PharmMapper (http://www.lilab-ecust.cn/pharmmapper/); 2) CTD (http://ctdbase.org/); 3) SwissTargetPrediction (http://www.swisstargetprediction.ch/); 4) Drug Gene Interaction Database (DGIdb, https://www.dgidb.org/); 5) TCMSP (https://tcmspw.com/tcmsp.php); 6) STITCH (http://stitch.embl.de/); 7) ECTM (http://www.tcmip.cn/ETCM/). To ensure the accuracy of subsequent analyses, targets that appeared at least twice in the database were considered to be therapeutic target genes for puerarin.

Construction of PPI Network and Analysis of Core Genes

We referred to the intersection of therapeutic target genes for puerarin, COVID-19-related genes and COAD-related genes as target genes of puerarin in COVID-19/COAD comorbidity. The protein-protein interaction (PPI) network was constructed by using the GeneMANIA database (http://genemania.org/). The GeneMANIA database is one of the recognized bioinformatics databases that helps to explain the molecular mechanisms involved in the target proteins. Cytoscape software was used to

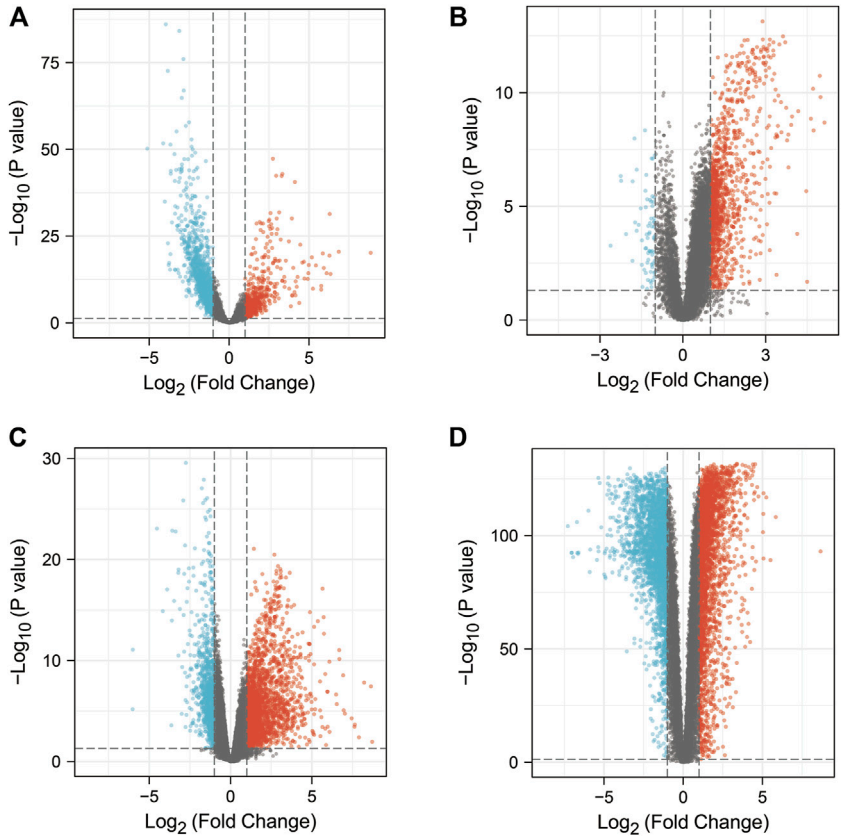


FIGURE 2 | DEGs from COAD patients or COVID-19 patients ($p < 0.05$ and absolute value of log fold change >1). The red and blue dots represented genes with increased or decreased expression in COVID-19 patients or COAD patients, respectively, while the black dots represented genes with no significant difference in expression between COVID-19 or COAD patients. **(A)** Volcano plot of DEGs in the GSE152075 dataset. **(B)** Volcano plot of DEGs in the GSE157103 dataset. **(C)** Volcano plot of DEGs in the GSE171110 dataset. **(D)** Volcano plot of DEGs in the TCGA-COAD dataset and the GTEx-CO dataset.

visualize the degree of target genes and to identify the core genes for puerarin treatment of COVID-19/COAD.

Bioinformatics Analysis

The uniport database was used to convert gene names to gene IDs for subsequent bioinformatic analysis. We called the "enrichGO" and "enrichKEGG" functions in R to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis on the target genes for puerarin treatment of COVID-19/COAD, and the "ggplot2" package in R to visualise the analysis results in R (only displayed the 20 results with the lowest q values). Finally, we used the "circlize" package in R to construct the circos plots showing the results of the top five GO and KEGG enrichment analyses with the corresponding genes.

RESULTS

Identification Results of COVID-19-Related and COAD-Related Targets

The study contained 3 GEO datasets associated with COVID-19: GSE152075, GSE157103, and GSE171110. In the GSE157103 dataset, we included sequencing data from 484 nasopharyngeal swab samples (containing 430 COVID-19 positive patients and 54 COVID-19 negative healthy individuals). In the GSE157103 dataset, we included sequencing data from 126 plasma samples (containing 100 COVID-19 positive patients and 26 COVID-19 negative healthy individuals). In the GSE171110 dataset, we included sequencing data from 54 whole blood samples (containing 44 COVID-19 positive patients and 10 COVID-19 negative healthy individuals). The DEGs in the GEO dataset were searched though the "limma" package in R. 2334, 1316, 3302 DEGs were obtained from the GSE152075, GSE157103, GSE171110 datasets respectively (**Figures Figure2A–C**). In addition, CTD, OMIM, DisGeNET, GeneCards, NCBI, TTD, and pharmaGKB databases were used to find 9,879, 2, 1843, 4,600, 346, 68, and 0 genes associated with COVID-19, respectively (**Figure Figure3A**, **Supplementary Table S1**). In the current study, we considered target genes that appeared in at least two databases to be COVID-19-related genes, resulting in 5968 COVID-19-related genes.

In this study, the TCGA and GTEx datasets were used to identify DEGs in COAD patients. We combined the data from TCGA-COAD and GTEx-colon and included sequencing data from a total of 349 normal colon samples and 471 COAD samples. The data were analyzed by using the "limma" package in R, resulting in 5,818 DEGs (**Figure Figure2D**).

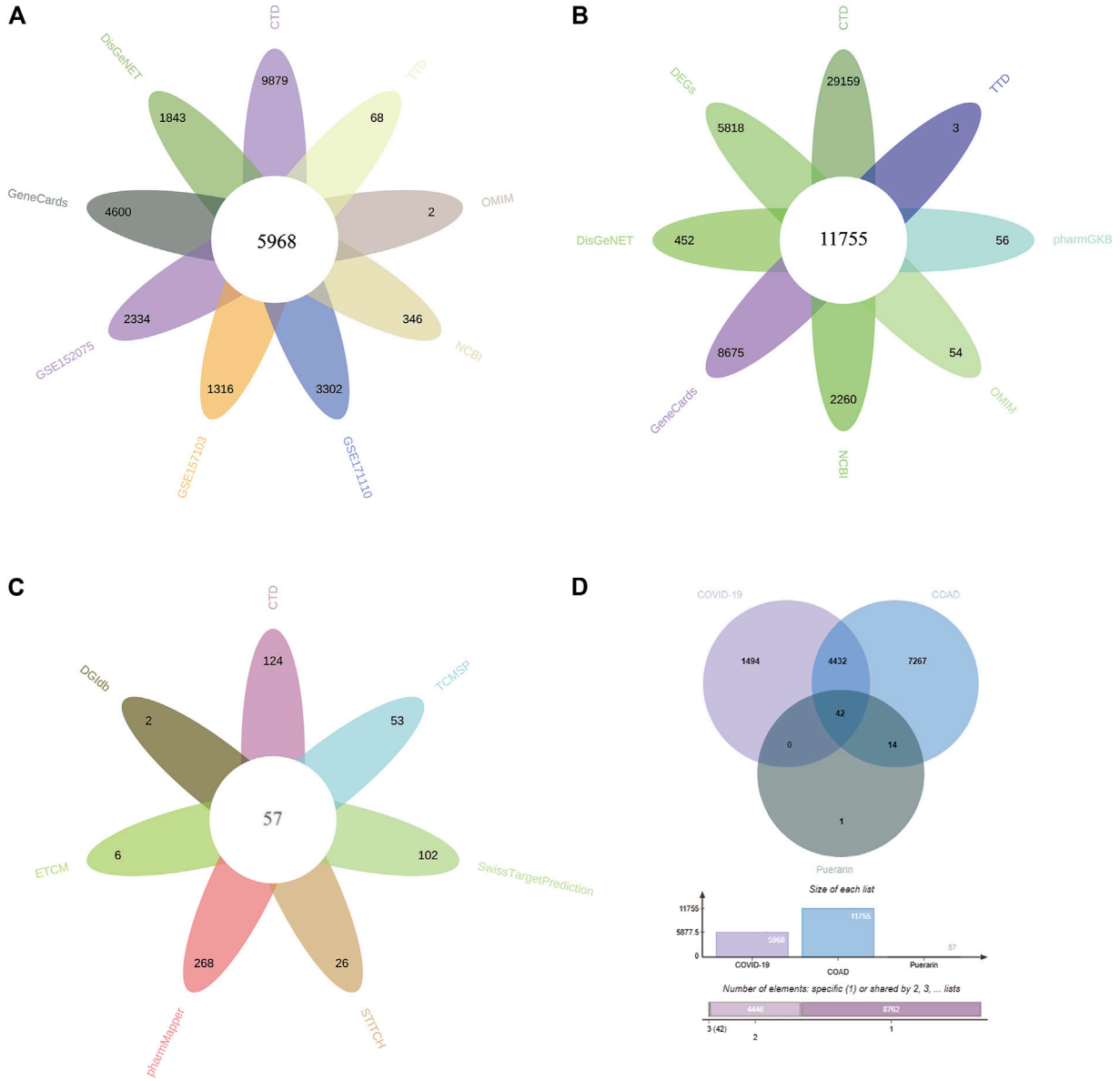


FIGURE 3 | Potential target genes of puerarin in the treatment of COVID-19/COAD comorbidity. **(A)** Genes associated with COVID-19 from 3 GEO datasets and six databases. **(B)** Genes associated with COAD from the TCGA-COAD dataset, GTEx-CO dataset, and seven databases. **(C)** Therapeutic target genes of Puerarin from seven databases. **(D)** Venn diagram of therapeutic target genes for puerarin, COVID-19-related genes and COAD-related genes.

Through CTD, OMIM, DisGeNET, GeneCards, NCBI, TTD, and pharmaGKB databases, we obtained 29,159, 54, 452, 8,675, 2,260, 3, 56 genes associated with COAD (**Figure Figure3B**, **Supplementary Table S2**). We referred to target genes that appeared in at least two databases as COAD-related genes, resulting in 11,755 COAD-related genes.

Identification Results of Puerarin Target Genes

To obtain therapeutic target genes for puerarin, seven databases containing PharmMapper, CTD, SwissTargetPrediction, DGIdb, TCMSP, STITCH, and ECTM were searched, resulting in 268, 124, 102, 2, 53, 26, and six therapeutic target genes respectively (**Figure 3C**, **Supplementary Table S3**). The therapeutic target genes were screened by using the presence of at least two databases as a screening condition, resulting in 57 therapeutic targets for puerarin.

Therapeutic Target Genes of Puerarin in COVID-19/COAD

We intersected the therapeutic target genes of puerarin, COAD-related genes, and COVID-19-related genes, and presented them

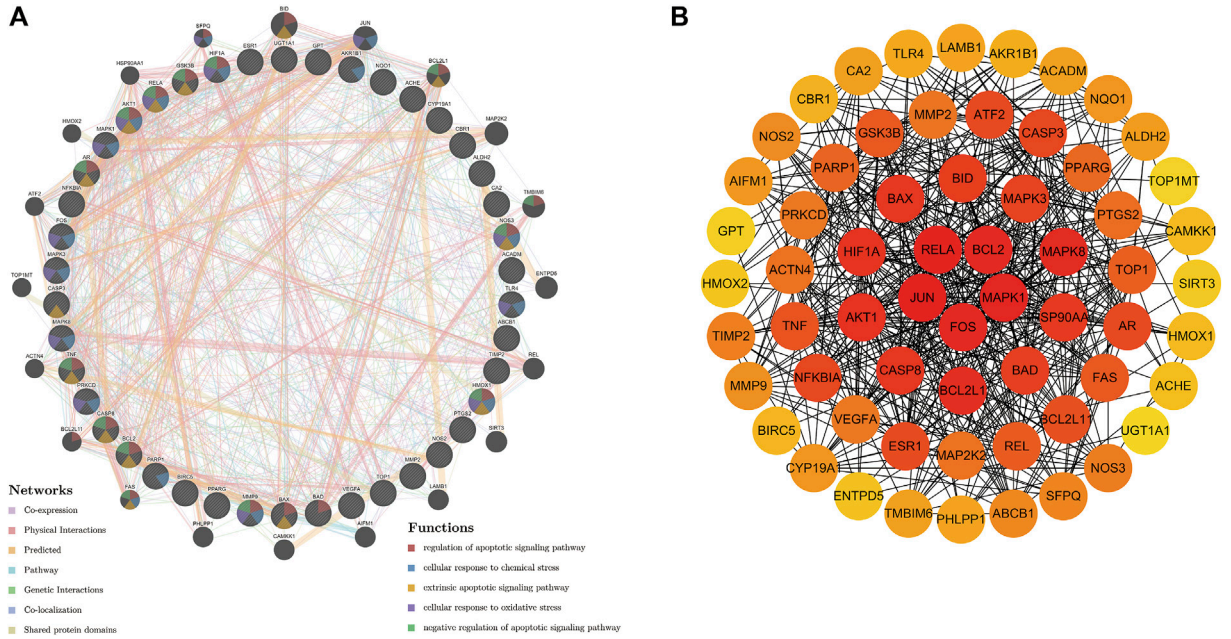


FIGURE 4 | PPI network of potential therapeutic target genes of puerarin in the treatment of COVID-19/COAD. **(A)** The PPI network was derived from the GeneMANIA database. The different relationships between nodes were indicated by the different colored connecting lines. Nodes were enriched in different functions indicated by the colors of the nodes. **(B)** PPI networks constructed from nodal degree values. The degree of the node was proportional to the depth of the node color.

by using Venn diagram (**Figure 3D**, **Supplementary Table S4**). We defined these 42 intersecting genes as gene set 1 and used them for subsequent bioinformatics analysis.

PPI Network

We used 42 genes from gene set 1 for PPI network construction. The GeneMANIA database, based on large amounts of genomics and proteomics data, is commonly used for the prediction of gene function and the construction of interactions between genes. In this study, we uploaded gene set 1 to the GeneMANIA database. The results of the analysis were shown in **Figure 4A** (**Supplementary Table S5**). The circular nodes represented genes, and the different colors of the node represented gene enriched in different functional analysis results. The different colored lines between the nodes represented the different interactions. The mechanism of puerarin in COVID-19/COAD comorbidity might be related to apoptotic, chemical stress, and oxidative stress. By using Cytoscape software to visualize the degree of each gene in the PPI network (**Figure 4B**), we found that RELA, BCL2, JUN, FOS, and MAPK1 had the highest degree, suggesting that the five genes might be core targets of puerarin in COVID-19/COAD comorbidity.

Results of Bioinformatics Analysis

To investigate the pharmacological mechanism of puerarin in the treatment of COVID-19/COAD, we performed bioinformatic analysis of 42 genes in gene set 1. The "cluster profile" package in R was used to perform enrichment analysis of the BP, CC, and MF aspects of GO (**Figures 5A–C**, **Supplementary Table S6**). The results of the top five BP, CC, MF, and KEGG enrichment analyses and the corresponding genes were presented using circus plots (**Figures 6A–D**). The results of the BP enrichment analysis mainly included regulation of apoptotic signaling pathway, response to the metal ion, response to lipopolysaccharide, response to molecule of bacterial origin, regulation of neuron death, productive system development, peptidyl-serine modification, and so on. The results of the CC enrichment analysis found that gene set 1 was mainly involved in caveola, plasma membrane raft, myelin sheath, mitochondrial outer membrane, pseudopodium, organelle outer membrane, protein-DNA complex, and so on. The results of the MF enrichment analysis found that gene set 1 mainly involved MAP kinase activity, phosphatase binding, NF-κB binding, ubiquitin-protein ligase binding, coenzyme binding, histone deacetylase binding, tyrosine kinase activity, etc.

In addition, the results of the KEGG pathway enrichment analysis mainly included Apoptosis, Colorectal cancer, IL-17 signaling pathway, TNF signaling pathway, Human immunodeficiency virus 1 infection, HIF-1 signaling pathway (**Figure 5D**, **Supplementary Table S7**).

DISCUSSION

The SARS-CoV-2 virus has been spreading globally for more than 2 years and has not yet shown signs of improvement, yet we still lack effective drugs against COVID-19. However, in China, protocols that combine Chinese medicine with Western medicine to treat patients with COVID-19 have achieved

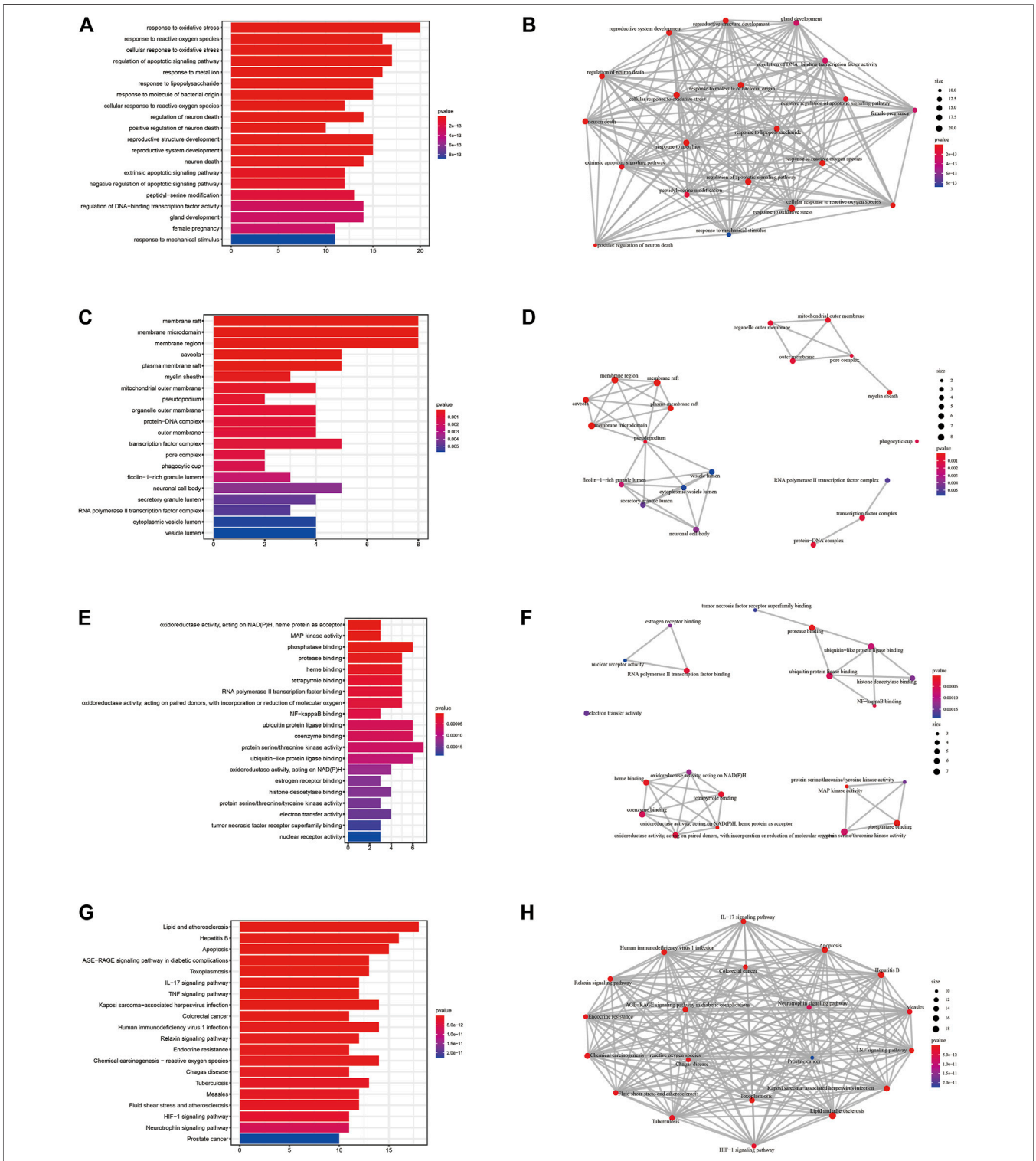


FIGURE 5 | GO and KEGG enrichment analysis of potential therapeutic target genes of puerarin for COVID-19/COAD (only the 20 pathways with the smallest *p*-values were shown). **(A)**, **(C)** and **(E)** The results of BP, CC, and MF term enrichment analysis, respectively. **(B)**, **(D)** and **(F)** The results of correlation analysis of BP, CC, MF term enrichment analysis, respectively. **(G)** The results of KEGG enrichment analysis. **(H)**The results of correlation analysis of KEGG enrichment analysis.

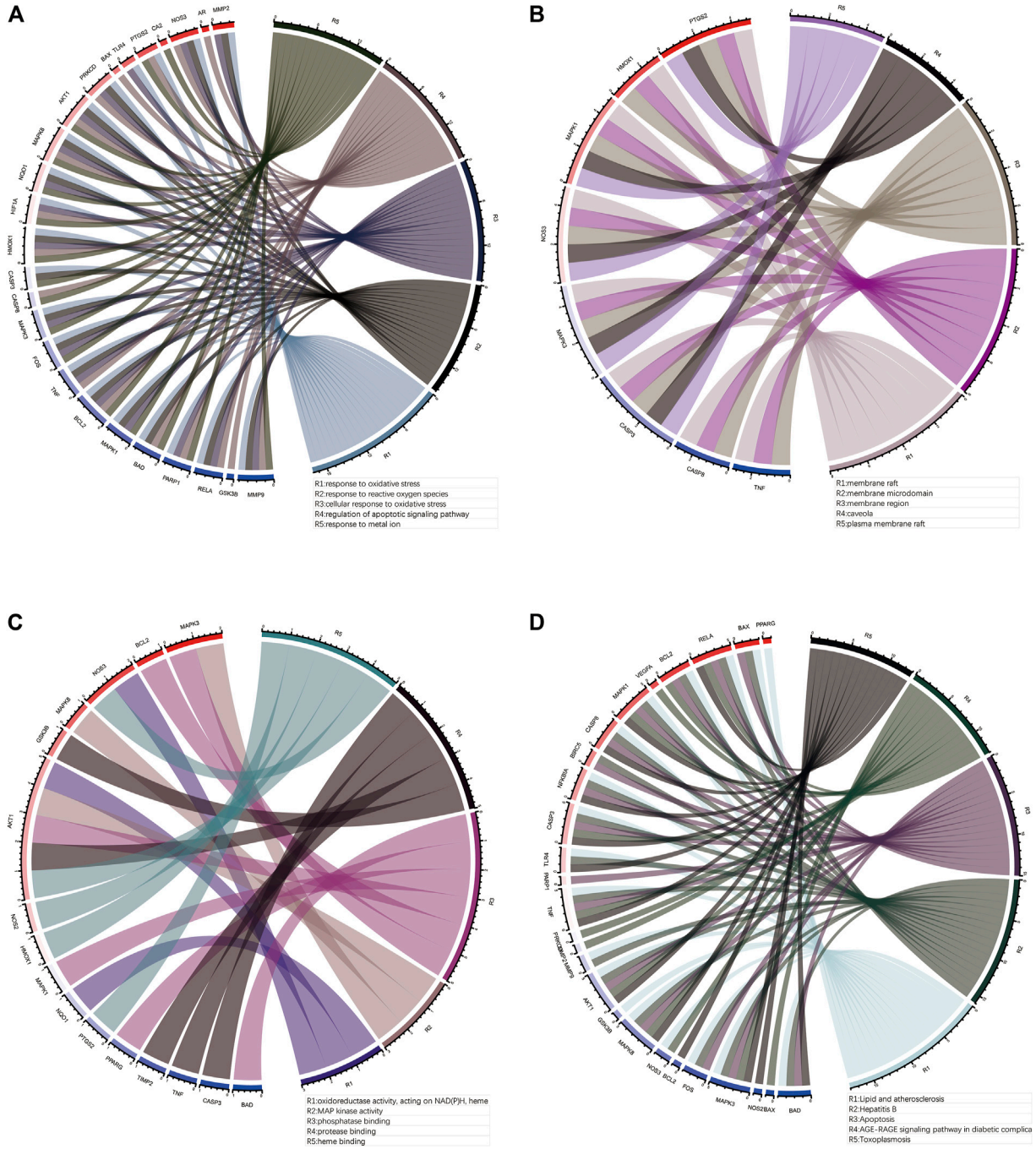


FIGURE 6 | Top five enrichment analysis results and corresponding genes. **(A)**, **(B)** and **(C)**The top five results of BP, CC, MF enrichment analysis and corresponding genes. **(D)** The top five results of KEGG enrichment analysis and the corresponding genes.

excellent results. Clinical studies have found that TCM treatment can reduce the rate of severe disease, decrease the use of antibiotics and alleviate clinical symptoms including fever and cough (Huang et al., 2021; Zhang et al., 2021). Cumulatively, one in twenty people worldwide has been infected with the SARS-CoV-2 virus, and patients with COAD are more susceptible to SARS-CoV-2 virus infection than healthy individuals due to their chronically depressed immune system. Based on the antiviral and anticancer properties of puerarin, we combined bioinformatics and network pharmacology approaches to investigate the target genes and mechanisms of puerarin in the treatment of COVID-19/COAD (Wei et al., 2014).

We analyzed data from open databases and defined the 42 potential target genes of puerarin in the treatment of COVID-19/

COAD as gene set 1. Through the GeneMANIA database to construct a PPI network and perform functional analysis, we found that gene set 1 mainly has the functions of chemical stress, apoptosis, and oxidative stress. Chemical stress in the body might trigger adverse drug reactions, and adverse reactions during the treatment of COAD patients often led to the inability of patients to tolerate subsequent treatment (Mutter et al., 2015). Apoptosis isthe normal physiological process that occurs within the body in a state of relative equilibrium. Disruption of this balance may lead to disease. A high number of apoptotic cells, for example, could lead to degenerative disorders, whereas a low number of apoptotic cells could contribute to tumor growth and progression (Wong, 2011). But apoptosis is a double-edged sword, and induction of apoptosis in tumor cells is one of the therapeutic mechanisms for tumor patients. Abnormal differentiation and apoptosis of T cells, one of the hallmarks of severe COVID-19, led to immune dysfunction in patients (Leonardi and Proenca, 2020). Persistent oxidative stress might lead to chronic inflammation, which in turn led to the development of diseases including cancer, lung disease, and diabetes (Reuter et al., 2010). Studies have confirmed that oxidative stress is positively related to the incidence of COAD (Barrett et al., 2017). SARS-CoV-2 virus induces the abnormal oxidative stress response in humans, which in turn can lead to the development of the respiratory syndrome and even death in patients. Therefore, drugs that improve the oxidative stress status of patients may improve prognosis and reduce mortality. We screened the genes based on their degree values and identified RELA, BCL2, JUN, FOS, and MAPK1 as possible core target genes for puerarin in treatment of COVID-19/COAD. The NF-κB signaling pathway is involved in several biological processes such as cellular transformation, and immune response in the body. Abnormal NF-κB directly contributed to tumorigenesis, growth, and metastasis, while NF-κB activity was controlled by phosphorylation of upstream genes containing RELA (Lu and Yarbrough, 2015). Inhibition of NF-κB pathway activity reduced the likelihood of severe disease in COVID-19 patients (Hariharan et al., 2021). As an important factor regulating apoptosis, BCL2 has been associated with the development and progression of cancer and autoimmune diseases, and several drugs targeting BCL2 have been developed (Siddiqui et al., 2015). Clinical studies have found that serum concentrations of BCL2 in COVID-19 patients correlate with patient prognosis, with high BCL2 concentrations predicting a better prognosis (Lorente et al., 2021). Activator protein 1, consisting of Jun-Jun dimer or Jun-Fos dimer, regulated the transcription of multiple genes required for biological processes and the development and progression of many tumors, including COAD (Ashida et al., 2005). As one of the complexes of activator protein 1, c-Jun was involved in the crosstalk, integration, and amplification of intercellular signaling pathways, which were involved in cellular activities including survival, apoptosis, proliferation, and tumorigenesis (Meng and Xia, 2011). MAPK signaling pathway controlled cell survival, senescence, and drug resistance by integrating extracellular signals (Braicu et al., 2019). MAPK1 was found to play an important role in the invasion and proliferation of COAD cell line SW480 (Yang et al., 2018). The severe inflammatory response that occurred in severe COVID-19 patients might be associated with activation of the p38 MAKP signaling pathway (Grimes and Grimes, 2020).

The results of bioinformatics analysis revealed that the mechanism of puerarin in COVID-19/COAD might be associated with NF-κB, MAPK, IL-17, TNF, and HIF-1 signaling pathway. Elevated pro-inflammatory cytokines, including IL-17 and TNF-α, were the main causes of disease deterioration in COVID-19 patients (Shibabaw, 2020). IL-17 was involved in the activation of multiple signaling pathways resulting to the emergence of multiple cytokines (e.g. TNF-α, IL-1β) and chemokines, which in turn led to cytokine storm (Ryzhakov et al., 2011; Veldhoen, 2017; Badawi, 2020). Inflammatory cytokines IL-17 and TNF-α upregulated PD-L1 expression in COAD cell line HCT116 through activation of NF-κB signaling pathways, leading to COAD progression (Wang et al., 2017). HIF-1 induced an adaptive response in tumor cells under localized hypoxic conditions in cancer patients and was associated with poor patient prognosis (Lu et al., 2016). Activation of HIF signaling pathway was related to tumor cell invasion and tumor cell drug resistance (Brocato et al., 2014). HIF-1α signaling pathway was involved in SARS-CoV-2 virus infection and subsequent inflammatory response, often leading to poor prognosis in COVID-19 patients (Tian et al., 2021).

CONCLUSION

In this study, a series of bioinformatics analysis were employed to explore that the potential treatment mechanisms of puerarin in COVID-19/COAD comorbidity. These mechanisms may be related to apoptosis, antiviral, antioxidant, IL-17, TNF, and HIF-1 signaling pathway. The present study provides the preliminary basis and directions for the study of puerarin in COVID-19/COAD. However, as the study is based on transcriptome sequencing data and multiple databases, experimental validation is still lacking. In the follow-up studies, we will use new coronavirus-infected immune cell lines to co-culture with COAD cell lines. The therapeutic effect of puerarin will be observed by simulating the *in vivo* condition of COVID-19/COAD patients.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/ **Supplementary Material**.

AUTHOR CONTRIBUTIONS

JX, HW, and WL developed the concept of the project. The data was collected and evaluated by WL, XL, and YY with the help of QM and XW. All authors reviewed and discussed the results and

contributed to the paper preparation. JX, HW, WL, and XL wrote the manuscript. All authors have read and approved the final manuscript.

FUNDING

This study was supportive by Hebei Funding Project for High-level Talents (A202101062); Training Project for Excellent Clinical Medical Talents of Hebei Provincial Department of Finance (361009); Hebei Provincial Health Commission Medical Science Research Key Project Program (20210954); Shenzhen Key Laboratory Foundation (ZDSYS20200811143757022); Project of Department of education of Guangdong province (No. 2021KQNCX074)

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.921517/full#supplementary-material

Supplementary Table S1 | Therapeutic target genes of Puerarin.

Supplementary Table S2 | COVID-19-related genes.

Supplementary Table S3 | COAD-related genes.

Supplementary Table S4 | Potential target genes of puerarin in the treatment of COVID-19/COAD.

Supplementary Table S5 | The results of PPI network.

Supplementary Table S6 | The results of GO enrichment analysis.

Supplementary Table S7 | The results of KEGG enrichment analysis.

REFERENCES

Ashida, R., Tominaga, K., Sasaki, E., Watanabe, T., Fujiwara, Y., Oshitani, N., et al. (2005). AP-1 and Colorectal Cancer. *Inflammopharmacology* 13 (1-3), 113–125. doi:10.1163/156856005774423935

Badawi, A. (2020). Hypercytokinemia and Pathogen-Host Interaction in COVID-19. *J. Inflamm. Res.* 13, 255–261. doi:10.2147/jir.S259096

Barrett, C. W., Short, S. P., and Williams, C. S. (2017). Selenoproteins and Oxidative Stress-Induced Inflammatory Tumorigenesis in the Gut. *Cell Mol. Life Sci.* 74 (4), 607–616. doi:10.1007/s00018-016-2339-2

Braicu, C., Buse, M., Busuioc, C., Drula, R., Gulei, D., Raduly, L., et al. (2019). A Comprehensive Review on MAPK: A Promising Therapeutic Target in Cancer. *Cancers (Basel)* 11 (10), 1618. doi:10.3390/cancers11101618

Brocato, J., Chervona, Y., and Costa, M. (2014). Molecular Responses to Hypoxia-Inducible Factor 1α and beyond. *Mol. Pharmacol.* 85 (5), 651–657. doi:10.1124/mol.113.089623

Brown, K. G. M., and Solomon, M. J. (2018). Progress and Future Direction in the Management of Advanced Colorectal Cancer. *Br. J. Surg.* 105 (6), 615–617. doi:10.1002/bjs.10759

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., et al. (2020). Epidemiological and Clinical Characteristics of 99 Cases of 2019 Novel Coronavirus Pneumonia in Wuhan, China: a Descriptive Study. *Lancet* 395 (10223), 507–513. doi:10.1016/s0140-6736(20)30211-7

Durinikova, E., Buzo, K., and Arena, S. (2021). Preclinical Models as Patients' Avatars for Precision Medicine in Colorectal Cancer: Past and Future Challenges. *J. Exp. Clin. Cancer Res.* 40 (1), 185. doi:10.1186/s13046-021-01981-z

Giacomelli, A., Pezzati, L., Conti, F., Bernacchia, D., Siano, M., Oreni, L., et al. (2020). Self-reported Olfactory and Taste Disorders in Patients With Severe Acute Respiratory Coronavirus 2 Infection: A Cross-Sectional Study. *Clin. Infect. Dis.* 71 (15), 889–890. doi:10.1093/cid/ciaa330

Grimes, J. M., and Grimes, K. V. (2020). p38 MAPK Inhibition: A Promising Therapeutic Approach for COVID-19. *J. Mol. Cell Cardiol.* 144, 63–65. doi:10.1016/j.yjmcc.2020.05.007

Hariharan, A., Hakeem, A. R., Radhakrishnan, S., Reddy, M. S., and Rela, M. (2021). The Role and Therapeutic Potential of NF-Kappa-B Pathway in Severe COVID-19 Patients. *Inflammopharmacology* 29 (1), 91–100. doi:10.1007/s10787-020-00773-9

Huang, K., Zhang, P., Zhang, Z., Youn, J. Y., Wang, C., Zhang, H., et al. (2021). Traditional Chinese Medicine (TCM) in the Treatment of COVID-19 and Other Viral Infections: Efficacies and Mechanisms. *Pharmacol. Ther.* 225, 107843. doi:10.1016/j.pharmthera.2021.107843

Jayaweera, M., Perera, H., Gunawardana, B., and Manatunge, J. (2020). Transmission of COVID-19 Virus by Droplets and Aerosols: A Critical Review on the Unresolved Dichotomy. *Environ. Res.* 188, 109819. doi:10.1016/j.envres.2020.109819

Kamboj, M., and Sepkowitz, K. A. (2009). Nosocomial Infections in Patients with Cancer. *Lancet Oncol.* 10 (6), 589–597. doi:10.1016/s1470-2045(09)70069-5

Lazarus, J. V., Ratzan, S. C., Palayew, A., Gostin, L. O., Larson, H. J., Rabin, K., et al. (2021). A Global Survey of Potential Acceptance of a COVID-19 Vaccine. *Nat. Med.* 27 (2), 225–228. doi:10.1038/s41591-020-1124-9

Leonardi, A. J., and Proenca, R. B. (2020). Akt-Fas to Quell Aberrant T Cell Differentiation and Apoptosis in Covid-19. *Front. Immunol.* 11, 600405. doi:10.3389/fimmu.2020.600405

Li, Q., Guan, X., Wu, P., Wang, X., Zhou, L., Tong, Y., et al. (2020). Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. *N. Engl. J. Med.* 382 (13), 1199–1207. doi:10.1056/NEJMoa2001316

Liang, W., Guan, W., Chen, R., Wang, W., Li, J., Xu, K., et al. (2020). Cancer Patients in SARS-CoV-2 Infection: a Nationwide Analysis in China. *Lancet Oncol.* 21 (3), 335–337. doi:10.1016/s1470-2045(20)30096-6

Liu, W. (2021). Long Non-coding RNA VPS9D1-AS1 Promotes Growth of Colon Adenocarcinoma by Sponging miR-1301-3p and CLDN1. *Hum. Cell* 34 (6), 1775–1787. doi:10.1007/s13577-021-00604-1

Liu, X., Liu, C., Liu, G., Luo, W., and Xia, N. (2020). COVID-19: Progress in Diagnostics, Therapy and Vaccination. *Theranostics* 10 (17), 7821–7835. doi:10.7150/thno.47987

Lorente, L., Martín, M. M., González-Rivero, A. F., Pérez-Cejas, A., Argueso, M., Perez, A., et al. (2021). Blood Concentrations of Proapoptotic sFas and Antiapoptotic Bcl2 and COVID-19 Patient Mortality. *Expert Rev. Mol. Diagn* 21 (8), 837–844. doi:10.1080/14737159.2021.1941880

Lu, X., and Yarbrough, W. G. (2015). Negative Regulation of RelA Phosphorylation: Emerging Players and Their Roles in Cancer. *Cytokine Growth Factor Rev.* 26 (1), 7–13. doi:10.1016/j.cytogfr.2014.09.003

Lu, Y., Wang, B., Shi, Q., Wang, X., Wang, D., and Zhu, L. (2016). Brusatol Inhibits HIF-1 Signaling Pathway and Suppresses Glucose Uptake under Hypoxic Conditions in HCT116 Cells. *Sci. Rep.* 6, 39123. doi:10.1038/srep39123

Meng, Q., and Xia, Y. (2011). c-Jun, at the Crossroad of the Signaling Network. *Protein Cell* 2 (11), 889–898. doi:10.1007/s13238-011-1113-3

Mutter, F. E., Park, B. K., and Copple, I. M. (2015). Value of Monitoring Nrf2 Activity for the Detection of Chemical and Oxidative Stress. *Biochem. Soc. Trans.* 43 (4), 657–662. doi:10.1042/bst20150044

Ren, J.-l., Zhang, A.-H., and Wang, X.-J. (2020). Traditional Chinese Medicine for COVID-19 Treatment. *Pharmacol. Res.* 155, 104743. doi:10.1016/j.phrs.2020.104743

Reuter, S., Gupta, S. C., Chaturvedi, M. M., and Aggarwal, B. B. (2010). Oxidative Stress, Inflammation, and Cancer: How Are They Linked? *Free Radic. Biol. Med.* 49 (11), 1603–1616. doi:10.1016/j.freeradbiomed.2010.09.006

Ryzhakov, G., Lai, C. C., Blazek, K., To, K. W., Hussell, T., and Udalova, I. (2011). IL-17 Boosts Proinflammatory Outcome of Antiviral Response in Human Cells. *J. Immunol.* 187 (10), 5357–5362. doi:10.4049/jimmunol.1100917

Shibabaw, T. (2020). Inflammatory Cytokine: IL-17A Signaling Pathway in Patients Present with COVID-19 and Current Treatment Strategy. *J. Inflamm. Res.* 13, 673–680. doi:10.2147/jir.S278335

Siddiqui, W. A., Ahad, A., and Ahsan, H. (2015). The Mystery of BCL2 Family: Bcl-2 Proteins and Apoptosis: an Update. *Arch. Toxicol.* 89 (3), 289–317. doi:10.1007/s00204-014-1448-7

Siegel, R. L., Miller, K. D., Fuchs, H. E., and Jemal, A. (2022). Cancer Statistics, 2022. *CA Cancer J. Clin.* 72 (1), 7–33. doi:10.3322/caac.21708

Tian, M., Liu, W., Li, X., Zhao, P., Shereen, M. A., Zhu, C., et al. (2021). HIF-1α Promotes SARS-CoV-2 Infection and Aggravates Inflammatory Responses to COVID-19. *Signal Transduct. Target Ther.* 6 (1), 308. doi:10.1038/s41392-021-00726-w

Veldhoen, M. (2017). Interleukin 17 Is a Chief Orchestrator of Immunity. *Nat. Immunol.* 18 (6), 612–621. doi:10.1038/ni.3742

Wang, H. X., Zeng, M. S., Ye, Y., Liu, J. Y., and Xu, P. P. (2021). Antiviral Activity of Puerarin as Potent Inhibitor of Influenza Virus Neuraminidase. *Phytother. Res.* 35 (1), 324–336. doi:10.1002/ptr.6803

Wang, S., Zhang, S., Wang, S., Gao, P., and Dai, L. (2020). A Comprehensive Review on Pueraria: Insights on its Chemistry and Medicinal Value. *Biomed. Pharmacother.* 131, 110734. doi:10.1016/j.biopha.2020.110734

Wang, X., Yang, L., Huang, F., Zhang, Q., Liu, S., Ma, L., et al. (2017). Inflammatory Cytokines IL-17 and TNF-α Up-Regulate PD-L1 Expression in Human Prostate and Colon Cancer Cells. *Immunol. Lett.* 184, 7–14. doi:10.1016/j.imlet.2017.02.006

Wang, Y., Ma, Y., Zheng, Y., Song, J., Yang, X., Bi, C., et al. (2013). *In Vitro* and *In Vivo* Anticancer Activity of a Novel Puerarin Nanosuspension against Colon Cancer, with High Efficacy and Low Toxicity. *Int. J. Pharm.* 441 (1-2), 728–735. doi:10.1016/j.ijpharm.2012.10.021

Wei, S. Y., Chen, Y., and Xu, X. Y. (2014). Progress on the Pharmacological Research of Puerarin: a Review. *Chin. J. Nat. Med.* 12 (6), 407–414. doi:10.1016/s1875-5364(14)60064-9

Wong, R. S. (2011). Apoptosis in Cancer: from Pathogenesis to Treatment. *J. Exp. Clin. Cancer Res.* 30 (1), 87. doi:10.1186/1756-9966-30-87

Xu, J., Zhao, S., Teng, T., Abdalla, A. E., Zhu, W., Xie, L., et al. (2020). Systematic Comparison of Two Animal-To-Human Transmitted Human Coronaviruses: SARS-CoV-2 and SARS-CoV. *Viruses* 12 (2), 244. doi:10.3390/v12020244

Yang, Y., Li, X. J., Li, P., and Guo, X. T. (2018). MicroRNA-145 Regulates the Proliferation, Migration and Invasion of Human Primary Colon Adenocarcinoma Cells by Targeting MAPK1. *Int. J. Mol. Med.* 42 (6), 3171–3180. doi:10.3892/ijmm.2018.3904

Zhang, X. Y., Lv, L., Zhou, Y. L., Xie, L. D., Xu, Q., Zou, X. F., et al. (2021). Efficacy and Safety of Xiyanping Injection in the Treatment of COVID-19: A Multicenter, Prospective, Open-Label and Randomized Controlled Trial. *Phytother. Res.* 35 (8), 4401–4410. doi:10.1002/ptr.7141

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Liang, Li, Yao, Meng, Wu, Wang and Xue. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers | Frontiers in Oncology

REVIEW
published: 30 May 2022
doi: 10.3389/fonc.2022.879848

The Interaction Between Epigenetic Changes, EMT, and Exosomes in Predicting Metastasis of Colorectal Cancers (CRC)

*Meiqi Yang, Mingjun Sun and Huijing Zhang**

Department of Endoscopy, The First Affiliated Hospital of China Medical University, Shenyang, China

Worldwide, colorectal cancer (CRC) ranks as the third most common malignancy, and the second most deadly with nearly one million attributable deaths in 2020. Metastatic disease is present in nearly 25% of newly diagnosed CRC, and despite advances in chemotherapy, less than 20% will remain alive at 5 years. Epigenetic change plays a key role in the epithelial-to-mesenchymal transition (EMT), which is a crucial phenotype for metastasis and mainly includes DNA methylation, non-coding RNAs (ncRNAs), and N^6-methyladenosine (m^6A) RNA, seemingly valuable biomarkers in CRCs. For ncRNAs, there exists a "molecular sponge effect" between long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), and microRNAs (miRNAs). The detection of exosomes is a novel method in CRC monitoring, especially for predicting metastasis. There is a close relationship between exosomes and EMT in CRCs. This review summarizes the close relationship between epigenetic changes and EMT in CRCs and emphasizes the crucial function of exosomes in regulating the EMT process.

Keywords: colorectal cancer (CRC), epithelial to mesenchymal transition (EMT), exosomes, DNA methylation, non-coding RNAs (ncRNAs), N^6-methyladenosine (m^6A) RNA, metastasis

OPEN ACCESS

Edited by:
Yongye Huang,
Northeastern University, China

Reviewed by:
Shang-Qian Xie,
Hainan University, China
Yi Qin,
Fudan University, China

***Correspondence:**
Huijing Zhang
zhj1223@hotmail.com

Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Oncology

Received: *20 February 2022*
Accepted: *01 April 2022*
Published: *30 May 2022*

Citation:
Yang M, Sun M and Zhang H (2022) The Interaction Between Epigenetic Changes, EMT, and Exosomes in Predicting Metastasis of Colorectal Cancers (CRC). Front. Oncol. 12:879848. doi: 10.3389/fonc.2022.879848

INTRODUCTION

In 2020, colorectal cancers (CRCs) have become significant public health problems worldwide, and their incidence has increased to twice in the top 10 cancers (1–3). Monitoring distant metastatic loci and recurrence *in situ* is the last and most crucial step for cancer treatment. Furthermore, it is the most common cause of concern and anxiety for patients with CRCs. Based on previous studies, the mortality of metastatic CRCs is much higher than that of primary CRCs, especially for liver metastasis. Metastatic cancers severely influence the 5-year survival rate and quality of life of patients (4). Therefore, it is pivotal to predict the metastasis and recurrence of CRCs in order to increase distal survival time and quality of life.

For CRC, epithelial–mesenchymal transition (EMT) is a vital phenotype in its metastasis. EMT is a reversible process that promotes tumor cells exfoliating into circulation and damaging the intercellular skeletal structure. These exfoliated tumor cells could reach distal cites and form metastatic loci. The transcription factors (TFs) *SNAI1*, *SNAI2*, *Zeb1*, *Zeb2*, and *Twist*, called EMT-TFs, and some non-coding RNAs (ncRNAs) can influence the EMT process to regulate the migration and infiltration of tumor cells (5, 6). EMT is a dynamic process that is affected by genetic, epigenetic, and immune environmental factors. N-cadherin, vimentin, matrix metalloproteinases (MMPs), E-cadherins, claudins, epithelial cell adhesion molecules (EpCAMs), and cytokeratins are common biomarkers for the detection of EMT or

its reverse process, mesenchymal–epithelial transition (MET). EMT is an essential phenotype in the study of tumor metastasis.

The liquid biopsy (LB) technology is a novel method used to detect the genesis, progress of recurrence, and metastasis in CRC patients. Circulating tumor cells (CTCs), circulating tumor DNAs (ctDNAs), and cell-free RNAs are detectable biomarkers in serum (7). In addition, the detection of exosomes in the urine, cerebrospinal fluid, and saliva is applied widely and clinically. Detecting genetic materials has more perspective as nano-scale biological vesicles that are stable, suitable for storage, and valuable as both biomarkers and therapeutic nano-drugs, exosomes likely have a brighter future than cells (8).

Exosomes are tiny vesicles derived from tumor cells that contain DNA, ncRNAs, epigenetic modulation, and metabolites. Especially in comparison to obtaining tissue biopsies, liquid biopsies, including the relatively noninvasive venipuncture, or sampling other bodily fluids is often safer and more convenient. According to previous studies, exosomes play a pivotal role in predicting cancer metastasis. Comparably, exosome detection is safer and more convenient in malignant cancers, especially for CRCs. LB has shown considerable accuracy in CRC testing. Its sensitivity and specificity can reach up to 60% and 90%, respectively (9, 10). Therefore, this review summarized the relationship between epigenetic changes and EMT in CRCs and emphasized the association between exosomes and EMT.

EPIGENETIC CHANGES AND THE EMT PROCESS IN CRC

The reverse EMT process is characterized by the downregulation of E-cadherin, desmoplakin, claudins, and β-catenin and the upregulation of N-cadherin, vimentin, fibronectin, and Snail1/Snail2 mediate the migration and invasive metastasis of cancer cells (11–13). Whether to promote or inhibit is a question that can be answered using qualified biomarkers in EMT and reflects their influence on tumor migration, infiltration, or more aggressive properties. This process may not be complete; partial EMT is a novel phenomenon that has been recently put forward and proven to be more related to a malignant phenotype. It provides a warning that paying more attention to the regulators during the EMT process is more critical than just the results.

The chromosomal instability (CIN), microsatellite instability (MSI), and the CpG island (CGI) methylator phenotype (CIMP) pathways are three principle pathways involved in the genesis and development of CRCs. CIMP is related to the hypermethylation of promoters or the upstream regulator regions that usually contain CGIs, reported to be related to the detection of CRCs (14, 15). The definition of CIMP depends on the number of methylated loci on these CGIs; these also decide the survival outcomes and long-term prognosis of CRCs (16–18). The CIMP pathway could predict metastasis and recurrence *via* the detection of epigenetic biomarkers, such as DNA methylation, overexpression of ncRNAs, and N^6-methyladenosine (m^6A) modification. These epigenetic changes are regulated by genes and can be inherited by the next generation, but they cannot be transmitted into proteins to form phenotypes. Their influence is so strong that they have gained wide attention (19, 20). Several signaling pathways such as the Wnt signaling pathway, Raf/MEK/ERK pathway, and the NF-κB pathway function in CRCs. Especially for the Wnt signaling pathway, canonical Wnt signaling is frequently activated in the tumorigenesis of CRCs, with loss of the adenomatous polyposis coli (APC) genes and the translocation of β-catenin into the nucleus (21–23).

Regarding epigenetic changes, 5-methylcytosine (5mC) methylation for DNA and m^6A modification for RNA are two epigenetic modifications affecting the transcription and expression of critical genes. In addition, ncRNA is another group that cannot be transcribed, but has high regulatory ability for coding genes. These two mechanisms can undoubtedly regulate the EMT process in CRC metastatic events.

DNA 5mC METHYLATION: A ROBUST PROGNOSTIC BIOMARKER IN CRC

DNA methylation occurs when a methyl group is transferred to the carbon-5′ position of the cytosine base (5-methylcytosine) concentrated in CGIs under the catalytic action of DNA methyltransferase enzymes (24). The hypermethylation of 5-mC can regulate not only the transcription elements but also alternative splicing, which can modulate the production of ncRNAs (25–27). The methylation of promoters could weaken the transcription activity into messenger RNA (mRNA) and affect the function of the translated proteins. The intensity of their influence depends on the methylated sites on CGIs, which is differentially sensitive to the distance between the CpG sites among groups (28, 29).

This review focuses on several common and proven effective DNA methylation indicators for CRCs, shown in **Table 1**. The secreted frizzled-related protein (SFRP) family is regarded as an antagonist of the Wnt signaling pathway for its similarity to frizzled (Fz) receptors with bidirectional function (41). SFRP1 and SFRP2 are expressed in stromal myofibroblasts and are downregulated from typical adjacent tumor (normal tissue adjacent to the tumor, NAT) tissues toward the tumor (30, 31, 42, 43). They are diagnostic and prognostic biomarkers and suppress the growth and metastasis of CRC tumor cells. SFRP4 and SFRP5 may also inhibit invasion in CRC, but the underlying mechanisms in regulating the signaling pathways are more complex by comparison (32, 33, 44). Additional studies focusing on the SFRPs family as it relates to CRC should be undertaken to clarify their overall role and potentially derive a novel therapeutic regimen. Different from that of SFRPs, *SDC2* methylation is a carcinogenic biomarker in CTCs tested in patients with CRC (45). Hua et al. conducted a study to prove that the upregulation of SDC2 promotes cell proliferation, migration, and invasion; inhibits apoptosis; and activates and promotes EMT through mitogen-activated protein kinase (MAPK) signaling pathways (34). Apart from that of SFRPs

TABLE 1 | Potential DNA methylation seen in colorectal cancers (CRCs).

DNA methylation	Expression		Function in CRCs	Reference
SFRP1	Down	Suppressor	Detection, prognosis, and metastasis	(30)
SFRP2	Down	Suppressor	Detection, prognosis, and metastasis	(31)
SFRP4	Up	Suppressor	Detection, prognosis, and metastasis	(32)
SFRP5	Up	Suppressor	Detection, prognosis, and metastasis	(33)
SDC2	Up	Carcinogenic	Detection, prognosis, and metastasis	(34)
HPP1	Up	Carcinogenic	Metastasis and pre-therapeutic outcomes	(35)
HLTF	Up	Carcinogenic	Metastasis and pre-therapeutic outcomes	(36, 37)
SEPT9	Up	Carcinogenic	Metastasis and pre-therapeutic outcomes	(37)
BCAT1	Up	Carcinogenic	Metastasis and pre-therapeutic outcomes	(38)
NPY	Up	Carcinogenic	Metastasis and pre-therapeutic outcomes	(39, 40)

Shown in the table are the usual DNA methylation markers for the prediction of metastasis in CRCs.

and *SDC2*, the methylation of *TAC1*, *SEPT9*, *HPP1*, *HLTF*, and *NPY* has also been proven to be related to the recurrence and metastasis of CRCs (39). Moreover, the methylation of two factors, hyperplastic polyposis protein 1 (*HPP1*) and helicase-like transcription factor (*HLTF*), has prognostic value for therapy and metastatic location in CRC patients (35, 36, 46, 47). Several DNA methylation have been applied clinically with high sensitivity and specificity. Overall, the panels of methylated *SEPT9* and *SDC2* (48, 49), *SEPT9* and *OSMR* (77.0%) (37), *SEPT9* and *OSMR* (37), *SEPT9* and *TAC1* and *HIC/CYCD2/VHL* (50), *APC/MGMT/RASSF2A/WIF1* (51), *SYNE1* and *FOXE1* (52), *WIF1/NPY/PENK* (53), *THBD* and *C9orf50* (54), and the combinations of *FAM123A/GLI3/PPP1R16B/SLIT3/TMEM90B* (55) and *MGMT/RASSF1A/SEPT9* (56) were reported to have diagnostic significance in early-stage CRCs and may be more promising than the use of single markers. The combinations of *BCAT1/IKZF1* and *BCAT1/IKZF1/IRF4* were exploited and were shown to perform better than individual markers (57, 58), with higher sensitivity (73.9%) and specificity (90.1%) and an area under the curve (AUC) of 0.82 (38, 59).

Methylated DNA analysis in exosomes has been reported for gastric cancer, prostate cancer, lymphoma, breast cancer, and gingivitis (60). Although an association between DNA methylation in vesicles and CRCs exists, no study on their relationship has been conducted. DNA methylation can regulate the expression of hypoxia-inducible factor (HIF), which can modulate the development and progression of hypoxic CRCs. It is a critical factor in the regulatory EMT process.

NCRNAS REGULATE THE EMT PROCESS IN CRC

NcRNAs are a class of small RNA fragments or circulars in the non-coding regions. Although an association between DNA methylation in vesicles and CRCs exists, no study on their relationship has been conducted. The ncRNAs in CTCs originate from three main pathways, namely, membrane-bound vesicles, apoptotic bodies, and RNA-binding proteins (RBPs), which have been elucidated in detail in a previous review (61). The ncRNAs can maintain highly stable in the bloodstream with the protection of exosomes or RBPs, such as argonaute-2 (AGO2) high-density lipoprotein (HDL), due to their small size and their ability to escape RNase-mediated degradation (62–66). NcRNAs include a number of RNA types, such as microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs).

MiRNAs were the first discovered ncRNAs that can regulate the functions of genes and proteins. The sequence of miRNAs is short and can form complementary pairs with mRNAs. Most miRNAs are inhibitory and can lead to the degradation of mRNAs, which decreases the expressions of target proteins. Many cytoplasmic miRNAs have been discovered that can regulate expression of N-cadherin, Vimentin, MMPs, E-cadherin, Claudin, and EpCAM, thereby influencing the direction of EMT through molecular regulation in targeted pathways (67–69). Go et al. provided proof that the maternally expressed gene 3 differentially methylated region (MEG3-DMR) can control liver metastasis by regulating miRNAs, such as miR-655-3p, in the 14q32 cluster in CRCs (70, 71). This confirms that circulating miRNAs have an endothelial cell and blood component origin (19, 63, 72). A molecular classification based on epigenetically regulated gene expression profiles was established by Wang et al. to provide a better understanding of the epigenetic mechanisms underlying CRC heterogeneity using The Cancer Genome Atlas (TCGA) dataset and validated across Gene Expression Omnibus (GEO) datasets (73–78).

The upregulation or the downregulation of miRNAs hinges on regulating genes and epigenetic modifications. This variability, the targets and resultant outcomes are exemplified by the following: miR192-2 promoter hypermethylation was shown to modulate tumor metastasis in CRC by regulating the expression of *SOX4* (80). MiR-31 has been reported to increase the sensitivity to 5-fluorouracil (5-FU) at an early stage (81). In the latest study, miR-31 has been proven to increase radiosensitivity in CRC cell lines through targeting *STK40* (80, 82–85).

LncRNAs can promote or suppress the metastasis of tumor cells *via* upregulating or downregulating the process of EMT. LncRNAs have RNA sequences longer than 200 nucleotides that are not translated into proteins (86), so they are more stable than miRNAs (87). Similar to miRNAs, detection of expression or methylation of lncRNAs in CRC has been reported (88). Several lncRNAs, such as *XIST*, *H19*, and *SPRY4-IT1*, are related to EB1, 2/E-cadherin, and vimentin and can be detected from CTCs in plasma (89–91). The lncRNA *H19* can promote CRC metastasis *via* inducing EMT (92). EMT promotes tumor cell growth, proliferation, and metastasis

(93). The upregulation of the lncRNA *TUG1* increases the risk of metastasis and further invasion in CRCs *via* activating the Wnt/β-catenin signaling pathway (94). The lncRNA *MALAT1* is a predictive biomarker for metastasis by regulating the epigenetic model (95). Urothelial carcinoma-associated 1 (*UCA1*), which is produced by cancer-associated fibroblasts (CAFs) and released into serum, is associated with the metastasis of CRCs. The upregulation of *UCA1* can change the expressions of the genes in EMT (96). The lncRNA *BANCR* is an oncogenic molecule that enhanced the aggressiveness of CRC metastasis (97). The TGF-β signaling pathway is a critical pathway in regulating cancer-associated expression. The lncRNA activated by TGF-β (*LNCRNA-ATB*) can suppress the expression of E-cadherin in EMT to promote CRC metastasis (98). The lncRNAs *HOTAIR* and *HOXA-AS2* in plasma correlate with the invasion of CRCs *via* EMT (99, 100). The role of lncRNAs in serum is shown in **Table 2**.

CircRNAs are novel derivatives from the alternative splicing of lncRNAs that have small ring structures (120, 121). Compared to miRNAs and lncRNAs, circRNAs have shown their fantastic advantage in forecasting the metastasis of CRCs. By analyzing the expression profiles of circRNAs in the six pairs of CRC tissues and adjacent normal tissues, Chen et al. found that about two in three circRNAs were upregulated and one in three circRNAs was downregulated (122). In CRC tissues, the recovery of some circRNAs hints at the possibility of metastasis and recurrence of CRCs. CircRNA-1662 is an oncogenic circRNA that promoted CRC cell invasion and migration through control of EMT, and was reported to be a robust predictor for cancer metastasis (122). CircCSPP1 can promote CRC metastasis by regulating *COL1A1*, which is composed of type I collagen (105). CircRNA_0001946, hsa_circRNA_102209, circPTK2, hsa_circ_0001178, circRNA_100876, circ-SIRT1, hsa_circRNA_002144, circRNA_101951, and circTBL1XR can be overexpressed in CRC to promote metastasis and proliferation (106, 109–111, 113, 114, 116–118). On the other hand, circ_0026344, circRNA-0074027, hsa_circ_0009361, and hsa_circ_0001666 are downregulated in CRC tissues and suppress the metastasis of tumor cells (107, 108, 112, 115, 119). The elevation of circPTK2 in CRC CTCs increases the possibility of tumor metastasis (110). The hsa_circ_0001821 (circ3823) from both CRC tissues and sera may lead to tumor cell infiltration by binding with miR-30c-5p and its target to regulate the expression of *TCF7* (123). The role of circRNAs in CRCs is shown in **Table 2**.

INTERACTIONS BETWEEN MIRNAS, LNCRNAS, AND CIRCRNAS—THE "SPONGE EFFECT"

There are reports that, at times, the outcomes of miRNA transcription and expression is less satisfactory than expected, which hints at the existence of miRNA inhibitors might be present in the tumor microenvironment of CRCs (124). Seitz et al. hypothesized this inhibitor as a "miRNA sponge" that competes with endogenous mRNAs for miRNAs (125). LncRNAs and circRNAs can regulate miRNAs from binding

TABLE 2 | Role of long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs) associated with epithelial–mesenchymal transition (EMT) in colorectal cancers (CRCs).

	Expression	Signaling pathway	Function in CRCs	Reference
		Long non-coding RNAs		
XIST	Up	ZEB1, 2/E-cadherin, vimentin	Promotes metastasis	(101)
H19	Up	ZEB1, 2/E-cadherin, vimentin	Promotes metastasis	(92)
SPRY4-IT1	Up	ZEB1, 2/E-cadherin, vimentin	Promotes metastasis	(91)
TUG1	Up	Wnt/β-catenin Signaling	Promotes metastasis	(102)
MALAT1	Up	Chromatin Remodeling and epigenetic modulation	Promotes metastasis	(103)
UCA1	Up	mTOR signaling	Promotes metastasis	(96)
BANCR	Up	MAPK/ERK	Promotes metastasis	(104)
LNCRNA-ATB	Up	Caspase signaling	Promotes metastasis	(98)
HOTAIR	Up	unclear	Promotes metastasis	(100)
HOXA-AS2	Up	unclear	Promotes metastasis	(99)
		Circular RNAs		
circCSPP1	Up	circCSPP1/miR-193a-5p/COL1A1 axis	Promotes metastasis	(105)
circRNA_0001946	Up	miR-135a-5p/EMT axis	Promotes metastasis	(106)
circ_0026344	Down	Wnt/β-catenin signaling	Inhibits metastasis	(107)
circRNA_0074027	Down	EMT signaling pathway	Inhibits metastasis	(108)
hsa_circRNA_102209	Up	Ras and Rab interactor 1 signaling	Promotes metastasis	(109)
circPTK2	Up	EMT signaling pathway	Promotes metastasis	(110)
hsa_circ_0001178	Up	ZEB1/miR-382/587/616 axis	Promotes metastasis	(111)
hsa_circ_0009361	Down	Wnt/β-catenin signaling	Inhibits metastasis	(112)
circRNA_100876	Up	EMT signaling pathway	Promotes metastasis	(113)
circ-SIRT1	Up	circ-SIRT1/EIF4A3/N-cadherin/vimentin pathway	Promotes metastasis	(114)
circRNA_104916	Down	EMT signaling pathway	Inhibits metastasis	(115)
hsa_circRNA_002144	Up	miR-615-5p/LARP1/mTOR pathway	Promotes metastasis	(116)
circRNA_101951	Up	KIF3A-mediated EMT pathway	Promotes metastasis	(117)
circTBL1XR1	Up	CircTBL1XR1/miR-424 axis	Promotes metastasis	(118)
hsa_circ_0001666	Down	miR-576-5p/PCDH10 axis	Inhibits metastasis	(119)

LncRNAs can regulate CRCs through modulating the EMT process, while circRNAs can regulate CRCs through modulating EMT in tumor tissues.

with the target genes by functioning as competing endogenous RNAs (ceRNAs), which can regulate the expressions of genes by competitively integrating with miRNAs (126). The "lncRNA/circRNAs/miRNA/mRNA/protein" pathway is also called the molecular sponge effect (126).

In contrast, miRNA modulation can regulate the expressions of lncRNAs as well. MiR-939-5p is a suppressive miRNA that can inhibit the invasion and metastasis of CRC cells by decreasing the level of *LIMK2*. LncRNA *LINC00460* reduced the inhibition of miR-939-5p on *LIMK2* and bound with miR-939-5p as a ceRNA (127). It is oncogenic through upregulation of annexin to promote the EMT process (128). The glycolysis-related lncRNA *MIR17HG* was found to competitively bind with miR-138-5p to increase glycolysis, which can promote CRC liver metastasis (129). The antisense lncRNA *MCM3AP-AS1* sponged miR-193a-5p through upregulating *SENP1* to promote the progression and migration of CRC (130). The lncRNA *SNHG7* sponges miR-216b to promote the proliferation and liver metastasis of colorectal cancer through upregulating *GALNT1* (131). In the cytoplasm, *LINC00460* is a type of lncRNA that can regulate the expression of high mobility group A1 (*HMGA1*) of m^6A modification under the catalytic function of methyltransferase 3 (*METTL3*) (132). The earliest found lncRNA, *H19*, functioned as a ceRNA that sequesters miR-138 and miR-200a and leads to the inhibition of vimentin and the protein expressions of *ZEB1* and *ZEB2* (90, 92). The regulatory pathway of lncRNAs on miRNAs is shown in **Figure 1**. Similarly, circRNAs can act as ceRNA sponges of miRNAs (133). Most circRNAs positively affect target genes and promote the metastasis of CRCs. A few circRNAs act as suppressors of tumor invasion and promotion. Circ_0026344, hsa_circ_0009361, and hsa_circ_0001666 are negative regulators of tumor proliferation and inhibit metastasis. The sponge mechanism of circRNAs on miRNAs is shown in **Figure 2**.

THE ROLE OF M6A METHYLATION OF RNAS IN EMT

M6A methylation is a novel and critical component of RNA modification related to the expressions of ncRNAs in epigenetics (134). M6A in RNA post-transcriptionally regulates mRNAs by affecting the splicing, export, stability, and translation of transcripts (135–138). Its methylation regulates miRNA synthesis, processing, and maturation, which are crucial in tumorigenesis and cancer progression (139). It has been reported that the m^6A modification could alter its local RNA structure at the terminal loop region of primary miRNAs (pri-miRNAs) to promote their processing through nuclear transcripts and alternative splicing by modulating RALY binding (140–142). It is promising that m^6A methylation can predict metastasis and the prognosis of therapy (143). The upregulation of *HMGA1* of m^6A modification in CRC cells enhances the probability of metastasis (132).

In total, 11 readers, 7 writers, and 2 erasers have already been found. The term "writers" often denotes methyltransferase as the beginning of m^6A methylation, and different methyltransferases form a complex to gain more powerful catalytic ability. The (143) methyltransferase complex (MTC) comprises the m^6A/METTL complex (MAC) and the m^6A/METTL-associated complex (MACOM). MAC consists of *METTL3* and *METTL14*, which can form stable heterodimers (144). Besides *METTL3* and *METTL14*, *WTAP* is another writer that can participate in the formation of complex to regulate m^6A. NcRNAs can affect the

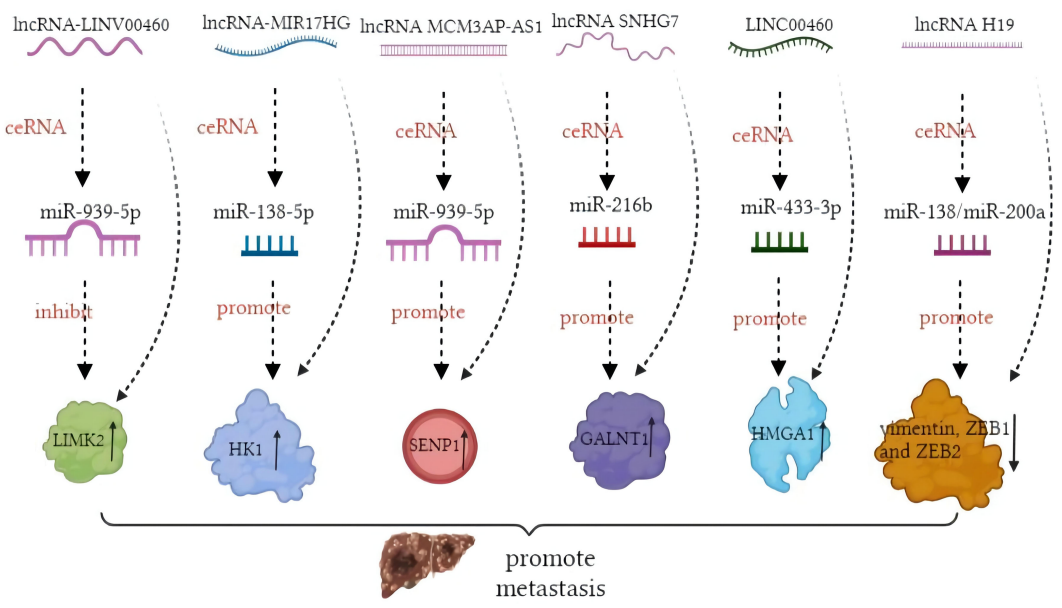


FIGURE 1 | Mechanism of long non-coding RNAs (lncRNAs) acting as competing endogenous RNAs (ceRNAs) in colorectal cancer (CRC) metastasis.

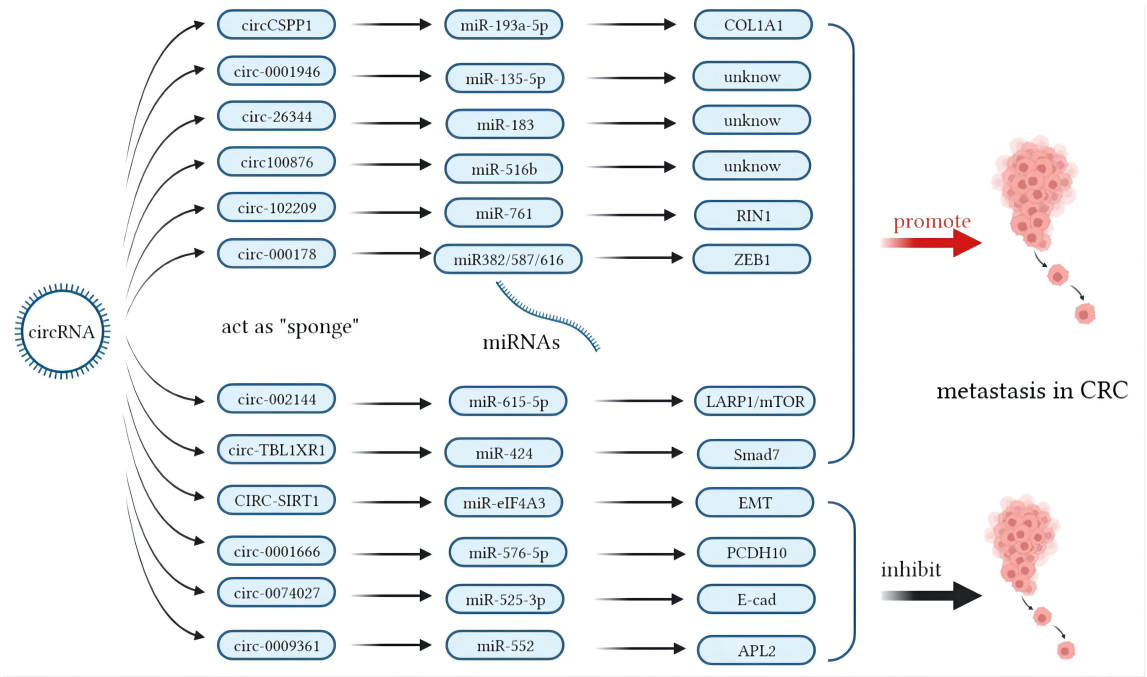


FIGURE 2 | Mechanisms of circular RNAs (circRNAs) acting as competing endogenous RNAs (ceRNAs) in colorectal cancer (CRC) metastasis.

process of m^{6}A by regulating the function of the "writer" complex. For example, *METTL3* is the first discovered RNA methylation-related mRNA with a catalytic subunit and is upregulated in metastasis to promote cell migration and invasion in CRCs by regulating miR-1246 (145). Contrarily, *METTL14* is regulated to suppress cell proliferation, invasion, and migration in CRCs *via* miR-375 (146–149). Furthermore, there exists mutual action between m6A modulation and ncRNAs. Prior studies demonstrated that knockdown of METTL3 downregulated the expression levels of miR-483, miR-676, miR-877 and circ1662-YAP1-SMAD3 axis in regulating CRC invasion (122, 142). The m^{6}A modification of circRNA_103783 (also called circNSUN2) could increase export into the cytoplasm in the circNSUN2/*IGF2BP2*/*HMGA2* ternary complex (150). In addition, the high expression of circNSUN2 predicts a more aggressive characteristic of CRC; thus, m^{6}A modification enhances the metastasis risk of CRC cells (150).

Beyond the methyltransferases, the "readers" that can recognize m^{6}A modulation sites, such as *YTHDF1*, *IGF2BP1*, *IGF2BP3*, and *EIF3B* (151), are also under the influence of ncRNAs. The tumor-suppressive miR-1266 promoted the occurrence and progression of CRCs by directly targeting *FTO* (152). Moreover, the expression of the lncRNA *GAS5* can reversely regulate *YAP1 via* m^{6}A (153). Surprisingly, the levels of m^{6}A can positively increase with the increased concentrations of circRNAs (154). The upregulation of the lncRNA *RP11* by m^{6}A methylation can accelerate the spread of CRC cells through the upregulation of *ZEB1* (155). Interestingly, it has been proven that m^{6}A circRNAs are different from m^{6}A mRNA sites. This indicates that disturbance in the m^{6}A modification of mRNA is not equivalent to that of m^{6}A circRNAs. Therefore, to abolish the effects of m^{6}A modification, then the m^{6}A of circRNAs must be considered as well (123, 154). Therefore, to abolish the effects of m^{6}A modification, then the m6A of circRNAs must be considered as well. NcRNAs can regulate the critical enzymes as the downstream target molecules or combined partners. Feedback of the essential enzymes affects the m^{6}A modulation of ncRNAs, and this process can change the cancer-related phenotype and lead to metastasis (155).

THE EXOSOMES AND EMT IN CRC

What is an exosome? Exosomes are microvesicles with diameters from 30 to 150 nm. They are derived from normal intestinal cells, CRC cells, and other cancer-related stromal cells (156). Exosomes have various physiological functions and participate in multiple cancer-related signaling pathways. Exosomes derived from CRCs cells are associated with a metastatic phenotype in CRCs, such as migration, infiltration, and EMT. For exosomes, surface antigens act as biomarkers that contribute to recognition, and their components determine the tasks exosomes carry out. Exosomes are secreted not only from cancer cells but also from normal intestinal cells or other tumor-related cells. Hepatocytes and fibroblasts can also produce exosomes to enter into the blood, urine, or saliva (157, 158). NcRNAs excreted from exosomes play an important role in regulating the signaling pathways in CRC metastasis (159). Studies that excluded the interference of miRNAs and lncRNAs

TABLE 3 | Functions of exosomes in colorectal cancer (CRC) metastasis *via* regulating the epithelial–mesenchymal transition (EMT) process.

Author	Source of exosomes	Components in exosomes	Target molecules	Regulated phenotype	Outcomes	Reference
J.L. Hu	Cancer-associated fibroblasts (CAFs)	miR-92a-3p	*FBXW7* and *MOAP1*	EMT	Metastasis	(160)
T. Liu	normal intestinal FHC cells	miR-128-3p	*BMI1* and *MRP5*	EMT	Metastasis and oxaliplatin resistance	(161)
Z. Liang	CRC cells	LncRNA RPPH1	*TUBB3* and macrophage M2 polarization	EMT	Metastasis	(162)
H. Xu	hepatocyte	miR-203a-3p	Src	EMT	Metastasis	(158)
D. Wang	CRC cells	miR-25-3p, miR-130b-3p, and miR-425-5p	PTEN through PI3K/Akt signaling pathway/macrophage M2 polarization	EMT	Metastasis	(164)
R. Rezaei	CRC cells	miRNA-375-3p	β-catenin, vimentin, *ZEB1*, and *SNAIL*	EMT	Metastasis	(165)
X. Zhang	CRC cells	miR-1255b-5p	Human telomerase reverse transcriptase (hTERT) and *BRG1*	EMT	Metastasis	(166)
T. Li	Mesenchymal stem cells	microRNA-3940-5p	Integrin α6 and TGF-β1	EMT	Metastasis	(157)
H. Liu	CRC cells	miR-106b-3p	DLC-1	EMT	Metastasis	(167)
L. Zhou	Cancer-associated fibroblasts (CAFs)	LncRNA LINC00659	miR-342-3p/*ANXA2* axis	Proliferation, invasion, migration, and EMT	Metastasis	(168)
Z. Xiao	CRC cells	miR-1915-3p	*PFKFB3* and *USP2*	EMT	Metastasis	(169)
F. Mannavola	CRC cells	miR-106b-3p	Deleted in liver cancer 1 (*DLC1*)	EMT	Metastasis	(170)
Y. Xu	CRC cells	circRNA FBXW7	miR-18b-5p	EMT and oxaliplatin resistance	Metastasis	(171)

in cells *via* the addition of anti-miRNAs and exogenous exosomes containing miRNAs confirmed that ncRNAs negatively regulated the downstream target proteins or mRNA molecules to promote tumor metastasis *via* EMT (160–162). In contrast, EMT cancer cells can promote exosome secretion *via* increasing the permeability of vascular epithelial layers, and miR-27b-3p can regulate this process through the STAT3 pathway (163). There is a mutual regulation between exosomes and the EMT process, but most studies have focused on the influence of exosome transportation in EMT, as shown in **Table 3**. While the ncRNAs in exosomes can negatively regulate the target genes and promote tumor metastasis, this also hints that they may be brilliant therapeutic targets for the prevention of metastasis. Besides CRC cells, tumor-associated macrophages (TAMs) comprise another class involved in the regulation of exosome and regulate the EMT process in inflammatory or immune diseases (164). The effects being positive or negative depend on the inflammatory cells and cytokines that TAMs secrete. The miRNAs or lncRNAs wrapped in vesicles could affect the M1 or M2 polarization of TAMs (172).

CONCLUSION

Cancer metastasis has always been a hot topic for scientists and clinicians, and discovering the risk factors and suitable therapeutic targets is a critical part of preventing metastasis. Epigenetic change is a popular context associated with the progression and metastasis of CRCs due to its variability and vulnerability. NcRNAs plays a crucial role in regulating epithelial tissues and the mesenchymal components. The dynamic process determines the tumor metastasis tendency. In this regulation, the sponge effect between lncRNAs/circRNAs/miRNAs/mRNA is critical. In addition, epigenetic modification is a crucial link. The 5mC methylation of DNA and the m^6A methylation of RNA in CRCs could enhance the invasive and migration ability to promote metastasis. The mechanisms of the mutual effect between methylation and ncRNAs may provide a novel direction for future studies.

The EMT is a vital regulator in metastasis, while the mechanism and influence factor of EMT is still unclear. As mentioned before, these epigenetic changes are not independent but interacting. The methylation of promoters can regulate the process of alternative splicing and production of ncRNAs. The review interprets a crosstalk interaction in epigenetic changes from DNA methylation, ncRNAs, and m6A methylation. The function of epigenetic changes is powerful and complex in regulating mechanisms in CRC metastasis. At the same time, EMT is a cross point that the DNA methylation, m6A methylation, and ncRNAs can all affect CRC metastasis. They can upregulate or downregulate the biomarkers in EMT such as N-cadherin, Vimentin, MMPs, E-cadherin, Claudin, and EpCAM to control the progress of the epithelial transition.

Another novelty of the review is that the exosomes can regulate EMT as a carrier for transporting ncRNAs. The function of exosomes has gained widespread attention in cancers. The exosomes have been proved to be related to cancers detection. This review summarizes the effect of exosomes in CRC metastasis related to EMT. That indicates that exosomes are a vital factor in the tumor micro-environment. The tumor cells or other stromal cells can secret exosomes to regulate EMT. And this review indicates that DNA methylation, ncRNAs, m6A methylation, exosomes, and EMT can all act as potential therapeutic targets in CRC metastasis. The associated relationship and mutual interaction among them are elucidated thoroughly in this review. While prior studies have been published elucidating the mechanisms of exosomes and EMT,

the field remains in its relative infancy, with the potential to discover important new therapeutic targets for CRC.

The detection of exosomes in LB is a novel method to predict metastasis in CRCs. The variety of stromal cells can produce exosomes to participate in the progression, proliferation, migration, and EMT in CRCs. Epigenetic changes, ncRNAs, and exosomes are not independent of each other; on the contrary, there exists an intrinsic interrelation between them. Different from previous studies, this review elaborated on the potential links between epigenetic changes, exosomes, and EMT in CRCs regarding metastasis. It provided novel sights for the study of probable mechanisms of metastasis in CRCs. There have already been several studies on the mechanisms of exosomes and EMT, but more studies are needed to discover new therapeutic targets for CRCs.

AUTHOR CONTRIBUTIONS

MY and HZ designed the study design and wrote the main manuscript. MS contributed to the final proof. All authors reviewed the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Conteduca V, Sansonno D, Russi S, Dammacco F. Precancerous Colorectal Lesions (Review). *Int J Oncol* (2013) 43(4):973–84. doi: 10.3892/ijo.2013.2041
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660
3. Barnell EK, Kang Y, Barnell AR, Kruse KR, Fiske J, Pittz ZR, et al. Multitarget Stool RNA Test for Noninvasive Detection of Colorectal Neoplasias in a Multicenter, Prospective, and Retrospective Cohort. *Clin Trans Gastroenterol* (2021) 12(5):e00360. doi: 10.14309/ctg.0000000000000360
4. Govaert KM, van Kessel CS, Steller EJ, Emmink BL, Molenaar IQ, Kranenburg O, et al. Recurrence Location After Resection of Colorectal Liver Metastases Influences Prognosis. *J Gastrointestinal Surg Off J Soc Surg Alimentary Tract* (2014) 18(5):952–60. doi: 10.1007/s11605-014-2461-0
5. Zhang N, Ng AS, Cai S, Li Q, Yang L, Kerr D. Novel Therapeutic Strategies: Targeting Epithelial–Mesenchymal Transition in Colorectal Cancer. *Lancet Oncol* (2021) 22(8):e358–68. doi: 10.1016/S1470-2045(21)00343-0
6. Rajamaki K, Taira A, Katainen R, Valimaki N, Kuosmanen A, Plaketti RM, et al. Genetic and Epigenetic Characteristics of Inflammatory Bowel Disease-Associated Colorectal Cancer. *Gastroenterology* (2021) 161 (2):592–607. doi: 10.1053/j.gastro.2021.04.042
7. Palanca-Ballester C, Rodriguez-Casanova A, Torres S, Calabuig-Farinas S, Exposito F, Serrano D, et al. Cancer Epigenetic Biomarkers in Liquid Biopsy for High Incidence Malignancies. *Cancers* (2021) 13(12). doi: 10.3390/cancers13123016
8. Barault L, Amatu A, Siravegna G, Ponzetti A, Moran S, Cassingena A, et al. Discovery of Methylated Circulating DNA Biomarkers for Comprehensive Non-Invasive Monitoring of Treatment Response in Metastatic Colorectal Cancer. *Gut* (2018) 67(11):1995–2005. doi: 10.1136/gutjnl-2016-313372
9. Marcuello M, Duran-Sanchon S, Moreno L, Lozano JJ, Bujanda L, Castells A, et al. Analysis of A 6-Mirna Signature in Serum From Colorectal Cancer Screening Participants as Non-Invasive Biomarkers for Advanced Adenoma and Colorectal Cancer Detection. *Cancers* (2019) 11(10). doi: 10.3390/cancers11101542
10. Xie W, Xie L, Song X. The Diagnostic Accuracy of Circulating Free DNA for the Detection of KRAS Mutation Status in Colorectal Cancer: A Meta-Analysis. *Cancer Med* (2019) 8(3):1218–31. doi: 10.1002/cam4.1989
11. Yeung KT, Yang J. Epithelial-Mesenchymal Transition in Tumor Metastasis. *Mol Oncol* (2017) 11(1):28–39. doi: 10.1002/1878-0261.12017
12. Gurzu S, Turdean S, Kovecsi A, Contac AO, Jung I. Epithelial-Mesenchymal, Mesenchymal-Epithelial, and Endothelial-Mesenchymal Transitions in Malignant Tumors: An Update. *World J Clin Cases* (2015) 3(5):393–404. doi: 10.12998/wjcc.v3.i5.393
13. Ye Z, Zhou M, Tian B, Wu B, Li J. Expression of lncRNA-CCAT1, E-Cadherin and N-Cadherin in Colorectal Cancer and Its Clinical Significance. (2015).
14. Luo1 H, Zhao Q, Wei1 W, Zheng L. Circulating Tumor DNA Methylation Profiles Enable Early Diagnosis, Prognosis Prediction, and Screening for Colorectal Cancer. *Sci Transl Med* (2020) 12:eaax7533. doi: 10.1126/scitranslmed.aax7533
15. Øster B, Thorsen K, Lamy P, Wojdacz TK, Hansen LL, Birkenkamp-Demtro K, et al. Identification and Validation of Highly Frequent CpG Island Hypermethylation in Colorectal Adenomas and Carcinomas. *Int J Cancer* (2011) 129:2855–66. doi: 10.1002/ijc.25951
16. Singh MP, Rai S, Suyal S, Singh SK, Singh NK, Agarwal A, et al. Genetic and Epigenetic Markers in Colorectal Cancer Screening: Recent Advances. *Expert Rev Mol Diagn* (2017) 17(7):665–85. doi: 10.1080/14737159.2017.1337511
17. Kang KJ, Min BH, Ryu KJ, Kim KM, Chang DK, Kim JJ, et al. The Role of the CpG Island Methylator Phenotype on Survival Outcome in Colon Cancer. *Gut Liver* (2015) 9(2):202–7. doi: 10.5009/gnl13352
18. Shiovitz S, Bertagnolli MM, Renfro LA, Nam E, Foster NR, Dzieciatkowski S, et al. CpG Island Methylator Phenotype Is Associated With Response to Adjuvant Irinotecan-Based Therapy for Stage III Colon Cancer. *Gastroenterology* (2014) 147(3):637–45. doi: 10.1053/j.gastro.2014.05.009
19. Kaur S, Lotsari-Salomaa JE, Seppanen-Kaijansinkko R, Peltomaki P. MicroRNA Methylation in Colorectal Cancer. *Adv Exp Med Biol* (2016) 937:109–22. doi: 10.1007/978-3-319-42059-2_6
20. Katada S, Imhof A, Sassone-Corsi P. Connecting Threads: Epigenetics and Metabolism. *Cell* (2012) 148(1-2):24–8. doi: 10.1016/j.cell.2012.01.001
21. Najdi R, Holcombe RF, Waterman ML. Wnt Signaling and Colon Carcinogenesis: Beyond APC. *J Carcinogenesis* (2011) 10:5. doi: 10.4103/1477-3163.78111
22. Amelio I, Bertolo R, Bove P, Buonomo OC, Candi E, Chiocchi M, et al. Liquid Biopsies and Cancer Omics. *Cell Death Discov* (2020) 6(1):131. doi: 10.1038/s41420-020-00373-0
23. Butz H, Nofech-Mozes R, Ding Q, Khella HWZ, Szabo PM, Jewett M, et al. Exosomal MicroRNAs Are Diagnostic Biomarkers and Can Mediate Cell-Cell Communication in Renal Cell Carcinoma. *Eur Urol Focus* (2016) 2 (2):210–8. doi: 10.1016/j.euf.2015.11.006
24. Holubekova V, Zubor P, Mendelova A. Epigenetic Regulation by DNA Methylation and miRNA Molecules in Cancer. (2017). doi: 10.2217/fon-2017-0363
25. Mazouji O, Ouhajjou A, Incitti R, Mansour H. Updates on Clinical Use of Liquid Biopsy in Colorectal Cancer Screening, Diagnosis, Follow-Up, and Treatment Guidance. *Front Cell Dev Biol* (2021) 9:660924. doi: 10.3389/fcell.2021.660924
26. Luo M, Yang X, Chen HN, Nice EC, Huang C. Drug Resistance in Colorectal Cancer: An Epigenetic Overview. *Biochim Biophys Acta Rev Cancer* (2021) 1876(2):188623. doi: 10.1016/j.bbcan.2021.188623
27. Ehrlich M. DNA Hypermethylation in Disease: Mechanisms and Clinical Relevance. *Epigenetics* (2019) 14(12):1141–63. doi: 10.1080/15592294.2019.1638701
28. Vinson C, Chatterjee R. CG Methylation. *Epigenomics* (2012) 4(6):655–63. doi: 10.2217/epi.12.55
29. Sancho E, Batlle E, Clevers H. Signaling Pathways in Intestinal Development and Cancer. *Annu Rev Cell Dev Biol* (2004) 20:695–723. doi: 10.1146/annurev.cellbio.20.010403.092805

30. Valcz G, Patai AV, Kalmar A, Peterfia B, Furi I, Wichmann B, et al. Myofibroblast-Derived SFRP1 as Potential Inhibitor of Colorectal Carcinoma Field Effect. *PloS One* (2014) 9(11):e106143. doi: 10.1371/journal.pone.0106143
31. Li H, Wang Z, Zhao G, Ma Y, Chen Y, Xue Q, et al. Performance of a MethyLight Assay for Methylated SFRP2 DNA Detection in Colorectal Cancer Tissue and Serum. *Int J Biol Markers* (2019) 34(1):54–9. doi: 10.1177/1724600818820536
32. Liu Y, Yu J, Xie Y, Li M, Wang F, Zhang J, et al. EZH2 Regulates Sfrp4 Expression Without Affecting the Methylation of Sfrp4 Promoter DNA in Colorectal Cancer Cell Lines. *Exp Ther Med* (2020) 20(5):33. doi: 10.3892/etm.2020.9160
33. Kirana C, Smith E. High Preoperative Levels of Circulating SFRP5 Predict Better Prognosis in Colorectal Cancer Patients. *Future Oncol* (2020) 16 (31):2499–509. doi: 10.2217/fon-2020-0356
34. Hua R, Yu J, Yan X, Ni Q, Zhi X, Li X, et al. Syndecan-2 in Colorectal Cancer Plays Oncogenic Role *via* Epithelial-Mesenchymal Transition and MAPK Pathway. *Biomed Pharmacother Biomed Pharmacother* (2020) 121:109630. doi: 10.1016/j.biopha.2019.109630
35. Herbst A, Vdovin N, Gacesa S, Ofner A, Philipp A, Nagel D, et al. Methylated Free-Circulating HPP1 DNA Is an Early Response Marker in Patients With Metastatic Colorectal Cancer. *Int J Cancer* (2017) 140 (9):2134–44. doi: 10.1002/ijc.30625
36. Helmer RA, Martinez-Zaguilan R, Kaur G, Smith LA, Dufour JM, Chilton BS. Helicase-Like Transcription Factor-Deletion From the Tumor Microenvironment in a Cell Line-Derived Xenograft Model of Colorectal Cancer Reprogrammed the Human Transcriptome-S-Nitroso-Proteome to Promote Inflammation and Redirect Metastasis. *PLoS One* (2021) 16: e0251132. doi: 10.17504/protocols.io.bs5xng7n
37. Yuan P, Cheng X, Wu X, Li L, Zhang L, Li Z, et al. OSMR and SEPT9: Promising Biomarkers for Detection of Colorectal Cancer Based on Blood-Based Tests. *Trans Cancer Res* (2016) 5(2):131–9. doi: 10.21037/tcr.2016.03.07
38. Young GP, Symonds EL, Nielsen HJ, Ferm L, Christensen IJ, Dekker E, et al. Evaluation of a Panel of Tumor-Specific Differentially-Methylated DNA Regions in IRF4, IKZF1 and BCAT1 for Blood-Based Detection of Colorectal Cancer. *Clin Epigenet* (2021) 13(1):14. doi: 10.1186/s13148-020-00999-y
39. Jensen LH, Olesen R, Petersen LN, Boysen AK, Andersen RF, Lindebjerg J, et al. NPY Gene Methylation as a Universal, Longitudinal Plasma Marker for Evaluating the Clinical Benefit From Last-Line Treatment With Regorafenib in Metastatic Colorectal Cancer. *Cancers* (2019) 11(11). doi: 10.3390/cancers11111649
40. Overs A, Flammang M, Hervouet E, Bermont L, Pretet JL, Christophe B, et al. The Detection of Specific Hypermethylated WIF1 and NPY Genes in Circulating DNA by Crystal Digital PCR Is a Powerful New Tool for Colorectal Cancer Diagnosis and Screening. *BMC Cancer* (2021) 21 (1):1092. doi: 10.1186/s12885-021-08816-2
41. Cruciat CM, Niehrs C. Secreted and Transmembrane Wnt Inhibitors and Activators. *Cold Spring Harbor Perspect Biol* (2013) 5(3):a015081. doi: 10.1101/cshperspect.a015081
42. Silva AL, Dawson SN, Arends MJ, Guttula K, Hall N, Cameron EA, et al. Boosting Wnt Activity During Colorectal Cancer Progression Through Selective Hypermethylation of Wnt Signaling Antagonists. *BMC Cancer* (2014) 14:891. doi: 10.1186/1471-2407-14-891
43. Bartak BK, Kalmar A, Peterfia B, Patai AV, Galamb O, Valcz G, et al. Colorectal Adenoma and Cancer Detection Based on Altered Methylation Pattern of SFRP1, SFRP2, SDC2, and PRIMA1 in Plasma Samples. *Epigenetics* (2017) 12(9):751–63. doi: 10.1080/15592294.2017.1356957
44. Huang D, Yu B, Deng Y, Sheng W, Peng Z, Qin W, et al. SFRP4 was Overexpressed in Colorectal Carcinoma. *J Cancer Res Clin Oncol* (2010) 136 (3):395–401. doi: 10.1007/s00432-009-0669-2
45. Molnar B, Galamb O, Kalmar A, Bartak BK, Nagy ZB, Toth K, et al. Circulating Cell-Free Nucleic Acids as Biomarkers in Colorectal Cancer Screening and Diagnosis - An Update. *Expert Rev Mol Diagn* (2019) 19 (6):477–98. doi: 10.1080/14737159.2019.1613891
46. Philipp AB, Nagel D, Stieber P, Lamerz R, Thalhammer I, Herbst A, et al. Circulating Cell-Free Methylated DNA and Lactate Dehydrogenase Release in Colorectal Cancer. (2014). doi: 10.1186/1471-2407-14-245
47. Ebert MP, Mooney SH, Tonnes-Priddy L, Lograsso J, Hoffmann J, Chen J, et al. Hypermethylation of the TPEF/HPP1 Gene in Primary and Metastatic Colorectal Cancers. *Neoplasia* (2005) 7(8):771–8. doi: 10.1593/neo.05235
48. Zhao G, Li H, Yang Z, Wang Z, Xu M, Xiong S, et al. Multiplex Methylated DNA Testing in Plasma With High Sensitivity and Specificity for Colorectal Cancer Screening. *Cancer Med* (2019) 8(12):5619–28. doi: 10.1002/cam4.2475
49. Chen Y, Wang Z, Zhao G, Sun C, Ma Y, Zhang L, et al. Performance of a Novel Blood-Based Early Colorectal Cancer Screening Assay in Remaining Serum After the Blood Biochemical Test. *Dis Markers* (2019) 2019:5232780. doi: 10.1155/2019/5232780
50. Melson J, Li Y, Cassinotti E, Melnikov A, Boni L, Ai J, et al. Commonality and Differences of Methylation Signatures in the Plasma of Patients With Pancreatic Cancer and Colorectal Cancer. *Int J Cancer* (2014) 134(11):2656–62. doi: 10.1002/ijc.28593
51. Lee BB, Lee EJ, Jung EH, Chun HK, Chang DK, Song SY, et al. Aberrant Methylation of APC, MGMT, RASSF2A, and Wif-1 Genes in Plasma as a Biomarker for Early Detection of Colorectal Cancer. *Clin Cancer Res Off J Am Assoc Cancer Res* (2009) 15(19):6185–91. doi: 10.1158/1078-0432.CCR-09-0111
52. Melotte V, Yi JM, Lentjes MH, Smits KM, Van Neste L, Niessen HE, et al. Spectrin Repeat Containing Nuclear Envelope 1 and Forkhead Box Protein E1 Are Promising Markers for the Detection of Colorectal Cancer in Blood. *Cancer Prev Res* (2015) 8(2):157–64. doi: 10.1158/1940-6207.CAPR-14-0198
53. Roperch J-P, Incitti R, Forbin S, Bard F, Mansour H, Mesli F, et al. Aberrant Methylation of NPY, PENK, and WIF1 as a Promising Marker for Blood-Based Diagnosis of Colorectal Cancer. *BMC Cancer* (2013) 13(1). doi: 10.1186/1471-2407-13-566
54. Lange CP, Campan M, Hinoue T, Schmitz RF, van der Meulen-de Jong AE, Slingerland H, et al. Genome-Scale Discovery of DNA-Methylation Biomarkers for Blood-Based Detection of Colorectal Cancer. *PloS One* (2012) 7(11):e50266. doi: 10.1371/journal.pone.0050266
55. Cho NY, Park JW, Wen X, Shin YJ, Kang JK, Song SH, et al. Blood-Based Detection of Colorectal Cancer Using Cancer-Specific DNA Methylation Markers. *Diagnostics* (2020) 11(1). doi: 10.3390/diagnostics11010051
56. Freitas M, Ferreira F, Carvalho S, Silva F, Lopes P, Antunes L, et al. A Novel DNA Methylation Panel Accurately Detects Colorectal Cancer Independently of Molecular Pathway. *J Trans Med* (2018) 16(1):45. doi: 10.1186/s12967-018-1415-9
57. Jedi M, Young GP, Pedersen SK, Symonds EL. Methylation and Gene Expression of BCAT1 and IKZF1 in Colorectal Cancer Tissues. *Clin Med Insights Oncol* (2018) 12:1179554918775064. doi: 10.1177/1179554918775064
58. Javierre BM, Rodriguez-Ubreva J, Al-Shahrour F, Corominas M, Graña O, Ciudad L, et al. Long-Range Epigenetic Silencing Associates With Deregulation of Ikaros Targets in Colorectal Cancer Cells. *Mol Cancer Res* (2011) 9(8):1139–51. doi: 10.1158/1541-7786.MCR-10-0515
59. Pedersen SK, Baker RT, McEvoy A, Murray DH, Thomas M, Molloy PL, et al. A Two-Gene Blood Test for Methylated DNA Sensitive for Colorectal Cancer. *PloS One* (2015) 10(4):e0125041. doi: 10.1371/journal.pone.0125041
60. Hur JY, Lee KY. Characteristics and Clinical Application of Extracellular Vesicle-Derived DNA. *Cancers* (2021) 13(15). doi: 10.3390/cancers13153827
61. Wang JJ, Wang X, Song YX, Zhao JH, Sun JX, Shi JX, et al. Circulating Noncoding RNAs Have a Promising Future Acting as Novel Biomarkers for Colorectal Cancer. *Dis Markers* (2019) 2019:2587109. doi: 10.1155/2019/2587109
62. Fernandez-Lazaro D, Garcia Hernandez JL, Garcia AC, Cordova Martinez A, Mielgo-Ayuso J, Cruz-Hernandez JJ. Liquid Biopsy as Novel Tool in Precision Medicine: Origins, Properties, Identification and Clinical Perspective of Cancer's Biomarkers. *Diagnostics* (2020) 10(4). doi: 10.3390/diagnostics10040215
63. Shigeyasu K, Toden S, Zumwalt TJ, Okugawa Y, Goel A. Emerging Role of MicroRNAs as Liquid Biopsy Biomarkers in Gastrointestinal Cancers. *Clin Cancer Res Off J Am Assoc Cancer Res* (2017) 23(10):2391–9. doi: 10.1158/1078-0432.CCR-16-1676
64. Cortez MA, Bueso-Ramos C, Ferdin J, Lopez-Berestein G, Sood AK, Calin GA. MicroRNAs in Body Fluids–the Mix of Hormones and Biomarkers. *Nat Rev Clin Oncol* (2011) 8(8):467–77. doi: 10.1038/nrclinonc.2011.76

65. Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, et al. Argonaute2 Complexes Carry a Population of Circulating microRNAs Independent of Vesicles in Human Plasma. *Proc Natl Acad Sci USA* (2011) 108(12):5003–8. doi: 10.1073/pnas.1019055108
66. Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs Are Transported in Plasma and Delivered to Recipient Cells by High-Density Lipoproteins. *Nat Cell Biol* (2011) 13(4):423–33. doi: 10.1038/ncb2210
67. Di Palo A, Siniscalchi C, Polito R, Nigro E, Russo A, Daniele A, et al. microRNA-377-3p Downregulates the Oncosuppressor T-Cadherin in Colorectal Adenocarcinoma Cells. *Cell Biol Int* (2021) 45(8):1797–803. doi: 10.1002/cbin.11605
68. Ye M, Huang T, Li J, Zhou C, Yang P, Ni C, et al. Role of CDH13 Promoter Methylation in the Carcinogenesis, Progression, and Prognosis of Colorectal Cancer: A Systematic Meta-Analysis Under PRISMA Guidelines. *Medicine* (2017) 96(4):e5956. doi: 10.1097/MD.0000000000005956
69. Duan BS, Xie LF, Wang Y. Aberrant Methylation of T-Cadherin Can Be a Diagnostic Biomarker for Colorectal Cancer. *Cancer Genomics Proteomics* (2017) 14(4):277–84. doi: 10.21873/cgp.20038
70. Oshima G, Poli EC, Bolt MJ, Chlenski A, Forde M, Jutzy JMS, et al. DNA Methylation Controls Metastasis-Suppressive 14q32-Encoded miRNAs. *Cancer Res* (2019) 79(3):650–62. doi: 10.1158/0008-5472.CAN-18-0692
71. Oshima G, Guo N, He C, Stack ME, Poon C, Uppal A, et al. *In Vivo* Delivery and Therapeutic Effects of a MicroRNA on Colorectal Liver Metastases. *Mol Ther J Am Soc Gene Ther* (2017) 25(7):1588–95. doi: 10.1016/j.ymthe.2017.04.005
72. He L, Hannon GJ. MicroRNAs: Small RNAs With a Big Role in Gene Regulation. *Nat Rev Genet* (2004) 5(7):522–31. doi: 10.1038/nrg1379
73. Wang X, Liu J, Wang D, Feng M, Wu X. Epigenetically Regulated Gene Expression Profiles Reveal Four Molecular Subtypes With Prognostic and Therapeutic Implications in Colorectal Cancer. *Briefings Bioinf* (2021) 22(4). doi: 10.1093/bib/bbaa309
74. Laiho P, Kokko A, Vanharanta S, Salovaara R, Sammalkorpi H, Jarvinen H, et al. Serrated Carcinomas Form a Subclass of Colorectal Cancer With Distinct Molecular Basis. *Oncogene* (2007) 26(2):312–20. doi: 10.1038/sj.onc.1209778
75. Kurozumi A, Goto Y, Okato A, Seki N. DNA Methylation and Dysregulation of miRNA in Cancer. *Mol Oncol* (2017) 6:281–96. doi: 10.1007/978-3-319-59786-7_10
76. Suzuki H, Takatsuka S, Akashi H, Yamamoto E, Nojima M, Maruyama R, et al. Genome-Wide Profiling of Chromatin Signatures Reveals Epigenetic Regulation of MicroRNA Genes in Colorectal Cancer. *Cancer Res* (2011) 71(17):5646–58. doi: 10.1158/0008-5472.CAN-11-1076
77. Croce CM. Causes and Consequences of microRNA Dysregulation in Cancer. *Nat Rev Genet* (2009) 10(10):704–14. doi: 10.1038/nrg2634
78. Lei L, An G, Zhu Z, Liu S, Fu Y, Zeng X, et al. C8orf48 Inhibits the Tumorigenesis of Colorectal Cancer by Regulating the MAPK Signaling Pathway. *Life Sci* (2021) 266:118872. doi: 10.1016/j.lfs.2020.118872
79. Ma Y, Zhang P, Yang J, Liu Z, Yang Z, Qin H. Candidate microRNA Biomarkers in Human Colorectal Cancer: Systematic Review Profiling Studies and Experimental Validation. *Int J Cancer* (2012) 130(9):2077–87. doi: 10.1002/ijc.26232
80. Zhang W, Zhu Y, Zhou Y, Wang J, Jiang P, Xue L. miRNA-31 Increases Radiosensitivity Through Targeting STK40 in Colorectal Cancer Cells. *Asia-Pacific J Clin Oncol* (2021). doi: 10.1111/ajco.13602
81. Wang CJ, Stratmann J, Zhou ZG, Sun XF. Suppression of microRNA-31 Increases Sensitivity to 5-FU at an Early Stage, and Affects Cell Migration and Invasion in HCT-116 Colon Cancer Cells. *BMC Cancer* (2010) 10:616. doi: 10.1186/1471-2407-10-616
82. Pinho SS, Reis CA. Glycosylation in Cancer: Mechanisms and Clinical Implications. *Nat Rev Cancer* (2015) 15(9):540–55. doi: 10.1038/nrc3982
83. Pucci M, Malagolini N, Dall'Olio F. Glycosyltransferase B4GALNT2 as a Predictor of Good Prognosis in Colon Cancer: Lessons From Databases. *Int J Mol Sci* (2021) 22(9). doi: 10.3390/ijms22094331
84. Kalluri R. The Biology and Function of Exosomes in Cancer. *J Clin Invest* (2016) 126(4):1208–15. doi: 10.1172/JCI81135
85. Hur K, Toiyama Y, Okugawa Y, Ide S, Imaoka H, Boland CR, et al. Circulating microRNA-203 Predicts Prognosis and Metastasis in Human Colorectal Cancer. *Gut* (2017) 66(4):654–65. doi: 10.1136/gutjnl-2014-308737
86. Orom UA, Derrien T, Beringer M, Gumireddy K, Gardini A, Bussotti G, et al. Long Noncoding RNAs With Enhancer-Like Function in Human Cells. *Cell* (2010) 143(1):46–58. doi: 10.1016/j.cell.2010.09.001
87. Han J, Rong LF, Shi CB, Dong XG, Wang J, Wang BL, et al. Screening of Lymph Nodes Metastasis Associated lncRNAs in Colorectal Cancer Patients. *World J Gastroenterol* (2014) 20(25):8139–50. doi: 10.3748/wjg.v20.i25.8139
88. Ye B, Li F, Chen M, Weng Y, Qi C, Xie Y, et al. A Panel of Platelet-Associated Circulating Long Non-Coding RNAs as Potential Biomarkers for Colorectal Cancer. *Genomics* (2022) 114(1):31–7. doi: 10.1016/j.ygeno.2021.11.026
89. Chen DL, Chen LZ, Lu YX, Zhang DS, Zeng ZL, Pan ZZ, et al. Long Noncoding RNA XIST Expedites Metastasis and Modulates Epithelial-Mesenchymal Transition in Colorectal Cancer. *Cell Death Dis* (2017) 8(8):e3011. doi: 10.1038/cddis.2017.421
90. Chen SW, Zhu J, Ma J, Zhang JL, Zuo S, Chen GW, et al. Overexpression of Long Non-Coding RNA H19 is Associated With Unfavorable Prognosis in Patients With Colorectal Cancer and Increased Proliferation and Migration in Colon Cancer Cells. *Oncol Lett* (2017) 14(2):2446–52. doi: 10.3892/ol.2017.6390
91. Jin J, Chu Z, Ma P, Meng Y, Yang Y. Long non-Coding RNA SPRY4-IT1 Promotes Proliferation and Invasion by Acting as a ceRNA of miR-101-3p in Colorectal Cancer Cells. *Tumour Biol J Int Soc Oncodevelopmental Biol Med* (2017) 39(7):1010428317716250. doi: 10.1177/1010428317716250
92. Zhang Y, Huang W, Yuan Y, Li J, Wu J, Yu J, et al. Long non-Coding RNA H19 Promotes Colorectal Cancer Metastasis *via* Binding to Hnrnpa2b1. *J Exp Clin Cancer Res CR* (2020) 39(1):141. doi: 10.1186/s13046-020-01619-6
93. Yagi T, Imaeda K, K E, Koyama H, Joh T. Glucagon Promotes Colon Cancer Cell Growth *via* Regulating AMPK and MAPK Pathways. *Oncotarget* (2018) 9:10650–64. doi: 10.18632/oncotarget.24367
94. Yue B, Liu C, Sun H, Liu M, Song C, Cui R, et al. A Positive Feed-Forward Loop Between LncRNA-CYTOR and Wnt/beta-Catenin Signaling Promotes Metastasis of Colon Cancer. *Mol Ther J Am Soc Gene Ther* (2018) 26(5):1287–98. doi: 10.1016/j.ymthe.2018.02.024
95. Ji Q, Cai G, Liu X, Zhang Y, Wang Y, Zhou L, et al. MALAT1 Regulates the Transcriptional and Translational Levels of Proto-Oncogene RUNX2 in Colorectal Cancer Metastasis. *Cell Death Dis* (2019) 10(6):378. doi: 10.1038/s41419-019-1598-x
96. Jahangiri B, Khalaj-Kondori M, Asadollahi E, Sadeghizadeh M. Cancer-Associated Fibroblasts Enhance Cell Proliferation and Metastasis of Colorectal Cancer SW480 Cells by Provoking Long Noncoding RNA Uca1. *J Cell Commun Signal* (2019) 13(1):53–64. doi: 10.1007/s12079-018-0471-5
97. Guo Q, Zhao Y, Chen J, Hu J, Wang S, Zhang D, et al. BRAF-Activated Long Non-Coding RNA Contributes to Colorectal Cancer Migration by Inducing Epithelial-Mesenchymal Transition. *Oncol Lett* (2014) 8(2):869–75. doi: 10.3892/ol.2014.2154
98. Yue B, Qiu S, Zhao S, Liu C, Zhang D, Yu F, et al. LncRNA-ATB Mediated E-Cadherin Repression Promotes the Progression of Colon Cancer and Predicts Poor Prognosis. *J Gastroenterol Hepatol* (2016) 31(3):595–603. doi: 10.1111/jgh.13206
99. Tong G, Liu Y, Wu X, Wang S. Knockdown of HOXA-AS2 Suppresses Proliferation and Induces Apoptosis in Colorectal Cancer. (2017).
100. Wu XL, Lu RY, Wang LK, Wang YY, Dai YJ, Wang CY, et al. Long Noncoding RNA HOTAIR Silencing Inhibits Invasion and Proliferation of Human Colon Cancer LoVo Cells *Via* Regulating IGF2BP2. *J Cell Biochem* (2018).
101. Liu X, Cui L, Hua D. Long Noncoding RNA XIST Regulates miR-137-EZH2 Axis to Promote Tumor Metastasis in Colorectal Cancer. *Oncol Res* (2018) 27(1):99–106. doi: 10.3727/096504018X15195193936573
102. Shen X, Hu X, Mao J, Wu Y, Liu H, Shen J, et al. The Long Noncoding RNA TUG1 Is Required for TGF-Beta/TWIST1/EMT-Mediated Metastasis in Colorectal Cancer Cells. *Cell Death Dis* (2020) 11(1):65. doi: 10.1038/s41419-020-2254-1
103. Ji Q, Zhang L, Liu X, Zhou L, Wang W, Han Z, et al. Long non-Coding RNA MALAT1 Promotes Tumour Growth and Metastasis in Colorectal Cancer Through Binding to SFPQ and Releasing Oncogene PTBP2 From SFPQ/PTBP2 Complex. *Br J Cancer* (2014) 111(4):736–48. doi: 10.1038/bjc.2014.383

104. Shen X, Bai Y, Luo B, Zhou X. Upregulation of lncRNA BANCR Associated With the Lymph Node Metastasis and Poor Prognosis in Colorectal Cancer. *Biol Res* (2017) 50(1):32. doi: 10.1186/s40659-017-0136-5
105. Wang Q, Shi L, Shi K, Yuan B, Cao G, Kong C, et al. CircCSPP1 Functions as a ceRNA to Promote Colorectal Carcinoma Cell EMT and Liver Metastasis by Upregulating Col1a1. *Front Oncol* (2020) 10:850. doi: 10.3389/fonc.2020.00850
106. Deng Z, Li X, Wang H, Geng Y, Cai Y, Tang Y, et al. Dysregulation of CircRNA_0001946 Contributes to the Proliferation and Metastasis of Colorectal Cancer Cells by Targeting MicroRNA-135a-5p. *Front Genet* (2020) 11:357. doi: 10.3389/fgene.2020.00357
107. Shen T, Cheng X, Liu X, Xia C, Zhang H, Pan D, et al. Circ_0026344 Restrains Metastasis of Human Colorectal Cancer Cells *via* miR-183. *Artif Cells Nanomed Biotechnol* (2019) 47(1):4038–45. doi: 10.1080/21691401.2019.1669620
108. Xiong G, Zhang J, Zhang Y, Pang X, Wang B, Zhang Y. Circular RNA_0074027 Participates in Cell Proliferation, Apoptosis and Metastasis of Colorectal Cancer Cells Through Regulation of Mir5253p. *Mol Med Rep* (2021) 23(5). doi: 10.3892/mmr.2021.11963
109. Li C, Zhou H. Circular RNA Hsa_circRNA_102209 Promotes the Growth and Metastasis of Colorectal Cancer Through miR-761-Mediated Ras and Rab Interactor 1 Signaling. *Cancer Med* (2020) 9(18):6710–25. doi: 10.1002/cam4.3332
110. Yang H, Li X, Meng Q, Sun H, Wu S, Hu W, et al. CircPTK2 (Hsa_Circ_0005273) as a Novel Therapeutic Target for Metastatic Colorectal Cancer. *Mol Cancer* (2020) 19(1):13. doi: 10.1186/s12943-020-1139-3
111. Rena C, Zhanga Z, Wang S, Zhu W, Zheng P, Wang W. Circular RNA Hsa_Circ_0001178 Facilitates the Invasion and Metastasis of Colorectal Cancer Through Upregulating ZEB1 *via* Sponging Multiple miRNAs. *Biol Chem* (2019) 401:487–96. doi: 10.1515/hsz-2019-0350
112. Geng Y, Zheng X, Hu W, Wang Q, Xu Y, He W, et al. Hsa_circ_0009361 Acts as the Sponge of miR-582 to Suppress Colorectal Cancer Progression by Regulating APC2 Expression. *Clin Sci* (2019) 133(10):1197–213. doi: 10.1042/CS20190286
113. Zhang J, Wang H, Wu K, Zhan F, Zeng H. Dysregulated circRNA_100876 Contributes to Proliferation and Metastasis of Colorectal Cancer by Targeting microRNA-516b (miR-516b). *Cancer Biol Ther* (2020) 21(8):733–40. doi: 10.1080/15384047.2020.1776075
114. Wang X, Liu S, Xu B, Liu Y, Kong P, Li C, et al. Circ-SIRT1 Promotes Colorectal Cancer Proliferation and EMT by Recruiting and Binding to Eif4a3. *Analytical Cell Pathol* (2021) 2021:5739769. doi: 10.1155/2021/5739769
115. Min L, Wang H, Zeng Y. CircRNA_104916 Regulates Migration, Apoptosis and Epithelial-Mesenchymal Transition in Colon Cancer Cells. *Front Biosci (Landmark Ed)* (2019) 24:819–32. doi: 10.2741/4753
116. Wu M, Kong C, Cai M, Huang W, Chen Y, Wang B, et al. Hsa_circRNA_002144 Promotes Growth and Metastasis of Colorectal Cancer Through Regulating miR-615-5p/LARP1/mTOR Pathway. *Carcinogenesis* (2021) 42(4):601–10. doi: 10.1093/carcin/bgaa140
117. Li Y, Pei F, Cao M. CircRNA_101951 Promotes Migration and Invasion of Colorectal Cancer Cells by Regulating the KIF3A-Mediated EMT Pathway. *Exp Ther Med* (2020) 19(5):3355–61.
118. Li N. CircTBL1XR1/miR-424 Axis Regulates Smad7 to Promote the Proliferation and Metastasis of Colorectal Cancer. *J Gastrointestinal Oncol* (2020) 11(5):918–31. doi: 10.21037/jgo-20-395
119. Zhou J, Wang L, Sun Q, Chen R, Zhang C, Yang P, et al. *Et Al*: Hsa_circ_0001666 Suppresses the Progression of Colorectal Cancer Through the miR-576-5p/PCDH10 Axis. *Clin Trans Med* (2021) 11(11):e565. doi: 10.1002/ctm2.565
120. Wang Y, Wang Z. Efficient Backsplicing Produces Translatable Circular mRNAs. *Rna* (2015) 21(2):172–9. doi: 10.1261/rna.048272.114
121. Li X, Yang L, Chen L-L. The Biogenesis, Functions, and Challenges of Circular RNAs. *Mol Cell* (2018) 71(3):428–42. doi: 10.1016/j.molcel.2018.06.034
122. Chen C, Yuan W, Zhou Q, Shao B, Guo Y, Wang W, et al. N6-Methyladenosine-Induced Circ1662 Promotes Metastasis of Colorectal Cancer by Accelerating YAP1 Nuclear Localization. *Theranostics* (2021) 11(9):4298–315. doi: 10.7150/thno.51342
123. Guo Y, Guo Y, Chen C, Fan D, Wu X, Zhao L, et al. Circ3823 Contributes to Growth, Metastasis and Angiogenesis of Colorectal Cancer: Involvement of miR-30c-5p/TCF7 Axis. *Mol Cancer* (2021) 20(1):93. doi: 10.1186/s12943-021-01372-0
124. Selbach M, Schwanhausser B, Thierfelder N, Fang Z, Khanin R, Rajewsky N. Widespread Changes in Protein Synthesis Induced by microRNAs. *Nature* (2008) 455(7209):58–63. doi: 10.1038/nature07228
125. Seitz H. Redefining microRNA Targets. *Curr Biol CB* (2009) 19(10):870–3. doi: 10.1016/j.cub.2009.03.059
126. Thomson DW, Dinger ME. Endogenous microRNA Sponges: Evidence and Controversy. *Nat Rev Genet* (2016) 17(5):272–83. doi: 10.1038/nrg.2016.20
127. Zhang Y, Liu X, Li Q, Zhang Y. lncRNA LINC00460 Promoted Colorectal Cancer Cells Metastasis *via* miR-939-5p Sponging. *Cancer Manage Res* (2019) 11:1779–89. doi: 10.2147/CMAR.S192452
128. Hong W, Ying H, Lin F, Ding R, Wang W, Zhang M. lncRNA LINC00460 Silencing Represses EMT in Colon Cancer Through Downregulation of ANXA2 *via* Upregulating miR-433-3p. *Mol Ther Nucleic Acids* (2020) 19:1209–18. doi: 10.1016/j.omtn.2019.12.006
129. Zhao S, Guan B, Mi Y, Shi D, Wei P, Gu Y, et al. LncRNA MIR17HG Promotes Colorectal Cancer Liver Metastasis by Mediating a Glycolysis-Associated Positive Feedback Circuit. *Oncogene* (2021) 40(28):4709–24. doi: 10.1038/s41388-021-01859-6
130. Zhou M, Bian Z, Liu B, Zhang Y, Cao Y, Cui K, et al. Long Noncoding RNA MCM3AP-AS1 Enhances Cell Proliferation and Metastasis in Colorectal Cancer by Regulating miR-193a-5p/SENP1. *Cancer Med* (2021) 10(7):2470–81. doi: 10.1002/cam4.3830
131. Shan Y, Ma J, Pan Y, Hu J, Liu B, Jia L. LncRNA SNHG7 Sponges miR-216b to Promote Proliferation and Liver Metastasis of Colorectal Cancer Through Upregulating GALNT1. *Cell Death Dis* (2018) 9(7):722. doi: 10.1038/s41419-018-0759-7
132. Hou P, Meng S, Li M, Lin T, Chu S, Li Z, et al. LINC00460/DHX9/IGF2BP2 Complex Promotes Colorectal Cancer Proliferation and Metastasis by Mediating HMGA1 mRNA Stability Depending on M6a Modification. *J Exp Clin Cancer Res CR* (2021) 40(1):52. doi: 10.1186/s13046-021-01857-2
133. Thomas LF, Saetrom P. Circular RNAs Are Depleted of Polymorphisms at microRNA Binding Sites. *Bioinformatics* (2014) 30(16):2243–6. doi: 10.1093/bioinformatics/btu257
134. Lence T, Paolantoni C, Worpenberg L, Roignant JY. Mechanistic Insights Into M(6)A RNA Enzymes. *Biochim Biophys Acta Gene Regul Mech* (2019) 1862(3):222–9. doi: 10.1016/j.bbagrm.2018.10.014
135. Tang C, Klukovich R, Peng H, Wang Z, Yu T, Zhang Y, et al. ALKBH5-Dependent M6a Demethylation Controls Splicing and Stability of Long 3'-UTR mRNAs in Male Germ Cells. *Proc Natl Acad Sci USA* (2018) 115(2):E325–33. doi: 10.1073/pnas.1717794115
136. Zhou KI, Shi H, Lyu R, Wylder AC, Matuszek Z, Pan JN, et al. Regulation of Co-Transcriptional Pre-mRNA Splicing by M(6)A Through the Low-Complexity Protein hnRNPG. *Mol Cell* (2019) 76(1):70–81.e79. doi: 10.1016/j.molcel.2019.07.005
137. Zhang. YTHDC1 Mediates Nuclear Export of N6-Methyladenosine Methylated mRNAs. (2017). doi: 10.7554/eLife.31311
138. Huang H, Weng H, Sun W, Qin X, Shi H, Wu H, et al. Recognition of RNA N(6)-Methyladenosine by IGF2BP Proteins Enhances mRNA Stability and Translation. *Nat Cell Biol* (2018) 20(3):285–95. doi: 10.1038/s41556-018-0045-z
139. Yao L, Yin H, Hong M, Wang Y, Yu T, Teng Y, et al. RNA Methylation in Hematological Malignancies and Its Interactions With Other Epigenetic Modifications. *Leukemia* (2021) 35(5):1243–57. doi: 10.1038/s41375-021-01225-1
140. Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, et al. Topology of the Human and Mouse M6a RNA Methylomes Revealed by M6a-Seq. *Nature* (2012) 485(7397):201–6. doi: 10.1038/nature11112
141. Alarcon CR, Lee H, Goodarzi H, Halberg N, Tavazoie SF. N6-Methyladenosine Marks Primary microRNAs for Processing. *Nature* (2015) 519(7544):482–5. doi: 10.1038/nature14281
142. Sun L, Wan A, Zhou Z, Chen D, Liang H, Liu C, et al. RNA-Binding Protein RALY Reprogrammes Mitochondrial Metabolism *via* Mediating miRNA Processing in Colorectal Cancer. *Gut* (2021) 70(9):1698–712. doi: 10.1136/gutjnl-2020-320652
143. Li Y, Xiao J, Bai J, Tian Y, Qu Y, Chen X, et al. Molecular Characterization and Clinical Relevance of M(6)A Regulators Across 33 Cancer Types. *Mol Cancer* (2019) 18(1):137. doi: 10.1186/s12943-019-1066-3

144. Liu J, Yue Y, Han D, Wang X, Fu Y, Zhang L, et al. A METTL3-METTL14 Complex Mediates Mammalian Nuclear RNA N6-Adenosine Methylation. *Nat Chem Biol* (2014) 10(2):93–5. doi: 10.1038/nchembio.1432
145. Peng W, Li J, Chen R, Gu Q, Yang P, Qian W, et al. Upregulated METTL3 Promotes Metastasis of Colorectal Cancer *via* miR-1246/SPRED2/MAPK Signaling Pathway. *J Exp Clin Cancer Res CR* (2019) 38(1):393. doi: 10.1186/s13046-019-1408-4
146. Chen X, Xu M, Xu X, Zeng K, Liu X, Sun L, et al. METTL14 Suppresses CRC Progression *via* Regulating N6-Methyladenosine-Dependent Primary miR-375 Processing. *Mol Ther J Am Soc Gene Ther* (2020) 28(2):599–612. doi: 10.1016/j.ymthe.2019.11.016
147. Xu L, L M, Wang M, Yan D, Feng G, An G. The Expression of microRNA-375 in Plasma and Tissue Is Matched in Human Colorectal Cancer. (2014).
148. Jepsen RK, N G, Klarskov LL, Christensen IJ, Riis LB, Høgdall E. Intra-Tumor Heterogeneity of microRNA-92a,microRNA-375 and microRNA-424 in Colorectal Cancer. *Exp Mol Pathol* (2016) 100:125–31. doi: 10.1016/j.yexmp.2015.12.004
149. Zaharie F, Muresan MS, Petrushev B, Berce C, Gafencu GA, Selicean S, et al. Exosome-Carried microRNA-375 Inhibits Cell Progression and Dissemination *via* Bcl-2 Blocking in Colon Cancer. *J Gastrointestinal Liver Dis JGLD* (2015) 24(4):435–43. doi: 10.15403/jgld.2014.1121.244.375
150. Chen RX, Chen X, Xia LP, Zhang JX, Pan ZZ, Ma XD, et al. N(6)-Methyladenosine Modification of Circnsun2 Facilitates Cytoplasmic Export and Stabilizes HMGA2 to Promote Colorectal Liver Metastasis. *Nat Commun* (2019) 10(1):4695. doi: 10.1038/s41467-019-12651-2
151. Kuai D, Zhu S, Shi H, Yang R, Liu T, Liu H, et al. Aberrant Expression of M(6)A mRNA Methylation Regulators in Colorectal Adenoma and Adenocarcinoma. *Life Sci* (2021) 273:119258. doi: 10.1016/j.lfs.2021.119258
152. SHEN X-P, L X, LU H, ZHOU C-X, ZHANG J-K, YU Q. Low Expression of microRNA-1266 Promotes Colorectal Cancer Progression *via* Targeting FTO. *Eur Rev Med Pharmacol Sci* (2018) 22:8220–6. doi: 10.26355/eurrev_201812_16516
153. Ni W, Yao S, Zhou Y, Liu Y, Huang P, Zhou A, et al. Long Noncoding RNA GAS5 Inhibits Progression of Colorectal Cancer by Interacting With and Triggering YAP Phosphorylation and Degradation and Is Negatively Regulated by the M(6)A Reader YTHDF3. *Mol Cancer* (2019) 18(1):143. doi: 10.1186/s12943-019-1079-y
154. Zhou C, Molinie B, Daneshvar K, Pondick JV, Wang J, Van Wittenberghe N, et al. Genome-Wide Maps of M6a circRNAs Identify Widespread and Cell-Type-Specific Methylation Patterns That Are Distinct From mRNAs. *Cell Rep* (2017) 20(9):2262–76. doi: 10.1016/j.celrep.2017.08.027
155. Wu Y, Yang X, Chen Z, Tian L, Jiang G, Chen F, et al. M6a-Induced lncRNA RP11 Triggers the Dissemination of Colorectal Cancer Cells *Via* Upregulation Zeb1. *Mol Cancer* (2019) 18(1). doi: 10.1186/s12943-019-1014-2
156. Liu J, Ren L, Li S, Li W, Zheng X, Yang Y, et al. The Biology, Function, and Applications of Exosomes in Cancer. *Acta Pharm Sin B* (2021) 11(9):2783–97. doi: 10.1016/j.apsb.2021.01.001
157. Li T, Wan Y, Su Z, Li J, Han M, Zhou C. Mesenchymal Stem Cell-Derived Exosomal microRNA-3940-5p Inhibits Colorectal Cancer Metastasis by Targeting Integrin Alpha6. *Digestive Dis Sci* (2021) 66(6):1916–27. doi: 10.1007/s10620-020-06458-1
158. Xu H, Lan Q, Huang Y, Zhang Y, Zeng Y, Su P, et al. The Mechanisms of Colorectal Cancer Cell Mesenchymal-Epithelial Transition Induced by Hepatocyte Exosome-Derived miR-203a-3p. *BMC Cancer* (2021) 21(1):718. doi: 10.1186/s12885-021-08419-x
159. Tovar-Camargo OA, Toden S, Goel A. Exosomal microRNA Biomarkers: Emerging Frontiers in Colorectal and Other Human Cancers. *Expert Rev Mol Diagn* (2016) 16(5):553–67. doi: 10.1586/14737159.2016.1156535
160. Hu JL, Wang W, Lan XL, Zeng ZC, Liang YS, Yan YR, et al. CAFs Secreted Exosomes Promote Metastasis and Chemotherapy Resistance by Enhancing Cell Stemness and Epithelial-Mesenchymal Transition in Colorectal Cancer. *Mol Cancer* (2019) 18(1):91. doi: 10.1186/s12943-019-1019-x
161. Liu T, Zhang X, Du L, Wang Y, Liu X, Tian H, et al. Exosome-Transmitted miR-128-3p Increase Chemosensitivity of Oxaliplatin-Resistant Colorectal Cancer. *Mol Cancer* (2019) 18(1):43. doi: 10.1186/s12943-019-0981-7
162. Liang ZX, Liu HS, Wang FW, Xiong L, Zhou C, Hu T, et al. LncRNA RPPH1 Promotes Colorectal Cancer Metastasis by Interacting With TUBB3 and by Promoting Exosomes-Mediated Macrophage M2 Polarization. *Cell Death Dis* (2019) 10(11):829. doi: 10.1038/s41419-019-2077-0
163. Dou R, Liu K, Yang C, Zheng J, Shi D, Lin X, et al. EMT-Cancer Cells-Derived Exosomal miR-27b-3p Promotes Circulating Tumour Cells-Mediated Metastasis by Modulating Vascular Permeability in Colorectal Cancer. *Clin Trans Med* (2021) 11(12):e595. doi: 10.1002/ctm2.595
164. Wang D, Wang X, Si M, Yang J, Sun S, Wu H, et al. Exosome-Encapsulated miRNAs Contribute to CXCL12/CXCR4-Induced Liver Metastasis of Colorectal Cancer by Enhancing M2 Polarization of Macrophages. *Cancer Lett* (2020) 474:36–52. doi: 10.1016/j.canlet.2020.01.005
165. Rezaei R, Baghaei K, Amani D, Piccin A, Hashemi SM, Asadzadeh Aghdaei H, et al. Exosome-Mediated Delivery of Functionally Active miRNA-375-3p Mimic Regulate Epithelial Mesenchymal Transition (EMT) of Colon Cancer Cells. *Life Sci* (2021) 269:119035. doi: 10.1016/j.lfs.2021.119035
166. Zhang X, Bai J, Yin H, Long L, Zheng Z, Wang Q, et al. Exosomal miR-1255b-5p Targets Human Telomerase Reverse Transcriptase in Colorectal Cancer Cells to Suppress Epithelial-to-Mesenchymal Transition. *Mol Oncol* (2020) 14(10):2589–608. doi: 10.1002/1878-0261.12765
167. Liu H, Liu Y, Sun P, Leng K, Xu Y, Mei L, et al. Colorectal Cancer-Derived Exosomal miR-106b-3p Promotes Metastasis by Down-Regulating DLC-1 Expression. *Clin Sci* (2020) 134(4):419–34. doi: 10.1042/CS20191087
168. Zhou L, Li J, Tang Y, Yang M. Exosomal LncRNA LINC00659 Transferred From Cancer-Associated Fibroblasts Promotes Colorectal Cancer Cell Progression *via* miR-342-3p/ANXA2 Axis. *J Trans Med* (2021) 19(1):8. doi: 10.1186/s12967-020-02648-7
169. Xiao Z, Liu Y, Li Q, Liu Q, Liu Y, Luo Y, et al. EVs Delivery of miR-1915-3p Improves the Chemotherapeutic Efficacy of Oxaliplatin in Colorectal Cancer. *Cancer Chemother Pharmacol* (2021) 88(6):1021–31. doi: 10.1007/s00280-021-04348-5
170. Mannavola F, Pezzicoli G, Tucci M. DLC-1 Down-Regulation *via* Exosomal miR-106b-3p Exchange Promotes CRC Metastasis by the Epithelial-to-Mesenchymal Transition. *Clin Sci* (2020) 134(8):955–9. doi: 10.1042/CS20200181
171. Xu Y, Qiu A, Peng F, Tan X, Wang J, Gong X. Exosomal Transfer of Circular RNA FBXW7 Ameliorates the Chemoresistance to Oxaliplatin in Colorectal Cancer by Sponging miR-18b-5p. *Neoplasma* (2021) 68(1):108–18. doi: 10.4149/neo_2020_200417N414
172. Larionova I, Tuguzbaeva G, Ponomaryova A, Stakheyeva M, Cherdyntseva N, Pavlov V, et al. Tumor-Associated Macrophages in Human Breast, Colorectal, Lung, Ovarian and Prostate Cancers. *Front Oncol* (2020) 10:566511. doi: 10.3389/fonc.2020.566511

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Yang, Sun and Zhang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers | Frontiers in Pharmacology

REVIEW
published: 16 June 2022
doi: 10.3389/fphar.2022.933332

The Role of m6A RNA Methylation in Cancer: Implication for Nature Products Anti-Cancer Research

Na Song[1,2], Kai Cui[2], Ke Zhang[2], Jie Yang[2], Jia Liu[2], Zhuang Miao[2], Feiyue Zhao[2], Hongjing Meng[2], Lu Chen[2], Chong Chen[2], Yushan Li[3], Minglong Shao[4], Jinghang Zhang[1] and Haijun Wang[1,2]**

[1]Department of Pathology, Key Laboratory of Clinical Molecular Pathology, The First Affiliated Hospital of Xinxiang Medical University, Xinxiang, China, [2]Department of Pathology, Xinxiang Medical University, Xinxiang, China, [3]School of Public Health, Xinxiang Medical University, Xinxiang, China, [4]The Second Affiliated Hospital of Xinxiang Medical University, Xinxiang, China

OPEN ACCESS

Edited by:
Yue Hou,
Northeastern University, China

Reviewed by:
Kunqi Chen,
Fujian Medical University, China
Weili Miao,
Stanford University, United States
Jiangbo Wei,
The University of Chicago, United States
Divya Jha,
Icahn School of Medicine at Mount Sinai, United States

***Correspondence:**
Jinghang Zhang
jinghangzhang_xxmu@163.com
Haijun Wang
wnavy200299@163.com

Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

Received: 30 April 2022
Accepted: 27 May 2022
Published: 16 June 2022

Citation:
Song N, Cui K, Zhang K, Yang J, Liu J, Miao Z, Zhao F, Meng H, Chen L, Chen C, Li Y, Shao M, Zhang J and Wang H (2022) The Role of m6A RNA Methylation in Cancer: Implication for Nature Products Anti-Cancer Research. Front. Pharmacol. 13:933332. doi: 10.3389/fphar.2022.933332

N6-methyladenosine (m6A) RNA methylation is identified as the most common, abundant and reversible RNA epigenetic modification in messenger RNA (mRNA) and non-coding RNA, especially within eukaryotic messenger RNAs (mRNAs), which post-transcriptionally directs many important processes of RNA. It has also been demonstrated that m6A modification plays a pivotal role in the occurrence and development of tumors by regulating RNA splicing, localization, translation, stabilization and decay. Growing number of studies have indicated that natural products have outstanding anti-cancer effects of their unique advantages of high efficiency and minimal side effects. However, at present, there are very few research articles to study and explore the relationship between natural products and m6A RNA modification in tumorigenesis. m6A is dynamically deposited, removed, and recognized by m6A methyltransferases (METTL3/14, METTL16, WTAP, RBM15/15B, VIRMA, CBLL1, and ZC3H13, called as "writers"), demethylases (FTO and ALKBH5, called as "erasers"), and m6A-specific binding proteins (YTHDF1/2/3, YTHDC1/2, IGH2BP1/2/3, hnRNPs, eIF3, and FMR1, called as "readers"), respectively. In this review, we summarize the biological function of m6A modification, the role of m6A and the related signaling pathway in cancer, such as AKT, NF-kB, MAPK, ERK, Wnt/β-catenin, STAT, p53, Notch signaling pathway, and so on. Furthermore, we reviewed the current research on nature products in anti-tumor, and further to get a better understanding of the anti-tumor mechanism, thus provide an implication for nature products with anti-cancer research by regulating m6A modification in the future.

Keywords: m6A, RNA methylation, molecular function, signaling pathway, implication, nature products, anti-cancer

INTRODUCTION

In recent years, N^6-methyladenosine (m6A) RNA modification, as a new regulatory mechanism for controlling gene expression in eukaryotes, has attracted more and more attention and gradually become a hot topic in biological research. Reversible epigenetic modification of m6A RNA was found not only in messenger RNA (mRNA) but also in non-coding RNA (ncRNA). m6A modification affects the biological fate of target RNA molecules and plays an important role in almost biological activities, including the occurrence, development and outcome of cancer (Chen et al., 2019a; He et al., 2019; Sun et al., 2019; Wang et al., 2020e; Huang et al., 2020). Among the more than 100 types of

post-transcriptional epitranscriptomic modifications identified in RNAs thus far, m6A is the most abundant internal modification of eukaryotic RNAs, including mRNA, tRNA, rRNA, snRNA, microRNA (miRNA), and long non-coding RNA (lncRNA), contributing to RNA splicing, localization, translation, stabilization, and decay (Gokhale et al., 2016; Wang et al., 2018; Koh et al., 2019; Meyer, 2019; Zhang et al., 2019; Gong et al., 2020). In recent years, it has been found that the abnormal modification of m6A RNA is closely related to the occurrence and development of a variety of tumors (Chen et al., 2018; Jin et al., 2019; Shi et al., 2020; Wang et al., 2020a; Zhang et al., 2021a). Therefore, it is necessary to conduct in-depth studies on the relationship between m6A and tumor, especially to harness the key mechanisms involved in m6A modification for anti-cancer research (Niu et al., 2018; Gao et al., 2021).

Natural products are essential to modern medicine, such as curcumin, chrysin, cucurbitacin B, cinnamaldehyde, bruceine D, baicalein, epigallocatechin gallate, fucoidan, resveratrol, rhein, and so on. Many of which have been shown to effectively regulate the progression of cancer and enhance the anticancer effects of various anticancer drugs (Li et al., 2020a; Chen et al., 2020b; Chen et al., 2021b; Zhang et al., 2021b; Wang et al., 2021). In addition, 73 percent of antibiotics, 49 percent of anticancer compounds, and 32 percent of all new drugs approved by the U.S. Food and Drug Administration (FDA) between 1980 and 2012 were natural products or derivatives of them (Newman and Cragg, 2016; Harvey et al., 2018). These natural products, including compounds with antibiotic, antifungal, immunosuppressant, and anticancer activity, are a rich source of anticancer agents and provide new and more effective anticancer agents for therapeutic use. However, there are few reports that natural products inhibit tumor genesis and development by regulating m6A RNA modification (Qian et al., 2021b; Feng et al., 2022; Jiang et al., 2022).

In this review, we summarize the biological functions of m6A modification, the role of m6A in tumors, and the related signal pathways of m6A in tumors, such as Akt, NF-kB, MAPK, ERK, and Wnt/β-Catenin, STAT, p53, Notch signaling pathway, etc. Moreover, we also review the research progress of natural products in antitumor, and further understands its antitumor mechanism, so as to provide an implication for the anti-cancer research of natural products *via* regulating m6A modification in the future.

PART 1: OVERVIEW OF M6A RNA METHYLATION

m6A RNA Methyltransferases—Writers

The RNA m6A modification is deposited by the m6A methyltransferase complex (called as "writer"), which mainly consist of a methyltransferases like 3 and 14 (METTL3 and METTL14) heterodimeric enzymatic core, and other cofactor protein, such as Wilm's tumor-1-associated protein (WTAP), RNA binding motif protein 15 (RBM15) and its paralogue RBM15B, vir like m6A methyltransferase associated (VIRMA, originally known as KIAA1429, Virilizer), zinc finger CCCH type containing 13 (ZC3H13), and CBLL1 (Patil et al., 2016; Roundtree et al., 2017a; **Table 1**).

METTL3 and METTL14 are essential components of methyltransferase complex (MTC), in which METTL3 is catalytically active while METTL14 has critical structural scaffold functions, isolated METTL3 without METTL14 is not bioactive (Roundtree et al., 2017a; Hsu et al., 2017). WTAP, as the key METTL3 adaptor, directly interacts with METTL3 and recruits METTL3-METTL14 heterodimeric complex to nuclear speckles for m6A modification (Ping et al., 2014). Meanwhile, WTAP also binds to the m6A consensus RRACH (R = A/G, H = A/C/U) motif of RNA and recruits catalytic subunits METTL3 and METTL14 to the vicinity of the target RNA sequence (Ping et al., 2014). In addition, studies have shown that knockdown of WTAP reduces METTL3 and METTL14, while knockdown of METTL3 or METTL14 does not reduce or even increase WTAP, although a single knockdown of METTL3 or METTL14 reduces each other (Ping et al., 2014; Kobayashi et al., 2018). Moreover, WTAP also may scaffold the MTC and RBM15/RBM15B recruits the MTC to target sites of modification (Kan et al., 2017). METTL16 is another independent m6A methyltransferase that can directly methylate UACAGAGAA motifs. In addition, METTL16 can also regulate the activity of all cellular methyltransferases including METTL3/METTL14, so the deletion of METTLl6 can reduce the methylation level of UACAGAGAA and/or RRACH motif regions (Fazi and Fatica, 2019; Koh et al., 2019; Aoyama et al., 2020). Recent studies have shown that METTL5 as the enzyme responsible for 18s rRNA m6A modification in site of 1831A, which must form a METTL5-TRMT112 (TRMT112, a methyltransferase activator) heterodimeric complex to maintain its biological stability (Van Tran et al., 2019; Rong et al., 2020).

RNA m6A modification is enriched in the 3′untranslated region (3′UTR) and near termination codon of mature polyadenylate mRNA in mammalian system, and plays a regulatory role in the transcription of eukaryotic mRNA. VIRMA, an important WTAP interactor, is essential for the deposition of m6A to the 3′UTR of mRNA and near stop codons and recruiting the methyltransferase core components METTL3/METTL14/WTAP (Horiuchi et al., 2013; Yue et al., 2018; Shi et al., 2019; UniProt, 2019); Knockdown of VIRMA resulted in reduced m6A methylation near the 3′UTR of the stop codon. In addition, VIRMA binds to the polyadenylation cleavage factors CPSF5 and CPSF6 in an RNA-dependent manner, and knockdown of CPSF5 also resulted in a significant shortening of the 3′UTR of the mRNA (Yue et al., 2018).

ZC3H13 could promote nuclear localization of the writer complex for RNA m6A modification, ZCCHC4 as the 28s rRNA modification enzyme for m6A modification at the site of 4220A (Ren et al., 2019). RBM15/15B binds to U-rich regions and cooperates with MTC for certain RNAs methylation (Shi et al., 2019). CBLL1 (Cbl proto-oncogene like 1), also known as Hakai, was originally found to encode the E3 ubiquitin ligase for the E-cadherin complex and mediate its ubiquitination, endocytosis, and degradation in lysosomes. Recent studies have shown that CBLL1 is required for stabilization of core components METTL3/14 of the m6A RNA methylation and

TABLE 1 | Functions of m6A regulators in RNA metabolism.

Type	Regulators	Biology function
m6A writer	METLL3	Catalyzes m6A modification
	METLL14	Cooperates METLL3 to recognize the subtract
	METLL16	Catalyzes m6A modification
	WTAP	Promotes METLL3/14 heterodimer to the nuclear speckle
	VIRMA	Recruits the m6A complex to the special RNA site and interacts with polyadenylation cleavage factors CPSF5 and CPSF6
	ZC3H13	Promotes the nuclear localization of the m6A complex
	RBM 15	Binds RNA and recruits m6A complex to special RNA site
	CBLL1/Hakai	Core component for METTL3/14 stabilization
m6A eraser	ALKBH5	Removes m6A modification
	FTO	Removes m6A modification
m6A reader	YTHDC1	Contributes to RNA splicing, stabilization, export
	YTHDC2	Enhances mRNA translation and reduces the abundance of target RNA
	YTHDF1	Enhances mRNA translation
	YTHDF2	Promotes mRNA degradation
	YTHDF3	Cooperates YTHDF1/2 for RNA translation/degradation
	IGF2BPs	Enhances mRNA stability and storage
	eIF3	Enhances mRNA translation
	hnRNPA2B1	Mediates pri-miRNA processing and pre-mRNA splicing
	hnRNPC	Mediates pre-mRNA splicing

plays a role in the efficiency of mRNA splicing and RNA processing (Yue et al., 2018; Bawankar et al., 2021).

m6A RNA Demethylases—Erasers

The intracellular RNA m6A demethylation is mainly accomplished by fat mass and obesity-associated protein (FTO) and the alkylation repair homolog protein 5 (ALKBH5), referred to as erasers, which cooperates with m6A methylases to maintain the dynamic balance of m6A modification in the cells (Jia et al., 2011; Zheng et al., 2013; Xue et al., 2019).

As the first m6A mRNA demethylase, FTO established the concept of reversible RNA modification (Jiang et al., 2021b). The whole transcriptome RNA demethylation analysis clarified that FTO is a potent nuclear mRNA processing regulator, which preferentially binds to the pre-mRNA in the intron region, in the proximity of alternative splicing exons and poly(A) sites, and participates in alternative splicing and 3′ end mRNA processing (Bartosovic et al., 2017). In addition, multiple evidences indicate that FTO does play a key role in regulating fat mass, fat production and body weight (Merritt et al., 2018). Epidemiological studies also show that FTO SNPs is strongly associated with increased risk of various cancers (Chen and Du, 2019). FTO has recently been shown to play an m6A-dependent role in multiple biological processes, such as cancer cell apoptosis, proliferation, migration, invasion, metastasis, cell-cycle, differentiation, stem cell self-renewal and so on (Deng et al., 2018; Wang et al., 2020d). In addition, studies have shown that FTO binds multiple RNA species, including mRNA, snRNA, and tRNA, and can demethylate internal m6A and cap m6Am in mRNA, internal and cap m6Am in snRNA, internal m6A in U6 RNA, and N1-methyladenosine (m1A) in tRNA (Wei et al., 2018).

ALKBH5 is another major m6A demethylase which plays a critical biological and pharmacological role in human cancer. ALKBH5 is an independent prognostic indicator in a variety of cancers. ALKBH5 can regulate various tumor biological processes, such as the cell proliferation, invasion, migration, metastasis, cancer stem cell self-renewal and tumor microenvironment (Li et al., 2020b; Shen et al., 2020). In addition, ALKBH5 also plays an important role in human non-cancer diseases, such as reproductive system diseases (Nettersheim et al., 2019). The potential regulatory mechanism of ALKBH5 relies on m6A-dependent modifications (Wang et al., 2020c). Moreover, it was found that ALKBH5 knockout resulted in increased mRNA m6A modification level in the nucleus, but a decrease in the amount of mRNA in the cytoplasm, suggesting that ALKBH5 may be involved in mRNA transport (Bartosovic et al., 2017). In addition, neither FTO nor ALKBH5 display a preference for RRACH m6A motif demethylation (Zou et al., 2016).

m6A RNA Binding Proteins—Readers

The m6A modification plays an important role in the production, processing, splicing and translation of mRNA (Huang et al., 2021a). m6A binding protein (also called: reader) is essential for the recognition of m6A RNA, including m6A direct binding proteins (such as: YTH domain family, IGF2BPs, eIF3, etc) and m6A indirect binding proteins (hnRNP family, etc).

At present, it is considered that the YT521-B homology (YTH) domain family consists of YTH domain containing protein 1-2 (YTHDC1-2) and YTH domain family protein 1-3 (YTHDF1-3). YTHDC1 mediates the splicing of m6A pre-mRNA *via* recruiting splicing factor and nuclear export adaptor SRSF3 while blocking SRSF10 (Xiao et al., 2016), and NXF1 also involved the nuclear exporting of m6A-containing mRNAs (Roundtree et al., 2017b); ELAVL1 (ELAV like RNA binding protein 1, also known as HuR) is known to be an RNA stabilizing protein that interacts with mRNAs containing m6A modifications and cooperates with YTHDC1 to enhance the stability and subsequent translation of target gene mRNAs (Liang et al., 2021). The m6A reader

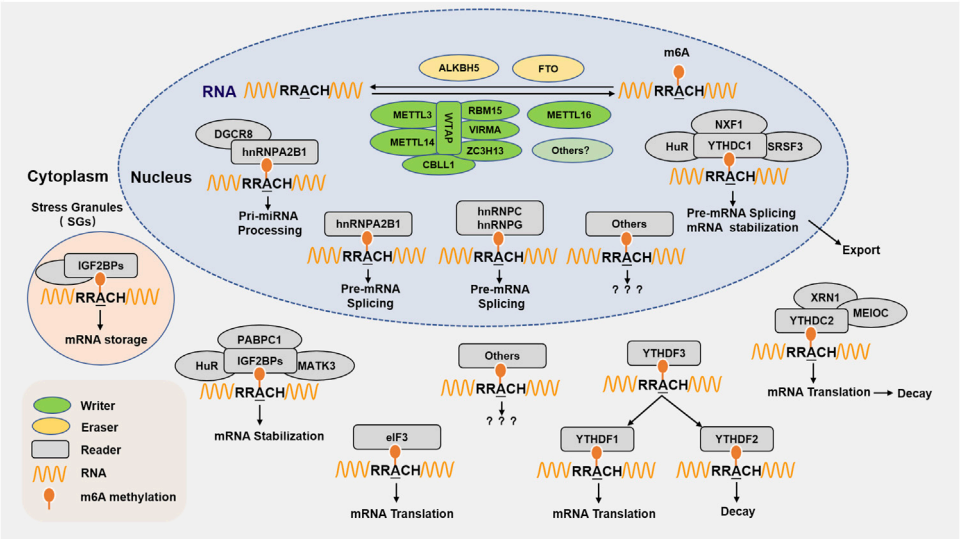


FIGURE 1 | m6A RNA methylation regulating proteins and molecular functions. m6A RNA methylation is performed by its writer, eraser, and reader to add, delete, recognize m6A, respectively. The writer refers to m6A methyltransferases including METTL3, METTL14, WTAP, RBM15/15B, VIRMA/KIAA1429/Virilizer, ZC3H13, CBLL1/Hakai, and METTL16. The m6A eraser is the demethylase FTO and ALKBH5. The m6A readers are proteins that recognize m6A sites and perform a variety of functions in the nucleus or cytoplasm, the currently identified m6A reader proteins including YTHDC1, YTHDC2, YTHDF1, YTHDF2, YTHDF3, IGF2BPs, eIF3, and hnRNPA2B1, HNRNPC, HNRNPG. m6A methylation is involved multiple functions in RNA metabolism, including RNA stabilization and splicing, miRNA processing, nuclear export, translation, RNA storage and decay, and so on.

YTHDC2 functions in the cytoplasm, affecting the m6A mRNA translation efficiency and abundance of its targets. It has been proposed that YTHDC2 interacts with m6A-containing mRNAs, ribosomes, and XRN1 to regulate mRNA stability and translation. In addition, MEIOC also can be bound to YTHDC2 in an RNA-independent manner, stabilize the binding of YTHDC2 to mRNA (Abby et al., 2016; Inada, 2020). YTHDF3 has been shown to promote the functions of YTHDF1 and YTHDF2, which promote m6A mRNA translation by binding to YTHDF1 and enhance RNA degradation by interacting with YTHDF2, respectively (Shi et al., 2017).

The insulin-like growth factor-2 (IGF2) mRNA-binding proteins 1, 2, and 3 (IGF2BP1/2/3) as a distinct family of m6A readers that regulate mRNA stability, transport, and translation (Uyar et al., 2017). Recent research shows that the association between IGF2BPs and target mRNAs, such as myc mRNA, is enhanced by m6A modification of target transcripts, indicating that IGF2BPs is an important m6A reader (Muller et al., 2019). In addition, IGF2BPs can stabilize the mRNA of target genes by recruiting and interacting with RNA stabilizing proteins, such as: ELAVL1/HuR, MATK3, PABPC1, etc (He et al., 2019; Zhu et al., 2020a), and also IGF2BPs can protect the target mRNA from stress condition (Huang et al., 2018). Eukaryotic initiation factor 3 (eIF3) can be regarded as a reader of 5′ UTR m6A, which can interact with m6A modified RNA, enhance ribosomal loading and promote the translation of target gene mRNA (Shah et al., 2017).

Heterogeneous ribonucleoproteins (hnRNPs) refer to a large family of RNA-binding proteins (RBPs) that are involved in multiple aspects of nucleic acid metabolism, including alternative splicing, processing, mRNA stabilization, and subsequent transcription and translation. Among them, hnRNPA2B1 can directly bind to m6A RNA to regulate RNA splicing and processing. In addition, hnRNPA2B1 can also cooperate with proteins, such as: DGCR8, etc., to promote the processing and formation of miRNA (Alarcon et al., 2015). The m6A reader protein hnRNPC or hnRNPG binds m6A RNAs through an "m6A switch" mechanism, in which m6A-mediated RNA hairpin destabilization exposes a single-stranded hnRNPC or hnRNPG-binding motif, and binds to m6A RNA and facilitates processing (Liu et al., 2017; **Figure 1**).

PART 2: THE ROLE OF M6A MODIFICATION IN CANCER

More and more research evidences show that m6A modification is related to the proliferation, differentiation, tumorigenesis, invasion and metastasis of tumors, and plays a key role as an oncogene or tumor suppressor gene in the occurrence and development of malignant tumors (**Table 2**).

Acute Myeloid Leukemia

The link between m6A and tumorigenesis was first demonstrated in AML, where overexpression of methyltransferases leads to increased RNA m6A modification, leading to abnormal hematopoietic stem cell differentiation and promoting leukemogenesis (Vu et al., 2017).

The abnormally high expression of METTL3 in AML can promote m6A modification and promote the occurrence and

TABLE 2 | Role of m6A modification in various cancers.

Cancer	Cell lines	Expression	Function	Role	Target/Pathway	Mechanism	References
AML	MOLM13	METTL3↑	Writer	Oncogene	c-Myc↑, BCL2↑, PTEN↑	Inhibit AML cell differentiation and apoptosis	Vu et al. (2017)
	K562, Kasumi-1	METTL3/14↑	Writer	Oncogene	MDM2↑, P21↓, P53↓	Promote cell proliferation and cell cycle, inhibit AML cell apoptosis and differentiation	Sang et al. (2022)
	AML-MSCs	METTL3↓	Writer	Suppressor	AKT↑, PI3K↑-AKT↑	Upregulate PI3K-AKT pathway activity and enhance chemoresistance	Pan et al. (2021b)
	MMC6, NOMO1	ALKBH5↑	Eraser	Oncogene	TACC3↑	Maintenance of AML and self-renewal of LSCs/LICs	Shen et al. (2020)
	MOLM13, THP1	ALKBH5↑	Eraser	Oncogene	AXL↑, p-SRC↑, p-AKT↑, p-ERK1/2↑, p-STAT3↑	Affects mRNA stability of AXL; Maintenance of AML and self-renewal of LSCs/LICs	Wang et al. (2020b)
	MonoMac-6, MV4-11, NB4	FTO↑	Eraser	Oncogene	ASB↓, RARA↓	Enhances cell transformation, leukemogenesis, and inhibits differentiation	Li et al. (2017)
	NB-4, MOLM13	YTHDC1↑	Reader	Oncogene	MCM4↑	Maintenance of AML and self-renewal of LSCs, YTHDC1 upregulates MCM4 expression and promotes AML	Sheng et al. (2021)
	MOLM13	YTHDC1↑	Reader	Oncogene	Myc↑, Phase seperation↑	Promotes liquid-liquid phase separation, maintains AML cell survival and the undifferentiated state	Cheng et al. (2021)
NSCLC	A549, A520; H1299, H1975	METTL3↑	Writer	Oncogene	c-Myc↑, BCL2↑	Promotes cell growth, invasion and migration	Wu et al. (2021a); Zhang et al. (2021a)
	A549	METTL3↑	Writer	Oncogene	EGFR↑, TAZ↑, YAP↑, MAPKAPK2↑, DNMT3A↑	Promotes cell growth, survival, invasion and migration	Lin et al. (2016); Jin et al. (2019)
	A549, LC-2/ad	METTL3↑	Writer	Oncogene	JUNB↑, E-Cadherin↓, FN1↑, VIM↑	Promotes EMT by up-regulating JUNB expression, which upregulates EMT-related protein expression	Wanna-Udom et al. (2020)
	A549, Calu1	METTL3↑	Writer	Oncogene	LCAT3↑-FUBP1↑-MYC↑	Enhances LCAT3 lncRNA stabilization Promotes cell proliferation, migration and invasion	Qian et al. (2021a)
	A549, H838	METTL3↑	Writer	Oncogene	DAPK2↓, NF-kB↑	Inhibiting DAPK2 expression and activating NF-κB pathway	Jin et al. (2019)
	A549, H1299, H1975	METTL3↑	Writer	Oncogene	miR-1246↑, PEG3↓; miR-143-3p↑, VASH1↓, dY-Tub↓	Promotes cell growth, EMT, invasion, migration and angiogenesis	Huang et al. (2021a) ; Wang et al. (2020b)
	A549, H1975	METTL3↑	Writer	Oncogene	miR-1915-3p↓, SET↑, JNK/Jun & NF-κB↑	Promotes cell growth, EMT, survival, invasion, and migration	Pan et al. (2021a)
	H1975, H322	METTL3↑	Writer	Oncogene	SLC7A11↑	Enhances the stability of SLC7A11 mRNA which promotes cell proliferation and inhibits ferroptosis	Xu et al. (2022)
	H1299, H1650	METTL3↑	Writer	Oncogene	LncRNA ABH11-AS1↑, EZH2-KLF4, Warburg effect↑	Enhance the Warburg effect and promote tumor progression	Xue et al. (2021)
	H1975, H1299	YTHDC2↓	Reader	Suppressor	SLC7A11↑	Downregulation of YTHDC2 expression leads to enhanced SLC7A11 mRNA stability and promotes tumorigenesis	Ma et al. (2021a)
	H441, H1299	YTHDC2↓	Reader	Suppressor	HOXA13↑, SLC3A2↑	Downregulation of YTHDC2 expression leads to enhanced HOXA13 mRNA stability and promotes tumorigenesis	Ma et al. (2021b)
	HCC827	METTL3↓	Writer	Suppressor	Bax↓, cleaved caspase 3↓, FBXW7↓, c-Myc↑, Mcl-1↑	Downregulation of METTL3 decreased apoptotic molecules and increased FBXW7 expression and its target genes Mcl-1 and c-Myc, and promotes tumorigenesis	Wu et al. (2021b)
	A549, H1299	YTHDF1,3↑	Reader	Oncogene	YAP↑, ABCG2↑, ERCC1↑, CTGF↑, Cyr61↑	Promotes cell growth, EMT, invasion, migration and anti-apoptosis	Jin et al. (2019)
HCC	HepG2, Huh7	METTL3↑	Writer	Oncogene	SOCS2↓, JAK/STAT↑	Promotes cell growth, migration, metastasis, tumorgenicity	Chen et al. (2018)
	Huh7, HCCLM3	WTAP↑	Writer	Oncogene	ETS1↓, ETS1-p21/p27↓	Promotes cell growth, tumorgenicity	Chen et al. (2019a)
	SK-Hep1, HCCLM3	VIRMA↑	Writer	Oncogene	GATA3↓	Enhances tumor growth and metastasis	Lan et al. (2019)

(Continued on following page)

TABLE 2 | (*Continued*) Role of m6A modification in various cancers.

Cancer	Cell lines	Expression	Function	Role	Target/Pathway	Mechanism	References
	HepG2, Huh7	METTL14↓	Writer	Suppressor	USP48↓, SIRT6↓, aerobic glycolysis↑	Down-regulation of METTL4 in HCC promotes aerobic glycolysis, cell proliferation, tumorigenesis	Du et al. (2021)
	HepG2	METTL14↓	Writer	Suppressor	miR-126↓	Downregulated METTL14 affects miR-126 processing and promotes tumor cell metastasis	Ma et al. (2017)
	Huh7, MHCC97H	ALKBH5↓	Eraser	Suppressor	LYPD1↑	Downregulated ALKBH5 enhances LYPD1 mRNA stability and translation, which promotes cell growth, invasion	Chen et al. (2020a)
	MHCC97H, HCCLM3	FTO↑	Eraser	Oncogene	SOX2↑, KLF4↑, NANOG↑	Promotes cell growth, metastasis, stemness	Bian et al. (2021)
	Huh7, MHCC97H	YTHDF1↑	Reader	Oncogene	PI3K-AKT-mTOR↑	Promotes cell proliferation, EMT, migration, invasion, and cell cycle process	Luo et al. (2021)
	HepG2, Huh7	IGF2BP2↑	Reader	Oncogene	FEN1↑	Enhances FEN1 mRNA stability and promotes cell proliferation	Pu et al. (2020)
Breast cancer	MDA-MB-231, MCF7, BT549	METTL3↑	Writer	Oncogene	BCL2↑; SOX2↑; KRT7↑; MALAT1↑, HMGA2↑; PD-L1↑	Promotes cell proliferation, EMT, metastasis, stemness; Anti-apoptosis, inhibits tumor immunity	Wang et al. (2020a) *etc*.
	—	METTL14↓ ZC3H13↓	Writer	Suppressor	APC↓, Wnt signaling↑	Promotes cell proliferation, EMT, migration, invasion, metastasis	Gong et al. (2020)
	MDA-MB-231, MCF7; SKBR3 MDA-MB-453	FTO↑	Eraser	Oncogene	BNIP3↓; miR-181-3P↓-ARL5B↑	Promotes cell proliferation, EMT, migration, invasion, metastasis	Niu et al. (2019); Xu et al. (2020)
	MDA-MB-231, MCF7	YTHDF1↑	Reader	Oncogene	FOXM1↑	Enhances FOXM1 translation, Promotes cell proliferation, invasion, metastasis	Chen et al. (2022)
	MDA-MB-231 4T1	YTHDF3↑	Reader	Oncogene	ST6GALNAC5↑, GJA1↑, EGFR↑, VEGFRA↑	Promotes brain metastasis, invasion, angiogenesis	Chang et al. (2020)

development of AML by enhancing the translation of c-MYC, BCL2, and PTEN mRNAs. Moreover, knockdown of METTL3 can lead to increased p-AKT levels and promote cell differentiation (Vu et al., 2017). In addition, METTL3 and METTL14 exert oncogenic roles in AML by increasing m6A levels in MDM2 mRNA and targeting the MDM2/p53 signaling pathway (Sang et al., 2022). However, the low expression of METTL3 in AML-MSCs (mesenchymal stem cells from AML patients) promotes the expression of AKT protein and upregulate the PI3K-AKT signaling pathway, which ultimately leads to the enhanced adipogenesis ability of AML-MSCs and facilitates the chemoresistance of AML (Pan et al., 2021b).

ALKBH5 is aberrantly overexpressed in AML and associated with poor prognosis. ALKBH5 exerts tumor-promoting roles in the development and maintenance of AML and self-renewal of leukemia stem/initiating cells (LSCs/LICs) through post-transcriptional regulation of its key target TACC3, AXL (Wang et al., 2020b; Shen et al., 2020). FTO is highly expressed in AML containing t (11q23)/MLL rearrangements, t (15;17)/PML-RARA, FLT3-ITD and/or NPM1 mutations. FTO inhibits the expression of ASB2 and RARA by reducing m6A levels of the target mRNA, which in turn enhances leukemic oncogene-mediated cell transformation and leukemogenesis, and inhibits all-trans retinoic acid (ATRA)-induced AML cell differentiation (Li et al., 2017).

YTHDC1 is overexpressed in AML and is required for proliferation, differentiation and development, maintenance of self-renewal, leukemogenesis, and survival (Sheng et al., 2021). Moreover, YTHDC1 can also promote the development of AML by promoting phase separation. In recent years, studies have shown that abnormal phase separation is closely related to the occurrence of tumors. YTHDC1 binds to m6A and forms nuclear condensates (nYACs) which are essential for maintaining targets mRNA stability and AML cell survival and the undifferentiated state (Cheng et al., 2021).

Non-Small-Cell Lung Cancer

In non-small-cell lung cancer (NSCLC), METTL3 promotes the translation of mRNAs of certain genes by increasing m6A levels, including c-Myc, BCL-2, epidermal growth factor receptor (EGFR), hippo pathway effector TAZ and YAP, MAPKAPK2 (MK2), and DNMT3A, thereby promoting lung adenocarcinoma (LUAD) cell proliferation, survival, invasion and metastasis (Lin et al., 2016; Jin et al., 2019; Wu et al., 2021a; Zhang et al., 2021c). High expression of METTL3 can improve the stability of JUNB mRNA and increase its expression. JUNB is an important transcriptional regulator of epithelial-mesenchymal transition (EMT), including CDH1/E-cadherin, FN1/Fibronectin and VIM/Vimentin (Wanna-Udom et al., 2020). Long non-coding RNAs (lncRNAs) play key roles in a variety of physiological and pathological processes. METTL3 enhances the stability of LCAT3

lncRNA through m6A modification, and then promotes the proliferation, invasion and metastasis of LUAD cells through the LCAT3-FUBP1-MYC axis (Qian et al., 2021a). Moreover, METTL3 also upregulates the level of miR-1246 which reduces the expression of the tumor suppressor PEG3 to promote the development of lung adenocarcinoma (Huang et al., 2021b); In addition, METTL3 can also promote tumor proliferation, EMT, invasion, and migration by down-regulating miRNAs, such as: miR-1915-3p (Pan et al., 2021a). Cell ferroptosis, a novel form of programmed cell death. The highly expressed METTL3 in LUAD can promote LUAD cell proliferation and inhibit ferroptosis, and its molecular mechanism is that METTL3-mediated m6A modification enhances the stability of SLC7A11 mRNA and promotes its translation (Xu et al., 2022). Metabolic reprogramming, known as the Warburg effect, is considered a key hallmark of cancer, including lung cancer. Studies have shown that METTL3 can enhance the stability of the lncRNA ABH11-AS1 transcript, thereby enhancing the Warburg effect of lung adenocarcinoma, and ultimately promoting the occurrence and development of tumors (Xue et al., 2021).

The m6A reader YTHDC2 is frequently repressed in LUAD, indicating a poor prognosis. Downregulation of YTHDC2 expression leads to enhanced SLC7A11 and SLC3A2 mRNA stability and promotes tumorigenesis (Ma et al., 2021a; Ma et al., 2021b). But there is an interesting study showing that METTL3 has an anti-tumor effect, downregulation of METTL3 decreased protein levels of apoptotic molecules and increased protein levels of the FBXW7 gene and its target genes Mcl-1 and c-Myc, and promotes tumorigenesis in LUAD which may be caused by the heterogeneity of tumor cells (Wu et al., 2021b). METTL3, YTHDF1/3 is highly expressed in LUAD, METTL3 promotes YAP mRNA translation by recruiting YTHDF1/3 and eIF3b to the translation start site and increased YAP mRNA stability by modulating the MALAT1-miR-1914-3p-YAP axis, ultimately resulting in increased YAP expression and activity that induces NSCLC drug resistance and metastasis (Jin et al., 2019).

Hepatocellular Carcinoma

METTL3 is highly expressed in HCC and is closely associated with poor prognosis of HCC patients, and promotes HCC cell proliferation, migration, metastasis and tumorgenicity *via* YTHDF2-dependent post-transcriptional silencing of SOCS2, which is a member of the suppressor of cytokine signaling (SOCS) family, and is a negative regulator of the JAK/STAT pathway (Chen et al., 2018). WTAP-guided m6A modification plays a key role in HCC oncogenesis through the HuR1-ETS1-P21/P27 axis (Chen et al., 2019b). High expression of VIRMA in HCC patients is associated with poor prognosis. VIRMA induces m6A methylation of the 3′ UTR of GATA3 pre-mRNA, leading to dissociation of the RNA-binding protein HuR and degradation of GATA3 pre-mRNA, promoting tumor growth and metastasis *in vivo* (Lan et al., 2019).

However, METTL14, as a tumor suppressor in HCC, is down-regulated. The stability of USP48 mRNA is reduced due to decreased m6A modification of METTL14 and leads to the degradation of its downstream target gene SIRT6, which ultimately enhances aerobic glycolysis in tumor cells (Du et al., 2021). In addition, METTL14, as an anti-metastatic factor, acts as a favorable factor for HCC by regulating m6A-dependent miRNA processing (Ma et al., 2017).

Downregulation of ALKBH5 is associated with poor prognosis in HCC. Decreased expression of ALKBH5 leads to increased 3′UTR m6A modification of LYPD1 mRNA, which in turn results in the increased expression of LYPD1, which promotes tumor cell growth, migration, invasion and metastasis (Chen et al., 2020c). FTO can increase the proportion of stem-like cells in HCC by enhancing the expression of SOX2, KLF4, and NANOG (Bian et al., 2021).

YTHDF1 is highly expressed in HCC and associated with poor survival. YTHDF1 participates in the progression of HCC by activating the PI3K/AKT/mTOR signaling pathway and inducing EMT (Luo et al., 2021). Overexpressed IGF2BP2 can directly recognize and bind to the m6A site of FEN1 mRNA, enhancing the stability of FEN1 mRNA and promoting the proliferation of liver cancer cells *in vitro* and *in vivo* (Pu et al., 2020).

Breast Cancer

m6A regulatory factor is an important participant in the malignant progression of breast cancer and may be a prognostic and therapeutic target for BCa. METTL3 is highly up-regulated in BCa and predicts a poor prognosis. High expression of METTL3 can increase the modification level of downstream target mRNA 3′UTR m6A and enhance the stability and translation of mRNA, such as BCL-2 (Wang et al., 2020a), SOX2 (Xie et al., 2021), KRT7 (Chen et al., 2021a), MALAT1 (Zhao et al., 2021), etc., which can promote BCa cell proliferation, EMT, invasion, metastasis, tumorigenicity and stem cell. Tumor immune deficiency is an important cause of tumorigenesis. PD-L1, the ligand of PD-1, is highly expressed in many cancers and is closely associated with mortality in cancer patients, establishing the role of PD-1 in cancer-induced immunosuppression. METTL3 enhances the stability of PD-L1 mRNA, promotes the expression of PD-L1 and induces tumor immunosuppression by relying on m6A modification (Wan et al., 2022). However, METTL3 is low expression in triple negative breast cancer (TNBC) and strongly associated with short-distance-metastasis-free survival. METTL3 knockout can enhance cell migration, invasion and adhesion by reducing m6A level (Shi et al., 2020). Downregulation of METTL14 and ZC3H13 in BCa, act as tumor suppressor, has been found in breast cancer and poor prognosis is predicted (Gong et al., 2020).

High levels of FTO were significantly associated with lower survival in BCa patients. FTO promotes BCa cell proliferation, colony formation and metastasis *in vitro* and *in vivo* (Niu et al., 2019; Xu et al., 2020).

YTHDF1 and YTHDF3 are highly expressed in BCa patients and with poor prognosis, which can enhance the expression of related genes, such as FOXM1, ST6GALNAC5, GJA1, EGFR, VEGFRA, etc., and promote the metastasis, invasion and angiogenesis of BCa cells (Chang et al., 2020; Chen et al., 2022).

The Diverse Role of m6A in Cancer

More recently, extensive efforts have been made to investigate the biological effects of dysregulated m6A modifications and related

mechanisms (i.e., m6A writers, erasers, and reader proteins) in various cancers, such as prostate cancer (Wang et al., 2022), colorectal cancer (Tanabe et al., 2016; Zhu et al., 2020b), gastric cancer (Xu et al., 2021), esophageal cancer (Guo et al., 2020), cervical cancer and endometrial cancer (Zhang and Yang, 2021; Huang et al., 2022), pancreatic cancer (Li et al., 2021), skin cancer (Yang et al., 2021), etc. Moreover, METTL16, as an independent regulator, plays an important role in RNA m6A modification. In the nucleus, METTL16 functions as a writer for the deposition of m6A in its specific mRNA; And in the in the cytoplasm, METTL16 could promote the translation of over 4000 mRNA transcripts *via* interacting with eIF3a and 3b (Su et al., 2022). In addition, m6A regulator also involved the immune response. There is evidence that the m6A eraser FTO can decrease the response of PD-1 inhibitor immunotherapy and promote tumorigenesis. Knockdown of FTO enhances the sensitivity to interferon gamma (IFNγ) and anti-PD-1 therapy (Yang et al., 2019). Furthermore, FTO could promote the malignant transformation and tumorigenesis *via* up-regulated the target gene NEDD4L in keratinocytes. Upon further investigation, it was found that the FTO expression was elevated after treated with low-level arsenic which is a human carcinogen (Cui et al., 2021).

Above all, different m6A regulators have different biological effects in various tumors. Even if the same regulator is in the same tumor but is of different molecular subtypes, the biological effects are also not the same, which may be related to tumor heterogeneity and tumor microenvironment. In general, m6A plays an important role in the regulation of tumor cell proliferation, cell cycle, EMT, invasion, metastasis, stemness maintenance, apoptosis, immune effect, and other aspects, which is of great significance for the occurrence and development of tumors.

PART 3: THE ANTI-CANCER EFFECT OF NATURE PRODUCTS BY REGULATING M6A MODIFICATION

Natural products and compounds derived from natural products account for about 40% of all drugs approved for clinical use, among which ~70% of anticancer drugs are derived from natural products. (Butler et al., 2014; Newman and Cragg, 2016; Bahman et al., 2018; Sahm et al., 2020). After years of efforts, natural products have also made great progress in anti-cancer, and many natural small molecules have been successfully marketed and become star therapeutic drugs in the corresponding field. For example, paclitaxel, camptothecin, homoharringtonine, ginsenoside Rg3, etc. In recent years, with the deepening of understanding and attention to natural products, many new opportunities have been brought to the development of natural anti-tumor molecules (Singla et al., 2021a; Singla et al., 2021b; Tan et al., 2021; Wang et al., 2021; Singla et al., 2022).

β-Elemene

Elemene, a sesquiterpenoid natural compound, is the extraction of traditional Chinese medicinal material herb Curcuma wenyujin, and which is a mixture of β-, γ-, δ-elemene with β-elemene as the main component. β-elemene, the most important component of elemene for pharmaceutical activity, has been shown to be effective *in vitro* and *in vivo* against a variety of cancers, such as lung, leukemia, liver cancer, gastric, cervical cancer, colorectal, ovarian, glioblastoma, and melanoma, and so on (Li et al., 2013; Bayala et al., 2014; Gong et al., 2015; Liu et al., 2016; Jiang et al., 2017; Fu et al., 2018; Chen et al., 2020a).

Gefitinib, a classic epidermal growth factor receptor (EGFR) tyrosine kinase receptor inhibitor, has been a bottleneck in the treatment of NSCLC due to the problem of drug resistance (Onitsuka et al., 2010; Sun et al., 2020). Studies have shown that METTL3 is highly expressed in NSCLC and promotes the occurrence and development of NSCLC by promoting tumor cell proliferation, EMT, invasion, metastasis, angiogenesis, and anti-apoptosis. Moreover, highly expressed METTL3 can increase autophagy in NSCLC by upregulating autophagy-related proteins ATG5 and ATG7, and eventually lead to resistance to gefitinib and other EGFR TKI drugs. β-elemene can directly inhibit the expression of METTL3, but not VIRMA and METTL14, causing the down-regulation of autophagy-related proteins LC3B, ATG5, and ATG7, thereby inhibiting autophagy, tumor cell proliferation and promoting cell apoptosis, and finally reversing the effect of gefitinib resistance in NSCLC (Liu et al., 2020). In addition, PTEN is a well-known tumor suppressor in various cancers and acts as a negative regulator of PI3K-Akt pathway. β-elemene can reduce the level of m6A modification of PTEN mRNA by downregulating the expression of METTL3, resulting in an increase in the expression of PTEN protein, which ultimately inhibits the growth of tumor cells and promotes apoptosis (Feng et al., 2022).

Soy Isoflavones Genistein

Genistein is a major active factor in soy isoflavones, the most effective functional component in soy isoflavone products, and has a variety of physiological functions. Genistein is similar in structure to mammalian estrogen-estradiol, and has the diphenolic hydroxyl active group of estrogen. So genistein has various physiological activities such as estrogen-like activity.

Studies have shown that the antioxidant and anti-proliferative properties of soy isoflavones are the main reasons for their anti-cancer effects. Soy isoflavones have obvious therapeutic effects on breast cancer, colon cancer, lung cancer, prostate cancer, skin cancer, and leukemia. Soy isoflavones can also prevent the occurrence of ovarian cancer, colon cancer, stomach cancer, and prostate cancer (Suthar et al., 2001; Sarkar et al., 2006; Kaushik et al., 2018; Park et al., 2018; Imai-Sumida et al., 2020; Aboushanab et al., 2021). In addition, down-regulation of ALKBH5 expression enhanced the expression of mesenchymal phenotype markers α-smooth muscle actin and snail, which are factors that promote EMT. Genistein can increase the expression of ALKBH5, reduce the level of RNA m6A and inhibit EMT (Ning et al., 2020).

Resveratrol

Resveratrol, a non-flavonoid polyphenolic organic compound, is a phytoalexin with the chemical formula $C_{14}H_{12}O_3$. It can be synthesized in grape leaves and grape skins, and is a biologically

active ingredient in wine and grape juice. Resveratrol has antioxidant, anti-inflammatory, anti-cancer and cardiovascular protection effects (Rauf et al., 2018a). The heavy metal cadmium (Cd) can promote the migration and invasion of colorectal cancer cells, and Cd could upregulate the expressions of N-cadherin, vimentin, and ZEB1 and downregulate the expression of E-cadherin in colorectal cancer cells. Resveratrol could reverse Cd-promoted migration, invasion, and EMT processes by modulating ZEB1 expression (Qian et al., 2021b). Moreover, resveratrol can reduce aflatoxin B1-induced ROS accumulation, and can also cause changes in m6A modification-related proteins, including: METTL3, FTO, YTHDF2 (Wu et al., 2020).

Rhein

Extracted from the rhizome of Rheum palmatum, Rhein is the main bioactive component of Dahuang (rhubarb) therapeutic laxatives. In tumors, Rhein mainly inhibits cell proliferation, promotes cell apoptosis, inhibits tumor cell invasion, and metastasis, and enhances hypoxia tolerance, and so on. Its target signaling pathways include: PI3K-Akt, MAPK (ERKs, JNKs, P38), Wnt, NF-kB, et al. (Henamayee et al., 2020). Rhein was the first FTO-competitive inhibitor discovered, which can increase the level of m6A modification on intracellular target mRNA. In addition, Rhein can reversibly bind to FTO or ALKBH5 to form a complex and prevent recognition of intracellular m6A substrates (Chen et al., 2012; Zannella et al., 2021).

Baicalin

Baicalin is a flavonoid compound extracted and isolated from the dry root of Scutellaria baicalensis Georgi (a dicotyledonous Lamiaceae plant), which has a variety of biological activities, including bacteriostatic, diuretic, anti-inflammatory, cholesterol-lowering, anti-thrombotic, anti-asthmatic, hemostatic, anti-allergic, and antispasmodic effects, and also has a strong anti-cancer biological effect (Wang et al., 2015; Gong et al., 2017; Lai et al., 2018).

Type 2 diabetes (T2D) is defined as a metabolic disorder characterized by hyperglycemia that can lead to abnormal organ metabolism or hormones, which in turn lead to a variety of diseases, including cancer. High glucose environment can induce hepatoma cells to up-regulate the expression of HKDC, which can promote the proliferation, invasion and metastasis of tumor cells by downregulating the HKDC/JAK2/STAT1/caspase-3 signaling pathway. Baicalin can reduce the m6A modification level of HKDC mRNA by inhibiting METTL3, which leads to the decrease of HKDC expression and the upregulation of HKDC/JAK2/STAT1/caspase-3 signaling pathway, and finally inhibits the invasion and metastasis of tumor cells (Jiang et al., 2022). In addition, Baicalein inhibits pancreatic cancer cell proliferation and invasion by inhibiting NEDD9 and its downstream Akt and ERK signaling pathways (Zhou et al., 2017).

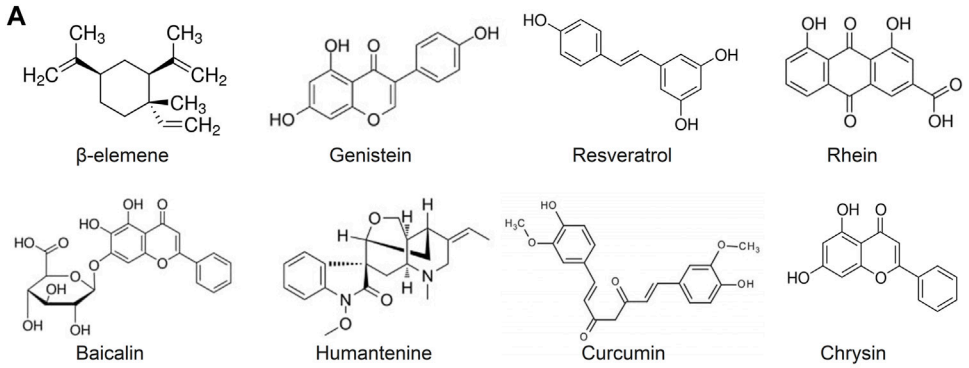
Others Natural Product

Humantenine, an indole alkaloid compound isolated from Gelsemium elegans, is a traditional medical herb with a drug use. Wu et al (2022) studies indicated that the humantenine leads to the colon cancer cell injury *via* affecting the gene transcriptome and m6A regulators, including RBM15, METTL3, YTHDF3, and ALKBH5 are up-regulated, and RBM15B, ZC3H13, hnRNPA2BA, YTHDC1, YTHDC2, YTHDF2, and IGF2BP3 are down-regulated.

Curcumin, a polyphenol extracted from turmeric in 1815, has attracted worldwide attention due to its biological activities (such as antioxidant, anti-inflammatory, antibacterial, and antiviral), among which its anticancer potential has been described the most, and the relevant anticancer mechanism is still being investigated (Mortezaee et al., 2019; Wang et al., 2021). Curcumin inhibits the occurrence and development of tumor cells by regulating the expression of growth factors, inflammatory factors, apoptosis-related proteins, protein phosphokinases, and receptors, and cell proliferation-related genes, such as FGFs, VEGF, EGF/EGFR, TNF, IFN, ILs, Caspase-3/6/8/10, FADD, MAPK, JNK, IKK, Survivin, MCL-1, BCL-xL, cIAP-1/2, BCL-2, c-Myc, PCNA, Cyclin D1, etc (Giordano and Tommonaro, 2019). Unfortunately, there is still no relevant report on the relationship between curcumin and m6A enzyme, which also provides a broad space for us to explore the correlation between curcumin and m6A enzyme and its anti-cancer mechanism.

Chrysin (5,7-dihydroxyflavonoid) is a natural, bioactive dietary flavonoid commonly found in a variety of plant extracts, including chamomile, pleurotusostreatus, and honeycombs, as well as in honey and propolis, which has a significant medicinal function and economic value. Chrysin has a variety of biological properties, including anti-cancer, antioxidant, anti-inflammatory, antibacterial, and other effects. Several recent studies have reported that chrysin exerts its anti-cancer effects on endometrial cancer, gastric cancer, breast, lung, cervical, bladder, breast cancer, and colorectal cancer, etc. *via* selectively inhibiting various cell signaling pathways, such as: Akt/mTOR, JNK1/2, ERK1/2, NF-κB, etc., and promoting tumor cells apoptosis and autophagy (Xia et al., 2015a; Xia et al., 2015b; Roy et al., 2019; He et al., 2021). However, the regulatory relationship between chrysin and m6A enzyme has not been reported.

In addition, there are a number of natural products with anticancer activities, such as: cucurbitacin B, ailanthone, fucoidan, casearlucin A, Wan-Nian-Qing prescription, bruceine D, cinnamaldehyde, fisetin, quercetin, betulinic acid, astragalus polysaccharide, ginsenosides, panax notoginseng saponins, and so on (Wong et al., 2015; Syed et al., 2016; Rauf et al., 2018b; Li et al., 2020c; Jiang et al., 2021a; Huang et al., 2021c; Imran et al., 2021; Yao et al., 2021). By regulating the whole process of tumor occurrence and development, such as cell cycle, apoptosis, autophagy, invasion, and migration, and cell metabolic reprogramming, etc. (**Figure 2**), these natural products have broad research value and clinical treatment in the field of anti-cancer.



B

Name	Target	Biology function
β-elemene	METTL3	Inhibits cell proliferation and autophagy, promotes tumor cell apoptosis.
Genistein	ALKBH5	Enhances the expression of ALKBH5, reduces the level of RNA m6A and inhibit EMT.
Resveratrol	ZEB1, FTO METTL3 YTHDF2	Reverses Cd-mediated tumor migration, invasion, and EMT processes; Reduces ROS accumulation.
Rhein	FTO ALKBH5	Rhein is competitively bind to FTO or ALKBH5, increases the level of m6A modification of target mRNA.
Baicalin	METTL3	Decreases HKDC expression via inhibiting METTL3 and finally inhibits the invasion and metastasis of tumor cells.

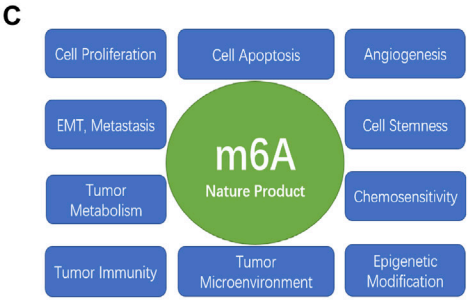


FIGURE 2 | Natural products and m6A in anti-cancer research. **(A)** The structure of the common natural anticancer products, follow as: β-elemene, genistein, resveratrol, rhein, baicalin, humantenine, curcumin, chrysin. **(B)** The anti-cancer effects of β-elemene, genistein, resveratrol, rhein, baicalin depended on m6A enzyme. **(C)** The possible mechanism of nature products mediated m6A modification in tumor cell proliferation, apoptosis, angiogenesis, EMT, invasion, metastasis, maintenance of tumor cell stemness, tumor metabolism, chemosensitivity, tumor immunity, tumor microenvironment, and epigenetic modification.

CONCLUSION

More and more studies have shown that m6A modification plays a key role in the tumorigenesis and progression of various cancers, and the abnormal expression of m6A modification-related proteins is closely related to the occurrence and development of tumors. Natural products have broad prospects and applications in the field of anti-tumor research. At present, some natural products have played an important role in clinical applications. There are relatively few studies on the regulation of m6A modification by natural products, and need more extensive and in-depth research in the field of anti-tumor. The role of natural products in tumors needs further study, m6A modification in cell proliferation, apoptosis, angiogenesis, EMT, invasion, metastasis, maintenance of tumor cell stemness, tumor metabolism, chemosensitivity, tumor immunity, tumor microenvironment, and the roles and mechanisms of epigenetic modification still need to be further explored.

AUTHOR CONTRIBUTIONS

NS, KC, JZ, KZ, JY, and HW wrote the article. JL, ZM, FZ, HM, LC, CC, MS, and YL reviewed and revised the article. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the National Natural Science Foundation of China (U1904133), Program for Young Key Teachers in Colleges and Universities in Henan Province (2020GGJS150, 2021GGJS104) and the Natural Science Foundation of Henan Province of China (212300410224, 212300410381). This work was also supported by Henan Province Training Programs of Innovation and Entrepreneurship for Undergraduates (202210472054).

REFERENCES

Abby, E., Tourpin, S., Ribeiro, J., Daniel, K., Messiaen, S., Moison, D., et al. (2016). Implementation of Meiosis Prophase I Programme Requires a Conserved Retinoid-independent Stabilizer of Meiotic Transcripts. *Nat. Commun.* 7, 10324. doi:10.1038/ncomms10324

Aboushanab, S. A., Khedr, S. M., Gette, I. F., Danilova, I. G., Kolberg, N. A., Ravishankar, G. A., et al. (2021). Isoflavones Derived from Plant Raw Materials: Bioavailability, Anti-cancer, Anti-aging Potentials, and Microbiome Modulation. *Crit. Rev. Food Sci. Nutr.* 1, 1–27. doi:10.1080/10408398.2021.1946006

Alarcón, C. R., Goodarzi, H., Lee, H., Liu, X., Tavazoie, S., and Tavazoie, S. F. (2015). HNRNPA2B1 Is a Mediator of m(6)A-dependent Nuclear RNA Processing Events. *Cell* 162 (6), 1299–1308. doi:10.1016/j.cell.2015.08.011

Aoyama, T., Yamashita, S., and Tomita, K. (2020). Mechanistic Insights into m6A Modification of U6 snRNA by Human METTL16. *Nucleic Acids Res.* 48 (9), 5157–5168. doi:10.1093/nar/gkaa227

Bahman, A. A., Abaza, M. S. I., Khoushiash, S. I., and Al-Attiyah, R. J. (2018). Sequence-dependent E-ffect of S-orafenib in C-ombination with N-atural P-henolic C-ompounds on H-epatic C-ancer C-ells and the P-ossible M-echanism of A-ction. *Int. J. Mol. Med.* 42 (3), 1695–1715. doi:10.3892/ijmm.2018.3725

Bartosovic, M., Molares, H. C., Gregorova, P., Hrossova, D., Kudla, G., and Vanacova, S. (2017). N6-methyladenosine Demethylase FTO Targets Pre-mRNAs and Regulates Alternative Splicing and 3'-end Processing. *Nucleic Acids Res.* 45 (19), 11356–11370. doi:10.1093/nar/gkx778

Bawankar, P., Lence, T., Paolantoni, C., Haussmann, I. U., Kazlauskiene, M., Jacob, D., et al. (2021). Hakai Is Required for Stabilization of Core Components of the m6A mRNA Methylation Machinery. *Nat. Commun.* 12 (1), 3778. doi:10.1038/s41467-021-23892-5

Bayala, B., Bassole, I. H., Scifo, R., Gnoula, C., Morel, L., Lobaccaro, J. M., et al. (2014). Anticancer Activity of Essential Oils and Their Chemical Components - a Review. *Am. J. Cancer Res.* 4 (6), 591–607.

Bian, X., Shi, D., Xing, K., Zhou, H., Lu, L., Yu, D., et al. (2021). AMD1 Upregulates Hepatocellular Carcinoma Cells Stemness by FTO Mediated mRNA Demethylation. *Clin. Transl. Med.* 11 (3), e352. doi:10.1002/ctm2.352

Butler, M. S., Robertson, A. A., and Cooper, M. A. (2014). Natural Product and Natural Product Derived Drugs in Clinical Trials. *Nat. Prod. Rep.* 31 (11), 1612–1661. doi:10.1039/c4np00064a

Chang, G., Shi, L., Ye, Y., Shi, H., Zeng, L., Tiwary, S., et al. (2020). YTHDF3 Induces the Translation of m6A-Enriched Gene Transcripts to Promote Breast Cancer Brain Metastasis. *Cancer Cell* 38 (6), 857–871. e857. doi:10.1016/j.ccell.2020.10.004

Chen, B., Ye, F., Yu, L., Jia, G., Huang, X., Zhang, X., et al. (2012). Development of Cell-Active N6-Methyladenosine RNA Demethylase FTO Inhibitor. *J. Am. Chem. Soc.* 134 (43), 17963–17971. doi:10.1021/ja3064149

Chen, F., Chen, Z., Guan, T., Zhou, Y., Ge, L., Zhang, H., et al. (2021a). N6 -Methyladenosine Regulates mRNA Stability and Translation Efficiency of KRT7 to Promote Breast Cancer Lung Metastasis. *Cancer Res.* 81 (11), 2847–2860. doi:10.1158/0008-5472.CAN-20-3779

Chen, H., Yu, Y., Yang, M., Huang, H., Ma, S., Hu, J., et al. (2022). YTHDF1 Promotes Breast Cancer Progression by Facilitating FOXM1 Translation in an m6A-dependent Manner. *Cell Biosci.* 12 (1), 19. doi:10.1186/s13578-022-00759-w

Chen, J., and Du, B. (2019). Novel Positioning from Obesity to Cancer: FTO, an m6A RNA Demethylase, Regulates Tumour Progression. *J. Cancer Res. Clin. Oncol.* 145 (1), 19–29. doi:10.1007/s00432-018-2796-0

Chen, L., Li, Q., Jiang, Z., Li, C., Hu, H., Wang, T., et al. (2021b). Chrysin Induced Cell Apoptosis through H19/let-7a/COPB2 Axis in Gastric Cancer Cells and Inhibited Tumor Growth. *Front. Oncol.* 11, 651644. doi:10.3389/fonc.2021.651644

Chen, M., Wei, L., Law, C. T., Tsang, F. H., Shen, J., Cheng, C. L., et al. (2018). RNA N6-Methyladenosine Methyltransferase-like 3 Promotes Liver Cancer Progression through YTHDF2-dependent Posttranscriptional Silencing of SOCS2. *Hepatology* 67 (6), 2254–2270. doi:10.1002/hep.29683

Chen, P., Li, X., Zhang, R., Liu, S., Xiang, Y., Zhang, M., et al. (2020a). Combinative Treatment of β-elemene and Cetuximab Is Sensitive to KRAS Mutant Colorectal Cancer Cells by Inducing Ferroptosis and Inhibiting Epithelial-Mesenchymal Transformation. *Theranostics* 10 (11), 5107–5119. doi:10.7150/thno.44705

Chen, R., Wu, J., Lu, C., Yan, T., Qian, Y., Shen, H., et al. (2020b). Systematic Transcriptome Analysis Reveals the Inhibitory Function of Cinnamaldehyde in Non-small Cell Lung Cancer. *Front. Pharmacol.* 11, 611060. doi:10.3389/fphar.2020.611060

Chen, X. Y., Zhang, J., and Zhu, J. S. (2019a). The Role of m6A RNA Methylation in Human Cancer. *Mol. Cancer* 18 (1), 103. doi:10.1186/s12943-019-1033-z

Chen, Y., Peng, C., Chen, J., Chen, D., Yang, B., He, B., et al. (2019b). WTAP Facilitates Progression of Hepatocellular Carcinoma via m6A-HuR-dependent Epigenetic Silencing of ETS1. *Mol. Cancer* 18 (1), 127. doi:10.1186/s12943-019-1053-8

Chen, Y., Zhao, Y., Chen, J., Peng, C., Zhang, Y., Tong, R., et al. (2020c). ALKBH5 Suppresses Malignancy of Hepatocellular Carcinoma via m6A-Guided Epigenetic Inhibition of LYPD1. *Mol. Cancer* 19 (1), 123. doi:10.1186/s12943-020-01239-w

Cheng, Y., Xie, W., Pickering, B. F., Chu, K. L., Savino, A. M., Yang, X., et al. (2021). N6-Methyladenosine on mRNA Facilitates a Phase-Separated Nuclear Body that Suppresses Myeloid Leukemic Differentiation. *Cancer Cell* 39 (7), 958–e8. doi:10.1016/j.ccell.2021.04.017

Cui, Y. H., Yang, S., Wei, J., Shea, C. R., Zhong, W., Wang, F., et al. (2021). Autophagy of the m6A mRNA Demethylase FTO Is Impaired by Low-Level Arsenic Exposure to Promote Tumorigenesis. *Nat. Commun.* 12 (1), 2183. doi:10.1038/s41467-021-22469-6

Deng, X., Su, R., Stanford, S., and Chen, J. (2018). Critical Enzymatic Functions of FTO in Obesity and Cancer. *Front. Endocrinol. (Lausanne)* 9, 396. doi:10.3389/fendo.2018.00396

Du, L., Li, Y., Kang, M., Feng, M., Ren, Y., Dai, H., et al. (2021). USP48 Is Upregulated by Mettl14 to Attenuate Hepatocellular Carcinoma via Regulating SIRT6 Stabilization. *Cancer Res.* 81 (14), 3822–3834. doi:10.1158/0008-5472.CAN-20-4163

Fazi, F., and Fatica, A. (2019). Interplay between N 6-Methyladenosine (m6A) and Non-coding RNAs in Cell Development and Cancer. *Front. Cell Dev. Biol.* 7, 116. doi:10.3389/fcell.2019.00116

Feng, Y., Li, C., Liu, S., Yan, F., Teng, Y., Li, X., et al. (2022). β-Elemene Restrains PTEN mRNA Degradation to Restrain the Growth of Lung Cancer Cells via METTL3-Mediated N6 Methyladenosine Modification. *J. Oncol.* 2022, 1–11. doi:10.1155/2022/3472745

Fu, B., Wang, N., Tan, H. Y., Li, S., Cheung, F., and Feng, Y. (2018). Multi-Component Herbal Products in the Prevention and Treatment of Chemotherapy-Associated Toxicity and Side Effects: A Review on Experimental and Clinical Evidences. *Front. Pharmacol.* 9, 1394. doi:10.3389/fphar.2018.01394

Gao, R., Ye, M., Liu, B., Wei, M., Ma, D., and Dong, K. (2021). m6A Modification: A Double-Edged Sword in Tumor Development. *Front. Oncol.* 11, 679367. doi:10.3389/fonc.2021.679367

Giordano, A., and Tommonaro, G. (2019). Curcumin and Cancer. *Nutrients* 11 (10), 2376. doi:10.3390/nu11102376

Gokhale, N. S., McIntyre, A. B. R., McFadden, M. J., Roder, A. E., Kennedy, E. M., Gandara, J. A., et al. (2016). N6-Methyladenosine in Flaviviridae Viral RNA Genomes Regulates Infection. *Cell Host Microbe* 20 (5), 654–665. doi:10.1016/j.chom.2016.09.015

Gong, M., Liu, Y., Zhang, J., Gao, Y.-j., Zhai, P.-p., Su, X., et al. (2015). β-Elemene Inhibits Cell Proliferation by Regulating the Expression and Activity of Topoisomerases I and IIαin Human Hepatocarcinoma HepG-2 Cells. *BioMed Res. Int.* 2015, 1–10. doi:10.1155/2015/153987

Gong, P. J., Shao, Y. C., Yang, Y., Song, W. J., He, X., Zeng, Y. F., et al. (2020). Analysis of N6-Methyladenosine Methyltransferase Reveals METTL14 and ZC3H13 as Tumor Suppressor Genes in Breast Cancer. *Front. Oncol.* 10, 578963. doi:10.3389/fonc.2020.578963

Gong, W. Y., Zhao, Z. X., Liu, B. J., Lu, L. W., and Dong, J. C. (2017). Exploring the Chemopreventive Properties and Perspectives of Baicalin and its Aglycone Baicalein in Solid Tumors. *Eur. J. Med. Chem.* 126, 844–852. doi:10.1016/j.ejmech.2016.11.058

Guo, H., Wang, B., Xu, K., Nie, L., Fu, Y., Wang, Z., et al. (2020). m6A Reader HNRNPA2B1 Promotes Esophageal Cancer Progression via Up-Regulation of ACLY and ACC1. *Front. Oncol.* 10, 553045. doi:10.3389/fonc.2020.553045

Harvey, C. J. B., Tang, M., Schlecht, U., Horecka, J., Fischer, C. R., Lin, H. C., et al. (2018). HEx: A Heterologous Expression Platform for the Discovery of Fungal Natural Products. *Sci. Adv.* 4 (4), eaar5459. doi:10.1126/sciadv.aar5459

He, L., Li, H., Wu, A., Peng, Y., Shu, G., and Yin, G. (2019). Functions of N6-Methyladenosine and its Role in Cancer. *Mol. Cancer* 18 (1), 176. doi:10.1186/s12943-019-1109-9

He, Y., Shi, Y., Huang, H., Feng, Y., Wang, Y., Zhan, L., et al. (2021). Chrysin Induces Autophagy through the Inactivation of the ROS-mediated Akt/mTOR S-ignaling P-athway in E-ndometrial C-ancer. *Int. J. Mol. Med.* 48 (3), 172. doi:10.3892/ijmm.2021.5005

Henamayee, S., Banik, K., Sailo, B. L., Shabnam, B., Harsha, C., Srilakshmi, S., et al. (2020). Therapeutic Emergence of Rhein as a Potential Anticancer Drug: A Review of its Molecular Targets and Anticancer Properties. *Molecules* 25 (10), 2278. doi:10.3390/molecules25102278

Horiuchi, K., Kawamura, T., Iwanari, H., Ohashi, R., Naito, M., Kodama, T., et al. (2013). Identification of Wilms' Tumor 1-associating Protein Complex and its

Role in Alternative Splicing and the Cell Cycle. *J. Biol. Chem.* 288 (46), 33292–33302. doi:10.1074/jbc.M113.500397

Hsu, P. J., Shi, H., and He, C. (2017). Epitranscriptomic Influences on Development and Disease. *Genome Biol.* 18 (1), 197. doi:10.1186/s13059-017-1336-6

Huang, H., Weng, H., and Chen, J. (2020). m6A Modification in Coding and Non-coding RNAs: Roles and Therapeutic Implications in Cancer. *Cancer Cell* 37 (3), 270–288. doi:10.1016/j.ccell.2020.02.004

Huang, H., Weng, H., Sun, W., Qin, X., Shi, H., Wu, H., et al. (2018). Recognition of RNA N6-Methyladenosine by IGF2BP Proteins Enhances mRNA Stability and Translation. *Nat. Cell Biol.* 20 (3), 285–295. doi:10.1038/s41556-018-0045-z

Huang, J., Shao, Y., and Gu, W. (2021a). Function and Clinical Significance of N6-Methyladenosine in Digestive System Tumours. *Exp. Hematol. Oncol.* 10 (1), 40. doi:10.1186/s40164-021-00234-1

Huang, S., Luo, S., Gong, C., Liang, L., Xiao, Y., Li, M., et al. (2021b). MTTL3 Upregulates microRNA-1246 to Promote Occurrence and Progression of NSCLC via Targeting Paternally Expressed Gene 3. *Mol. Ther. Nucleic Acids* 24, 542–553. doi:10.1016/j.omtn.2021.02.020

Huang, W., Kong, F., Li, R., Chen, X., and Wang, K. (2022). Emerging Roles of m6A RNA Methylation Regulators in Gynecological Cancer. *Front. Oncol.* 12, 827956. doi:10.3389/fonc.2022.827956

Huang, Y., Hou, Y., Qu, P., and Cai, Y. (2021c). Editorial: Combating Cancer with Natural Products: What Would Non-coding RNAs Bring? *Front. Oncol.* 11, 747586. doi:10.3389/fonc.2021.747586

Imai-Sumida, M., Dasgupta, P., Kulkarni, P., Shiina, M., Hashimoto, Y., Shahryari, V., et al. (2020). Genistein Represses HOTAIR/Chromatin Remodeling Pathways to Suppress Kidney Cancer. *Cell Physiol. Biochem.* 54 (1), 53–70. doi:10.33594/000000205

Imran, M., Saeed, F., Gilani, S. A., Shariati, M. A., Imran, A., Afzaal, M., et al. (2021). Fisetin: An Anticancer Perspective. *Food Sci. Nutr.* 9 (1), 3–16. doi:10.1002/fsn3.1872

Inada, T. (2020). Quality Controls Induced by Aberrant Translation. *Nucleic Acids Res.* 48 (3), 1084–1096. doi:10.1093/nar/gkz1201

Jia, G., Fu, Y., Zhao, X., Dai, Q., Zheng, G., Yang, Y., et al. (2011). N6-methyladenosine in Nuclear RNA Is a Major Substrate of the Obesity-Associated FTO. *Nat. Chem. Biol.* 7 (12), 885–887. doi:10.1038/nchembio.687

Jiang, H., Yao, Q., An, Y., Fan, L., Wang, J., and Li, H. (2022). Baicalin Suppresses the Progression of Type 2 Diabetes-Induced Liver Tumor through Regulating METTL3/m6A/HKDC1 axis and Downstream P-JAK2/STAT1/clevaged Capase3 Pathway. *Phytomedicine* 94, 153823. doi:10.1016/j.phymed.2021.153823

Jiang, W., Li, X., Dong, S., and Zhou, W. (2021a). Betulinic Acid in the Treatment of Tumour Diseases: Application and Research Progress. *Biomed. Pharmacother.* 142, 111990. doi:10.1016/j.biopha.2021.111990

Jiang, X., Liu, B., Nie, Z., Duan, L., Xiong, Q., Jin, Z., et al. (2021b). The Role of m6A Modification in the Biological Functions and Diseases. *Sig Transduct. Target Ther.* 6 (1), 74. doi:10.1038/s41392-020-00450-x

Jiang, Z., Jacob, J. A., Loganathachetti, D. S., Nainangu, P., and Chen, B. (2017). β-Elemene: Mechanistic Studies on Cancer Cell Interaction and its Chemosensitization Effect. *Front. Pharmacol.* 8, 105. doi:10.3389/fphar.2017.00105

Jin, D., Guo, J., Wu, Y., Du, J., Yang, L., Wang, X., et al. (2019). m6A mRNA Methylation Initiated by METTL3 Directly Promotes YAP Translation and Increases YAP Activity by Regulating the MALAT1-miR-1914-3p-YAP axis to Induce NSCLC Drug Resistance and Metastasis. *J. Hematol. Oncol.* 12 (1), 135. doi:10.1186/s13045-019-0830-6

Kan, L., Grozhik, A. V., Vedanayagam, J., Patil, D. P., Pang, N., Lim, K. S., et al. (2017). The m6A Pathway Facilitates Sex Determination in Drosophila. *Nat. Commun.* 8, 15737. doi:10.1038/ncomms15737

Kaushik, S., Shyam, H., Sharma, R., and Balapure, A. K. (2018). Dietary Isoflavone Daidzein Synergizes Centchroman Action via Induction of Apoptosis and Inhibition of PI3K/Akt Pathway in MCF-7/MDA MB-231 Human Breast Cancer Cells. *Phytomedicine* 40, 116–124. doi:10.1016/j.phymed.2018.01.007

Kobayashi, M., Ohsugi, M., Sasako, T., Awazawa, M., Umehara, T., Iwane, A., et al. (2018). The RNA Methyltransferase Complex of WTAP, METTL3, and METTL14 Regulates Mitotic Clonal Expansion in Adipogenesis. *Mol. Cell Biol.* 38 (16), e00116–18. doi:10.1128/MCB.00116-18

Koh, C. W. Q., Goh, Y. T., and Goh, W. S. S. (2019). Atlas of Quantitative single-Base-Resolution N6-Methyl-Adenine Methylomes. *Nat. Commun.* 10 (1), 5636. doi:10.1038/s41467-019-13561-z

Lai, W., Jia, J., Yan, B., Jiang, Y., Shi, Y., Chen, L., et al. (2018). Baicalin Hydrate Inhibits Cancer Progression in Nasopharyngeal Carcinoma by Affecting Genome Instability and Splicing. *Oncotarget* 9 (1), 901–914. doi:10.18632/oncotarget.22868

Lan, T., Li, H., Zhang, D., Xu, L., Liu, H., Hao, X., et al. (2019). KIAA1429 Contributes to Liver Cancer Progression through N6-methyladenosine-dependent Post-transcriptional Modification of GATA3. *Mol. Cancer* 18 (1), 186. doi:10.1186/s12943-019-1106-z

Li, J., Wang, F., Liu, Y., Wang, H., and Ni, B. (2021). N6-methyladenosine (m6A) in Pancreatic Cancer: Regulatory Mechanisms and Future Direction. *Int. J. Biol. Sci.* 17 (9), 2323–2335. doi:10.7150/ijbs.60115

Li, L., Dong, Z., Shi, P., Tan, L., Xu, J., Huang, P., et al. (2020a). Bruceine D Inhibits Cell Proliferation through Downregulating LINC01667/MicroRNA-138-5p/Cyclin E1 Axis in Gastric Cancer. *Front. Pharmacol.* 11, 584960. doi:10.3389/fphar.2020.584960

Li, N., Kang, Y., Wang, L., Huff, S., Tang, R., Hui, H., et al. (2020b). ALKBH5 Regulates Anti-PD-1 Therapy Response by Modulating Lactate and Suppressive Immune Cell Accumulation in Tumor Microenvironment. *Proc. Natl. Acad. Sci. U. S. A.* 117 (33), 20159–20170. doi:10.1073/pnas.1918986117

Li, Q. Q., Lee, R. X., Liang, H., Wang, G., Li, J. M., Zhong, Y., et al. (2013). β-Elemene Enhances Susceptibility to Cisplatin in Resistant Ovarian Carcinoma Cells via Downregulation of ERCC-1 and XIAP and Inactivation of JNK. *Int. J. Oncol.* 43 (3), 721–728. doi:10.3892/ijo.2013.1996

Li, W., Hu, X., Wang, S., Jiao, Z., Sun, T., Liu, T., et al. (2020c). Characterization and Anti-tumor Bioactivity of astragalus Polysaccharides by Immunomodulation. *Int. J. Biol. Macromol.* 145, 985–997. doi:10.1016/j.ijbiomac.2019.09.189

Li, Z., Weng, H., Su, R., Weng, X., Zuo, Z., Li, C., et al. (2017). FTO Plays an Oncogenic Role in Acute Myeloid Leukemia as a N6-Methyladenosine RNA Demethylase. *Cancer Cell* 31 (1), 127–141. doi:10.1016/j.ccell.2016.11.017

Liang, D., Lin, W. J., Ren, M., Qiu, J., Yang, C., Wang, X., et al. (2021). m6A Reader YTHDC1 Modulates Autophagy by Targeting SQSTM1 in Diabetic Skin. *Autophagy* 1, 1–20. doi:10.1080/15548627.2021.1974175

Lin, S., Choe, J., Du, P., Triboulet, R., and Gregory, R. I. (2016). The M(6)A Methyltransferase METTL3 Promotes Translation in Human Cancer Cells. *Mol. Cell* 62 (3), 335–345. doi:10.1016/j.molcel.2016.03.021

Liu, M., Zhao, G., Cao, S., Zhang, Y., Li, X., and Lin, X. (2016). Development of Certain Protein Kinase Inhibitors with the Components from Traditional Chinese Medicine. *Front. Pharmacol.* 7, 523. doi:10.3389/fphar.2016.00523

Liu, N., Zhou, K. I., Parisien, M., Dai, Q., Diatchenko, L., and Pan, T. (2017). N6-methyladenosine Alters RNA Structure to Regulate Binding of a Low-Complexity Protein. *Nucleic Acids Res.* 45 (10), 6051–6063. doi:10.1093/nar/gkx141

Liu, S., Li, Q., Li, G., Zhang, Q., Zhuo, L., Han, X., et al. (2020). The Mechanism of m6A Methyltransferase METTL3-Mediated Autophagy in Reversing Gefitinib Resistance in NSCLC Cells by β-elemene. *Cell Death Dis.* 11 (11), 969. doi:10.1038/s41419-020-03148-8

Luo, X., Cao, M., Gao, F., and He, X. (2021). YTHDF1 Promotes Hepatocellular Carcinoma Progression via Activating PI3K/AKT/mTOR Signaling Pathway and Inducing Epithelial-Mesenchymal Transition. *Exp. Hematol. Oncol.* 10 (1), 35. doi:10.1186/s40164-021-00227-0

Ma, J. Z., Yang, F., Zhou, C. C., Liu, F., Yuan, J. H., Wang, F., et al. (2017). METTL14 Suppresses the Metastatic Potential of Hepatocellular Carcinoma by Modulating N6 -methyladenosine-dependent Primary MicroRNA Processing. *Hepatology* 65 (2), 529–543. doi:10.1002/hep.28885

Ma, L., Chen, T., Zhang, X., Miao, Y., Tian, X., Yu, K., et al. (2021a). The m6A Reader YTHDC2 Inhibits Lung Adenocarcinoma Tumorigenesis by Suppressing SLC7A11-dependent Antioxidant Function. *Redox Biol.* 38, 101801. doi:10.1016/j.redox.2020.101801

Ma, L., Zhang, X., Yu, K., Xu, X., Chen, T., Shi, Y., et al. (2021b). Targeting SLC3A2 Subunit of System XC- Is Essential for m6A Reader YTHDC2 to Be an Endogenous Ferroptosis Inducer in Lung Adenocarcinoma. *Free Radic. Biol. Med.* 168, 25–43. doi:10.1016/j.freeradbiomed.2021.03.023

Merritt, D. C., Jamnik, J., and El-Sohemy, A. (2018). FTO Genotype, Dietary Protein Intake, and Body Weight in a Multiethnic Population of Young Adults: a Cross-Sectional Study. *Genes Nutr.* 13, 4. doi:10.1186/s12263-018-0593-7

Meyer, K. D. (2019). DART-seq: an Antibody-free Method for Global m6A Detection. *Nat. Methods* 16 (12), 1275–1280. doi:10.1038/s41592-019-0570-0

Mortezaee, K., Salehi, E., Mirtavoos-Mahyari, H., Motevaseli, E., Najafi, M., Farhood, B., et al. (2019). Mechanisms of Apoptosis Modulation by Curcumin: Implications for Cancer Therapy. *J. Cell Physiol.* 234 (8), 12537–12550. doi:10.1002/jcp.28122

Müller, S., Glass, M., Singh, A. K., Haase, J., Bley, N., Fuchs, T., et al. (2019). IGF2BP1 Promotes SRF-dependent Transcription in Cancer in a m6A- and miRNA-dependent Manner. *Nucleic Acids Res.* 47 (1), 375–390. doi:10.1093/nar/gky1012

Nettersheim, D., Berger, D., Jostes, S., Kristiansen, G., Lochnit, G., and Schorle, H. (2019). N6-Methyladenosine Detected in RNA of Testicular Germ Cell Tumors Is Controlled by METTL3, ALKBH5, YTHDC1/F1/F2, and HNRNPC as Writers, Erasers, and Readers. *Andrology* 7 (4), 498–506. doi:10.1111/andr.12612

Newman, D. J., and Cragg, G. M. (2016). Natural Products as Sources of New Drugs from 1981 to 2014. *J. Nat. Prod.* 79 (3), 629–661. doi:10.1021/acs.jnatprod.5b01055

Ning, Y., Chen, J., Shi, Y., Song, N., Yu, X., Fang, Y., et al. (2020). Genistein Ameliorates Renal Fibrosis through Regulation Snail via m6A RNA Demethylase ALKBH5. *Front. Pharmacol.* 11, 579265. doi:10.3389/fphar.2020.579265

Niu, Y., Lin, Z., Wan, A., Chen, H., Liang, H., Sun, L., et al. (2019). RNA N6-Methyladenosine Demethylase FTO Promotes Breast Tumor Progression through Inhibiting BNIP3. *Mol. Cancer* 18 (1), 46. doi:10.1186/s12943-019-1004-4

Niu, Y., Wan, A., Lin, Z., Lu, X., and Wan, G. (2018). N 6-Methyladenosine Modification: a Novel Pharmacological Target for Anti-cancer Drug Development. *Acta Pharm. Sin. B* 8 (6), 833–843. doi:10.1016/j.apsb.2018.06.001

Onitsuka, T., Uramoto, H., Nose, N., Takenoyama, M., Hanagiri, T., Sugio, K., et al. (2010). Acquired Resistance to Gefitinib: the Contribution of Mechanisms Other Than the T790M, MET, and HGF Status. *Lung Cancer* 68 (2), 198–203. doi:10.1016/j.lungcan.2009.05.022

Pan, H., Pan, Z., Guo, F., Meng, F., Zu, L., Fan, Y., et al. (2021a). MicroRNA-1915-3p Inhibits Cell Migration and Invasion by Targeting SET in Non-small-cell Lung Cancer. *BMC Cancer* 21 (1), 1218. doi:10.1186/s12885-021-08961-8

Pan, Z. P., Wang, B., Hou, D. Y., You, R. L., Wang, X. T., Xie, W. H., et al. (2021b). METTL3 Mediates Bone Marrow Mesenchymal Stem Cell Adipogenesis to Promote Chemoresistance in Acute Myeloid Leukaemia. *FEBS Open Bio* 11 (6), 1659–1672. doi:10.1002/2211-5463.13165

Park, S., Bazer, F. W., Lim, W., and Song, G. (2018). The O-Methylated Isoflavone, Formononetin, Inhibits Human Ovarian Cancer Cell Proliferation by Sub G0/G1 Cell Phase Arrest through PI3K/AKT and ERK1/2 Inactivation. *J. Cell Biochem.* 119 (9), 7377–7387. doi:10.1002/jcb.27041

Patil, D. P., Chen, C. K., Pickering, B. F., Chow, A., Jackson, C., Guttman, M., et al. (2016). m(6)A RNA Methylation Promotes XIST-Mediated Transcriptional Repression. *Nature* 537 (7620), 369–373. doi:10.1038/nature19342

Ping, X. L., Sun, B. F., Wang, L., Xiao, W., Yang, X., Wang, W. J., et al. (2014). Mammalian WTAP Is a Regulatory Subunit of the RNA N6-Methyladenosine Methyltransferase. *Cell Res.* 24 (2), 177–189. doi:10.1038/cr.2014.3

Pu, J., Wang, J., Qin, Z., Wang, A., Zhang, Y., Wu, X., et al. (2020). IGF2BP2 Promotes Liver Cancer Growth through an m6A-FEN1-dependent Mechanism. *Front. Oncol.* 10, 578816. doi:10.3389/fonc.2020.578816

Qian, X., Yang, J., Qiu, Q., Li, X., Jiang, C., Li, J., et al. (2021a). LCAT3, a Novel m6A-Regulated Long Non-coding RNA, Plays an Oncogenic Role in Lung Cancer via Binding with FUBP1 to Activate C-MYC. *J. Hematol. Oncol.* 14 (1), 112. doi:10.1186/s13045-021-01123-0

Qian, Y., Wang, R., Wei, W., Wang, M., and Wang, S. (2021b). Resveratrol Reverses the Cadmium-Promoted Migration, Invasion, and Epithelial-Mesenchymal Transition Procession by Regulating the Expression of ZEB1. *Hum. Exp. Toxicol.* 40 (12_Suppl. l), S331–S338. doi:10.1177/09603271211041678

Rauf, A., Imran, M., Butt, M. S., Nadeem, M., Peters, D. G., and Mubarak, M. S. (2018a). Resveratrol as an Anti-cancer Agent: A Review. *Crit. Rev. Food Sci. Nutr.* 58 (9), 1428–1447. doi:10.1080/10408398.2016.1263597

Rauf, A., Imran, M., Khan, I. A., Ur-Rehman, M., Gilani, S. A., Mehmood, Z., et al. (2018b). Anticancer Potential of Quercetin: A Comprehensive Review. *Phytother. Res.* 32 (11), 2109–2130. doi:10.1002/ptr.6155

Ren, W., Lu, J., Huang, M., Gao, L., Li, D., Wang, G. G., et al. (2019). Structure and Regulation of ZCCHC4 in m6A-Methylation of 28S rRNA. *Nat. Commun.* 10 (1), 5042. doi:10.1038/s41467-019-12923-x

Rong, B., Zhang, Q., Wan, J., Xing, S., Dai, R., Li, Y., et al. (2020). Ribosome 18S m6A Methyltransferase METTL5 Promotes Translation Initiation and Breast Cancer Cell Growth. *Cell Rep.* 33 (12), 108544. doi:10.1016/j.celrep.2020.108544

Roundtree, I. A., Evans, M. E., Pan, T., and He, C. (2017a). Dynamic RNA Modifications in Gene Expression Regulation. *Cell* 169 (7), 1187–1200. doi:10.1016/j.cell.2017.05.045

Roundtree, I. A., Luo, G. Z., Zhang, Z., Wang, X., Zhou, T., Cui, Y., et al. (2017b). YTHDC1 Mediates Nuclear Export of N6-Methyladenosine Methylated mRNAs. *Elife* 6, e31311. doi:10.7554/eLife.31311

Roy, S., Sil, A., and Chakraborty, T. (2019). Potentiating Apoptosis and Modulation of P53, Bcl2, and Bax by a Novel Chrysin Ruthenium Complex for Effective Chemotherapeutic Efficacy against Breast Cancer. *J. Cell Physiol.* 234 (4), 4888–4909. doi:10.1002/jcp.27287

Sahm, B. D. B., Peres, J., Rezende-Teixeira, P., Santos, E. A., Branco, P. C., Bauermeister, A., et al. (2020). Targeting the Oncogenic TBX2 Transcription Factor with Chromomycins. *Front. Chem.* 8, 110. doi:10.3389/fchem.2020.00110

Sang, L., Wu, X., Yan, T., Naren, D., Liu, X., Zheng, X., et al. (2022). The m6A RNA Methyltransferase METTL3/METTL14 Promotes Leukemogenesis through the Mdm2/p53 Pathway in Acute Myeloid Leukemia. *J. Cancer* 13 (3), 1019–1030. doi:10.7150/jca.60381

Sarkar, F. H., Adsule, S., Padhye, S., Kulkarni, S., and Li, Y. (2006). The Role of Genistein and Synthetic Derivatives of Isoflavone in Cancer Prevention and Therapy. *Mini Rev. Med. Chem.* 6 (4), 401–407. doi:10.2174/138955706776361439

Shah, A., Rashid, F., Awan, H. M., Hu, S., Wang, X., Chen, L., et al. (2017). The DEAD-Box RNA Helicase DDX3 Interacts with m6A RNA Demethylase ALKBH5. *Stem Cells Int.* 2017, 8596135. doi:10.1155/2017/8596135

Shen, C., Sheng, Y., Zhu, A. C., Robinson, S., Jiang, X., Dong, L., et al. (2020). RNA Demethylase ALKBH5 Selectively Promotes Tumorigenesis and Cancer Stem Cell Self-Renewal in Acute Myeloid Leukemia. *Cell Stem Cell* 27 (1), 64–e9. e69. doi:10.1016/j.stem.2020.04.009

Sheng, Y., Wei, J., Yu, F., Xu, H., Yu, C., Wu, Q., et al. (2021). A Critical Role of Nuclear m6A Reader YTHDC1 in Leukemogenesis by Regulating MCM Complex-Mediated DNA Replication. *Blood* 138 (26), 2838–2852. doi:10.1182/blood.2021011707

Shi, H., Wang, X., Lu, Z., Zhao, B. S., Ma, H., Hsu, P. J., et al. (2017). YTHDF3 Facilitates Translation and Decay of N6-Methyladenosine-Modified RNA. *Cell Res.* 27 (3), 315–328. doi:10.1038/cr.2017.15

Shi, H., Wei, J., and He, C. (2019). Where, when, and How: Context-dependent Functions of RNA Methylation Writers, Readers, and Erasers. *Mol. Cell* 74 (4), 640–650. doi:10.1016/j.molcel.2019.04.025

Shi, Y., Zheng, C., Jin, Y., Bao, B., Wang, D., Hou, K., et al. (2020). Reduced Expression of METTL3 Promotes Metastasis of Triple-Negative Breast Cancer by m6A Methylation-Mediated COL3A1 Up-Regulation. *Front. Oncol.* 10, 1126. doi:10.3389/fonc.2020.01126

Singla, R. K., Behzad, S., Khan, J., Tsagkaris, C., Gautam, R. K., Goyal, R., et al. (2022). Natural Kinase Inhibitors for the Treatment and Management of Endometrial/Uterine Cancer: Preclinical to Clinical Studies. *Front. Pharmacol.* 13, 801733. doi:10.3389/fphar.2022.801733

Singla, R. K., Sai, C. S., Chopra, H., Behzad, S., Bansal, H., Goyal, R., et al. (2021a). Natural Products for the Management of Castration-Resistant Prostate Cancer: Special Focus on Nanoparticles Based Studies. *Front. Cell Dev. Biol.* 9, 745177. doi:10.3389/fcell.2021.745177

Singla, R. K., Sharma, P., Dubey, A. K., Gundamaraju, R., Kumar, D., Kumar, S., et al. (2021b). Natural Product-Based Studies for the Management of Castration-Resistant Prostate Cancer: Computational to Clinical Studies. *Front. Pharmacol.* 12, 732266. doi:10.3389/fphar.2021.732266

Su, R., Dong, L., Li, Y., Gao, M., He, P. C., Liu, W., et al. (2022). METTL16 Exerts an m6A-independent Function to Facilitate Translation and Tumorigenesis. *Nat. Cell Biol.* 24 (2), 205–216. doi:10.1038/s41556-021-00835-2

Sun, C., Gao, W., Liu, J., Cheng, H., and Hao, J. (2020). FGL1 Regulates Acquired Resistance to Gefitinib by Inhibiting Apoptosis in Non-small Cell Lung Cancer. *Respir. Res.* 21 (1), 210. doi:10.1186/s12931-020-01477-y

Sun, T., Wu, R., and Ming, L. (2019). The Role of m6A RNA Methylation in Cancer. *Biomed. Pharmacother.* 112, 108613. doi:10.1016/j.biopha.2019.108613

Suthar, A. C., Banavalikar, M. M., and Biyani, M. K. (2001). Pharmacological Activities of Genistein, an Isoflavone from Soy (Glycine Max): Part I-AantiCcancer Activity. *Indian J. Exp. Biol.* 39 (6), 511–519.

Syed, D. N., Adhami, V. M., Khan, N., Khan, M. I., and Mukhtar, H. (2016). Exploring the Molecular Targets of Dietary Flavonoid Fisetin in Cancer. *Semin. Cancer Biol.* 40-41, 130–140. doi:10.1016/j.semcancer.2016.04.003

Tan, X., Fu, J., Yuan, Z., Zhu, L., and Fu, L. (2021). ACNPD: The Database for Elucidating the Relationships between Natural Products, Compounds, Molecular Mechanisms, and Cancer Types. *Front. Pharmacol.* 12, 746067. doi:10.3389/fphar.2021.746067

Tanabe, A., Tanikawa, K., Tsunetomi, M., Takai, K., Ikeda, H., Konno, J., et al. (2016). RNA Helicase YTHDC2 Promotes Cancer Metastasis via the Enhancement of the Efficiency by Which HIF-1α mRNA Is Translated. *Cancer Lett.* 376 (1), 34–42. doi:10.1016/j.canlet.2016.02.022

UniProt, C. (2019). UniProt: a Worldwide Hub of Protein Knowledge. *Nucleic Acids Res.* 47 (D1), D506–D515. doi:10.1093/nar/gky1049

Uyar, B., Yusuf, D., Wurmus, R., Rajewsky, N., Ohler, U., and Akalin, A. (2017). RCAS: an RNA Centric Annotation System for Transcriptome-wide Regions of Interest. *Nucleic Acids Res.* 45 (10), e91. doi:10.1093/nar/gkx120

van Tran, N., Ernst, F. G. M., Hawley, B. R., Zorbas, C., Ulryck, N., Hackert, P., et al. (2019). The Human 18S rRNA m6A Methyltransferase METTL5 Is Stabilized by TRMT112. *Nucleic Acids Res.* 47 (15), 7719–7733. doi:10.1093/nar/gkz619

Vu, L. P., Pickering, B. F., Cheng, Y., Zaccara, S., Nguyen, D., Minuesa, G., et al. (2017). The N6-Methyladenosine (m6A)-Forming Enzyme METTL3 Controls Myeloid Differentiation of Normal Hematopoietic and Leukemia Cells. *Nat. Med.* 23 (11), 1369–1376. doi:10.1038/nm.4416

Wan, W., Ao, X., Chen, Q., Yu, Y., Ao, L., Xing, W., et al. (2022). METTL3/IGF2BP3 axis Inhibits Tumor Immune Surveillance by Upregulating N6-Methyladenosine Modification of PD-L1 mRNA in Breast Cancer. *Mol. Cancer* 21 (1), 60. doi:10.1186/s12943-021-01447-y

Wang, C. Z., Zhang, C. F., Chen, L., Anderson, S., Lu, F., and Yuan, C. S. (2015). Colon Cancer Chemopreventive Effects of Baicalein, an Active Enteric Microbiome Metabolite from Baicalin. *Int. J. Oncol.* 47 (5), 1749–1758. doi:10.3892/ijo.2015.3173

Wang, H., Xu, B., and Shi, J. (2020a). N6-methyladenosine METTL3 Promotes the Breast Cancer Progression via Targeting Bcl-2. *Gene* 722, 144076. doi:10.1016/j.gene.2019.144076

Wang, H., Zhang, K., Liu, J., Yang, J., Tian, Y., Yang, C., et al. (2021). Curcumin Regulates Cancer Progression: Focus on ncRNAs and Molecular Signaling Pathways. *Front. Oncol.* 11, 660712. doi:10.3389/fonc.2021.660712

Wang, J., Li, Y., Wang, P., Han, G., Zhang, T., Chang, J., et al. (2020b). Leukemogenic Chromatin Alterations Promote AML Leukemia Stem Cells via a KDM4C-ALKBH5-AXL Signaling Axis. *Cell Stem Cell* 27 (1), 81–e8. e88. doi:10.1016/j.stem.2020.04.001

Wang, J., Wang, J., Gu, Q., Ma, Y., Yang, Y., Zhu, J., et al. (2020c). The Biological Function of m6A Demethylase ALKBH5 and its Role in Human Disease. *Cancer Cell Int.* 20, 347. doi:10.1186/s12935-020-01450-1

Wang, J. Y., Chen, L. J., and Qiang, P. (2020d). The Potential Role of N6-Methyladenosine (m6A) Demethylase Fat Mass and Obesity-Associated Gene (FTO) in Human Cancers. *Onco Targets Ther.* 13, 12845–12856. doi:10.2147/OTT.S283417

Wang, T., Kong, S., Tao, M., and Ju, S. (2020e). The Potential Role of RNA N6-Methyladenosine in Cancer Progression. *Mol. Cancer* 19 (1), 88. doi:10.1186/s12943-020-01204-7

Wang, Y., Chen, J., Gao, W. Q., and Yang, R. (2022). METTL14 Promotes Prostate Tumorigenesis by Inhibiting THBS1 via an m6A-YTHDF2-dependent Mechanism. *Cell Death Discov.* 8 (1), 143. doi:10.1038/s41420-022-00939-0

Wang, Y., Li, Y., Yue, M., Wang, J., Kumar, S., Wechsler-Reya, R. J., et al. (2018). N6-methyladenosine RNA Modification Regulates Embryonic Neural Stem Cell Self-Renewal through Histone Modifications. *Nat. Neurosci.* 21 (2), 195–206. doi:10.1038/s41593-017-0057-1

Wanna-Udom, S., Terashima, M., Lyu, H., Ishimura, A., Takino, T., Sakari, M., et al. (2020). The m6A Methyltransferase METTL3 Contributes to Transforming Growth Factor-Beta-Induced Epithelial-Mesenchymal Transition of Lung Cancer Cells through the Regulation of JUNB. *Biochem. Biophys. Res. Commun.* 524 (1), 150–155. doi:10.1016/j.bbrc.2020.01.042

Wei, J., Liu, F., Lu, Z., Fei, Q., Ai, Y., He, P. C., et al. (2018). Differential m6A, m6Am, and m1A Demethylation Mediated by FTO in the Cell Nucleus and Cytoplasm. *Mol. Cell* 71 (6), 973–e5. doi:10.1016/j.molcel.2018.08.011

Wong, A. S., Che, C. M., and Leung, K. W. (2015). Recent Advances in Ginseng as Cancer Therapeutics: a Functional and Mechanistic Overview. *Nat. Prod. Rep.* 32 (2), 256–272. doi:10.1039/c4np00080c

Wu, H., Li, F., and Zhu, R. (2021a). miR-338-5p Inhibits Cell Growth and Migration via Inhibition of the METTL3/m6A/c-Myc Pathway in Lung Cancer. *Acta Biochim. Biophys. Sin. (Shanghai)* 53 (3), 304–316. doi:10.1093/abbs/gmaa170

Wu, J., Gan, Z., Zhuo, R., Zhang, L., Wang, T., and Zhong, X. (2020). Resveratrol Attenuates Aflatoxin B1-Induced ROS Formation and Increase of m6A RNA Methylation. *Anim. (Basel)* 10 (4), 677. doi:10.3390/ani10040677

Wu, Y., Chang, N., Zhang, Y., Zhang, X., Xu, L., Che, Y., et al. (2021b). METTL3-mediated m6A mRNA Modification of FBXW7 Suppresses Lung Adenocarcinoma. *J. Exp. Clin. Cancer Res.* 40 (1), 90. doi:10.1186/s13046-021-01880-3

Wu, Y., Chen, X., Bao, W., Hong, X., Li, C., Lu, J., et al. (2022). Effect of Humantenine on mRNA m6A Modification and Expression in Human Colon Cancer Cell Line HCT116. *Genes* 13 (5), 781. doi:10.3390/genes13050781

Xia, Y., Lian, S., Khoi, P. N., Yoon, H. J., Han, J. Y., Chay, K. O., et al. (2015a). Chrysin Inhibits Cell Invasion by Inhibition of Recepteur D'origine Nantais via Suppressing Early Growth Response-1 and NF-Kb Transcription Factor Activities in Gastric Cancer Cells. *Int. J. Oncol.* 46 (4), 1835–1843. doi:10.3892/ijo.2015.2847

Xia, Y., Lian, S., Khoi, P. N., Yoon, H. J., Joo, Y. E., Chay, K. O., et al. (2015b). Chrysin Inhibits Tumor Promoter-Induced MMP-9 Expression by Blocking AP-1 via Suppression of ERK and JNK Pathways in Gastric Cancer Cells. *PLoS One* 10 (4), e0124007. doi:10.1371/journal.pone.0124007

Xiao, W., Adhikari, S., Dahal, U., Chen, Y. S., Hao, Y. J., Sun, B. F., et al. (2016). Nuclear M(6)A Reader YTHDC1 Regulates mRNA Splicing. *Mol. Cell* 61 (4), 507–519. doi:10.1016/j.molcel.2016.01.012

Xie, J., Ba, J., Zhang, M., Wan, Y., Jin, Z., and Yao, Y. (2021). The m6A Methyltransferase METTL3 Promotes the Stemness and Malignant Progression of Breast Cancer by Mediating m6A Modification on SOX2. *J. BUON* 26 (2), 444–449.

Xu, X., Zhou, E., Zheng, J., Zhang, C., Zou, Y., Lin, J., et al. (2021). Prognostic and Predictive Value of m6A "Eraser" Related Gene Signature in Gastric Cancer. *Front. Oncol.* 11, 631803. doi:10.3389/fonc.2021.631803

Xu, Y., Lv, D., Yan, C., Su, H., Zhang, X., Shi, Y., et al. (2022). METTL3 Promotes Lung Adenocarcinoma Tumor Growth and Inhibits Ferroptosis by Stabilizing SLC7A11 m6A Modification. *Cancer Cell Int.* 22 (1), 11. doi:10.1186/s12935-021-02433-6

Xu, Y., Ye, S., Zhang, N., Zheng, S., Liu, H., Zhou, K., et al. (2020). The FTO/miR-181b-3p/ARL5B Signaling Pathway Regulates Cell Migration and Invasion in Breast Cancer. *Cancer Commun. (Lond)* 40 (10), 484–500. doi:10.1002/cac2.12075

Xue, L., Li, J., Lin, Y., Liu, D., Yang, Q., Jian, J., et al. (2021). m6 A Transferase METTL3-Induced lncRNA ABHD11-AS1 Promotes the Warburg Effect of Non-small-cell Lung Cancer. *J. Cell Physiol.* 236 (4), 2649–2658. doi:10.1002/jcp.30023

Xue, M., Zhao, B. S., Zhang, Z., Lu, M., Harder, O., Chen, P., et al. (2019). Viral N6-Methyladenosine Upregulates Replication and Pathogenesis of Human Respiratory Syncytial Virus. *Nat. Commun.* 10 (1), 4595. doi:10.1038/s41467-019-12504-y

Yang, S., Wei, J., Cui, Y. H., Park, G., Shah, P., Deng, Y., et al. (2019). m6A mRNA Demethylase FTO Regulates Melanoma Tumorigenicity and Response to Anti-PD-1 Blockade. *Nat. Commun.* 10 (1), 2782. doi:10.1038/s41467-019-10669-0

Yang, Z., Yang, S., Cui, Y.-H., Wei, J., Shah, P., Park, G., et al. (2021). METTL14 Facilitates Global Genome Repair and Suppresses Skin Tumorigenesis. *Proc. Natl. Acad. Sci. U.S.A.* 118 (35), e2025948118. doi:10.1073/pnas.2025948118

Yao, L. C., Wu, L., Wang, W., Zhai, L. L., Ye, L., Xiang, F., et al. (2021). Panax Notoginseng Saponins Promote Cell Death and Chemosensitivity in Pancreatic Cancer through the Apoptosis and Autophagy Pathways. *Anticancer Agents Med. Chem.* 21 (13), 1680–1688. doi:10.2174/1871520620999201110191459

Yue, Y., Liu, J., Cui, X., Cao, J., Luo, G., Zhang, Z., et al. (2018). VIRMA Mediates Preferential m6A mRNA Methylation in 3'UTR and Near Stop Codon and Associates with Alternative Polyadenylation. *Cell Discov.* 4, 10. doi:10.1038/s41421-018-0019-0

Zannella, C., Rinaldi, L., Boccia, G., Chianese, A., Sasso, F. C., De Caro, F., et al. (2021). Regulation of m6A Methylation as a New Therapeutic Option against COVID-19. *Pharm. (Basel)* 14 (11), 1135. doi:10.3390/ph14111135

Zhang, C., Fu, J., and Zhou, Y. (2019). A Review in Research Progress Concerning m6A Methylation and Immunoregulation. *Front. Immunol.* 10, 922. doi:10.3389/fimmu.2019.00922

Zhang, H., Zhao, L., Li, S., Wang, J., Feng, C., Li, T., et al. (2021a). N6-Methylandenosine-Related lncRNAs in Tumor Microenvironment Are Potential Prognostic Biomarkers in Colon Cancer. *Front. Oncol.* 11, 697949. doi:10.3389/fonc.2021.697949

Zhang, X., Liu, X., Zhang, Y., Yang, A., Zhang, Y., Tong, Z., et al. (2021b). Wan-Nian-Qing, a Herbal Composite Prescription, Suppresses the Progression of Liver Cancer in Mice by Regulating Immune Response. *Front. Oncol.* 11, 696282. doi:10.3389/fonc.2021.696282

Zhang, Y., Liu, S., Zhao, T., and Dang, C. (2021c). METTL3-mediated m6A M-odification of Bcl-2 mRNA P-romotes N-on-small C-ell L-ung C-ancer P-rogression. *Oncol. Rep.* 46 (2), 163. doi:10.3892/or.2021.8114

Zhang, Y., and Yang, Y. (2021). Effects of m6A RNA Methylation Regulators on Endometrial Cancer. *J. Clin. Lab. Anal.* 35 (9), e23942. doi:10.1002/jcla.23942

Zhao, C., Ling, X., Xia, Y., Yan, B., and Guan, Q. (2021). The m6A Methyltransferase METTL3 Controls Epithelial-Mesenchymal Transition, Migration and Invasion of Breast Cancer through the MALAT1/miR-26b/HMGA2 axis. *Cancer Cell Int.* 21 (1), 441. doi:10.1186/s12935-021-02113-5

Zheng, G., Dahl, J. A., Niu, Y., Fedorcsak, P., Huang, C. M., Li, C. J., et al. (2013). ALKBH5 Is a Mammalian RNA Demethylase that Impacts RNA Metabolism and Mouse Fertility. *Mol. Cell* 49 (1), 18–29. doi:10.1016/j.molcel.2012.10.015

Zhou, R. T., He, M., Yu, Z., Liang, Y., Nie, Y., Tai, S., et al. (2017). Baicalein Inhibits Pancreatic Cancer Cell Proliferation and Invasion via Suppression of NEDD9 Expression and its Downstream Akt and ERK Signaling Pathways. *Oncotarget* 8 (34), 56351–56363. doi:10.18632/oncotarget.16912

Zhu, S., Wang, J. Z., Chen, D., He, Y. T., Meng, N., Chen, M., et al. (2020a). An Oncopeptide Regulates m6A Recognition by the m6A Reader IGF2BP1 and Tumorigenesis. *Nat. Commun.* 11 (1), 1685. doi:10.1038/s41467-020-15403-9

Zhu, W., Si, Y., Xu, J., Lin, Y., Wang, J. Z., Cao, M., et al. (2020b). Methyltransferase like 3 Promotes Colorectal Cancer Proliferation by Stabilizing CCNE1 mRNA in an m6A-dependent Manner. *J. Cell Mol. Med.* 24 (6), 3521–3533. doi:10.1111/jcmm.15042

Zou, S., Toh, J. D., Wong, K. H., Gao, Y. G., Hong, W., and Woon, E. C. (2016). N(6)-Methyladenosine: a Conformational Marker that Regulates the Substrate Specificity of Human Demethylases FTO and ALKBH5. *Sci. Rep.* 6, 25677. doi:10.1038/srep25677

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Song, Cui, Zhang, Yang, Liu, Miao, Zhao, Meng, Chen, Chen, Li, Shao, Zhang and Wang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers | Frontiers in Pharmacology

ORIGINAL RESEARCH
published: 12 July 2022
doi: 10.3389/fphar.2022.887387

Solamargine Inhibits the Development of Hypopharyngeal Squamous Cell Carcinoma by Decreasing LncRNA *HOXA11-As* Expression

Ying Meng[1†], Mengli Jin[1†], Dai Yuan[2†], Yicheng Zhao[1,3], Xiangri Kong[4], Xuerui Guo[5], Xingye Wang[2], Juan Hou[2], Bingmei Wang[1], Wu Song[1]* and Yong Tang[1]**

[1]College of Clinical Medicine, Changchun University of Chinese Medicine, Changchun, China, [2]College of Integrated Chinese and Western Medicine, College of Rehabilitation, Changchun University of Chinese Medicine, Changchun, China, [3]Center of Infections Diseases and Pathogen Biology, Key Laboratory of Organ Regeneration and Transplantation of the Ministry of Education, First Hospital of Jilin University, Changchun, China, [4]Affiliated Hospital to Changchun University of Chinese Medicine, Changchun University of Chinese Medicine, Changchun, China, [5]School of Pharmacy, Jilin University, Changchun, China

OPEN ACCESS

Edited by:
Yue Hou,
Northeastern University, China

Reviewed by:
Doan Duy Hai Tran,
Hannover Medical School, Germany
Shuqing Wang,
Shanghai Jiao Tong University School of Medicine, China
Enhong Xu,
Shanghai Changzheng Hospital, China
Bo Hu,
Fudan University, China

***Correspondence:**
Bingmei Wang
bingmeiwang1970@163.com
Wu Song
five841110@126.com
Yong Tang
15943012058@163.com

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

Received: 01 March 2022
Accepted: 09 June 2022
Published: 12 July 2022

Citation:
Meng Y, Jin M, Yuan D, Zhao Y, Kong X, Guo X, Wang X, Hou J, Wang B, Song W and Tang Y (2022) Solamargine Inhibits the Development of Hypopharyngeal Squamous Cell Carcinoma by Decreasing LncRNA HOXA11-As Expression. Front. Pharmacol. 13:887387. doi: 10.3389/fphar.2022.887387

Hypopharyngeal squamous cell carcinoma (HSCC) is one of the high mortality cancers with a poor prognosis, which is driving the development of new chemotherapeutic agents. We identified the anticancer effects of a natural compound, solamargine (SM), on FaDU cells and explored its mechanism in terms of non-coding RNA. It was observed that SM inhibited the proliferation of FaDU cells with an IC_{50} of 5.17 μM. High-throughput sequencing data revealed that lncRNA *HOXA11-AS* was significantly downregulated in cells co-incubated with SM. Further assays demonstrated that SM-induced downregulation of lncRNA *HOXA11-AS* showed important implications for apoptosis. Given the properties of *HOXA11-AS* as a *miR-155* sponge, we further confirmed that SM upregulated the expression of *miR-155* in FaDU cells. *C-Myc* is a transcription factor that regulates cell differentiation and apoptosis, whose mRNA is considered to be targeted by *miR-155*. We showed that *c-Myc* expression was downregulated by SM and accompanied by increased apoptosis, which was consistent with the findings of transcriptome sequencing. Furthermore, SM administration suppressed xenograft tumor growth in a xenograft mouse model *in vivo.* In the light of the aforementioned findings, our results suggested that SM downregulated the expression of *HOXA11-AS*, which in turn induces apoptosis by downregulating *c-Myc* in FaDU, providing evidence for the anticancer effect of SM on HSCC and uncovering the effect of SM on non-coding RNAs as, at least partly, a mechanism of action.

Keywords: solamargine, hypopharyngeal squamous cell carcinoma, HOXA11-AS, miR-155, c-Myc

INTRODUCTION

Hypopharyngeal cancer (HPC) is a subtype of head and neck cancer, which has a poor prognosis than other head and neck cancers, with an overall survival rate of only 20% (Hoffman et al., 1997; Iwae et al., 2017). In the United States, the annual incidence of HPC is approximately 3,000 cases per year, with the majority of patients in advanced/late stages (Kuo et al., 2014). Hypopharyngeal squamous cell carcinoma (HSCC) is a predominant histotype of HPC that accounts for 95% of the

total HPC types (Gang et al., 2022). There are significant regional variations in the epidemiological distribution of HSCC, with the highest incidence in South–Central Asia (Bray et al., 2018), which may be related to smoking, alcohol consumption, and dietary habits (Kwon and Miles, 2019). What is more worrying is that HSCC is prone to recurrence, and nearly 50% of patients recur within 1 year of diagnosis, often accompanied by metastatic (Hall et al., 2008).

LncRNA, a class of RNA transcripts greater than 200 bp length, has been a hot topic in tumor research (Peng et al., 2017). It is involved in the regulation of cancer development (Ijaz et al., 2018), including cell proliferation, migration, invasion, and apoptosis. *HOXA11-AS* (NCRNA 00076), the antisense strand of the *HOXA11* gene, regulates the growth and metastasis of cancer cells and is currently considered a cancer biomarker and therapeutic target (Xue et al., 2018). Recently, *HOXA11-AS* has been recognized to hold application potential as a circulating biomarker for early detection of HSCC, which promoted HSCC development by sponge to tumor suppressor *miR-155* (Xu et al., 2020).

Natural products have long been an essential source of anticancer agents (Rastelli et al., 2020). Solamargine (SM), a steroidal alkaloid glycoside derived from plants of the Solanum species such as *Solanum nigrum*, has been considered to carry the anticancer activity for decades (Kalalinia and Karimi-Sani, 2017). SM has shown potent anticancer effects exhibiting a very low IC_{50}, against a variety of cancers including liver cancer (Sani et al., 2015), lung cancer (Liang et al., 2004), breast cancer (Shiu et al., 2007), melanoma, prostate cancer (Xiang et al., 2016), colon cancer (Rastelli et al., 2020), and cervical cancer (Kalalinia and Karimi-Sani, 2017). One study comparing the sensitivity of SM to conventional chemotherapy agents on lung cancer cells showed that SM was the most sensitive of these chemotherapy agents including paclitaxel and cisplatin (Liu et al., 2004). In another study on human melanoma cancer cells, SM was shown to exert anticancer effects with a low impact on normal and benign WM35 cells, suggesting its selectivity for primary melanoma cells WM239 (Al Sinani et al., 2016). These characteristics of SM prompted us to explore its anticancer activity against HSCC and the involvement of non-protein-coding RNAs (ncRNA).

In the present study, we preliminarily evaluated the effect of SM on FaDU cell phenotypes. High-throughput sequencing was utilized to examine the regulation of lncRNAs by SM treatment (Booton and Lindsay, 2014). According to the vital role of lncRNA *HOXA11-AS* in HSCC and our data obtained from high-throughput sequencing, we revealed the involvement of *HOXA11-AS* in the inhibitory effect of SM on the proliferation of FaDU cells. These findings may contribute to the development of SM as an anticancer compound for HSCC applications.

MATERIALS AND METHODS

Cell Culture

The FaDU cell lines were obtained from the Affiliated Hospital of Changchun University of Chinese Medicine. FaDU cells were cultured in RPMI-1640 (Servicebio, Wuhan, China) supplemented with 10% fetal bovine serum (Servicebio, Wuhan, China), and 1% penicillin and streptomycin (Servicebio, Wuhan, China). The cells were cultured at 37°C in a 5% CO_2 incubator.

MTT Assay

The MTT assay was applied to determine the effect of SM on FaDU cell viability (Jin et al., 2014). FaDU cells were seeded into 96-well culture plates at 8,000 cells per well. The adherent cells were treated with 2 and 5 μM SM for 24, 48, and 72 h. After that, 10 μl of MTT solution (5 mg/ml) was added to each well and incubated at 37°C for 4 h. Then, the MTT solution was removed, and DMSO was used to dissolve formazan. The optical density ($OD_{490\ nm}$) value was detected using a microplate reader (Multiskan FC, Thermo Fisher) at 490 nm.

EdU Staining

The cell proliferation ability was detected by 5-ethynyl-2′-deoxyuridine (EdU) staining (Kazda et al., 2016). FaDU cells were plated into 24-well culture plates at a density of 1×10^4 cells/well. After 24 h, an equal volume of culture medium containing EdU (10 μM) was added to the cells and incubated for 2 h at 37°C, 5% CO_2. Cells were fixed and permeabilized following EdU kit (Beyotime, Beijing, China) according to the manufacturer's instructions. The cell nuclei were labeled with Hoechst 33342 stain. The results were photographed using an inverted fluorescence microscope, and the values of EdU-positive were analyzed by ImageJ software.

Live/Dead Cell Staining

Live/dead cell staining was performed to evaluate the effects of SM on FaDU cell viability. FaDU cells were cultured in 24-well plates at 5×10^4 cells per well. After treatment with SM (2 μM and 5 μM) for 24 h, cells were stained following the live/dead cell staining kit instructions (Beyotime, Beijing, China), at room temperature, away from light, for 30 min. Later, the cells were washed with phosphate-buffered saline (PBS). Fluorescence images were captured by inverted fluorescence microscopy (Leica, Wetzlar, Germany).

Transwell Assay

To examine the invasive ability of FaDU cells, we performed a transwell assay. In total, 1×10^5 cells per well were seeded in the upper compartment of Matrigel-coated invasion chambers in FBS-free media. Next, 500 μl RPMI-1640 medium containing 10% FBS was added to the lower chamber. After 24 h, migrated cells were fixed with 4% paraformaldehyde, stained with crystal violet, and counted with microscopy and ImageJ software.

Clone Formation Assay

For colony formation assay, FaDU cells were seeded in six-well cell culture plates at 2,000 cells per well overnight. Then, the medium was replaced by the culture medium containing 2 μM SM for 10 days. Every 3 days, the culture medium in the wells was replaced. All cells were washed with PBS, fixed with 4%

paraformaldehyde, and stained with crystal violet (Beyotime, Beijing, China). The images of the colony were photographed using the camera, and ImageJ was used to count them.

Wound Healing Assay

The migration activity of FaDU cells was detected using wound healing assay. FaDU cells were seeded at 6×10^5 cells per well of a six-well plate. When the cells reached 90% confluence, a 200-μL pipette tip was used to produce scratch wounds. Then, cells were treated with 2 and 5 μM SM. The area of the FaDU migrating into the wound was recorded with microscopy at time points of 0, 24, and 48 h and analyzed with ImageJ software.

Cell Cycle and Apoptosis Assay

FaDU cells were cultured at a density of 8×10^5 cells per well in six-well plates. Cells were then treated for 24 h with 2 and 5 μM SM. For assay of the cell cycle, Cell Cycle and Apoptosis Analysis Kit (Beyotime, Shanghai, China) was applied. Cells were stained according to the kit instructions after being fixed overnight at 4°C in 70% ethanol. Detection of experimental results was carried out using flow cytometry. For the assay of cell apoptosis, Annexin V-FITC apoptosis detection kit (Beyotime, Shanghai, China) was applied. The drug-treated cells were collected and stained according to the kit instructions. Detection of experimental results was carried out using flow cytometry and analysis with FlowJo software.

LncRNA-Seq and Data Analysis

FaDU cells were cultured in 10-cm Petri dishes. After attachment, cells were treated with 2 and 5 μM SM for 24 h. LncRNA sequencing was performed on total RNA from cells isolated with TRIzol solution. After removal of ribosomal RNA, the next-generation sequencing (NGS) library was constructed, and sequencing of the constructed libraries was performed with Illumina NovaSeq. Trim_galore (0.6.4) was used to remove spliced sequences, and sequences with QCs less than 25 from the raw sequencing data were read to obtain clean data. Clean data were aligned to the human genome (hg38) using HISAT2 (2.1.0). Transcripts were quantified and annotated using feature counts (1.3.3) to obtain counts for each transcript. Transcripts were normalized using DESeq2, and KEGG enrichment analysis was performed for differentially expressed genes. The KEGG enrichment analysis of differentially expressed genes was performed using the R package clusterProfiler. Tumor tissues refer to head and neck tumor tissues, and normal tissues refer to the tissues of patients without head and neck cancer. Gene expression data from patients with HPC and clinical data were downloaded from TCGA. The R package "survival" was used to calculate the survival rate of patients and construct Kaplan–Meier curves.

qRT-PCR

FaDU cells were cultured in 6-cm Petri dishes. After attachment, cells were treated with 2 and 5 μM SM for 24 h. Total RNA was extracted using TRIzol (Invitrogen). Then, using the SweScript All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Remover) (Servicebio, Wuhan, China) to synthesize cDNA according to the instructions. In addition, real-time fluorescent quantitative PCR was performed on a PikoReal 96 Real-Time PCR system using 2 × Fast SYBR Green qPCR Master Mix (None ROX) (Servicebio, Wuhan, China). GAPDH was used as an internal control, and the relative gene expression was calculated with the $2^{-\Delta\Delta Ct}$ method.

SiRNA Transfection

To further investigate the molecular mechanism of SM-regulated lncRNA in HSCC, we performed the knockdown assay. FaDU cells were cultured in 10-cm dishes. When cell confluency met approximately 75%, cells were transfected with *si-HOXA11-AS* or si-NC using Lipofectamine™ 3000 (Invitrogen). The expression level of lncRNA HOXA11-AS in transfected FaDU was verified by qPCR. The siRNA oligonucleotide sequences were 5′-AAUGAGAGAGUGUAAUCAAGAUUdTdT-3' (*si-HOXA11-AS*) and 5′-UCGUCAACGUUGCAUUGCGAUAUdTdT-3' (si-NC).

Dual-Luciferase Reporter Gene Assay

The luciferase reporter plasmid (lncRNA HOXA11-AS-WT or lncRNA HOXA11-AS-MUT) and miR-155 mimics were transfected into 293T cells, respectively. After 24 h of transfection, cells were lysed. Following the instructions of the Dual-Luciferase Reporter Gene Assay Kit (Beyotime, Shanghai, China), we set up different groups. After adding the firefly luciferase reaction solution, we detected the firefly luciferase fluorescence intensity using a microplate reader (Multiskan FC, Thermo Fisher). Then, we added the Renilla luciferase reaction solution and detected the Renilla luciferase fluorescence intensity.

Immunoblotting Assay

Drug-treated FaDU cells were collected, and the proteins were isolated by lysis in RIPA buffer. Subsequently, protein concentrations were determined using a BCA protein reagent assay kit (Beyotime, Shanghai, China). Next, 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed. Primary antibodies against c-Myc, P53, and anti-β-actin (Proteintech, 60004-1-Ig) were used overnight at 4°C. The secondary antibody was a goat anti-mouse HRP secondary antibody. The Western blot image was captured by Fusion FX Imaging systems (VILBER Lourmat, Collégien, France), and densitometric analysis was performed by ImageJ.

Xenograft Tumor Model

Six-week-old male BALB/c mice right axillae were shaved. FaDU cells (2×10^6) were injected subcutaneously into the right axilla of the mice. After 1 week of inoculation, 10 mice were randomly divided into two groups; the control group was injected with saline, and the SM group was injected with 10 mg/kg/d SM. Mice in each group were injected once daily for 30 days. The weight of the mice was recorded every 3 days. At the end of the treatment, the mice were sacrificed, the transplanted tumor was removed, and the tumor volume was recorded and weighed.

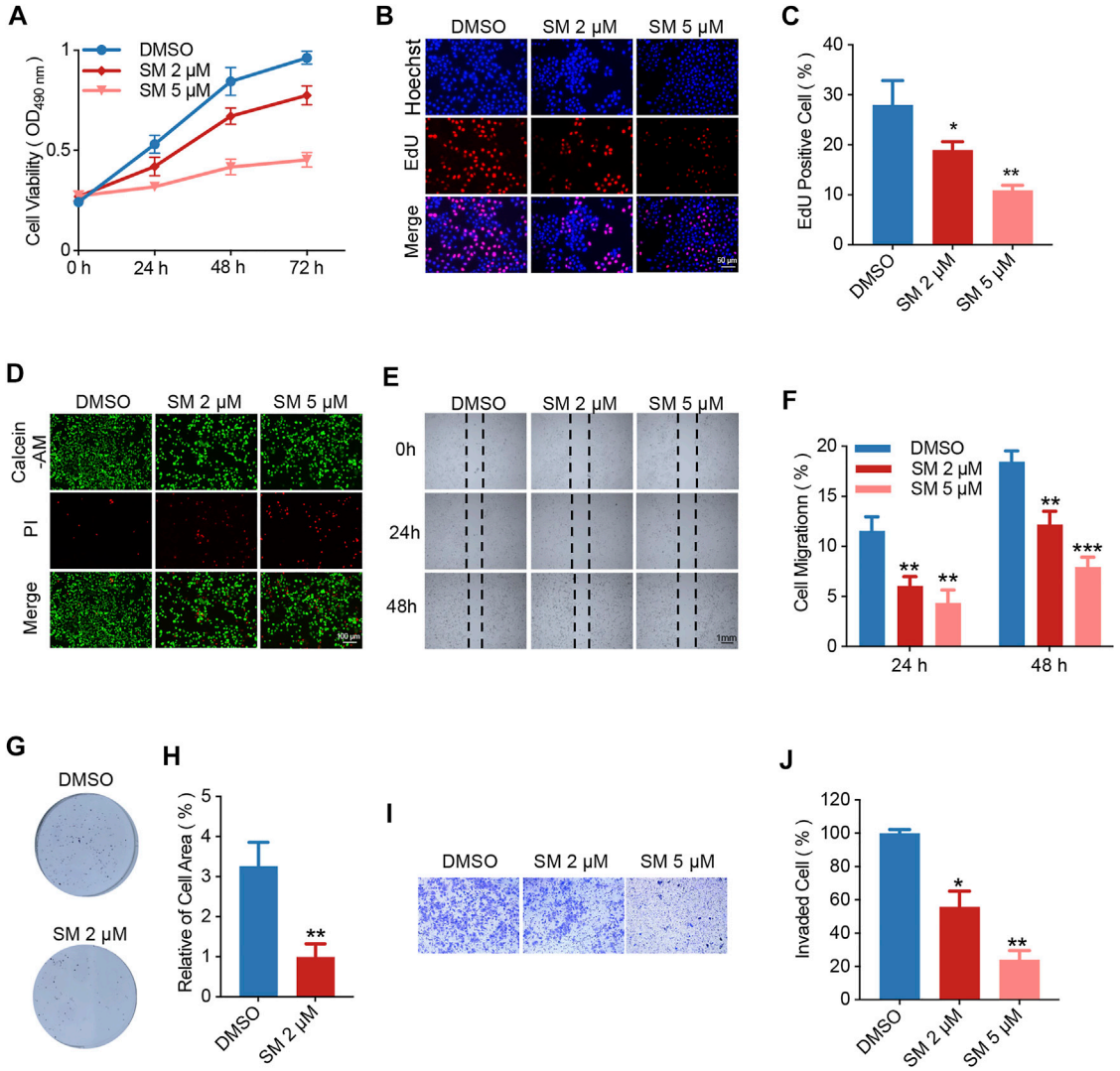


FIGURE 1 | SM Inhibits FaDU cell proliferative and migration ability. **(A)** Cell viability of FaDU cells treated with DMSO and 2 and 5 μM SM was analyzed by MTT assay at 0, 24, 48, and 72 h ($n = 5$). **(B,C)** EdU/Hoechst staining of FaDU cells treated with SM (2 and 5 μM) for 48 h. The ratio of EdU^+ cells calculated using ImageJ software was used to characterize cell proliferation ($n = 3$). **(D)** Calcein AM/PI staining of FaDU cells treated with SM (2 and 5 μM) for 48 h. **(E,F)** Cell migration of FaDU cells was tested by wound healing assay after treatment with DMSO and 2 and 5 μM SM, and the ratio was calculated by ImageJ software. **(G,H)** Colony formation of FaDU cells was performed with DMSO and 2 and 5 μM SM. Relative of cell area statistic with ImageJ software. **(I,J)** Cell invasion was analyzed using the transwell assay of FaDU cells treated with DMSO and 2 and 5 μM SM. The relative migration rate was calculated with ImageJ software. *($p < 0.05$), **($p < 0.01$), and ***($p < 0.001$) indicate statistically significant differences.

Statistical Analysis

Data were statistically analyzed with GraphPad Prism 9.3.0. All experiments were performed in triplicate. Data are expressed as mean ± SD, and a two-sided $p < 0.05$ was considered significant.

RESULTS

SM Inhibits FaDU Cell Proliferative and Migration Ability

In order to clarify the cytotoxic effect of SM on FaDU cells, the MTT assay was carried out on FaDU cells treated with SM (2 and 5 μM) for 72 h. The results showed that SM significantly inhibited the cell viability in a dose-dependent and time-dependent manner, and 5 μM SM inhibited cell proliferation by 50.68% for 48 h in MTT assays (**Figure 1A**). The IC_{50} (48 h) value of SM was 5.17 μM. EdU staining showed that novel proliferating cells were significantly reduced after SM treatment, as exhibited by the red fluorescence of EdU-positive cells attenuated in SM-treated FaDU cells (**Figures 1B,C**). The cytotoxic effect of SM on FaDU cells was then demonstrated by Calcein-AM/propidium iodide (PI) staining, as shown by an increase in red fluorescence of dead cells labeled by PI (**Figure 1D**). To evaluate the effect of SM on the migratory capacity of cells, wound healing assay was performed. We observed that SM

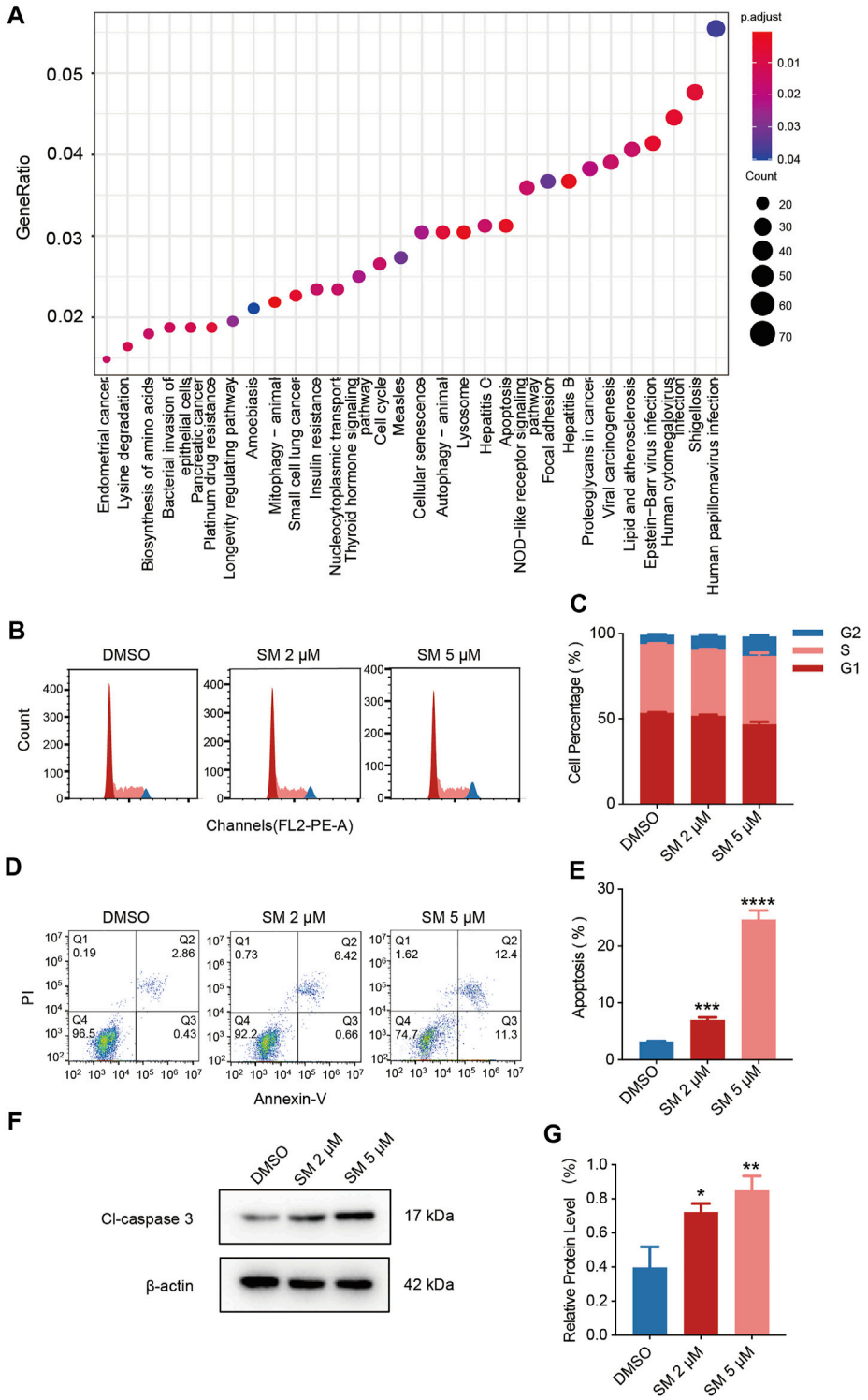


FIGURE 2 | SM mediates apoptosis and cycle arrest in FaDU cells. **(A)** Enriched KEGG pathways of differentially expressed mRNAs in FaDU cells after 5 μM SM treatment. p-value < 0.05. **(B,C)** Cell cycle assay was examined by flow cytometry in FaDU cells treated with DMSO and 2 and 5 μM SM. The result was analyzed using FlowJo software. **(D,E)** Apoptosis of FaDU cells treated with DMSO and 2 and 5 μM SM was analyzed using flow cytometry, and the total apoptosis proportion was calculated by FlowJo software. **(F,G)** Effect of SM on apoptosis determined by the level of the cleaved caspase-3 protein. *(p < 0.05), **(p < 0.01), ***(p < 0.001), and ****(p < 0.0001) indicate statistically significant differences.

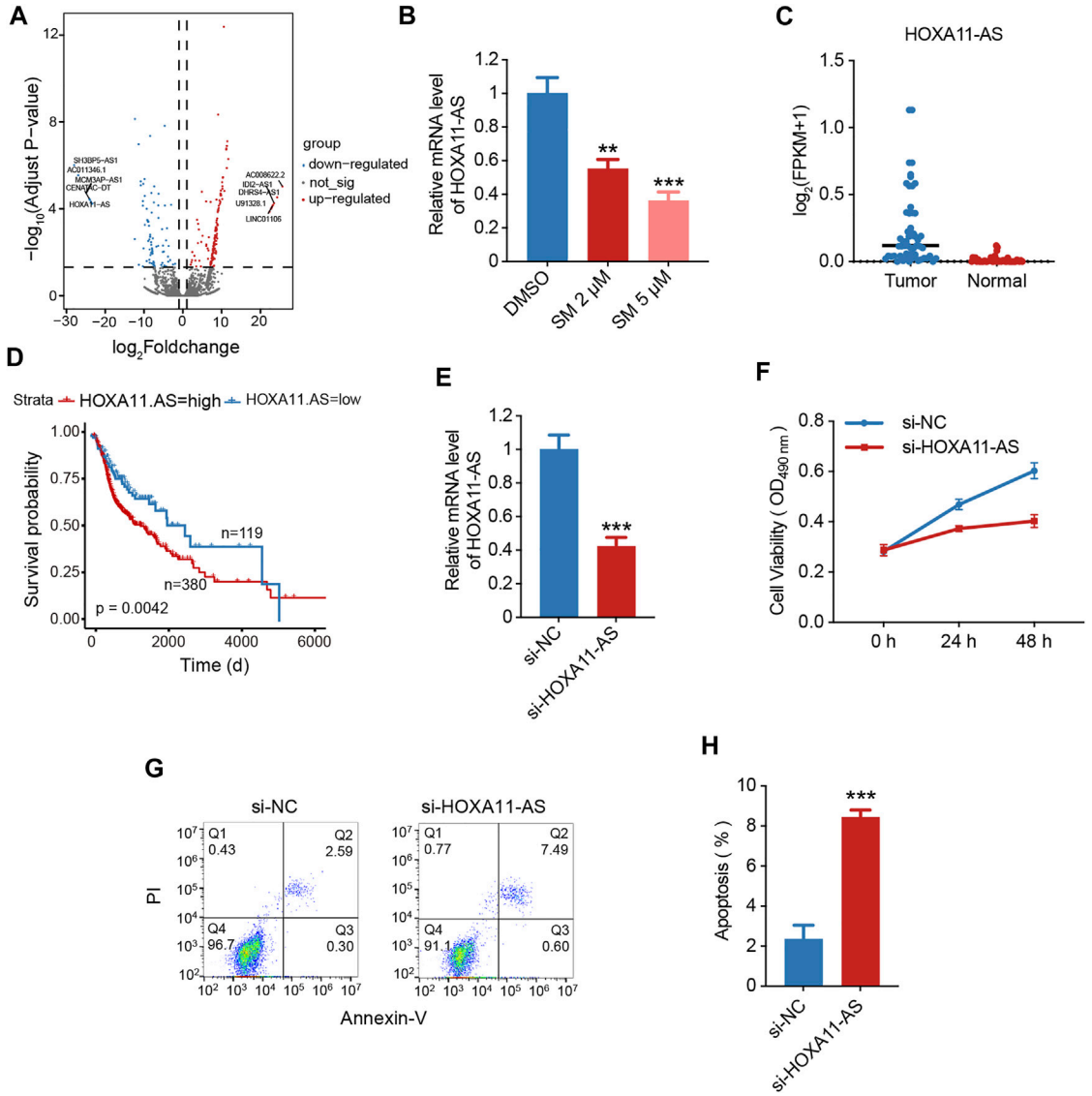


FIGURE 3 | SM suppresses the expression of lncRNA *HOXA11-AS,* which is responsible for the proliferation of FaDU cells. **(A)** Volcano plot shows differentially expressed lncRNAs in FaDU cells treated with DMSO and 5 μM SM. Blue dots represent the downregulated lncRNAs, while red dots represent the upregulated ones. **(B)** Relative expression of lncRNA *HOXA11-AS* was analyzed by RT-PCR after DMSO and 2 and 5 μM SM treatment in FaDU cells. **(C)** Expression of *HOXA1-AS* in head and neck tumor tissue and in normal tissue. The cut-off value for low and high *HOXA11-AS* expression was 0.03143. **(D)** Impact of lncRNA *HOXA11-AS* expression levels on survival of patients with hypopharyngeal cancer in TCGA database. **(E)** Relative expression of lncRNA *HOXA11-AS* in FaDU cells transfected with *si-Nc* and *si-HOXA11-AS* was detected by RT-PCR. **(F)** Cell viability of FaDU cells transfected with *si-Nc* and *si-HOXA11-AS* was analyzed by MTT assay at 0, 24, and 48 h ($n = 5$). **(G,H)** Apoptosis of FaDU cells transfected with *si-Nc* and *si-HOXA11-AS* was analyzed by flow cytometry, and the total apoptotic percentage was calculated using FlowJo software. **($p < 0.01$) and ***($p < 0.001$) indicated statistically significant differences.

inhibited cell migration and that SM (2 and 5 μM) increased the area of the wound compared to the DMSO group, indicating that cell migration was stifled by SM (**Figures 1E,F**). Clonogenic assays were performed to examine the impact of SM on the tumorigenicity of FaDU cells. The results showed that 2 μM SM greatly reduced the number of cell clones compared with the DMSO group, whereas FaDU cells treated with 5 μM had almost no clones visible after 10 days of incubation (data not shown) (**Figures 1G,H**). To examine the effect of SM on the invasion and migration ability of FaDU, the transwell assay was conducted. The results showed that SM (2 and 5 μM, for 24 h) had a significant inhibitory effect on the invasion ability of FaDU (**Figures 1I,J**). In summary, we verified for the first time the killing effect of SM on FaDU cells as well as its effect on cell migration and invasion capacity.

SM Mediates Apoptosis and Cycle Arrest in FaDU Cells

To uncover the potential mechanism of SM, we performed transcriptome sequencing and lncRNA high-throughput

sequencing of 5 μM SM-treated FaDU. KEGG and GO enrichment analyses were performed on transcriptome sequencing results, in which a multitude of altered cellular pathways, apoptosis, and cycle arrest were floated. In the following assays, we examined the effect of SM on apoptosis and cycle in FaDU cells by flow cytometry (**Figure 2A**). Flow cytometry results for the cell cycle showed that the proportion of cells in the G2 phase increased and that of G1 phase cells decreased in SM-treated FaDU cells, indicating that SM resulted in the G2 phase block in FaDU cells (**Figures 2B,C**). The proportion of apoptosis in FaDU cells was increased by SM treatment, in which the proportion of early apoptosis increased (Q3) from 0.41 ± 0.02% to 11.73 ± 0.91% while that of late apoptosis (Q2) increased from 2.80 ± 0.06% to 12.93 ± 0.45% in FaDU cells treated with 5 μM SM for 48 h (**Figures 2D,E**).

SM Suppresses the Expression of LncRNA *HOXA11-As,* Which Is Responsible for the Proliferation of FaDU Cells

Regarding lncRNA sequencing, the sequencing data showed that 244 differentially expressed lncRNAs were found in the SM-treated FaDU cells compared to that of untreated cells, of which 94 lncRNAs were downregulated and 150 lncRNAs were upregulated. From the significantly downregulated lncRNA, we focused on the *HOXA1-AS* (**Figure 3A**). Previous studies have suggested that *HOXA11-AS* is located in the *HOXA* gene cluster and its aberrant expression plays an important role in the development of various cancers such as non-small cell lung cancer, hepatocellular carcinoma, gastric cancer, and breast cancer (Wei et al., 2020). We performed the qRT-PCR assay for validation of lncRNA high-throughput sequencing findings, showing that SM was able to reduce *HOXA1-AS* expression in FaDU (**Figure 3B**). According to The Cancer Genome Atlas (TCGA) database, it was shown that the expression of *HOXA1-AS* was higher in head and neck tumor tissue than that in normal tissue (**Figure 3C**). Furthermore, patients with hypopharyngeal cancer with high *HOXA1-AS* expression were associated with lower survival rates (p = 0.0042), suggesting that the lncRNA *HOXA1-AS* represents a key point in the regulation of HSCC development (**Figure 3D**). The following experiments were carried out to identify the effect of *HOXA1-AS* on the proliferation and apoptosis of the HSCC cell line FaDU. As shown in **Figure 3E**, FaDU cells were transfected with *si-HOXA11-AS.* After transfection, qRT-PCR detection showed that the expression inhibition rate of *HOXA11-AS* in FaDU cells was 57.65%. To verify the role of *HOXA11-AS* in FaDU cell viability, we performed MTT assays on transfected cells, which showed a decrease in cell viability after 48 h of transfection, with 33.24% reduction compared to the control group (**Figure 3F**). We performed the cell counting test and confirmed consistent results that inhibition of *HOXA11-AS* led to a decrease in cell proliferation (**Figure 3G**). In order to verify the effect of *HOXA11-AS* on apoptosis in FaDU cells, apoptosis was measured by flow cytometry using Annexin-V/PI double staining and showed that transfection with *si-HOXA11-AS* caused an increase in the proportion of apoptotic cells, from 2.37 ± 0.56% to 8.46 ± 0.2% in the *si-HOXA11-AS* group compared to the si-NC group (**Figure 3H**). The aforementioned results suggested that downregulation of lncRNA *HOXA11-AS* by SM may be a prominent mechanism for SM to inhibit FaDU cell proliferation.

SM Regulates *miR-155*-Induced Apoptosis by Reducing LncRNA *HOXA1-AS* Expression

The base pairing pattern diagram is shown in **Figure 4A** to predict lncRNA *HOXA11-AS* targeting *miR-155*. This finding is supported by a previous study that lncRNA *HOXA11-AS* promoted the proliferation of HSCC through the sponge *miR-155* (Xu et al., 2020). The results of the dual-luciferase reporter gene assay confirmed that lncRNA *HOXA11-AS* could bind to *miR-155* and negatively relate with the expression of *miR-155*. In addition, the wiggle plot of lncRNA *HOXA11-AS* showed that the RNA-seq signal covers the potential *miR-155* binding site (**Supplementary Figure S1A**). To investigate whether *miR-155* is involved in the regulation of SM inhibition of FaDU cell proliferation, the qRT-PCR assay was carried out, showing *miR-155* was elevated by 1.65-fold when treated with 5 μM SM for 48 h compared to control cells (**Figure 4B**). The relative expression of *miR-155* was analyzed by RT-PCR transfected with si-NC or *si-HOXA11-AS* in FaDU cells (**Figure 4C**). FaDU cells were transfected with *miR-155* mimics and inhibitors to alter *miR-155* expression for subsequent assays with the purpose of testing the effect of differences in the *miR-155* expression on the proliferation and apoptosis of FaDU cells (**Figure 4D**). Data of MTT assays demonstrated that cells transfected with *miR-155* inhibitors displayed higher cell viability than the negative control, while lower viable in *miR-155* mimics-transfected cells (**Figure 4E**). In the apoptosis assay, a 21.07% increase in the apoptosis proportion was observed in cells transfected with *miR-155* mimics (**Figures 4F,G**). These results suggested that increasing *miR-155* expression suppressed cell viability and induced apoptosis. Furthermore, we evaluated the proliferation and apoptosis effects of 5 μM SM treatment on cells transfected with the *miR-155* inhibitor which showed a 41.58% increase in cell viability compared to SM treatment alone (**Figure 4H**), as well as a 44.29% decrease in apoptosis proportion (**Figures 4I,J**). The proliferation-inhibiting and apoptosis-inducing effects of SM on FaDU cells were attenuated by the inhibition of *miR-155* expression, suggesting that *miR-155* may be involved in the mechanism of SM combating FaDU cells. Moreover, SM could significantly upregulate the expression level of the cleaved caspase-3 protein (**Figures 2F,G**). According to the fact that lncRNA *HOXA1-AS* sponges *miR-155*, combined with the regulatory effect of SM on the expression of lncRNA *HOXA1-AS* and *miR-155*, we considered that downregulation of lncRNA *HOXA1-AS* and upregulation of *miR-155* were, at least partly, involved in inhibition of the proliferation of FaDU cells and apoptosis induction of SM.

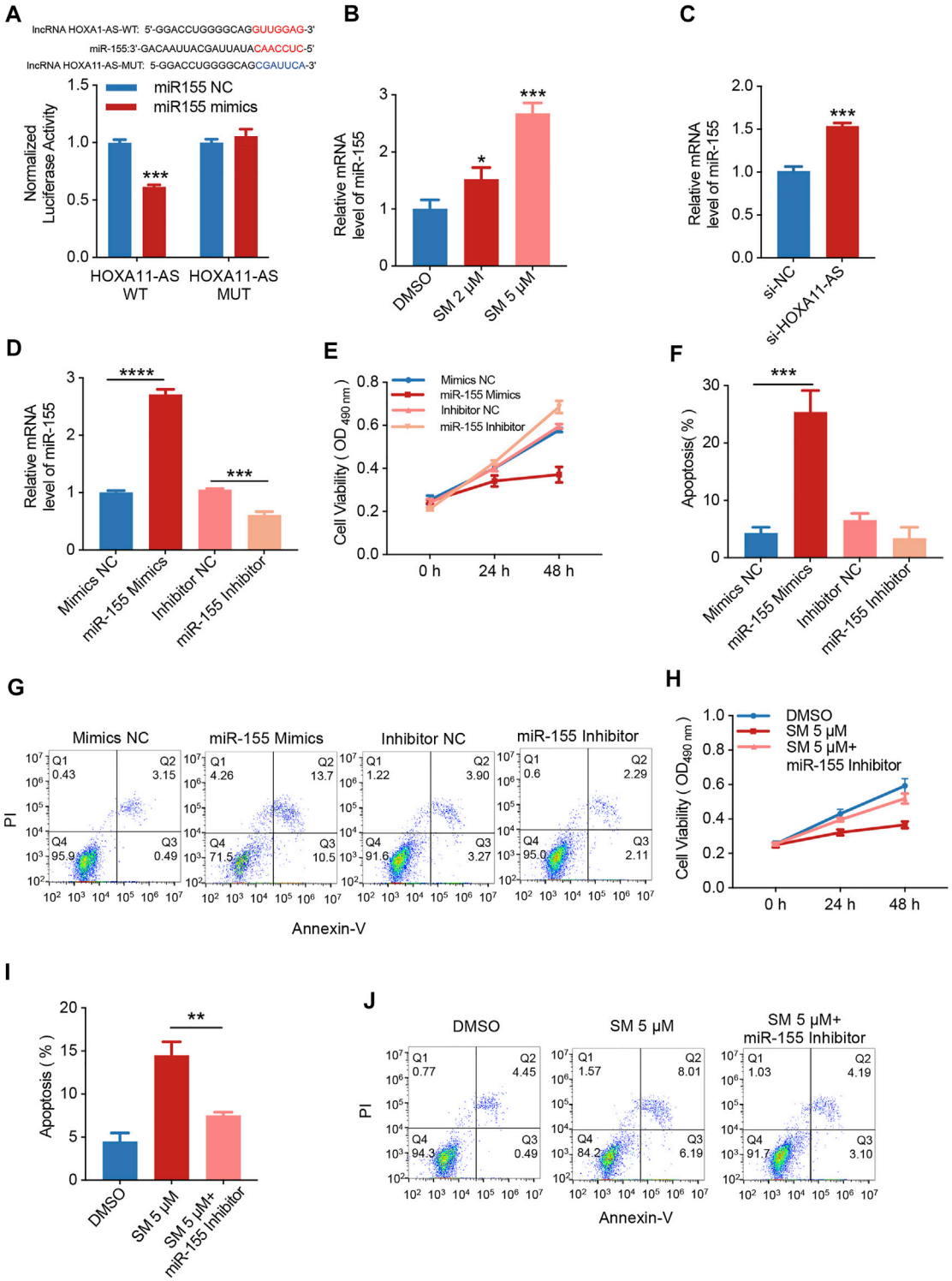


FIGURE 4 | SM regulates *miR-155*-induced apoptosis by reducing lncRNA *HOXA1-AS* expression. **(A)** Relative luciferase activity of 293 cells co-transfected with *miR-155* NC or *miR-155* mimics and the wild-type or mutated lncRNA *HOXA11-AS*. **(B)** Relative expression of *miR-155* was analyzed by RT-PCR after DMSO and 2 and 5 μM SM treatment in FaDU cells. **(C)** Relative expression of *miR-155* analyzed by RT-PCR transfected with *si-NC* or *si-HOXA11-AS* in FaDU cells. **(D)** Relative expression of *miR-155* in FaDU cells was analyzed by RT-PCR after being transfected with the *miR-155* mimic and inhibitor. **(E)** Cell viability of FaDU cells transfected with the *miR-155* mimic and inhibitor was analyzed by MTT assay at 0, 24, and 48 h ($n = 5$). **(F,G)** Apoptosis of FaDU cells transfected with the *miR-155* mimic and inhibitor was analyzed by flow cytometry, and the total apoptotic percentage was calculated using FlowJo software. **(H)** Cell viability of FaDU cells transfected with the *miR-155* inhibitor and treated with 5 μM SM was analyzed by MTT assay at 0, 24, and 48 h ($n = 5$). **(I,J)** Apoptosis of FaDU cells transfected with the *miR-155* inhibitor and treated with 5 μM SM was analyzed using flow cytometry, and the total apoptosis proportion was calculated by FlowJo software. *($p < 0.05$), **($p < 0.01$), ***($p < 0.001$), and ****($p < 0.0001$) indicate statistically significant differences.

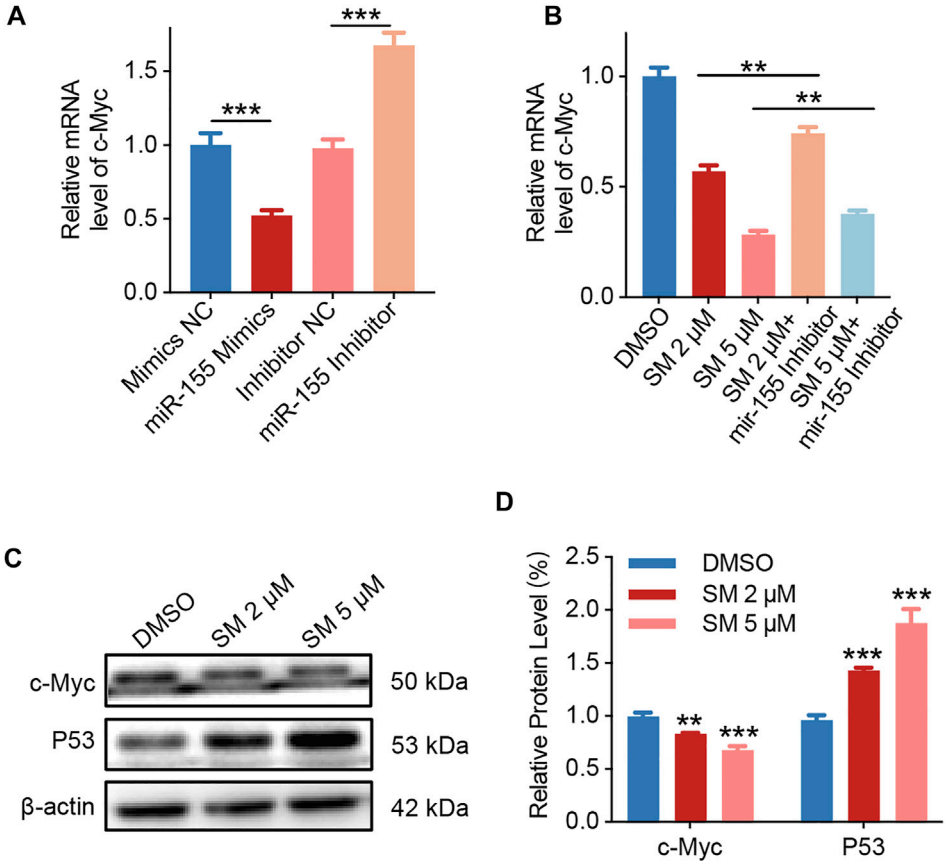


FIGURE 5 | SM induces apoptosis via lncRNA *HOXA11-AS/miR-155/c-Myc*. **(A)** Relative expression of *c-Myc* in FaDU cells was analyzed by RT-PCR after being transfected with the *miR-155* mimic and inhibitor. **(B)** Relative expression of *c-Myc* in FaDU cells was analyzed by RT-PCR after being transfected with the *miR-155* inhibitor and treated with 2 and 5 μM SM. **(C,D)** Immunoblotting assay analysis of c-Myc and P53 in FaDU cells treated with DMSO and 2 and 5 μM SM. The grayscale values of the strips were analyzed with ImageJ software. **($p < 0.01$) and ***($p < 0.001$) indicate statistically significant differences.

SM Induces Apoptosis via LncRNA *HOXA11-AS/miR-155/c-Myc*/p53

Studies have shown that the transcription factor *c-Myc*, which regulates cell apoptosis, is an important target gene for *miR-155*. The base pairing pattern diagram of *miR-155* and *c-Myc* sequence is shown in **Supplementary Figure S1B**. Furthermore, the qRT-PCR results showed that *miR-155* mimics decreased *c-Myc* expression and vice versa, while *miR-155* inhibitors increased *c-Myc* expression (**Figure 5A**). Then, we examined whether *c-Myc* was involved in the regulatory effect of SM on cells. The results obtained from qRT-PCR assay showed that the expression of *c-Myc* decreased by 71.66% after 5 μM SM treatment for 48 h, and the *miR-155* inhibitor was able to significantly weaken the inhibitory effect of SM on *c-Myc* expression (**Figure 5B**). Immunoblotting assay verified that SM (2 and 5 μM) downregulated c-Myc protein expression and upregulated P53 protein (**Figures 5C,D**). Taken together, these results suggested that SM downregulated lncRNA *HOXA11-AS,* which in turn regulated *c-Myc* gene expression through sponge *miR-155*.

SM Inhibits FaDU Development *In Vivo*

To investigate the tumor growth inhibitory effect of SM *in vivo*, a mouse xenograft tumor model was established, in which FaDU cells (2×10^6) were injected subcutaneously into the right axillary fossa of six-week-old female BALB/c mice. One week later, SM or saline was administrated. The mice in the SM group were injected with SM (10 mg/kg, i.v.) once daily, and the mice that were injected with saline served as controls. Body weights of the mice were recorded weekly. After 5 weeks of administration, tumors were separated, and their volume and weight were recorded. The data showed that there was no significant difference in body weight between the SM group and control, suggesting that there was no obvious toxicity in SM-treated mice (**Figure 6A**). The tumor volume and tumor weight decreased successively from 204.10 ± 80.01 mm^3 to 20.53 ± 10.80 mm^3 and from 73.6 ± 7.94 mg to 19.4 ± 5.31 mg, respectively, by SM treatment for 5 weeks, indicating that SM exerted a strong inhibitory effect on tumor development *in vivo* (**Figures 6B–D**).

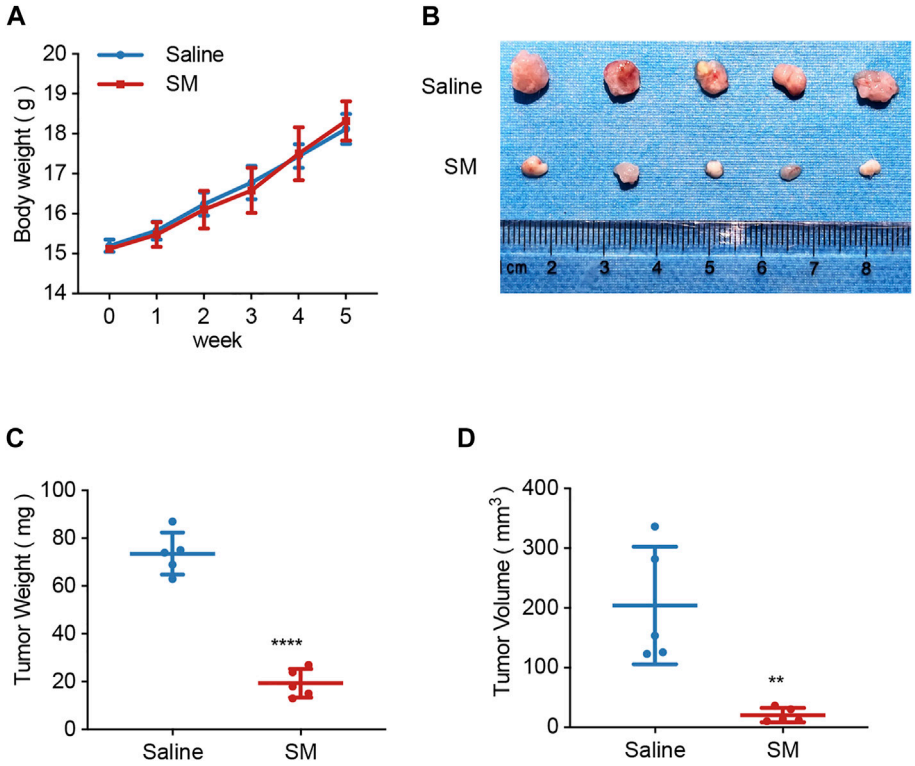


FIGURE 6 | SM inhibits FaDU xenograft tumor development *in vivo*. **(A)** Mice in the SM group were injected with SM (10 mg/kg, i. v.) once daily, and the mice that were injected with saline served as controls. The body weights of the mice were recorded weekly. After 5 weeks, tumors were separated, and their volume and weight were recorded. **(B)** Xenograft tumor morphology of the saline group and SM group. **(C)** Tumor weight of the saline group and SM group. **(D)** Tumor volume of the saline group and SM group. **($p < 0.01$) and ****($p < 0.0001$) indicate statistically significant differences.

DISCUSSION

HSCC is one of the head and neck cancers with a low incidence rate, but the prognosis is poor (Kwon and Miles, 2019). As its early stage is not easy to be detected, most patients are at an advanced stage, which increases the difficulty of treatment (Kuo et al., 2014). Although surgical treatment is capable of gaining sound local control, it will affect the normal speech function of patients and reduce their quality of life (Pracy et al., 2016; Wu et al., 2021). Chemotherapy remains one of the main strategies for HSCC, mainly including cisplatin (Janoray et al., 2020). The challenges of HSCC treatment necessitate the urgent search for a chemotherapeutic agent with few side effects and notable efficacy for patients.

LncRNAs were previously considered a byproduct of the transcription process, but with the in-depth into their research (Ma et al., 2013), their essential role in the development of many diseases becomes increasingly recognized, especially in cancer research. The aberrant expression or mutation of lncRNAs participates in a variety of tumor pathogenesis mechanisms, which include the regulation of proliferation, apoptosis, invasion, and migration of tumor cells (Xu et al., 2014; Bhan et al., 2017). One study of lncRNA and mRNA expression profiles in patients with HSCC identified differential expression of lncRNAs and mRNAs in cancer compared to para-cancer tissues, suggesting a potential regulatory role for multiple lncRNAs during the development of HSCC (Wang et al., 2016; Zhang et al., 2021).

We observed that SM exhibited a strong killing effect on HSCC cells, FaDU. The inhibitory effect of SM on tumor development was verified by establishing a xenograft tumor model. Then, high-throughput sequencing data clarified a key target of SM to inhibit cell proliferation, lncRNA *HOXA11-AS*. We verified that SM downregulated lncRNA *HOXA11-AS*, which could sponge *miR-155*, in turn, inhibited *c-Myc* and *P53* expression, resulting in suppression of cell proliferation inhibition and apoptosis. *P53* is a classic tumor suppressor gene, and *MYC* is the best-characterized proto-oncogene. The proto oncoprotein *c-Myc* and the tumor suppressor *P53* are inextricably linked in regulating cell survival and proliferation (Huang et al., 2021). Therefore, we examined the protein expression of P53 in this study to observe the anticancer effect of SM more comprehensively.

HOXA11-AS is the antisense lncRNA of *HOXA11* that regulates the proliferation and invasion of cancer cells (Xue et al., 2018). MicroRNA *miR-155* exerts significant functions in physiological processes such as cell cycle, apoptosis, and proliferation, which are involved in regulating the development of multiple cancers (Yang et al., 2018; Michaille et al., 2019). Liu et al. demonstrated that *miR-155* directly targeted

programmed cell death 4 (PDCD4) to promote lung cancer cell growth in a model of nude mice (Xia and Zhao, 2020). Xu et al. (2020) pointed out that as a molecular sponge for *miR-155*, *HOXA11-AS* was significantly upregulated in HSCC and exerted a pro-tumorigenic effect by sponging a tumor suppressor *miR-155* in HSCC. Our results indicated that SM was able to inhibit the development of FaDU xenograft tumors through suppression of HOXA11-AS, also providing evidence for *HOXA11-AS* on tumor regulation.

There is no direct experimental evidence about the molecular mechanism responsible for SM-induced *HOXA11-AS* downregulation in HSCC yet. Recently, growing evidence proves that transcription factors play a vital role in up- or downregulated lncRNAs in multiple cancers (Chen et al., 2019; Shuai et al., 2020). One study found that transcription factor homeobox B13 (*HOXB13*), identified as an upstream regulator of *HOXA11-AS*, could positively regulate the expression of *HOXA11-AS* in prostate cancer. Also, the *HOXA11-AS* combination with *HOXB13* manipulates the level of bone-specific metastasis-related genes of prostate cancer (Misawa et al., 2021). SM regulates multiple signaling pathways related to tumor progression and drug resistance, such as tumor suppressor pathways, mitochondrial pathways, and death receptor pathways (Wu et al., 2020; Tang et al., 2022). In this study, SM induces apoptosis via the lncRNA *HOXA11-AS/miR-155/c-Myc/p53* pathway. Therefore, a potential mechanism for SM downregulating *HOXA11-AS* expression is that SM may activate the upstream transcription factors of *HOXA11-AS* at the transcriptional level.

Natural compounds provide vital resources for the development of antitumor agents (Ijaz et al., 2018). Solamargine (SM), the extract of *Solanum nigrum*, exhibits a variety of therapeutic activities (Kalalinia and Karimi-Sani, 2017). Dozens of studies have demonstrated its potent killing effect on a variety of cancer cell lines. It is worth mentioning that SM shows an extremely low IC_{50} compared to most natural compounds. Liang et al. (2004) showed that the IC_{50} of SM on A549 cells was 2.9 μM, and Kuo et al. (2000) showed that the IC_{50} of SM on Hep3B cells was 5 μg/ml. We demonstrated for the first time the killing effect of SM on FaDU cells with an IC_{50} of 5.17 μM, which is comparable to other cancer cell lines, suggesting that SM has no obvious significantly selective killing effect on tumor cells. Considering the safety profile, Al Sinani et al. (2016) demonstrated that the cytotoxic effect of SM on human cancer cells was selective, evidenced by that it was able to selectively kill rapidly proliferating tumor cells with less damage to normal cells, indicating the promise of SM as an antitumor agent with few side effects and robust tumor-killing activity. The main mechanisms by which SM kills tumors include apoptosis, cycle arrest, and inhibition of tumor cell migration and invasion (Kalalinia and Karimi-Sani, 2017). Although the antitumor effect of SM has been widely recognized, the study of its effect on HSCC therapy is still blank. Our study confirmed the anti-HSCC tumor efficacy of SM for the first time, which revealed and broadened its application. We also explored the mechanism of SM killing FaDU cells with regard to ncRNA.

CONCLUSION

In this study, we revealed the effect of SM on FaDU cell phenotypes. According to the vital role of lncRNA *HOXA11-AS* in HSCC and our data obtained from high-throughput sequencing, we revealed the involvement of *HOXA11-AS* in the inhibitory effect of SM on the proliferation of FaDU cells. In conclusion, SM could be used in the development of an anticancer compound for HSCC, which has great prospects in the future.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are publicly available. These data can be found at: https://www.ncbi.nlm.nih.gov/sra, PRJNA821688

ETHICS STATEMENT

Animal experiments were performed in strict accordance with the guidelines of the Experimental Animal Ethics Committee of the Changchun University of Chinese Medicine.

AUTHOR CONTRIBUTIONS

WS and YZ designed the study. YM participated in the EdU staining and qRT-PCR. XW participated in the animal experiment and immunoblotting assay. DY and MJ participated in the wound healing assay and clone formation assay. XK, XG, and TY conducted the analysis. WS, YZ, YM, and JH drafted the manuscript. All authors read and approved the final manuscript.

FUNDING

This work was mainly supported by a grant from the Project of Jilin Provincial Department of Science and Technology: Mechanism of acupuncture on pterygopalatine ganglion in the treatment of allergic rhinitis by remodeling TLR2/4-MYD88 signaling pathway (20210101194JC).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.887387/full#supplementary-material

REFERENCES

Al Sinani, S. S., Eltayeb, E. A., Coomber, B. L., and Adham, S. A. (2016). Solamargine Triggers Cellular Necrosis Selectively in Different Types of Human Melanoma Cancer Cells through Extrinsic Lysosomal Mitochondrial Death Pathway. *Cancer Cell. Int.* 16, 11. doi:10.1186/s12935-016-0287-4

Bhan, A., Soleimani, M., and Mandal, S. S. (2017). Long Noncoding RNA and Cancer: A New Paradigm. *Cancer Res.* 77, 3965–3981. doi:10.1158/0008-5472.CAN-16-2634

Booton, R., and Lindsay, M. A. (2014). Emerging Role of MicroRNAs and Long Noncoding RNAs in Respiratory Disease. *Chest* 146, 193–204. doi:10.1378/chest.13-2736

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., and Jemal, A. (2018). Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.* 68, 394–424. doi:10.3322/caac.21492

Chen, L., Chen, Q., Kuang, S., Zhao, C., Yang, L., Zhang, Y., et al. (2019). USF1-induced Upregulation of LINC01048 Promotes Cell Proliferation and Apoptosis in Cutaneous Squamous Cell Carcinoma by Binding to TAF15 to Transcriptionally Activate YAP1. *Cell. Death Dis.* 10, 296. doi:10.1038/s41419-019-1516-2

Gang, G., Xinwei, C., Lixiao, C., Yu, Z., Cheng, Z., and Pin, D. (2022). Risk Factors of Lymphovascular Invasion in Hypopharyngeal Squamous Cell Carcinoma and its Influence on Prognosis. *Eur. Arch. Otorhinolaryngol.* 279, 1473–1479. doi:10.1007/s00405-021-06906-2

Hall, S. F., Groome, P. A., Irish, J., and O'sullivan, B. (2008). The Natural History of Patients with Squamous Cell Carcinoma of the Hypopharynx. *Laryngoscope* 118, 1362–1371. doi:10.1097/MLG.0b013e318173dc4a

Hoffman, H. T., Karnell, L. H., Shah, J. P., Ariyan, S., Brown, G. S., Fee, W. E., et al. (1997). Hypopharyngeal Cancer Patient Care Evaluation. *Laryngoscope* 107, 1005–1017. doi:10.1097/00005537-199708000-00001

Huang, Y., Yuan, K., Tang, M., Yue, J., Bao, L., Wu, S., et al. (2021). Melatonin Inhibiting the Survival of Human Gastric Cancer Cells under ER Stress Involving Autophagy and Ras-Raf-MAPK Signalling. *J. Cell. Mol. Med.* 25, 1480–1492. doi:10.1111/jcmm.16237

Ijaz, S., Akhtar, N., Khan, M. S., Hameed, A., Irfan, M., Arshad, M. A., et al. (2018). Plant Derived Anticancer Agents: A Green Approach towards Skin Cancers. *Biomed. Pharmacother.* 103, 1643–1651. doi:10.1016/j.biopha.2018.04.113

Iwae, S., Fujii, M., Hayashi, R., Hasegawa, Y., Fujii, T., Okami, K., et al. (2017). Matched-pair Analysis of Patients with Advanced Hypopharyngeal Cancer: Surgery versus Concomitant Chemoradiotherapy. *Int. J. Clin. Oncol.* 22, 1001–1008. doi:10.1007/s10147-017-1151-9

Janoray, G., Pointreau, Y., Alfonsi, M., Sire, C., Geoffrois, L., De Raucourt, D., et al. (2020). Induction Chemotherapy Followed by Cisplatin or Cetuximab Concomitant to Radiotherapy for Laryngeal/hypopharyngeal Cancer: Long-Term Results of the TREMPLIN Randomised GORTEC Trial. *Eur. J. Cancer* 133, 86–93. doi:10.1016/j.ejca.2020.04.009

Jin, Y. M., Xu, T. M., Zhao, Y. H., Wang, Y. C., and Cui, M. H. (2014). *In Vitro* and *In Vivo* Anti-cancer Activity of Formononetin on Human Cervical Cancer Cell Line HeLa. *Tumour Biol.* 35, 2279–2284. doi:10.1007/s13277-013-1302-1

Kalalinia, F., and Karimi-Sani, I. (2017). Anticancer Properties of Solamargine: A Systematic Review. *Phytother. Res.* 31, 858–870. doi:10.1002/ptr.5809

Kazda, A., Akimcheva, S., Watson, J. M., and Riha, K. (2016). Cell Proliferation Analysis Using EdU Labeling in Whole Plant and Histological Samples of Arabidopsis. *Methods Mol. Biol.* 1370, 169–182. doi:10.1007/978-1-4939-3142-2_13

Kuo, K. W., Hsu, S. H., Li, Y. P., Lin, W. L., Liu, L. F., Chang, L. C., et al. (2000). Anticancer Activity Evaluation of the Solanum Glycoalkaloid Solamargine. Triggering Apoptosis in Human Hepatoma Cells. *Biochem. Pharmacol.* 60, 1865–1873. doi:10.1016/s0006-2952(00)00506-2

Kuo, P., Chen, M. M., Decker, R. H., Yarbrough, W. G., and Judson, B. L. (2014). Hypopharyngeal Cancer Incidence, Treatment, and Survival: Temporal Trends in the United States. *Laryngoscope* 124, 2064–2069. doi:10.1002/lary.24651

Kwon, D. I., and Miles, B. A. (2019). Hypopharyngeal Carcinoma: Do You Know Your Guidelines? *Head. Neck* 41, 569–576. doi:10.1002/hed.24752

Liang, C. H., Liu, L. F., Shiu, L. Y., Huang, Y. S., Chang, L. C., and Kuo, K. W. (2004). Action of Solamargine on TNFs and Cisplatin-Resistant Human Lung Cancer Cells. *Biochem. Biophys. Res. Commun.* 322, 751–758. doi:10.1016/j.bbrc.2004.07.183

Liu, L. F., Liang, C. H., Shiu, L. Y., Lin, W. L., Lin, C. C., and Kuo, K. W. (2004). Action of Solamargine on Human Lung Cancer Cells-Eenhancement of the Susceptibility of Cancer Cells to TNFs. *FEBS Lett.* 577, 67–74. doi:10.1016/j.febslet.2004.09.064

Ma, L., Bajic, V. B., and Zhang, Z. (2013). On the Classification of Long Non-Coding RNAs. *RNA Biol.* 10, 925–933. doi:10.4161/rna.24604

Michaille, J. J., Awad, H., Fortman, E. C., Efanov, A. A., and Tili, E. (2019). miR-155 Expression in Antitumor Immunity: The Higher the Better? *Genes. Chromosom. Cancer* 58, 208–218. doi:10.1002/gcc.22698

Misawa, A., Kondo, Y., Takei, H., and Takizawa, T. (2021). Long Noncoding RNA HOXA11-AS and Transcription Factor HOXB13 Modulate the Expression of Bone Metastasis-Related Genes in Prostate Cancer. *Genes. (Basel)* 12, 182. doi:10.3390/genes12020182

Niyazi, M., Marini, P., Daniel, P. T., Humphreys, R., Jendrossek, V., and Belka, C. (2009). Efficacy of a Triple Treatment with Irradiation, Agonistic TRAIL Receptor Antibodies and EGFR Blockade. *Strahlenther. Onkol.* 185, 8–18. doi:10.1007/s00066-009-1856-4

Peng, W. X., Koirala, P., and Mo, Y. Y. (2017). LncRNA-mediated Regulation of Cell Signaling in Cancer. *Oncogene* 36, 5661–5667. doi:10.1038/onc.2017.184

Pracy, P., Loughran, S., Good, J., Parmar, S., and Goranova, R. (2016). Hypopharyngeal Cancer: United Kingdom National Multidisciplinary Guidelines. *J. Laryngol. Otol.* 130, S104–S110. doi:10.1017/S0022215116000529

Rastelli, G., Pellati, F., Pinzi, L., and Gamberini, M. C. (2020). Repositioning Natural Products in Drug Discovery. *Molecules* 25, 1154. doi:10.3390/molecules25051154

Sani, I. K., Marashi, S. H., and Kalalinia, F. (2015). Solamargine Inhibits Migration and Invasion of Human Hepatocellular Carcinoma Cells through Down-Regulation of Matrix Metalloproteinases 2 and 9 Expression and Activity. *Toxicol. Vitro* 29, 893–900. doi:10.1016/j.tiv.2015.03.012

Shiu, L. Y., Chang, L. C., Liang, C. H., Huang, Y. S., Sheu, H. M., and Kuo, K. W. (2007). Solamargine Induces Apoptosis and Sensitizes Breast Cancer Cells to Cisplatin. *Food Chem. Toxicol.* 45, 2155–2164. doi:10.1016/j.fct.2007.05.009

Shuai, Y., Ma, Z., Liu, W., Yu, T., Yan, C., Jiang, H., et al. (2020). TEAD4 Modulated LncRNA MNX1-AS1 Contributes to Gastric Cancer Progression Partly through Suppressing BTG2 and Activating BCL2. *Mol. Cancer* 19, 6. doi:10.1186/s12943-019-1104-1

Tang, Q., Li, X., Chen, Y., Long, S., Yu, Y., Sheng, H., et al. (2022). Solamargine Inhibits the Growth of Hepatocellular Carcinoma and Enhances the Anticancer Effect of Sorafenib by Regulating HOTTIP-TUG1/miR-4726-5p/MUC1 Pathway. *Mol. Carcinog.* 61, 417–432. doi:10.1002/mc.23389

Wang, P., Fu, H., Cui, J., and Chen, X. (2016). Differential lncRNA-mRNA Co-Expression Network Analysis Revealing the Potential Regulatory Roles of lncRNAs in Myocardial Infarction. *Mol. Med. Rep.* 13, 1195–1203. doi:10.3892/mmr.2015.4669

Wei, C., Zhao, L., Liang, H., Zhen, Y., and Han, L. (2020). Recent Advances in Unraveling the Molecular Mechanisms and Functions of HOXA11AS in Human Cancers and Other Diseases (Review). *Oncol. Rep.* 43, 1737–1754. doi:10.3892/or.2020.7552

Wu, J., Tang, X., Ma, C., Shi, Y., Wu, W., and Hann, S. S. (2020). The Regulation and Interaction of Colon Cancer-Associated Transcript-1 and miR7-5p Contribute to the Inhibition of SP1 Expression by Solamargine in Human Nasopharyngeal Carcinoma Cells. *Phytother. Res.* 34, 201–213. doi:10.1002/ptr.6555

Wu, S., Li, T., Liu, W., and Huang, Y. (2021). Ferroptosis and Cancer: Complex Relationship and Potential Application of Exosomes. *Front. Cell. Dev. Biol.* 9, 733751. doi:10.3389/fcell.2021.733751

Xia, H., and Zhao, Y. (2020). miR-155 Is High-Expressed in Polycystic Ovarian Syndrome and Promotes Cell Proliferation and Migration through Targeting PDCD4 in KGN Cells. *Artif. Cells Nanomed. Biotechnol.* 48, 197–205. doi:10.1080/21691401.2019.1699826

Xiang, S., Zhang, Q., Tang, Q., Zheng, F., Wu, J., Yang, L., et al. (2016). Activation of AMPKα Mediates Additive Effects of Solamargine and Metformin on Suppressing MUC1 Expression in Castration-Resistant Prostate Cancer Cells. *Sci. Rep.* 6, 36721. doi:10.1038/srep36721

Xu, M. D., Qi, P., and Du, X. (2014). Long Non-coding RNAs in Colorectal Cancer: Implications for Pathogenesis and Clinical Application. *Mod. Pathol.* 27, 1310–1320. doi:10.1038/modpathol.2014.33

Xu, J., Bo, Q., Zhang, X., Lei, D., Wang, J., and Pan, X. (2020). lncRNA HOXA11-AS Promotes Proliferation and Migration via Sponging miR-155 in Hypopharyngeal Squamous Cell Carcinoma. *Oncol. Res.* 28, 311–319. doi:10.3727/096504020X15801233454611

Xue, J. Y., Huang, C., Wang, W., Li, H. B., Sun, M., and Xie, M. (2018). HOXA11-AS: a Novel Regulator in Human Cancer Proliferation and Metastasis. *Onco Targets Ther.* 11, 4387–4393. doi:10.2147/OTT.S166961

Yang, N., Zhu, S., Lv, X., Qiao, Y., Liu, Y. J., and Chen, J. (2018). MicroRNAs: Pleiotropic Regulators in the Tumor Microenvironment. *Front. Immunol.* 9, 2491. doi:10.3389/fimmu.2018.02491

Zhang, G., Tan, G., Li, T., Ai, J., Song, Y., Zhou, Z., et al. (2021). Analysis of ceRNA Network of Differentially Expressed Genes in FaDu Cell Line and a Cisplatin-Resistant Line Derived from it. *PeerJ* 9, e11645. doi:10.7717/peerj.11645

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors, and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Meng, Jin, Yuan, Zhao, Kong, Guo, Wang, Hou, Wang, Song and Tang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 10 August 2022
DOI 10.3389/fphar.2022.934396

Check for updates

OPEN ACCESS

EDITED BY
Yue Hou,
Northeastern University, China

REVIEWED BY
Deniz Bartsch,
University Hospital of Cologne,
Germany
Gabriel Tao,
Merck (United States), United States

*CORRESPONDENCE
Shenglong Li,
lishenglong@cancerhosp-ln-cmu.com

SPECIALTY SECTION
This article was submitted to
Pharmacology of Anti-Cancer Drugs,
a section of the journal
Frontiers in Pharmacology

RECEIVED 02 May 2022
ACCEPTED 11 July 2022
PUBLISHED 10 August 2022

CITATION
Liu F and Li S (2022), Non-coding RNAs in skin cancers:Biological roles and molecular mechanisms.
Front. Pharmacol. 13:934396.
doi: 10.3389/fphar.2022.934396

COPYRIGHT
© 2022 Liu and Li. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Non-coding RNAs in skin cancers:Biological roles and molecular mechanisms

Fei Liu and Shenglong Li*

Department of Bone and Soft Tissue Tumor Surgery, Cancer Hospital of China Medical University, Liaoning Cancer Hospital & Institute, Shenyang, China

Cutaneous malignancies, including basal cell carcinoma, cutaneous squamous cell carcinoma, and cutaneous melanoma, are common human tumors. The incidence of cutaneous malignancies is increasing worldwide, and the leading cause of death is malignant invasion and metastasis. The molecular biology of oncogenes has drawn researchers' attention because of the potential for targeted therapies. Noncoding RNAs, including microRNAs, long noncoding RNAs, and circular RNAs, have been studied extensively in recent years. This review summarizes the aspects of noncoding RNAs related to the metastasis mechanism of skin malignancies. Continuous research may facilitate the identification of new therapeutic targets and help elucidate the mechanism of tumor metastasis, thus providing new opportunities to improve the survival rate of patients with skin malignancies.

KEYWORDS

skin cancers, non-coding RNA, malignant progression, molecular biology, metastasis

1 Introduction

Skin cancer is a common malignancy, and the most common types are basal cell carcinoma (BCC), cutaneous squamous cell carcinoma (CSCC), and cutaneous malignant melanoma (CMM) (Sato et al., 2021) (Figure 1). BCC and CSCC are more prevalent than melanoma, and most patients receive prompt treatment leading to a better long-term prognosis (Bednarski et al., 2021; Reddy et al., 2021). BCC and CSCC originate from epidermal keratin-forming cells and have a lower mortality than melanoma; the lesion is confined to the site of origin, and the treatment is thus straightforward (Cives et al., 2020; Krasowska et al., 2021). CMM is a difficult-to-treat metastatic malignancy that originates from epidermal melanocytes and is associated with a high mortality (Fujimura and Aiba 2020). When detected early, melanoma is treatable by surgical excision; however, rapid invasion and metastasis are the main reasons for the lower survival time after advanced treatment (Allegra et al., 2020). Genetic changes can induce the transformation of normal cells into cancer cells, and cancer cells can become malignant after cell division.

Noncoding RNAs (ncRNAs) include microRNAs (miRNAs), long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs) (He et al., 2021; Mercer et al., 2022). NcRNAs perform their respective biological functions at the RNA level (Xiaoqing Li et al., 2021). NcRNAs bind to various molecular targets to initiate specific cellular responses and

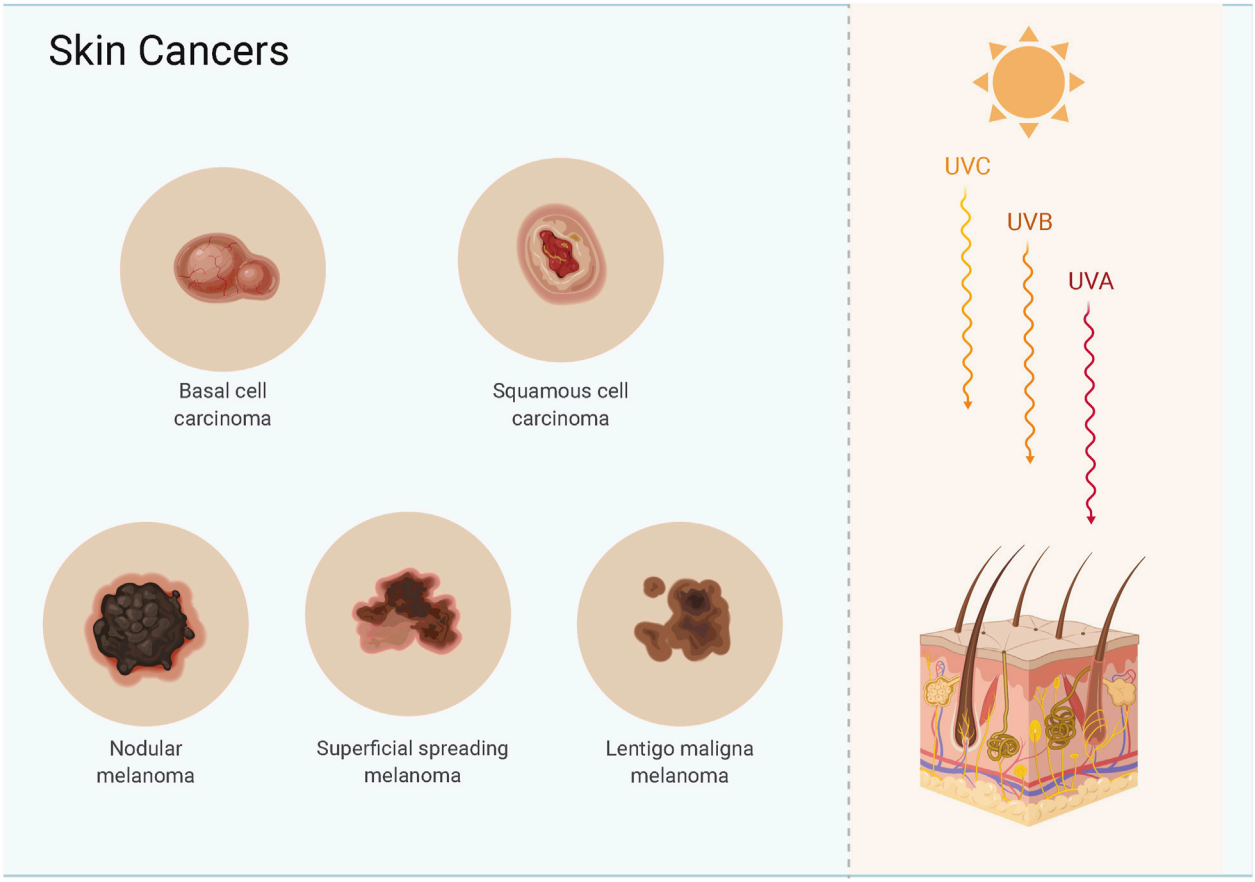


FIGURE 1
Skin cancers include basal cell carcinoma, cutaneous squamous cell carcinoma and melanoma.

regulate gene expression, intracellular signaling, epigenetic modifications, and other functions. The ncRNA is vital in the occurrence and development of tumors including skin cancer (Ding et al., 2021; Winkle et al., 2021). Accumulating evidence highlights the importance of ncRNAs in skin cancer. For example, circRNA_0016418 cross-talks with miR-625 and suppresses the progression of human skin melanoma (Zou et al., 2019). LncRNA MEG3 acts as a sponge for miR-21 and promotes melanoma growth and metastasis (Wu et al., 2020). The function of ncRNAs in cutaneous cancers was described previously (Zhou et al., 2022). Here, we provide an update of recent findings on the role of ncRNAs in skin cancer.

2 Noncoding RNAs in cancer

2.1 MiRNAs

MiRNAs are essential ncRNAs that regulate protein biosynthesis by modulating transcription (Kakumani 2022; Singh et al., 2022). The synthesis of miRNAs is catalyzed by RNA polymerase II, which produces primary miRNAs that enter the nucleus. Drosha, a family of ribonucleases, further catalyzes the production of precursor miRNAs with a hairpin structure (Asakiya et al., 2022; Quirico et al., 2022). This double-stranded product consists of a mature miRNA guide strand and a miRNA guest strand. Both miRNAs can be loaded into the RNA-induced silencing complex to degrade or inhibit translation of mRNA, thereby affecting protein expression (Shunhao Zhang et al., 2021; Das et al., 2022; Diener et al., 2022). A single miRNA can target hundreds of mRNAs, affecting the expression and interactions of many genes and participating in the regulation of numerous physiological and pathological processes. The biogenesis and biological function of miRNAs is described in Figure 2.

2.2 LncRNAs

LncRNAs are defined as non-protein coding RNAs with transcripts longer than 200 bp (Huang et al., 2022; Yang

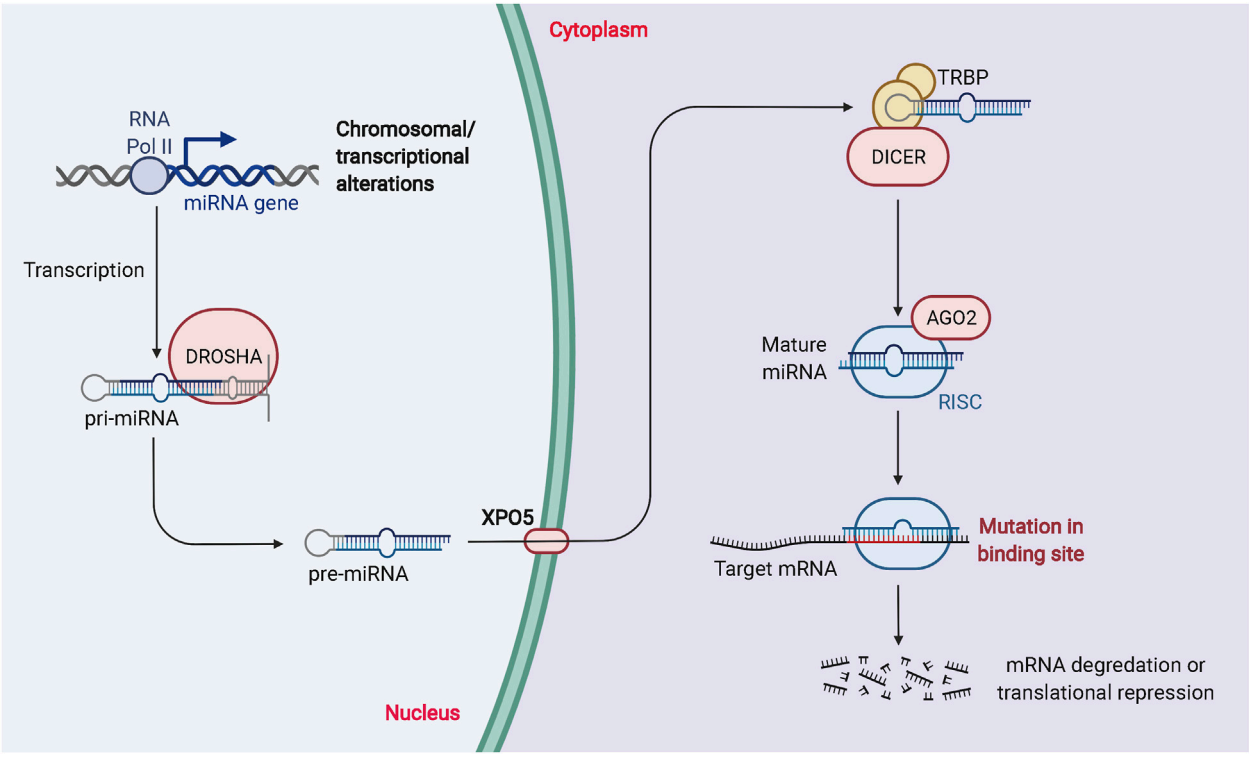


FIGURE 2
The biogenesis and biological function of miRNAs. RNA Pol II or III can regulate the transcription of pri-miRNAs, and DROSHA and DGCR8 can process the nuclear processing of pri-miRNAs into pre-miRNAs. Exportin 5 is involved in the processing of pre-miRNAs for nuclear export. Dicer and TRBP modulation regulate the cytoplasmic processing of pre-miRNAs into mature miRNA duplexes. miRNA duplexes include passenger strands and mature miRNAs. The passenger strand is degraded, and the mature miRNA strand is integrated into RISC to mediate translational repression or mRNA degradation depending on the degree of complementarity to the mRNA target.

et al., 2022), and they are considered byproducts of RNA polymerase II transcription with no biological function (Fernandes et al., 2021; Kong et al., 2021; Nojima and Proudfoot 2022). At present, there is no unified classification standard for lncRNAs (Nojima and Proudfoot 2022). According to the localization, lncRNAs are divided into cytoplasmic lncRNAs and nuclear lncRNAs, some of which are located in both the nucleus and the cytoplasm (Jianfei Tang et al., 2021). LncRNAs play different regulatory functions according to cellular localization (Cui et al., 2021). In the cytoplasm, lncRNAs act as competing endogenous RNAs (ceRNAs) to compete with miRNAs for binding and contribute to the release of target mRNAs. In tumors, abnormally expressed lncRNAs may break this balance, resulting in the abnormal expression of tumor-promoting genes or tumor-suppressor genes, and promoting the malignant progression of tumors. The ceRNA mechanism of lncRNAs is displayed in Figure 3. LncRNAs can be classified into five types according to the location in the genome relative to the protein-coding genes. The righteous lncRNAs overlap with exon regions of code-capable genes. The transcription of antisense lncRNAs begins with the reverse transcription of protein-coding genes. Bidirectional lncRNA expression start sites are very close to those of neighboring coding genes on the antisense strands. Basal lncRNAs originate from intronic regions, and intergenic lncRNAs are located in the interval between two genes on the chromosome (Cui et al., 2021; Jin et al., 2021). Based on their molecular function, lncRNAs are classified into decoy, guide, and backbone molecules. Decoy molecules are sufficient to induce transcription factors and inhibit the transcription of downstream genes. Guide molecules can bind to DNA or proteins at the same site and guide them to the site of action, enhancing the transcriptional activity of genes. The backbone molecules act as scaffolds for protein complexes, forming nucleic acid-protein complexes with target proteins and transferring the enzyme molecules involved in epigenetics to the protein complexes (Liu et al., 2022; Mercer et al., 2022). LncRNAs play critical regulatory roles in life activities and biological processes such as development, gene expression, stem cell differentiation, cell proliferation, and metastasis, indicating the close correlation to the occurrence and development of human diseases (DeSouza et al., 2021; Di Lu et al., 2021).

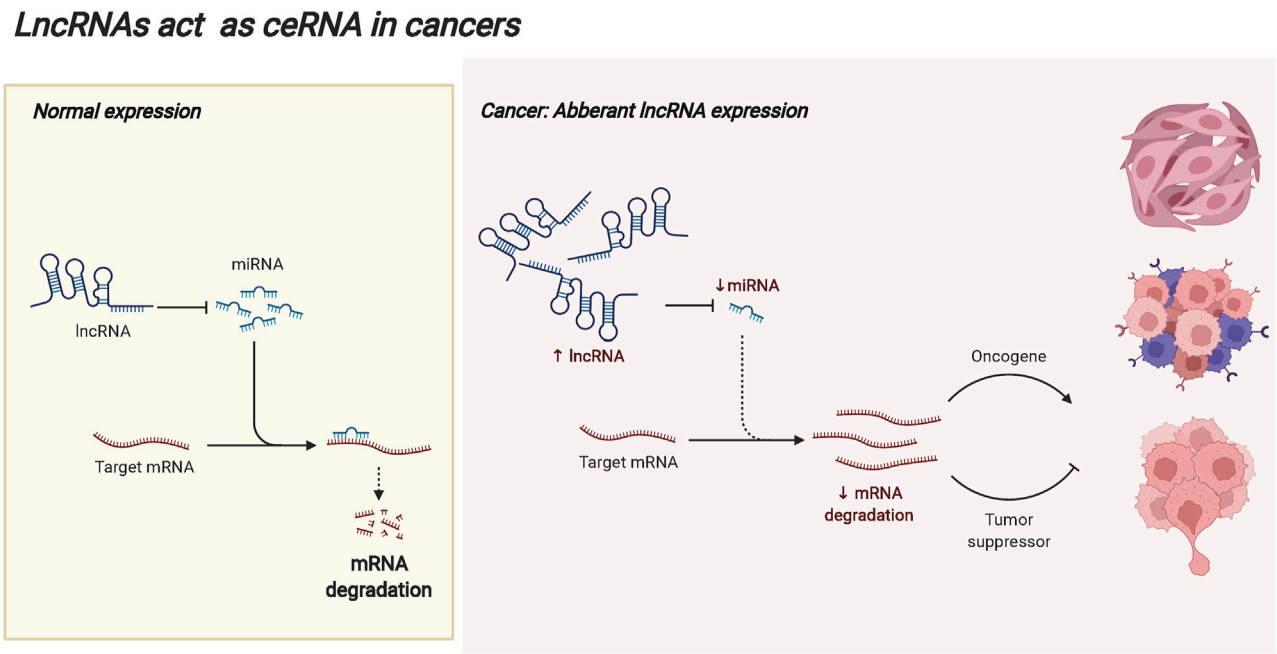


FIGURE 3
The ceRNA mechanism of lncRNAs. In the cytoplasm, lncRNAs can act as competing endogenous RNAs (ceRNAs) to compete with miRNAs for binding and contribute to the release of target mRNAs. In the occurrence and progression of tumors, abnormally expressed lncRNAs may break this balance, resulting in the abnormal expression of tumor-promoting genes or tumor suppressor genes, and promoting the malignant progression of tumors.

2.3 CircRNAs

CircRNAs are closed-loop molecules with unique structures that are widely distributed in animal and plant cells and regulate gene expression. They exist independently of proteins without being affected by exonucleases (Yue Zhang et al., 2022). Because of their low abundance and the limitation of detection technology, circRNAs were initially ignored as abnormal products of RNA splicing (Wen et al., 2022). The development of high-throughput sequencing technology has led to the identification of an increasing number of circRNAs (Kai Wang et al., 2022). Most circRNAs originate from exons in the coding regions of genes, whereas others originate from the 3′-UTR, 5′-UTR, introns, intergenic regions and antisense RNAs (Caba et al., 2021). CircRNAs are classified into four types, namely, exonic circRNAs (ecircRNAs), circular intronic RNAs (ciRNAs), exonic-intronic circRNAs (eiciRNAs), and tRNA intronic circRNAs (tricRNAs) (Wenqing Zhang et al., 2021). The most abundant and well studied circRNAs are exonucleotide circRNAs, which account for more than 80% of the total and are mainly localized in the cytoplasm. CiRNAs and eiciRNAs are abundant in the nucleus, and most circRNAs produced by different species are relatively evolutionarily conserved. The expression of the same circRNA varies greatly between diseased and non-diseased tissues and between different tissues or periods because of trans-shear (Tian Tian et al., 2021). In addition, circRNAs with covalent closed-loop structures are more stable than related linear mRNAs *in vivo* because they do not have 5′ caps or 3′ Poly A tails, which makes them highly resistant to the nucleic acid exonuclease (RNAse) (Zeng et al., 2021). Currently, there are three hypothetical models to explain the possible mechanisms of exon circRNA production, RNA binding protein (RBP)-mediated cyclization, intron pairing-driven cyclization, and lasso-driven cyclization (Kim et al., 2021; Sinha et al., 2021). The RBP connects two non-adjacent introns at both ends of the sequence, promoting the formation of a loop by bringing the two ends close together and then forming an exonic loop RNA by splicing. This model is defined as intron pairing-driven cyclization. The lasso-driven cyclization suggests that the pre-mRNA is in a half-folded state. During the transcription process, the non-adjacent exons approach each other and are driven by transacting factors to create a lasso. The intron sequence is removed by splicing in the lasso structure to form the circRNA. The biogenesis and biological function of circRNAs are shown in Figure 4.

3 Noncoding RNAs in skin cancer

NcRNAs play an essential role in the occurrence and development of skin cancers. This review summarizes the noncoding RNA aspects related to the metastasis mechanism of skin malignancies. The information may help identify new

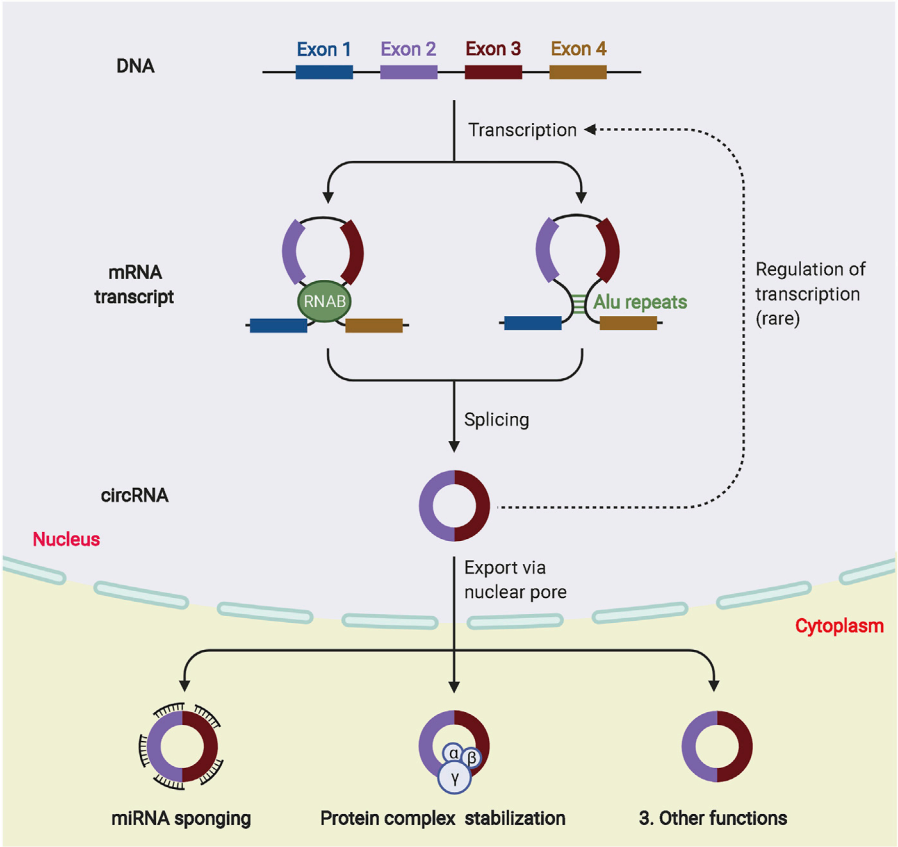


FIGURE 4
The biogenesis and biological function of circRNAs. Different circRNAs can be generated from pre-mRNA by back-splicing. According to the synthesis mechanism, circRNA can be divided into ecircRNA, ciRNA and eiciRNA. In the nucleus, circRNAs can participate in transcriptional regulation. In the cytoplasm, circRNAs are involved in many biological processes, such as miRNA sponge formation and binding to RBP.

therapeutic targets related to the mechanism of tumor metastasis and provide new opportunities to improve the survival rate and quality of life of patients with skin malignancies.

3.1 MiRNAs in skin cancer

Sand et al. (Sand et al., 2012) identified 16 miRNAs that are highly expressed in BCC tissues and 10 miRNAs significantly downregulated in BCC tissues compared with adjacent skin by microarray miRNA analysis. MiR-203 inhibits BCC cell proliferation, differentiation, and tumor growth by targeting c-JUN as an oncogenic factor in BCC (Sonkoly et al., 2012). MiR-451a inhibits BCC cell growth and colony formation by targeting TBX1, which may serve as an effective therapeutic target for BCC (Sun and Jiang 2018). MiR-18a expression is significantly increased in BCC tissues and cells and promotes BCC malignant progression by targeting the Akt/mTOR/Beclin 1/LC3 axis (Mi et al., 2020). MiR-17-92 cluster members and miR-143–145 cluster members are involved in BCC progression as pro-oncogenes and oncogenes, respectively (Sand et al., 2017). The miRNAs involved in BCC are listed in Table 1.

Various miRNAs are upregulated in CSCC and function as oncogenes to promote the malignant behavior of CSCC (Table 2 and Table 3). MiR-365 promotes the malignant progression of CSCC by targeting nuclear factor I B (NFIB), which may serve as a potential therapeutic target (Meijuan Zhou et al., 2014). MiR-31 expression promotes the malignant progression of tumors by increasing migration, invasion, and colony formation (Shao Hua Wang et al., 2014). MiR-365b and miR-135b target leucine zipper tumor suppressor 1 in CSCC tissues to promote migration and invasiveness (Olasz et al., 2015). Increased miR-365 expression promotes the malignant progression of tumors by targeting cyclin-dependent kinase 6 (Zhou et al., 2015). MiR-346 inhibits SRC kinase signaling inhibitor 1 expression and promotes CSCC cell proliferation and migration (Chen et al., 2016). Cadherin 1 and matrix metaloproteases (MMPs) are the downstream targets of miR-199a-5p and miR-217 in CSCC tissues, and their upregulation promotes malignant progression (Wang et al., 2016). Multiple miRNAs are

TABLE 1 Functional characterization of miRNAs in basal cell carcinoma.

miRNAs	Expression	Function role	Related genes	References
miR-203	Down	Inhibit cell proliferation and differentiation and tumor growth	c-JUN	Sonkoly et al. (2012)
miR-451a	Down	Inhibit cell growth and colony formation	TBX1	Sun and Jiang, (2018)
miR-18a	Up	Promote tumor malignant progression	Akt/mTOR/Beclin 1/LC3	Mi et al. (2020)
miR-17-92	Up	Promote tumor malignant progression	/	Sand et al. (2017)
miR-143-145	Down	Inhibit tumor malignant progression	/	Sand et al. (2017)

TABLE 2 Functional characterization of upregulated miRNAs in cutaneous squamous cell carcinoma.

miRNAs	Expression	Function role	Related genes	References
miR-365	Up	Promote tumor malignant progression	NFIB/ CDK6/BAX	(Meijuan Zhou et al., 2014; Zhou et al., 2015)
miR-31	Up	Promote cell migration, invasion, and colony formation	RhoBTB1	(Aoxue Wang et al., 2014; Lin et al., 2017)
miR-135b	Up	Increase the motility and aggressiveness of cancer cells	LZTS1	Olasz et al. (2015)
miR-346	Up	Promote cell proliferation and migration	SRCIN1	Chen et al. (2016)
miR-199a-5p	Up	Promote tumor malignant progression	CDH1/MMPs	Wang et al. (2016)
miR-217	Up	Promote cell growth, cell cycle and invasion	PTRF	Bai et al. (2017)
miR-142-5p	Up	Promote cell growth and inhibit cell apoptosis	PTEN	Bai et al. (2018)
miR-506	Up	Promote cell viability, migration, and invasion	LAMC1	Wu Zhou et al. (2019)
miR-186	Up	Promote cell growth and inhibit cell apoptosis	RETREG1	Yan Hu et al. (2019)
miR-766	Up	Promote cell proliferation, migration, invasion and inhibit cell apoptosis	PDCD5/MMPs	Liu et al. (2020)
miR-320a	Up	Promote cell growth and inhibit cell apoptosis	ATG2B	Bi et al. (2021)
miR-22	Up	Promote chemosensitivity	Wnt/β-catenin	Yuan et al. (2021)
miR-486-3p	Up	Promote cell proliferation, migration and tumorigenicity	FLOT2	Li et al. (2022)
miR-196a	Up	Promote cell proliferation, migration, invasion	—	Wei Zhang et al. (2022)

upregulated in CSCC and regulate various downstream signaling targets to promote tumor cell proliferation, migration, and invasion. MiR-27 regulates PTRF (Bai et al., 2017), miR-31 regulates rhobtb1 (Lin et al., 2017), miR-506 regulates laminin subunit gamma 1 (Jian Zhou et al., 2019), miR-125b regulates MMP 13 (Xu et al., 2012), miR-124/214 regulates ERK1/2 (Yamane et al., 2013), and miR-196a promotes malignant behavior in the same cellular signaling pattern (Wei Zhang et al., 2022). In addition to promoting proliferation and migration, numerous miRNAs inhibit cancer cell apoptosis to enhance the malignant behavior. MiR-365 expression is upregulated in CSCC and inhibits apoptosis by targeting BAX (Zhou et al., 2017). MiR-142-5p targets PTEN (Bai et al., 2018), miR-186 targets reticulophagy regulator 1 (Xinde Hu et al., 2019), and miR-320a regulates autophagy related 2B (Bi et al., 2021) to inhibit cancer cell apoptosis. Programmed cell death 5 is the dominant downstream target of miR-766, the upregulation of which inhibits the apoptosis of CSCC cells (Liu et al., 2020). Increased expression of miR-22 decreases CSCC chemosensitivity by targeting the Wnt/β-catenin signaling axis, thus promoting malignant progression (Yuan et al., 2021). MiR-486-3p targets flotillin 2 (FLOT2) to promote CSCC cell proliferation, migration, and tumorigenicity (Li et al., 2022). Angiogenesis is a common antineoplastic target for drugs and ncRNAs. MiR-361-5p inhibits SCC cell proliferation and angiogenesis by targeting VEGFA (Kanitz et al., 2012).

Decreased expression of various miRNAs results in CSCC suppression. MiR-20a is downregulated in CSCC tissues and inhibits cell proliferation and metastasis by targeting LIM domain kinase 1 (Jianda Zhou et al., 2014). Decreased expression of miR-199a affects the interaction between CD44 and Ezrin and inhibits CSCC proliferation and metastasis (Kanitz et al., 2012). MiR-199a-5p targets BCAM, FZD6, and DDR1 (Kim et al., 2015), and miR-148a targets MAP3K4 and MAP3K9 to inhibit the proliferation,

TABLE 3 Functional characterization of downregulated miRNAs in cutaneous squamous cell carcinoma.

miRNAs	Expression	Function role	Related genes	References
miR-125b	Down	Inhibit proliferation, colony formation, migration and invasion	MMP13	Xu et al. (2012)
miR-124/-214	Down	Inhibit cell proliferation	ERK1/2	Yamane et al. (2013)
miR-361-5p	Down	Inhibit cell invasion and angiogenesis	VEGFA	Kanitz et al. (2012)
miR-20a	Down	Suppress cell proliferation and metastasis	LIMK1	Jianda Zhou et al. (2014)
miR-199a	Down	Inhibit tumor growth and metastasis	CD44	Shao Hua Wang et al. (2014)
miR-199a-5p	Down	Inhibit cell invasion	BCAM, FZD6 and DDR1	Kim et al. (2015)
miR-148a	Down	Inhibit cell proliferation and metastasis	MAP3K4 and MAP3K9	Bai et al. (2018)
miR-204	Down	Inhibit tumor malignant progression	PTPN11	Toll et al. (2016)
miR-497	Down	Inhibit tumor malignant progression	SERPINE-1	Mizrahi et al. (2018)
miR-497	Down	Inhibit cell proliferation	FAM114A2	Wei et al. (2018)
miR-3619-5p	Down	Inhibit cell proliferation and cisplatin resistance	KPNA4	Zhang et al. (2019)
miR-30a-5p	Down	Inhibit tumor malignant progression	FOXG1	Zhang et al. (2019)
miR-199a-5p	Down	Inhibit tumor malignant progression	Sirt1/CD44ICD	Lu et al. (2020)
miR-27a	Down	Inhibit tumor malignant progression	EGFR	Yinghui Wang et al. (2019)
miR-125b	Down	Inhibit cell proliferation and promote cell apoptosis	STAT3	Ke Tian et al. (2020)
miR-216b	Down	Inhibit tumor malignant progression	TPX2	Feng et al. (2020)
miR-10a	Down	Inhibit tumor malignant progression	SDC-1	Xiong et al. (2020)
miR-214	Down	Inhibit tumor malignant progression	VEGFA and Bcl-2	Ma et al. (2020)
miR-451a	Down	Inhibit tumor malignant progression	PDPK1	Fu et al. (2021)
miR-130a	Down	Inhibit tumor malignant progression	ACVR1	Lohcharoenkal et al. (2021)
miR-573	Down	Inhibit cell proliferation, migration and invasion	PICSAR	Kai Wang et al. (2022)
miR-30c	Down	Inhibit cell proliferation and cisplatin resistance	SIRT1	Liu et al. (2022)

invasiveness, and metastasis of CSCC cells (Luo et al., 2015). MiR-204 is downregulated in CSCC tissues and regulates protein tyrosine phosphatase non-receptor type 11 (PTPN11), modulating the signal transducer and activator of transcription 3 (STAT3) and MAPK signaling pathways to inhibit the malignant progression of CSCC (Toll et al., 2016). MiR-497 expression is decreased in CSCC tissues and inhibits the malignant progression of CSCC by targeting PTPN11. Decreased expression of miR-497 inhibits the malignant progression of CSCC by targeting PTPN11 and SERPINE-1 in the AKT/mTOR and EMT processes (Mizrahi et al., 2018) and the FAM114A2 signaling pathway (Wei et al., 2018). MiR-3619-5p is downregulated in CSCC and modulates karyopherin subunit alpha four to inhibit proliferation and cisplatin resistance (Zhang et al., 2019). MiR-30a-5p (Shao et al., 2019) and miR-199a-5p are downregulated in CSCC tissues and modulate forkhead box G1 (FOXG1) to inhibit CSCC cell migration, invasion, and colony formation. MiR-199a-5p also inhibits the migration and tumorigenicity of CSCC by targeting Sirt1/CD44ICD (Lu et al., 2020). MiR-27a regulates EGFR (Yinghui Wang et al., 2019), miR-125b regulates STAT3 (Ke Tian et al., 2020), miR-216b regulates TPX2 (Feng et al., 2020), and miR-10a regulates SDC-1 (Xiong et al., 2020) to inhibit CSCC cell proliferation, migration, and invasion. MiR-214 is downregulated in CSCC tissues and inhibits migration and invasion by regulating VEGFA, Bcl-2, and the Wnt/β-catenin pathway (Feng et al., 2020). Downregulation of miR-451a and miR-130a inhibits CSCC cell proliferation, migration, invasion, and EMT by regulating the PI3K/AKT signaling pathway through 3-phosphoinositide dependent protein kinase 1 (Fu et al., 2021). Decreased miR-130a expression inhibits CSCC proliferation, cell motility, and invasion by regulating the ACVR1 and BMP/SMAD pathways (Lohcharoenkal et al., 2021). MiR-573 and miR-30c are downregulated in CSCC tissues and inhibit proliferation, migration, and invasion by modulating PICSAR (Lipeng Wang et al., 2022) and SIRT1 (Liu et al., 2022).

In summary, miRNAs play an essential role in the development and progression of CMM, promoting or inhibiting malignant progression by regulating cell proliferation, invasion, metastasis, drug resistance, the immune microenvironment, cell cycle progression, and apoptosis. Detailed information is provided in Table 4, Table 5, Table 6.

3.2 LncRNAs in skin cancer

Sand et al. (2016a) identified 1851 upregulated lncRNAs and 2165 downregulated lncRNAs in microarrays of BCC compared

TABLE 4 Functional characterization of miRNAs in cell proliferation, cell cycle and apoptosis of melanoma.

Cellular process	miRNAs	Related genes	References
Cell proliferation	miR-377	E2F3 and MAP3K7	Zehavi et al. (2015)
	miR-664	PLP2	Ding et al. (2015)
	miR-21	PTEN	Saldanha et al. (2016)
	miR-330-5p	TYR and PDIA3	Su et al. (2016)
	miR-4262	KLF6	Zhang et al. (2016)
	miR-135b	LATS2	Xinde Hu et al. (2019)
	miR-181	STAT3-AKT	He et al. (2020a)
	miR-140-3p	ABHD2	He et al. (2020b)
	miR-34a	ZEB1	Xu et al. (2021)
Cell cycle	miR-193b	CCND1	Chen et al. (2010)
	miR-186	—	Su et al. (2018)
	miR-21-5p	CDKN2C	Yang et al. (2020)
Cell apoptosis	miR-125b	—	(Glud et al., 2011; Nyholm et al., 2014)
	miR-92a-3p	MYCBP2	Venza et al. (2016)
	miR-365	BCL2	Zhu et al. (2018)
	miR-128	CCL18	Song et al. (2018)

TABLE 5 Functional characterization of miRNAs in cell migration and metastasis of melanoma.

Cellular process	miRNAs	Related genes	References
Migration	miR-532-5p	RUNX3	Kitago et al. (2009)
	MiR-125b	/	Glud et al. (2010)
	miR-200 and miR-203	E-cadherin	van Kempen et al. (2012)
	miR-21	TIMP3	Martin del Campo et al. (2015)
	miR-21	PDCD4	Jiao et al. (2015)
Metastasis	miR-23a	ATG12	Guo et al. (2017)
	miR-367	PTEN	Long et al. (2018)
	miR-509-3p	GPC6	Li et al. (2019)
	miR-152-5p	TXNIP	Li et al. (2020)

TABLE 6 Functional characterization of miRNAs in drug resistance and the tumor microenvironment of melanoma.

Cellular process	miRNAs	Related genes	References
Drug resistance	miR-514a	NF1	Stark et al. (2015)
	miR-579-3p	Verofini	Wang et al. (2018)
	miR-26a	HMGB1- dabrafenib	Yan Yu et al. (2019)
	miR-205	INPPL1	Sánchez-Sendra et al. (2020)
	miR-92a-3p	Dasatinib	Yuxiong Jiang et al. (2021)
Tumor microenvironment	miR-125b-5p	LIPA	Gerloff et al. (2020)

with non-lesioned skin. Further exploration demonstrated the biological roles and molecular mechanisms of these differentially expressed lncRNAs. Lnc-PICSAR is upregulated in DDP-resistant CSCC cells and regulates the miR-485-5p/REV3-like DNA directed polymerase zeta catalytic subunit (REV3L) signaling axis (Wang et al., 2020). LINC00963 is increased in

CSCC tissues and promotes malignant progression by upregulating SOX4 expression through the uptake of miR-1193 (Jingwen Wang et al., 2019). LINC00641 is downregulated in CSCC cell lines and inhibits CSCC growth and metastasis by downregulating miR-424 expression (Quan Liu et al., 2021). LINC00162 is specifically expressed in CSCC but not in keratin-forming cells in normal skin. Overexpression of LINC00162 promotes CSCC tumorigenesis *in vitro* and *in vivo* (Piipponen et al., 2016). LINC00319 is increased in CSCC and inhibits apoptosis, promoting cell proliferation, cell cycle progression, cell migration, and invasion. Mechanistic studies indicate that LINC00319 may exert an oncogenic function in CSCC by binding to miR-1207-5p and promoting the expression of cyclin-dependent kinase 3 (Li et al., 2018). LINC00520 regulates EGFR expression and inactivates the PI3K/Akt pathway, thereby inhibiting the development of CSCC (Mei and Zhong, 2019). USF1 activates LINC01048 to promote CSCC proliferation and inhibit apoptosis by interacting with TATA-box binding protein associated factor 15 (TAF15) to upregulate YAP1 (Chen et al., 2019). ALA-PDT promotes TINCR expression through the ERK1/2-SP3 pathway to induce apoptosis and autophagy, leading to the malignant progression of CSCC (Xiaobo Zhou et al., 2019). HOX transcript antisense RNA (HOTAIR) is elevated in CSCC cells and promotes migration, proliferation, and EMT, possibly by binding to miR-326 and promoting PRA1 domain family member 2 (PRAF2) expression (Guo Jun Yu et al., 2019). HOTAIR promotes CSCC stemness and progression by upregulating Sp1 and modulating miR-199a (Chen et al., 2022). LINC00346 is highly expressed in CSCC tissues to promote CSCC malignant progression by activating STAT3 and MMP expression (Piipponen et al., 2020). Small Cajal body-specific RNA 2 is upregulated in CSCC tissues and promotes proliferation and invasion by suppressing miR-342-3p expression (Zhang et al., 2020a). PICSAR functions as an oncogene by regulating the miR-125b/YAP1 signaling axis, and EZR-AS1 regulates the PI3K/AKT signaling pathway to promote CSCC cell proliferation and invasion and inhibit apoptosis (Qirong Lu et al., 2021). Overexpression of H19 promotes malignant behavior and induces apoptosis. H19 promotes the expression of the EMT-related marker miR-675 and inhibits p53 expression (Shunhao Zhang et al., 2021). Hcp5 can upregulate EZH2 expression by competitively binding to miR-138-5p, promoting autophagy and suppressing apoptosis by regulating the STAT3/VEGFR2 pathway, leading to malignant progression of CSCC (Zou et al., 2021). Neat1 promotes autophagy and decreases apoptosis by binding to miR-361-5p, promoting CSCC proliferation and invasion by activating the Wnt pathway (Shiqiu Jiang et al., 2021). NEAT1 promotes CSCC proliferation, invasion, and metastasis by regulating the expression of metalloplasmic proteins (Gong et al., 2022).

FOXD2-AS1 expression is increased in cutaneous melanoma tissue specimens and cell lines (Ren et al., 2019), inhibiting proliferation, migration, and invasion of cutaneous melanoma cells by regulating phospho-Akt expression (Wu Zhou et al., 2019). CPS1-IT1 inhibits melanoma metastasis by competitively binding to BRG1 and impairing CYR61 expression. Linc00961 is decreased in cutaneous melanoma tissues and functions as a ceRNA to regulate the miR-367/PTEN axis and inhibit cell proliferation and invasion (Mu et al., 2019). LncRNA FOXD3-AS1 promotes the proliferation, invasion, and migration of CMM by binding to miR-325 and promoting MAP3K2 expression as a potential cause of cutaneous melanoma (Wei et al., 2019). FENDRR suppresses MMP2 and MMP9 and antagonizes the JNK/c-Jun pathway to promote proliferation, migration, and invasion of melanoma (Shuang Chen et al., 2020). LncRNA-TTN-AS1 promotes TTN transcription and increases the stability of TTN mRNA thus inducing tumor progression. LINC00518 is upregulated in CMM and induces radioresistance of CMM by regulating glycolysis through a miR-33a-3p/HIF-1α negative feedback loop (Yan Liu et al., 2021). LINC01116 promotes cell proliferation, migration, invasion and EMT to sponge miR-3612 by regulating GDF11 and SDC3, thereby promoting melanoma progression (Yanhua Wang et al., 2022). LncRNA Tincr is downregulated in CMM, which promotes CTGF, CCN1, and AXL expression, leading to cell proliferation, invasion, and apoptosis inhibition in CMM cell lines. p53 upregulates PURPL in melanoma to promote cell proliferation, colony formation, migration, and invasion, and inhibiting apoptosis (Shuo Han et al., 2021). Mechanistic studies show that PURPL promotes mTOR-mediated phosphorylation of ULK1 at Ser757 by physically interacting with mTOR and ULK1, limiting the autophagic response to suppress apoptosis. TEX41 is activated by IRF4 and binds to miR-103a-3p to upregulate C1QB, thereby promoting cell proliferation, migration, and invasion while inhibiting apoptosis, suggesting that TEX41 is a potential therapeutic target for melanoma (Zheng et al., 2021). Linc00518 is upregulated in CMM tissues and promotes malignant progression via the miR-526b-3p/EIF5A2 axis (Xu et al., 2022).

Taken together, these data indicate that lncRNAs function as tumor-promoters or tumor-suppressors in CMM by interacting with miRNAs and regulating protein expression.

3.3 CircRNAs in skin cancer

The detailed information of circRNAs in skin cancers is shown in Table 7.

A circRNA microarray identified 23 upregulated and 48 downregulated circRNAs in BCC with a potential function in regulating the development of BCC. However, the underlying mechanism needs to be examined (Sand et al., 2016b).

TABLE 7 Functional characterization of circRNAs in skin cancers.

Cancer	circRNAs	Expression	Function role	Related genes	References
Basal cell carcinoma	Circ_0005795	Up	Promote cell viability, colony formation, and suppress cell apoptosis	miR-1231 and caspase-3	Yating Li et al. (2021)
	Circ_NCKAP1	Up	Promote cell proliferation, inhibit cell apoptosis	miR-148b-5p/HSP90 axis	Fan et al. (2021)
Malignant melanoma	circRNA_0084043	Up	promote melanoma cell proliferation, invasion and migration	miR-153-3p/Snail axis	Luan et al. (2018)
	hsa_circ_0025039	Up	promote cell proliferation, colony formation ability, invasion and glucose metabolism in melanoma cells	miR-198/CDK4	Bian et al. (2018)
	circMTUS1	Up	promote cell proliferation	hsa-miR-622/hsa-miR-1208	Shang et al. (2019)
	Circular RNA ITCH	Up	downregulate GLUT1 and suppresses glucose uptake in melanoma to inhibit cancer cell proliferation	GLUT1	Lin et al. (2021)
	circMYC	Up	promote the proliferation of human melanoma cells and Mel-CV cells. repress Mel-CV cell glycolysis and LDHA activities	Mel-CV cells/miR-1236/c-MYC-SRSF1 axis	Jin et al. (2020)
	CDR1as	Down	inhibit malignant progression	miR-7/LINC00632/IGF2BP3	Hanniford et al. (2020)
	circ_0084043	Up	promote cell proliferation, migration and invasion, facilitate apoptosis in A375 and SK-MEL-28 cells	miRNA (miR)-429/TRIB2	Zhibing Chen et al. (2020)
	circ-FOXM1	Up	promote cell proliferation, invasion, and glycolysis and facilitated cell apoptosis	miR-143-3p/FLOT2/MTT assay	Ke Tian et al. (2020)
	circ_0002770	Up	promote cell invasion, migration, and proliferation	miR-331-3p/DUSP5 and TGFBR1	Qian et al. (2020)
	circ_0020710	Up	promoted melanoma cell proliferation, migration and invasion	miR-370-3p/CXCL12	Wei et al. (2020)
	circ 0001591	Up	promoted cell growth and cell invasion and reduced apoptotic rate of melanoma	ROCK1/PI3K/AKT/ROCK1/miR-431-5p	Yin et al. (2021)
	circ_0079593	Up	Promotes cell proliferation, cell cycle progression, migration, invasion, inhibits cell apoptosis, and promotes tumor growth	miR-573/ABHD2	Zhao et al. (2021)
	CircRNA_0082835	Up	Promotes proliferation, invasion and migration of melanoma cells and regulates cell cycle levels	EZH2/miR-429	Sun et al. (2021)
	circZNF609	Up	Promotes the invasion, migration and proliferation of melanoma cells and inhibits apoptosis	miR-138-5p/SIRT7 axis	Yan Liu et al. (2021)
	circVANGL1	Up	Promotes proliferation, migration and invasion of melanoma cells	miR-150-5p/TGF-β	Zhou et al. (2021)
Cutaneous squamous cell carcinoma	circ_0070934	Up	associated with tumor aggressiveness	miR-1238/miR-1247–5p	An et al. (2019)
	hsa_circ_0070934	Up	Promotes the invasion and proliferation potential of CSCC cells and inhibits apoptosis	miR-1236-3p/HOXB7	Zhang et al. (2020a)
	circPVT1	Up	Promote CSCC cell proliferation and migration	/	Shuang Chen et al. (2020)
	circRNA_001937	Up	Promote CSCC progression and inhibit apoptosis	miRNA-597-3p/FOSL2	Gao et al. (2020)
	circSEC24A	Up	Promotes cell proliferation, migration, invasion and glycolysis, inhibits apoptosis	miR-1193/MAP3K9 axis	Xiaoyan Lu et al. (2021a)
	hsa_circ_0001360	Down	Inhibits the proliferation, migration and invasion of SCL-1 cells and promotes apoptosis	/	Chen et al. (2021)
	Circ_0067772	Up	Promotes proliferation, migration and invasion of CSCC cells	miR-1238-3p/FOXG1 axis	Ziwei Li et al. (2021)
	hsa_circ_0070934	Up	Promote CSCC cell proliferation, cell cycle process, migration, invasion, and inhibit apoptosis	miR-136-5p/PRAF2 axis	Xiong et al. (2021)
	circ-CYP24A1	Up	Promote cell proliferation, migration and invasion, inhibit apoptosis	CDS2、MAVS 和 SOGA	Zhang et al. (2021a)
	hsa_circ_0008234	Up	Increased the cell viability and colony formation of cSCC cells	miR-127-5p/ADCY7	Cai et al. (2021)
	circFADS2	Down	Inhibits CSCC cell proliferation, metastasis and glycolysis	miR-766-3p/HOXA9	Zhang et al. (2021b)

(Continued on following page)

TABLE 7 (*Continued*) Functional characterization of circRNAs in skin cancers.

Cancer	circRNAs	Expression	Function role	Related genes	References
	circ-LARP1B	Up	Promotes cell viability, colony-forming ability, migration, invasion, cell cycle progression and glycolysis of CSCC cells, and inhibits apoptosis	miR-515-5p/TPX2 axis	Yan Wang et al. (2022)
	circ_0001821	Up	Promotes cell viability, colony formation, cell cycle progression and metastasis, and inhibits apoptosis *in vitro* and promotes tumor growth *in vivo*	miR-148a-3p/EGFR axis, PI3K/Akt pathway	Yue Zhang et al. (2022)

Circ_0005795 expression is increased in BCC tissues, and knockdown of circ_0005795 inhibits cell viability, colony formation, and anti-apoptotic protein levels while increasing caspase-3 activity (Yating Li et al., 2021). Circ_0005795 exerts its pro-tumor effects by binding to miR-1231. Circ_NCKAP1 promotes the malignant progression of cutaneous BCC by regulating the miR-148b-5p/HSP90 signaling axis (Fan et al., 2021).

Circ_0070934 is expressed aberrantly in CSCC. Overexpression of circ_0070934 promotes cell proliferation, invasion, and migration, and inhibits apoptosis (An et al., 2019). The hsa_circ_0070934 binds to miR-1236-3p and regulates homeobox B7 expression to promote the malignant progression of CSCC (Zhang et al., 2020b). GSPS downregulates the expression of hsa_circ_0070934 and inhibits CSCC cell proliferation, cell cycle progression, migration, and invasion, and promotes apoptosis. However, GSPS also plays an oncogene role in CSCC by regulating the hsa_circ_0070934/miR-136-5p/PRA1 domain family member 2 (PRAF2) axis (Xiong et al., 2021). CircRNA_001937 promotes the malignant progression of CSCC by regulating the miR-597-3p/FOSL2 pathway as determined through *ex vivo* assays (Gao et al., 2020). CircSEC24A and MAP3K9 are upregulated in CSCC tissues, whereas the expression of miR-1193 is decreased (Ziwei Lu et al., 2021). Inhibition of circSEC24A inhibits malignant behavior and glycolysis to induced apoptosis, whereas overexpression of circSEC24A promotes tumor growth. CircSEC24A may act as a molecular sponge for miR-1193 and thus regulate the expression of MAP3K9 (Chen et al., 2021). The hsa_circ_0001360 inhibits cell proliferation, migration, and invasion and promotes apoptosis, acting as an antioncogene in CSCC. Circ_0067772 is upregulated in CSCC tissues and cells, and overexpression of circ_0067772 regulates FOXG1 by binding to miR-1238-3p, thereby promoting cell proliferation, migration, and invasion. Inhibition of circ_0067772 suppresses tumor growth (Xiaoqing Li et al., 2021).

Zhang et al. (2021c) also screened differentially expressed circRNAs in CSCC exosomes by RNA-seq analysis and identified 25 upregulated and 76 downregulated exosomal circRNAs. Circ-CYP24A1 promotes CSCC malignant progression by inducing the expression of CDS2, MAVS, and SOGA. Hsa_circ_0008234 promotes CSCC malignant progression by targeting miR-127-5p to regulate adenylate cyclase 7 expression (Cai et al., 2021). Circfads2 is downregulated in CSCC tissues and inhibits CSCC cell proliferation, metastasis, and glycolysis (Wenqing Zhang et al., 2021). Mechanistic studies suggest that circfads2 regulates the miR-766-3p/HOXA9 axis to inhibit CSCC progression, and is thus a potential therapeutic target for CSCC. Circ-LARP1B/miR-515-5p/TPX2 microtubule nucleation factor regulatory axis is involved in the malignant progression of CSCC by promoting cell viability, colony formation, migration, invasion, cell cycle progression, and glycolysis and inhibiting apoptosis (Yan Wang et al., 2022). Circ_0001821 plays a pro-oncogenic role in CSCC development by regulating the miR-148a-3p/EGFR signaling axis and PI3K/Akt pathway (Yue Zhang et al., 2022).

Luan et al. (Luan et al., 2018) found that Circ_0084043 is upregulated in melanoma tissues and functions as a sponge for miR-153-3p to upregulate Snail expression and thus promote melanoma cell proliferation, invasion, and migration. The hsa_circ_0025039 promotes CDK4 expression by binding to miR-198, promoting cell proliferation, colony-forming ability, invasion, and glucose metabolism (Bian et al., 2018). Circ_0084043 expression is increased in melanoma tissues and promotes the malignant development of melanoma by regulating the miR-429/TRIB2 signaling axis and Wnt/β-catenin signaling pathway (Xu E Chen et al., 2020). Circ-FOXM1 upregulates FLOT2 by binding to miR-143-3p, promoting cell proliferation, invasion, and glycolysis, playing a tumorigenic role *in vivo* (Shan Tian et al., 2020). The circRNA circ_0002770 promotes dual specificity phosphatase 5 and transforming growth factor beta receptor 1 expression by binding to miR-331-3p, thereby promoting the proliferation, invasion, and migration of melanoma cells, leading to malignant tumor progression (Qian et al., 2020). Wei et al. (Wei et al., 2020) showed that circ_0020710 expression is increased in melanoma tissues and promotes proliferation, migration, and invasion. increased circ_0020710 upregulates CXCL12 expression by sponging miR-370-3p, which is associated with cytotoxic lymphocyte depletion. Circ 0001591 expression is increased in melanoma patient sera, which promotes cell growth and invasion and inhibits apoptosis (Yin et al., 2021). Circ 0001591 upregulates ROCK1 expression by binding to miR-431-5p and promotes malignant progression of melanoma by regulating the ROCK1/

PI3K/AKT signaling pathway. Circ_0079593 regulates ABHD2 expression through miR-573 and promotes cell proliferation, migration, and invasion and inhibits apoptosis (Zhao et al., 2021). Circ_0082835 is upregulated in melanoma tissues and binds to miR-429 to promote the proliferation, invasion, and migration of melanoma cells, as well as regulating cell cycle progression (Sun et al., 2021). CircZNF609 inhibits DNA damage and promotes the malignant progression of melanoma by regulating the miR-138-5p/SIRT7 signaling axis (Quan Liu et al., 2021). The expression of circVANGL1 is increased in melanoma tissues and cell lines, and overexpression of circVANGL1 promotes the proliferation, migration, and invasion of melanoma cells (Zhou et al., 2021). Taken together, these findings indicate that lncRNAs function as tumor-promoters or tumor-suppressors involved in the malignancy of cutaneous melanoma through their ceRNA activity for miRNAs.

4 Future prospects and conclusion

Various miRNAs are involved in the regulation of skin cancer development and metastasis. The differential expression of miRNAs at different stages of skin cancer provides a new basis and novel direction for developing biomarkers and therapeutic targets. Further studies on the mechanism underlying the function of miRNAs will facilitate the development of new therapeutic strategies for the treatment of skin cancers, which will improve the outcomes of patients with advanced skin cancer. LncRNAs are critical epigenetic factors that regulate the development, progression, metastasis, and resistance to therapeutic agents in skin cancers. Although the majority of lncRNAs are expressed at low levels, the value of lncRNAs as early diagnostic, predictive, and therapeutic targets for skin cancer is becoming increasingly evident. The complexity of lncRNAs increases the difficulty of their biological investigation because the same lncRNAs function differently in distinct tumor types. Exploring the functions of lncRNAs and elucidating the molecular regulatory mechanisms involved in the development and progression of skin cancer is a new research direction.

CircRNAs have drawn increased attention in life science and medical research. CircRNAs competitively bind to miRNAs and regulate multiple tumor signaling pathways, suggesting the potential of circRNAs as biomarkers for detecting related diseases. Research on circRNAs in tumors is still in its infancy, with only a fraction of functional circRNAs available. The current circRNA database is incomplete, and there are no databases of circRNAs related to tumor prognosis for functional prediction after RNA sequencing. In addition, the mechanism of circRNAs in tumorigenesis remains to be fully elucidated. Their interaction with mRNA and its diagnostic applications show potential for clinical practice. CircRNAs show great potential in tumor diagnosis, prognosis, and treatment with high clinical value. We believe that continuing efforts and practical investigation will clarify the value of circRNAs for precision medicine in clinical diagnosis and treatment. The cross-talk of ncRNAs is complex and highly tissue specific. Therefore, exploring their functions in many cell types or tissues is essential to determine specificity and therapeutic effects. Despite the challenges associated with the application of ncRNAs, extensive research efforts have been devoted to extend investigation to ncRNA clinical trials.

This review summarized the mechanisms underlying the role of ncRNAs in the occurrence, metastasis, and drug resistance of skin cancers. Their potential application for the early diagnosis, prognosis assessment, and targeted therapy has been suggested. Several ncRNAs have undergone different phases of clinical trials. For example, a miR-122-related drug (miravirsen) and ncRNA-associated nanoparticles have been the subject of clinical trials (Gebert et al., 2014). The miRNA mimic MIRX34 was used in a phase I trial in patients with primary liver cancer (Bouchie 2013). Therefore, further exploration of clinical biosignatures and functional cell assays is needed. In addition, non-invasive methods should be considered for ncRNA harvesting.

Author contributions

Original draft preparation, allocation, supplementation and editing: SL and FL. All authors have read and agreed to the published version of the manuscript.

Funding

This work was supported by the Liaoning Cancer Hospital and Institute (Shenyang) and China Medical University (Shenyang).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Allegra, A., Pioggia, G., Tonacci, A., Musolino, C., and Gangemi, S. (2020). Oxidative stress and photodynamic therapy of skin cancers: Mechanisms, challenges and promising developments. *Antioxidants (Basel)* 9 (5), E448. doi:10.3390/antiox9050448

An, X., Liu, X., Ma, G., and Li, C. (2019). Upregulated circular RNA circ_0070934 facilitates cutaneous squamous cell carcinoma cell growth and invasion by sponging miR-1238 and miR-1247-5p. *Biochem. Biophys. Res. Commun.* 513 (2), 380–385. doi:10.1016/j.bbrc.2019.04.017

Aoxue Wang, A., Landén, N. X., Meisgen, F., Lohcharoenkal, W., Ståhle, M., Sonkoly, E., et al. (2014). MicroRNA-31 is overexpressed in cutaneous squamous cell carcinoma and regulates cell motility and colony formation ability of tumor cells. *PLoS One* 9 (7), e103206. doi:10.1371/journal.pone.0103206

Asakiya, C., Zhu, L., Yuhan, J., Zhu, L., Huang, K., Xu, W., et al. (2022). Current progress of miRNA-derivative nucleotide drugs: Modifications, delivery systems, applications. *Expert Opin. Drug Deliv.* 19 (4), 435–450. doi:10.1080/17425247.2022.2063835

Bai, M., Zhang, M., Long, F., Yu, N., Zeng, A., Wang, X., et al. (2017). MiR-217 promotes cutaneous squamous cell carcinoma progression by targeting PTRF. *Am. J. Transl. Res.* 9 (2), 647–655.

Bai, X., Zhou, Y., Chen, P., Yang, M., and Xu, J. (2018). MicroRNA-142-5p induces cancer stem cell-like properties of cutaneous squamous cell carcinoma via inhibiting PTEN. *J. Cell. Biochem.* 119 (2), 2179–2188. doi:10.1002/jcb.26379

Bednarski, I. A., Ciążyńska, M., Wódz, K., Dróżdż, I., Skibińska, M., Narbutt, J., et al. (2021). Hippo signaling pathway as a new potential target in non-melanoma skin cancers: A narrative review. *Life (Basel)* 11 (7), 680. doi:10.3390/life11070680

Bi, X., Jiang, Z., Luan, Z., and Qiu, D. (2021). Crocin exerts anti-proliferative and apoptotic effects on cutaneous squamous cell carcinoma via miR-320a/ATG2B. *Bioengineered* 12 (1), 4569–4580. doi:10.1080/21655979.2021.1955175

Bian, D., Wu, Y., and Song, G. (2018). Novel circular RNA, hsa_circ_0025039 promotes cell growth, invasion and glucose metabolism in malignant melanoma via the miR-198/CDK4 axis. *Biomed. Pharmacother.* 108, 165–176. doi:10.1016/j.biopha.2018.08.152

Bouchie, A. (2013). First microRNA mimic enters clinic. *Nat. Biotechnol.* 31 (7), 577. doi:10.1038/nbt0713-577

Caba, L., Florea, L., Gug, C., Dimitriu, D. C., and Gorduza, E. V. (2021). Circular RNA-is the circle perfect? *Biomolecules* 11 (12), 1755. doi:10.3390/biom11121755

Cai, L., Wang, Y., Wu, J., and Wu, G. (2021). Hsa_circ_0008234 facilitates proliferation of cutaneous squamous cell carcinoma through targeting miR-127-5p to regulate ADCY7. *Arch. Dermatol. Res.* 314, 541–551. doi:10.1007/s00403-021-02261-8

Chen, J., Feilotter, H. E., Paré, G. C., Zhang, X., Pemberton, J. G., Garady, C., et al. (2010). MicroRNA-193b represses cell proliferation and regulates cyclin D1 in melanoma. *Am. J. Pathol.* 176 (5), 2520–2529. doi:10.2353/ajpath.2010.091061

Chen, B., Pan, W., Lin, X., Hu, Z., Jin, Y., Chen, H., et al. (2016). MicroRNA-346 functions as an oncogene in cutaneous squamous cell carcinoma. *Tumour Biol.* 37 (2), 2765–2771. doi:10.1007/s13277-015-4046-2

Chen, L., Chen, Q., Kuang, S., Zhao, C., Yang, L., Zhang, Y., et al. (2019). USF1-induced upregulation of LINC01048 promotes cell proliferation and apoptosis in cutaneous squamous cell carcinoma by binding to TAF15 to transcriptionally activate YAP1. *Cell Death Dis.* 10 (4), 296. doi:10.1038/s41419-019-1516-2

Chen, P., Li, C., Huang, H., Liang, L., Zhang, J., Li, Q., et al. (2021). Circular RNA profiles and the potential involvement of down-expression of hsa_circ_0001360 in cutaneous squamous cell carcinogenesis. *FEBS Open Bio* 11 (4), 1209–1222. doi:10.1002/2211-5463.13114

Chen, J., Hou, S. F., Tang, F. J., Liu, D. S., Chen, Z. Z., Zhang, H. L., et al. (2022). HOTAIR/Sp1/miR-199a critically regulates cancer stemness and malignant progression of cutaneous squamous cell carcinoma. *Oncogene* 41 (1), 99–111. doi:10.1038/s41388-021-02014-x

Cives, M., Mannavola, F., Lospalluti, L., Sergi, M. C., Cazzato, G., Filoni, E., et al. (2020). Non-melanoma skin cancers: Biological and clinical features. *Int. J. Mol. Sci.* 21 (15), E5394. doi:10.3390/ijms21155394

Cui, X. Y., Zhan, J. K., and Liu, Y. S. (2021). Roles and functions of antisense lncRNA in vascular aging. *Ageing Res. Rev.* 72, 101480. doi:10.1016/j.arr.2021.101480

Das, M. K., Haugen Ø, P., and Haugen, T. B. (2022). Diverse roles and targets of miRNA in the pathogenesis of testicular germ cell tumour. *Cancers (Basel)* 14 (5), 1190. doi:10.3390/cancers14051190

DeSouza, P. A., Qu, X., Chen, H., Patel, B., Maher, C. A., Kim, A. H., et al. (2021). Long, noncoding RNA dysregulation in glioblastoma. *Cancers (Basel)* 13 (7), 1604. doi:10.3390/cancers13071604

Di Lu, D., Sun, L., Li, Z., and Mu, Z. (2021). lncRNA EZR-AS1 knockdown represses proliferation, migration and invasion of cSCC via the PI3K/AKT signaling pathway. *Mol. Med. Rep.* 23 (1), 76. doi:10.3892/mmr.2020.11714

Diener, C., Keller, A., and Meese, E. (2022). Emerging concepts of miRNA therapeutics: From cells to clinic. *Trends Genet.* 38, 613–626. doi:10.1016/j.tig.2022.02.006

Ding, Z., Jian, S., Peng, X., Liu, Y., Wang, J., Zheng, L., et al. (2015). Loss of MiR-664 expression enhances cutaneous malignant melanoma proliferation by upregulating PLP2. *Med. Baltim.* 94 (33), e1327. doi:10.1097/md.0000000000001327

Ding, L., Wang, R., Shen, D., Cheng, S., Wang, H., Lu, Z., et al. (2021). Role of noncoding RNA in drug resistance of prostate cancer. *Cell Death Dis.* 12 (6), 590. doi:10.1038/s41419-021-03854-x

Fan, Z. X., Xi, W., Miao, X. Y., Li, L. Y., and Miao, G. Y. (2021). Circ_NCKAP1 promotes skin basal cell carcinoma progression by sponging the miR-148b-5p/HSP90 axis. *Eur. Rev. Med. Pharmacol. Sci.* 25 (17), 5355–5364. doi:10.26355/eurrev_202109_26642

Feng, C., Zhang, H. L., Zeng, A., Bai, M., and Wang, X. J. (2020). Tumor-Suppressive MicroRNA-216b binds to TPX2, activating the p53 signaling in human cutaneous squamous cell carcinoma. *Mol. Ther. Nucleic Acids* 20, 186–195. doi:10.1016/j.omtn.2020.01.022

Fernandes, M., Marques, H., Teixeira, A. L., and Medeiros, R. (2021). miRNA- and lncRNA-based therapeutics for non-hodgkin's lymphoma: Moving towards an RNA-guided precision medicine. *Cancers (Basel)* 13 (24), 6324. doi:10.3390/cancers13246324

Fu, J., Zhao, J., Zhang, H., Fan, X., Geng, W., Qiao, S., et al. (2021). MicroRNA-451a prevents cutaneous squamous cell carcinoma progression via the 3-phosphoinositide-dependent protein kinase-1-mediated PI3K/AKT signaling pathway. *Exp. Ther. Med.* 21 (2), 116. doi:10.3892/etm.2020.9548

Fujimura, T., and Aiba, S. (2020). Significance of immunosuppressive cells as a target for immunotherapies in melanoma and non-melanoma skin cancers. *Biomolecules* 10 (8), E1087. doi:10.3390/biom10081087

Gao, L., Jin, H. J., Zhang, D., and Lin, Q. (2020). Silencing circRNA_001937 may inhibit cutaneous squamous cell carcinoma proliferation and induce apoptosis by preventing the sponging of the miRNA-597-3p/FOSL2 pathway. *Int. J. Mol. Med.* 46 (5), 1653–1660. doi:10.3892/ijmm.2020.4723

Gebert, L. F., Rebhan, M. A., Crivelli, S. E., Denzler, R., Stoffel, M., Hall, J., et al. (2014). Miravirsen (SPC3649) can inhibit the biogenesis of miR-122. *Nucleic Acids Res.* 42 (1), 609–621. doi:10.1093/nar/gkt852

Gerloff, D., Lützkendorf, J., Moritz, R. K. C., Wersig, T., Mäder, K., Müller, L. P., et al. (2020). Melanoma-derived exosomal miR-125b-5p educates tumor associated macrophages (TAMs) by targeting lysosomal acid lipase A (lipa). *Cancers (Basel)* 12 (2), E464. doi:10.3390/cancers12020464

Glud, M., Rossing, M., Hother, C., Holst, L., Hastrup, N., Nielsen, F. C., et al. (2010). Downregulation of miR-125b in metastatic cutaneous malignant melanoma. *Melanoma Res.* 20 (6), 479–484. doi:10.1097/CMR.0b013e32833e32a1

Glud, M., Manfé, V., Biskup, E., Holst, L., Dirksen, A. M., Hastrup, N., et al. (2011). MicroRNA miR-125b induces senescence in human melanoma cells. *Melanoma Res.* 21 (3), 253–256. doi:10.1097/CMR.0b013e328345333b

Gong, Z., Shen, G., Huang, C., Zhang, J., and Ji, J. (2022). Downregulation of lncRNA NEAT1 inhibits the proliferation of human cutaneous squamous cell carcinoma *in vivo* and *in vitro*. *Ann. Transl. Med.* 10 (2), 79. doi:10.21037/atm-21-6916

Guo Jun Yu, G. J., Sun, Y., Zhang, D. W., and Zhang, P. (2019). Long non-coding RNA HOTAIR functions as a competitive endogenous RNA to regulate PRAF2 expression by sponging miR-326 in cutaneous squamous cell carcinoma. *Cancer Cell Int.* 19, 270. doi:10.1186/s12935-019-0992-x

Guo, W., Wang, H., Yang, Y., Guo, S., Zhang, W., Liu, Y., et al. (2017). Down-regulated miR-23a contributes to the metastasis of cutaneous melanoma by promoting autophagy. *Theranostics* 7 (8), 2231–2249. doi:10.7150/thno.18835

Hanniford, D., Ulloa-Morales, A., Karz, A., Berzoti-Coelho, M. G., Moubarak, R. S., Sánchez-Sendra, B., et al. (2020). Epigenetic silencing of CDR1as drives IGF2BP3-mediated melanoma invasion and metastasis. *Cancer Cell* 37 (1), 55–70. e15. doi:10.1016/j.ccell.2019.12.007

He, Y., Yang, Y., Liao, Y., Xu, J., Liu, L., Li, C., et al. (2020a). miR-140-3p inhibits cutaneous melanoma progression by disrupting AKT/p70S6K and JNK pathways through ABHD2. *Mol. Ther. Oncolytics* 17, 83–93. doi:10.1016/j.omto.2020.03.009

He, Y., Yang, Y., Xu, J., Liao, Y., Liu, L., Deng, L., et al. (2020b). IL22 drives cutaneous melanoma cell proliferation, migration and invasion through activation of miR-181/STAT3/AKT axis. *J. Cancer* 11 (9), 2679–2687. doi:10.7150/jca.40974

He, C., Wang, K., Gao, Y., Wang, C., Li, L., Liao, Y., et al. (2021). Roles of noncoding RNA in reproduction. *Front. Genet.* 12, 777510. doi:10.3389/fgene.2021.777510

Huang, W., Li, H., Yu, Q., Xiao, W., and Wang, D. O. (2022). LncRNA-mediated DNA methylation: An emerging mechanism in cancer and beyond. *J. Exp. Clin. Cancer Res.* 41 (1), 100. doi:10.1186/s13046-022-02319-z

Jian Zhou, J., Zhang, Y., Han, Z., Dong, Z., Cao, T., Wei, A., et al. (2019). miR-506 contributes to malignancy of cutaneous squamous cell carcinoma via targeting of P65 and LAMC1. *Cell Cycle* 18 (3), 333–345. doi:10.1080/15384101.2019.1568747

Jianda Zhou, J., Liu, R., Luo, C., Zhou, X., Xia, K., Chen, X., et al. (2014). MiR-20a inhibits cutaneous squamous cell carcinoma metastasis and proliferation by directly targeting LIMK1. *Cancer Biol. Ther.* 15 (10), 1340–1349. doi:10.4161/cbt.29821

Jianfei Tang, J., Fang, X., Chen, J., Zhang, H., and Tang, Z. (2021). Long non-coding RNA (lncRNA) in oral squamous cell carcinoma: Biological function and clinical application. *Cancers (Basel)* 13 (23), 5944. doi:10.3390/cancers13235944

Jiao, J., Fan, Y., and Zhang, Y. (2015). Expression and clinicopathological significance of microRNA-21 and programmed cell death 4 in malignant melanoma. *J. Int. Med. Res.* 43 (5), 672–678. doi:10.1177/0300060515583707

Jin, C., Dong, D., Yang, Z., Xia, R., Tao, S., Piao, M., et al. (2020). CircMYC regulates glycolysis and cell proliferation in melanoma. *Cell biochem. Biophys.* 78 (1), 77–88. doi:10.1007/s12013-019-00895-0

Jin, H., Du, W., Huang, W., Yan, J., Tang, Q., Chen, Y., et al. (2021). lncRNA and breast cancer: Progress from identifying mechanisms to challenges and opportunities of clinical treatment. *Mol. Ther. Nucleic Acids* 25, 613–637. doi:10.1016/j.omtn.2021.08.005

Jingwen Wang, J., Li, C., Xu, L., Yang, C., and Zhang, X. (2019). MiR-1193 was sponged by LINC00963 and inhibited cutaneous squamous cell carcinoma progression by targeting SOX4. *Pathol. Res. Pract.* 215 (10), 152600. doi:10.1016/j.prp.2019.152600

Kai Wang, K., Li, M., Zhang, T., Xu, C., Yu, F., Duan, H., et al. (2022). LINC01116 facilitates melanoma 1 progression via sequestering miR-3612 and up-regulating GDF11 and SDC3. *Arch. Med. Res.* 53 (1), 44–50. doi:10.1016/j.arcmed.2021.06.008

Kakumani, P. K. (2022). AGO-RBP crosstalk on target mRNAs: Implications in miRNA-guided gene silencing and cancer. *Transl. Oncol.* 21, 101434. doi:10.1016/j.tranon.2022.101434

Kanitz, A., Imig, J., Dziunycz, P. J., Primorac, A., Galgano, A., Hofbauer, G. F., et al. (2012). The expression levels of microRNA-361-5p and its target VEGFA are inversely correlated in human cutaneous squamous cell carcinoma. *PLoS One* 7 (11), e49568. doi:10.1371/journal.pone.0049568

Ke Tian, K., Liu, W., Zhang, J., Fan, X., Liu, J., Zhao, N., et al. (2020). MicroRNA-125b exerts antitumor functions in cutaneous squamous cell carcinoma by targeting the STAT3 pathway. *Cell. Mol. Biol. Lett.* 25, 12. doi:10.1186/s11658-020-00207-y

Kim, B. K., Kim, I., and Yoon, S. K. (2015). Identification of miR-199a-5p target genes in the skin keratinocyte and their expression in cutaneous squamous cell carcinoma. *J. Dermatol. Sci.* 79 (2), 137–147. doi:10.1016/j.jdermsci.2015.05.005

Kim, E., Kim, Y. K., and Lee, S. V. (2021). Emerging functions of circular RNA in aging. *Trends Genet.* 37, 819–829. doi:10.1016/j.tig.2021.04.014

Kitago, M., Martinez, S. R., Nakamura, T., Sim, M. S., and Hoon, D. S. (2009). Regulation of RUNX3 tumor suppressor gene expression in cutaneous melanoma. *Clin. Cancer Res.* 15 (9), 2988–2994. doi:10.1158/1078-0432.Ccr-08-3172

Kong, H., Sun, M. L., Zhang, X. A., and Wang, X. Q. (2021). Crosstalk among circRNA/lncRNA, miRNA, and mRNA in osteoarthritis. *Front. Cell Dev. Biol.* 9, 774370. doi:10.3389/fcell.2021.774370

Krasowska, D., Gerkowicz, A., Mlak, R., Leziak, M., Małecka-Massalska, T., Krasowska, D., et al. (2021). Risk of nonmelanoma skin cancers and Parkinson's disease-meta-analysis and systematic review. *Cancers (Basel)* 13 (4), 587. doi:10.3390/cancers13040587

Li, F., Liao, J., Duan, X., He, Y., and Liao, Y. (2018). Upregulation of LINC00319 indicates a poor prognosis and promotes cell proliferation and invasion in cutaneous squamous cell carcinoma. *J. Cell. Biochem.* 119 (12), 10393–10405. doi:10.1002/jcb.27388

Li, Y., Li, M., Shats, I., Krahn, J. M., Flake, G. P., Umbach, D. M., et al. (2019). Glypican 6 is a putative biomarker for metastatic progression of cutaneous melanoma. *PLoS One* 14 (6), e0218067. doi:10.1371/journal.pone.0218067

Li, K., Tang, M., Tong, S., Wang, C., Sun, Q., Lv, M., et al. (2020). BRAFi induced demethylation of miR-152-5p regulates phenotype switching by targeting TXNIP in cutaneous melanoma. *Apoptosis* 25 (3-4), 179–191. doi:10.1007/s10495-019-01586-0

Li, X., Yuan, Y., Wang, Y., Xie, K., Lu, S., Chen, F., et al. (2022). MicroRNA-486-3p promotes the proliferation and metastasis of cutaneous squamous cell carcinoma by suppressing flotillin-2. *J. Dermatol. Sci.* 105 (1), 18–26. doi:10.1016/j.jdermsci.2021.11.005

Lin, N., Zhou, Y., Lian, X., and Tu, Y. (2017). MicroRNA-31 functions as an oncogenic microRNA in cutaneous squamous cell carcinoma cells by targeting RhoTBT1. *Oncol. Lett.* 13 (3), 1078–1082. doi:10.3892/ol.2017.5554

Lin, Q., Jiang, H., and Lin, D. (2021). Circular RNA ITCH downregulates GLUT1 and suppresses glucose uptake in melanoma to inhibit cancer cell proliferation. *J. Dermatol. Treat.* 32 (2), 231–235. doi:10.1080/09546634.2019.1654069

Lipeng Wang, L., Hou, S., Li, J., Tian, T., Hu, R., Yu, N., et al. (2022). Circular RNA circ-LARP1B contributes to cutaneous squamous cell carcinoma progression by targeting microRNA-515-5p/TPX2 microtubule nucleation factor axis. *Bioengineered* 13 (1), 1209–1223. doi:10.1080/21655979.2021.2019172

Liu, P., Shi, L., Ding, Y., Luan, J., Shan, X., Li, Q., et al. (2020). MicroRNA-766 promotes the proliferation, migration and invasion, and inhibits the apoptosis of cutaneous squamous cell carcinoma cells by targeting PDCD5. *Onco. Targets. Ther.* 13, 4099–4110. doi:10.2147/ott.S222821

Liu, T., Jiang, F., Yu, L. Y., and Wu, Y. Y. (2022). Lidocaine represses proliferation and cisplatin resistance in cutaneous squamous cell carcinoma via miR-30c/SIRT1 regulation. *Bioengineered* 13 (3), 6359–6370. doi:10.1080/21655979.2022.2031419

Lohcharoenkal, W., Li, C., Das Mahapatra, K., Lapins, J., Homey, B., Sonkoly, E., et al. (2021). MiR-130a acts as a tumor suppressor MicroRNA in cutaneous squamous cell carcinoma and regulates the activity of the BMP/SMAD pathway by suppressing ACVR1. *J. Invest. Dermatol.* 141 (8), 1922–1931. doi:10.1016/j.jid.2021.01.028

Long, J., Luo, J., and Yin, X. (2018). miR-367 enhances the proliferation and invasion of cutaneous malignant melanoma by regulating phosphatase and tensin homolog expression. *Mol. Med. Rep.* 17 (5), 6526–6532. doi:10.3892/mmr.2018.8663

Lu, R. H., Xiao, Z. Q., Zhou, J. D., Yin, C. Q., Chen, Z. Z., Tang, F. J., et al. (2020). MiR-199a-5p represses the stemness of cutaneous squamous cell carcinoma stem cells by targeting Sirt1 and CD44ICD cleavage signaling. *Cell Cycle* 19 (1), 1–14. doi:10.1080/15384101.2019.1689482

Luan, W., Shi, Y., Zhou, Z., Xia, Y., and Wang, J. (2018). circRNA_0084043 promote malignant melanoma progression via miR-153-3p/Snail axis. *Biochem. Biophys. Res. Commun.* 502 (1), 22–29. doi:10.1016/j.bbrc.2018.05.114

Luo, Q., Li, W., Zhao, T., Tian, X., Liu, Y., Zhang, X., et al. (2015). Role of miR-148a in cutaneous squamous cell carcinoma by repression of MAPK pathway. *Arch. Biochem. Biophys.* 583, 47–54. doi:10.1016/j.abb.2015.07.022

Ma, X., Wu, D., Zhang, X., Shao, X., and Hu, G. (2020). microRNA-214 prevents traits of cutaneous squamous cell carcinoma via VEGFA and bcl-2. *Technol. Cancer Res. Treat.* 19, 1533033820980098. doi:10.1177/1533033820980098

Martin del Campo, S. E., Latchana, N., Levine, K. M., Grignol, V. P., Fairchild, E. T., Jaime-Ramirez, A. C., et al. (2015). MiR-21 enhances melanoma invasiveness via inhibition of tissue inhibitor of metalloproteinases 3 expression: *In vivo* effects of MiR-21 inhibitor. *PLoS One* 10 (1), e0115919. doi:10.1371/journal.pone.0115919

Mei, X. L., and Zhong, S. (2019). Long noncoding RNA LINC00520 prevents the progression of cutaneous squamous cell carcinoma through the inactivation of the PI3K/Akt signaling pathway by downregulating EGFR. *Chin. Med. J.* 132 (4), 454–465. doi:10.1097/cm9.0000000000000070

Meijuan Zhou, M., Zhou, L., Zheng, L., Guo, L., Wang, Y., Liu, H., et al. (2014). miR-365 promotes cutaneous squamous cell carcinoma (CSCC) through targeting nuclear factor I/B (NFIB). *PLoS One* 9 (6), e100620. doi:10.1371/journal.pone.0100620

Mercer, T. R., Munro, T., and Mattick, J. S. (2022). The potential of long noncoding RNA therapies. *Trends Pharmacol. Sci.* 43 (4), 269–280. doi:10.1016/j.tips.2022.01.008

Mi, X., Lai, K., Yan, L., Xie, S., Qiu, X., Xiao, S., et al. (2020). miR-18a expression in basal cell carcinoma and regulatory mechanism on autophagy through mTOR pathway. *Clin. Exp. Dermatol.* 45 (8), 1027–1034. doi:10.1111/ced.14322

Mizrahi, A., Barzilai, A., Gur-Wahnon, D., Ben-Dov, I. Z., Glassberg, S., Meningher, T., et al. (2018). Alterations of microRNAs throughout the malignant evolution of cutaneous squamous cell carcinoma: The role of miR-497 in epithelial to mesenchymal transition of keratinocytes. *Oncogene* 37 (2), 218–230. doi:10.1038/onc.2017.315

Mu, X., Mou, K. H., Ge, R., Han, D., Zhou, Y., Wang, L. J., et al. (2019). Linc00961 inhibits the proliferation and invasion of skin melanoma by targeting the miR-367/PTEN axis. *Int. J. Oncol.* 55 (3), 708–720. doi:10.3892/ijo.2019.4848

Nojima, T., and Proudfoot, N. J. (2022). Mechanisms of lncRNA biogenesis as revealed by nascent transcriptomics. *Nat. Rev. Mol. Cell Biol.* 23, 389–406. doi:10.1038/s41580-021-00447-6

Nyholm, A. M., Lerche, C. M., Manfé, V., Biskup, E., Johansen, P., Morling, N., et al. (2014). miR-125b induces cellular senescence in malignant melanoma. *BMC Dermatol.* 14, 8. doi:10.1186/1471-5945-14-8

Olasz, E. B., Seline, L. N., Schock, A. M., Duncan, N. E., Lopez, A., Lazar, J., et al. (2015). MicroRNA-135b regulates leucine zipper tumor suppressor 1 in cutaneous squamous cell carcinoma. *PLoS One* 10 (5), e0125412. doi:10.1371/journal.pone.0125412

Piipponen, M., Nissinen, L., Farshchian, M., Riihilä, P., Kivisaari, A., Kallajoki, M., et al. (2016). Long noncoding RNA PICSAR promotes growth of cutaneous squamous cell carcinoma by regulating ERK1/2 activity. *J. Invest. Dermatol.* 136 (8), 1701–1710. doi:10.1016/j.jid.2016.03.028

Piipponen, M., Nissinen, L., Riihilä, P., Farshchian, M., Kallajoki, M., Peltonen, J., et al. (2020). p53-Regulated long noncoding RNA PRECSIT promotes progression of cutaneous squamous cell carcinoma via STAT3 signaling. *Am. J. Pathol.* 190 (2), 503–517. doi:10.1016/j.ajpath.2019.10.019

Qian, P., Linbo, L., Xiaomei, Z., and Hui, P. (2020). Circ_0002770, acting as a competitive endogenous RNA, promotes proliferation and invasion by targeting miR-331-3p in melanoma. *Cell Death Dis.* 11 (4), 264. doi:10.1038/s41419-020-2444-x

Qirong Lu, Q., Guo, P., Liu, A., Ares, I., Martinez-Larranaga, M. R., Wang, X., et al. (2021). The role of long noncoding RNA in lipid, cholesterol, and glucose metabolism and treatment of obesity syndrome. *Med. Res. Rev.* 41 (3), 1751–1774. doi:10.1002/med.21775

Quan Liu, Q., Cui, W., Yang, C., and Du, L. P. (2021). Circular RNA ZNF609 drives tumor progression by regulating the miR-138-5p/SIRT7 axis in melanoma. *Aging (Albany NY)* 13 (15), 19822–19834. doi:10.18632/aging.203394

Quirico, L., Orso, F., Cucinelli, S., Paradzik, M., Natalini, D., Centonze, G., et al. (2022). miRNA-guided reprogramming of glucose and glutamine metabolism and its impact on cell adhesion/migration during solid tumor progression. *Cell. Mol. Life Sci.* 79 (4), 216. doi:10.1007/s00018-022-04228-y

Reddy, P., Yao, M., and Patel, M. (2021). Investigative landscape in advanced non-melanoma skin cancers. *Curr. Treat. Options Oncol.* 22 (7), 56. doi:10.1007/s11864-021-00853-0

Ren, W., Zhu, Z., and Wu, L. (2019). FOXD2-AS1 correlates with the malignant status and regulates cell proliferation, migration, and invasion in cutaneous melanoma. *J. Cell. Biochem.* 120 (4), 5417–5423. doi:10.1002/jcb.27820

Saldanha, G., Potter, L., Lee, Y. S., Watson, S., Shendge, P., Pringle, J. H., et al. (2016). MicroRNA-21 expression and its pathogenetic significance in cutaneous melanoma. *Melanoma Res.* 26 (1), 21–28. doi:10.1097/cmr.0000000000000216

Sánchez-Sendra, B., Serna, E., Navarro, L., González-Muñoz, J. F., Portero, J., Ramos, A., et al. (2020). Transcriptomic identification of miR-205 target genes potentially involved in metastasis and survival of cutaneous malignant melanoma. *Sci. Rep.* 10 (1), 4771. doi:10.1038/s41598-020-61637-4

Sand, M., Skrygan, M., Sand, D., Georgas, D., Hahn, S. A., Gambichler, T., et al. (2012). Expression of microRNAs in basal cell carcinoma. *Br. J. Dermatol.* 167 (4), 847–855. doi:10.1111/j.1365-2133.2012.11022.x

Sand, M., Bechara, F. G., Sand, D., Gambichler, T., Hahn, S. A., Bromba, M., et al. (2016a). Circular RNA expression in basal cell carcinoma. *Epigenomics* 8 (5), 619–632. doi:10.2217/epi-2015-0019

Sand, M., Bechara, F. G., Sand, D., Gambichler, T., Hahn, S. A., Bromba, M., et al. (2016b). Long-noncoding RNAs in basal cell carcinoma. *Tumour Biol.* 37 (8), 10595–10608. doi:10.1007/s13277-016-4927-z

Sand, M., Hessam, S., Amur, S., Skrygan, M., Bromba, M., Stockfleth, E., et al. (2017). Expression of oncogenic miR-17-92 and tumor suppressive miR-143-145 clusters in basal cell carcinoma and cutaneous squamous cell carcinoma. *J. Dermatol. Sci.* 86 (2), 142–148. doi:10.1016/j.jdermsci.2017.01.012

Sato, S., Sawada, Y., and Nakamura, M. (2021). STING signaling and skin cancers. *Cancers (Basel)* 13 (22), 5603. doi:10.3390/cancers13225603

Shan Tian, S., Han, G., Lu, L., and Meng, X. (2020). Circ-FOXM1 contributes to cell proliferation, invasion, and glycolysis and represses apoptosis in melanoma by regulating miR-143-3p/FLOT2 axis. *World J. Surg. Oncol.* 18 (1), 56. doi:10.1186/s12957-020-01832-9

Shang, Q., Li, Y., Wang, H., Ge, S., and Jia, R. (2019). Altered expression profile of circular RNAs in conjunctival melanoma. *Epigenomics* 11 (7), 787–804. doi:10.2217/epi-2019-0029

Shao Hua Wang, S. H., Zhou, J. D., He, Q. Y., Yin, Z. Q., Cao, K., Luo, C. Q., et al. (2014). MiR-199a inhibits the ability of proliferation and migration by regulating CD44-Ezrin signaling in cutaneous squamous cell carcinoma cells. *Int. J. Clin. Exp. Pathol.* 7 (10), 7131–7141.

Shao, J., Liang, J., and Zhong, S. (2019). miR-30a-5p modulates traits of cutaneous squamous cell carcinoma (cSCC) via forkhead box protein G1 (FOXG1). *Neoplasma* 66 (6), 908–917. doi:10.4149/neo_2018_181205N923

Shiqiu Jiang, S., Liu, H., Zhang, J., Zhang, F., Fan, J., Liu, Y., et al. (2021). MMP1 regulated by NEAT1/miR-361-5p axis facilitates the proliferation and migration of cutaneous squamous cell carcinoma via the activation of Wnt pathway. *Cancer Biol. Ther.* 22 (5-6), 381–391. doi:10.1080/15384047.2021.1941583

Shuang Chen, S., Ding, J., Wang, Y., Lu, T., Wang, L., Gao, X., et al. (2020). RNA-seq profiling of circular RNAs and the oncogenic role of circPVT1 in cutaneous squamous cell carcinoma. *Onco. Targets. Ther.* 13, 6777–6788. doi:10.2147/ott.S252233

Shunhao Zhang, S., Sun, J., Gu, M., Wang, G., and Wang, X. (2021). Circular RNA: A promising new star for the diagnosis and treatment of colorectal cancer. *Cancer Med.* 10 (24), 8725–8740. doi:10.1002/cam4.4398

Shuo Han, S., Li, X., Wang, K., Zhu, D., Meng, B., Liu, J., et al. (2021). PURPL represses autophagic cell death to promote cutaneous melanoma by modulating ULK1 phosphorylation. *Cell Death Dis.* 12 (11), 1070. doi:10.1038/s41419-021-04362-8

Singh, A., Jain, D., Pandey, J., Yadav, M., Bansal, K. C., Singh, I. K., et al. (2022). Deciphering the role of miRNA in reprogramming plant responses to drought stress. *Crit. Rev. Biotechnol.* [Epub ahead of print], 1–15. doi:10.1080/07388551.2022.2047880

Sinha, T., Panigrahi, C., Das, D., and Chandra, P. A. (2021). Circular RNA translation, a path to hidden proteome. *Wiley Interdiscip. Rev. RNA* 13, e1685. doi:10.1002/wrna.1685

Song, H., Tao, Y., Ni, N., Zhou, X., Xiong, J., Zeng, X., et al. (2018). miR-128 targets the CC chemokine ligand 18 gene (CCL18) in cutaneous malignant melanoma progression. *J. Dermatol. Sci.* 91 (3), 317–324. doi:10.1016/j.jdermsci.2018.06.011

Sonkoly, E., Lovén, J., Xu, N., Meisgen, F., Wei, T., Brodin, P., et al. (2012). MicroRNA-203 functions as a tumor suppressor in basal cell carcinoma. *Oncogenesis* 1 (3), e3. doi:10.1038/oncsis.2012.3

Stark, M. S., Bonazzi, V. F., Boyle, G. M., Palmer, J. M., Symmons, J., Lanagan, C. M., et al. (2015). miR-514a regulates the tumour suppressor NF1 and modulates BRAFi sensitivity in melanoma. *Oncotarget* 6 (19), 17753–17763. doi:10.18632/oncotarget.3924

Su, B. B., Zhou, S. W., Gan, C. B., and Zhang, X. N. (2016). MiR-330-5p regulates tyrosinase and PDIA3 expression and suppresses cell proliferation and invasion in cutaneous malignant melanoma. *J. Surg. Res.* 203 (2), 434–440. doi:10.1016/j.jss.2016.03.021

Su, B. B., Zhou, S. W., Gan, C. B., and Zhang, X. N. (2018). MiR-186 inhibits cell proliferation and invasion in human cutaneous malignant melanoma. *J. Cancer Res. Ther.* 14, S60–S64. doi:10.4103/0973-1482.157340

Sun, H., and Jiang, P. (2018). MicroRNA-451a acts as tumor suppressor in cutaneous basal cell carcinoma. *Mol. Genet. Genomic Med.* 6 (6), 1001–1009. doi:10.1002/mgg3.473

Sun, Y., Hou, Z., Luo, B., Li, C., Liu, J., Liu, J., et al. (2021). Circular RNA circRNA_0082835 promotes progression and lymphatic metastasis of primary melanoma by sponging microRNA miRNA-429. *Bioengineered* 12 (1), 4159–4173. doi:10.1080/21655979.2021.1953822

Tian Tian, T., Zhao, Y., Zheng, J., Jin, S., Liu, Z., Wang, T., et al. (2021). Circular RNA: A potential diagnostic, prognostic, and therapeutic biomarker for human triple-negative breast cancer. *Mol. Ther. Nucleic Acids* 26, 63–80. doi:10.1016/j.omtn.2021.06.017

Toll, A., Salgado, R., Espinet, B., Díaz-Lagares, A., Hernández-Ruiz, E., Andrades, E., et al. (2016). MiR-204 silencing in intraepithelial to invasive cutaneous squamous cell carcinoma progression. *Mol. Cancer* 15 (1), 53. doi:10.1186/s12943-016-0537-z

van Kempen, L. C., van den Hurk, K., Lazar, V., Michiels, S., Winnepenninckx, V., Stas, M., et al. (2012). Loss of microRNA-200a and c, and microRNA-203 expression at the invasive front of primary cutaneous melanoma is associated with increased thickness and disease progression. *Virchows Arch.* 461 (4), 441–448. doi:10.1007/s00428-012-1309-9

Venza, M., Visalli, M., Beninati, C., Benfatto, S., Teti, D., Venza, I., et al. (2016). miR-92a-3p and MYCBP2 are involved in MS-275-induced and c-myc-mediated TRAIL-sensitivity in melanoma cells. *Int. Immunopharmacol.* 40, 235–243. doi:10.1016/j.intimp.2016.09.004

Wang, S., Cao, K. E., He, Q., Yin, Z., and Zhou, J. (2016). miR-199a-5p induces cell invasion by suppressing E-cadherin expression in cutaneous squamous cell carcinoma. *Oncol. Lett.* 12 (1), 97–101. doi:10.3892/ol.2016.4602

Wang, X., Qu, H., Dong, Y., Wang, G., Zhen, Y., Zhang, L., et al. (2018). Targeting signal-transducer-and-activator-of-transcription 3 sensitizes human cutaneous melanoma cells to BRAF inhibitor. *Cancer Biomark.* 23 (1), 67–77. doi:10.3233/cbm-181365

Wang, D., Zhou, X., Yin, J., and Zhou, Y. (2020). Lnc-PICSAR contributes to cisplatin resistance by miR-485-5p/REV3L axis in cutaneous squamous cell carcinoma. *Open Life Sci.* 15 (1), 488–500. doi:10.1515/biol-2020-0049

Wei, X. H., Gu, X. L., Zhou, X. T., Ma, M., and Lou, C. X. (2018). miR-497 promotes the progression of cutaneous squamous cell carcinoma through FAM114A2. *Eur. Rev. Med. Pharmacol. Sci.* 22 (21), 7348–7355. doi:10.26355/eurrev_201811_16272

Wei, X., Gu, X., Ma, M., and Lou, C. (2019). Long noncoding RNA HCP5 suppresses skin cutaneous melanoma development by regulating RARRES3 gene expression via sponging miR-12. *Onco. Targets. Ther.* 12, 6323–6335. doi:10.2147/ott.S195796

Wei, C. Y., Zhu, M. X., Lu, N. H., Liu, J. Q., Yang, Y. W., Zhang, Y., et al. (2020). Circular RNA circ_0020710 drives tumor progression and immune evasion by regulating the miR-370-3p/CXCL12 axis in melanoma. *Mol. Cancer* 19 (1), 84. doi:10.1186/s12943-020-01191-9

Wei Zhang, W., Yang, X., Lv, J., and An, S. (2022). Research on correlations of miR-196a expression with progression and prognosis of cutaneous squamous cell carcinoma. *Clin. Cosmet. Investig. Dermatol.* 15, 97–105. doi:10.2147/ccid.S323414

Wen, S. Y., Qadir, J., and Yang, B. B. (2022). Circular RNA translation: Novel protein isoforms and clinical significance. *Trends Mol. Med.* 28 (5), 405–420. doi:10.1016/j.molmed.2022.03.003

Wenqing Zhang, W., Zhou, K., Zhang, X., Wu, C., Deng, D., Yao, Z., et al. (2021). Roles of the H19/microRNA-675 axis in the proliferation and epithelial-mesenchymal transition of human cutaneous squamous cell carcinoma cells. *Oncol. Rep.* 45 (4), 39. doi:10.3892/or.2021.7990

Winkle, M., El-Daly, S. M., Fabbri, M., and Calin, G. A. (2021). Noncoding RNA therapeutics - challenges and potential solutions. *Nat. Rev. Drug Discov.* 20 (8), 629–651. doi:10.1038/s41573-021-00219-z

Wu, L., Zhu, L., Li, Y., Zheng, Z., Lin, X., Yang, C., et al. (2020). LncRNA MEG3 promotes melanoma growth, metastasis and formation through modulating miR-21/E-cadherin axis. *Cancer Cell Int.* 20, 12. doi:10.1186/s12935-019-1087-4

Wu Zhou, W., Zhang, S., Li, J., Li, Z., Wang, Y., Li, X., et al. (2019). lncRNA TINCR participates in ALA-PDT-induced apoptosis and autophagy in cutaneous squamous cell carcinoma. *J. Cell. Biochem.* 120 (8), 13893–13902. doi:10.1002/jcb.28662

Xiaobo Zhou, X., Rao, Y., Sun, Q., Liu, Y., Chen, J., Bu, W., et al. (2019). Long noncoding RNA CPS1-IT1 suppresses melanoma cell metastasis through inhibiting Cyr61 via competitively binding to BRG1. *J. Cell. Physiol.* 234 (12), 22017–22027. doi:10.1002/jcp.28764

Xiaoqing Li, X., Kong, Y., Li, H., Xu, M., Jiang, M., Sun, W., et al. (2021). CircRNA circ_0067772 aggravates the malignant progression of cutaneous squamous cell carcinoma by regulating miR-1238-3p/FOXG1 axis. *Genes Genomics* 43 (5), 491–501. doi:10.1007/s13258-021-01074-3

Xiaoyan Lu, X., Gan, Q., and Gan, C. (2021a). Circular RNA circSEC24A promotes cutaneous squamous cell carcinoma progression by regulating miR-1193/MAP3K9 Axis. *Onco. Targets. Ther.* 14, 653–666. doi:10.2147/ott.S275691

Xinde Hu, X., Liu, Y., Ai, P., He, S., Liu, L., Chen, C., et al. (2019). MicroRNA-186 promotes cell proliferation and inhibits cell apoptosis in cutaneous squamous cell carcinoma by targeting RETREG1. *Exp. Ther. Med.* 17 (3), 1930–1938. doi:10.3892/etm.2019.7154

Xiong, Z., Jiang, B., and Li, G. (2020). Downregulation of miR-10a inhibits cutaneous squamous cell carcinoma cell proliferation, migration, and invasion by targeting Syndecan-1. *Int. J. Clin. Exp. Pathol.* 13 (10), 2502–2512.

Xiong, W., Wu, L., Tang, R., Zhang, Q., Guo, Q., and Song, S. (2021). Grape seed proanthocyanidins (GSPs) inhibit the development of cutaneous squamous cell carcinoma by regulating the hsa_circ_0070934/miR-136-5p/PRAF2 Axis. *Cancer Manag. Res.* 13, 4359–4371. doi:10.2147/cmar.S302084

Xu E Chen, X. E., Chen, P., Chen, S., Lu, J., Ma, T., Shi, G., et al. (2020). Long non-coding RNA FENDRR inhibits migration and invasion of cutaneous malignant melanoma cells. *Biosci. Rep.* 40 (3), BSR20191194. doi:10.1042/bsr20191194

Xu, N., Zhang, L., Meisgen, F., Harada, M., Heilborn, J., Homey, B., et al. (2012). MicroRNA-125b down-regulates matrix metallopeptidase 13 and inhibits cutaneous squamous cell carcinoma cell proliferation, migration, and invasion. *J. Biol. Chem.* 287 (35), 29899–29908. doi:10.1074/jbc.M112.391243

Xu, Y., Guo, B., Liu, X., and Tao, K. (2021). miR-34a inhibits melanoma growth by targeting ZEB1. *Aging (Albany NY)* 13 (11), 15538–15547. doi:10.18632/aging.203114

Xu, J., Zhang, F., Lin, M., and Tong, Y. (2022). LINC00518 affects the proliferation, invasion and migration of cutaneous malignant melanoma cells via miR-526b-3p/EIF5A2 axis. *Acta Biochim. Pol.* 69 (1), 101–111. doi:10.18388/abp.2020_5746

Yamane, K., Jinnin, M., Etoh, T., Kobayashi, Y., Shimozono, N., Fukushima, S., et al. (2013). Down-regulation of miR-124/-214 in cutaneous squamous cell carcinoma mediates abnormal cell proliferation via the induction of ERK. *J. Mol. Med.* 91 (1), 69–81. doi:10.1007/s00109-012-0935-7

Yan Hu, Y., Wang, Q., and Zhu, X. H. (2019). MiR-135b is a novel oncogenic factor in cutaneous melanoma by targeting LATS2. *Melanoma Res.* 29 (2), 119–125. doi:10.1097/cmr.0000000000000524

Yan Liu, Y., He, D., Xiao, M., Zhu, Y., Zhou, J., Cao, K., et al. (2021). Long noncoding RNA LINC00518 induces radioresistance by regulating glycolysis through an miR-33a-3p/HIF-1α negative feedback loop in melanoma. *Cell Death Dis.* 12 (3), 245. doi:10.1038/s41419-021-03523-z

Yan Wang, Y., Wu, C., Du, Y., Li, Z., Li, M., Hou, P., et al. (2022). Expanding uncapped translation and emerging function of circular RNA in carcinomas and noncarcinomas. *Mol. Cancer* 21 (1), 13. doi:10.1186/s12943-021-01484-7

Yan Yu, Y., Xiang, N., Lin, M., Huang, J. W., Zhang, J., Cheng, B., et al. (2019). miR- 26a sensitizes melanoma cells to dabrafenib via targeting HMGB1-dependent autophagy pathways. *Drug Des. devel. Ther.* 13, 3717–3726. doi:10.2147/dddt.S225671

Yang, Z., Liao, B., Xiang, X., and Ke, S. (2020). miR-21-5p promotes cell proliferation and G1/S transition in melanoma by targeting CDKN2C. *FEBS Open Bio* 10 (5), 752–760. doi:10.1002/2211-5463.12819

Yang, L., Li, L. P., and Yi, H. C. (2022). DeepWalk based method to predict lncRNA-miRNA associations via lncRNA-miRNA-disease-protein-drug graph. *BMC Bioinforma.* 22 (12), 621. doi:10.1186/s12859-022-04579-0

Yanhua Wang, Y., Tang, S., and Lv, J. (2022). MicroRNA-573 inhibits cell proliferation, migration and invasion and is downregulated by PICSAR in cutaneous squamous cell carcinoma. *Bosn. J. Basic Med. Sci.* 22 (2), 229–237. doi:10.17305/bjbms.2021.6301

Yating Li, Y., Li, Y., and Li, L. (2021). Circular RNA hsa_Circ_0005795 mediates cell proliferation of cutaneous basal cell carcinoma via sponging miR-1231. *Arch. Dermatol. Res.* 313 (9), 773–782. doi:10.1007/s00403-020-02174-y

Yin, D., Wei, G., Yang, F., and Sun, X. (2021). Circular RNA has circ 0001591 promoted cell proliferation and metastasis of human melanoma via ROCK1/PI3K/AKT by targeting miR-431-5p. *Hum. Exp. Toxicol.* 40 (2), 310–324. doi:10.1177/0960327120950014

Yinghui Wang, Y., Deng, X., Dai, Y., Niu, X., and Zhou, M. (2019). miR-27a downregulation promotes cutaneous squamous cell carcinoma progression via targeting EGFR. *Front. Oncol.* 9, 1565. doi:10.3389/fonc.2019.01565

Yuan, S., Zhang, P., Wen, L., Jia, S., Wu, Y., Zhang, Z., et al. (2021). miR-22 promotes stem cell traits via activating Wnt/β-catenin signaling in cutaneous squamous cell carcinoma. *Oncogene* 40 (39), 5799–5813. doi:10.1038/s41388-021-01973-5

Yue Zhang, Y., Zhang, X., Xu, Y., Fang, S., Ji, Y., Lu, L., et al. (2022). Circular RNA and its roles in the occurrence, development, diagnosis of cancer. *Front. Oncol.* 12, 845703. doi:10.3389/fonc.2022.845703

Yuxiong Jiang, Y., Zhang, C., Zhang, J., Han, D., and Shi, X. (2021). Comprehensive analysis of the prognosis and biological significance for IFIT family in skin cutaneous melanoma. *Int. Immunopharmacol.* 101, 108344. doi:10.1016/j.intimp.2021.108344

Zehavi, L., Schayek, H., Jacob-Hirsch, J., Sidi, Y., Leibowitz-Amit, R., Avni, D., et al. (2015). MiR-377 targets E2F3 and alters the NF-kB signaling pathway through MAP3K7 in malignant melanoma. *Mol. Cancer* 14, 68. doi:10.1186/s12943-015-0338-9

Zeng, X., Yuan, X., Cai, Q., Tang, C., and Gao, J. (2021). Circular RNA as an epigenetic regulator in chronic liver diseases. *Cells* 10 (8), 1945. doi:10.3390/cells10081945

Zhang, D., Li, Z., Zhang, Y., Tu, C., Huo, J., Liu, Y., et al. (2016). miR-4262 promotes the proliferation of human cutaneous malignant melanoma cells through KLF6-mediated EGFR inactivation and p21 upregulation. *Oncol. Rep.* 36 (6), 3657–3663. doi:10.3892/or.2016.5190

Zhang, M., Luo, H., and Hui, L. (2019). MiR-3619-5p hampers proliferation and cisplatin resistance in cutaneous squamous-cell carcinoma via KPNA4. *Biochem. Biophys. Res. Commun.* 513 (2), 419–425. doi:10.1016/j.bbrc.2019.03.203

Zhang, D. W., Wu, H. Y., Zhu, C. R., and Wu, D. D. (2020a). CircRNA hsa_circ_0070934 functions as a competitive endogenous RNA to regulate

HOXB7 expression by sponging miR-1236-3p in cutaneous squamous cell carcinoma. *Int. J. Oncol.* 57 (2), 478–487. doi:10.3892/ijo.2020.5066

Zhang, Z., Jia, M., Wen, C., He, A., and Ma, Z. (2020b). Long non-coding RNA SCARNA2 induces cutaneous squamous cell carcinoma progression via modulating miR-342-3p expression. *J. Gene Med.* 22 (12), e3242. doi:10.1002/jgm.3242

Zhang, Z., Guo, H., Yang, W., and Li, J. (2021a). Exosomal circular RNA RNA-seq profiling and the carcinogenic role of exosomal circ-cyp24a1 in cutaneous squamous cell carcinoma. *Front. Med.* 8, 675842. doi:10.3389/fmed.2021.675842

Zhang, Z., Huang, Q., Yu, L., Zhu, D., Li, Y., Xue, Z., et al. (2021b). The role of miRNA in tumor immune escape and miRNA-based therapeutic strategies. *Front. Immunol.* 12, 807895. doi:10.3389/fimmu.2021.807895

Zhang, Z., Sun, H., Hou, J., Li, L., and Wu, L. (2021c). Circular RNA circFADS2 inhibits the progression of cutaneous squamous cell carcinoma by regulating miR-766-3p/HOXA9 axis. *Histol. Histopathol.* 37 (4), 335–348. doi:10.14670/hh-18-409

Zhao, F., Jia, Z., Feng, Y., Li, Z., and Feng, J. (2021). Circular RNA circ_0079593 enhances malignant melanoma progression by the regulation of the miR-573/ABHD2 axis. *J. Dermatol. Sci.* 102 (1), 7–15. doi:10.1016/j.jdermsci.2021.01.008

Zheng, Y., Zhou, W., Li, M., Xu, R., Zhang, S., Liu, Y., et al. (2021). IRF4-activated TEX41 promotes the malignant behaviors of melanoma cells by targeting miR-103a-3p/C1QB axis. *BMC Cancer* 21 (1), 1339. doi:10.1186/s12885-021-09039-1

Zhibing Chen, Z., Chen, J., Wa, Q., He, M., Wang, X., Zhou, J., et al. (2020). Knockdown of circ_0084043 suppresses the development of human melanoma cells through miR-429/tribbles homolog 2 axis and Wnt/β-catenin pathway. *Life Sci.* 243, 117323. doi:10.1016/j.lfs.2020.117323

Zhou, L., Wang, Y., Ou, C., Lin, Z., Wang, J., Liu, H., et al. (2015). microRNA-365-targeted nuclear factor I/B transcriptionally represses cyclin-dependent kinase 6 and 4 to inhibit the progression of cutaneous squamous cell carcinoma. *Int. J. Biochem. Cell Biol.* 65, 182–191. doi:10.1016/j.biocel.2015.06.009

Zhou, L., Gao, R., Wang, Y., Zhou, M., and Ding, Z. (2017). Loss of BAX by miR-365 promotes cutaneous squamous cell carcinoma progression by suppressing apoptosis. *Int. J. Mol. Sci.* 18 (6), E1157. doi:10.3390/ijms18061157

Zhou, H., Wu, J., Leng, S., Hou, C., Mo, L., Xie, X., et al. (2021). Knockdown of circular RNA VANGL1 inhibits TGF-β-induced epithelial-mesenchymal transition in melanoma cells by sponging miR-150-5p. *J. Cell. Mol. Med.* 25 (23), 10837–10845. doi:10.1111/jcmm.16887

Zhou, W. D., Shao, L., Dong, L., Zhang, R. H., Li, Y. F., Li, H. Y., et al. (2022). Circulating MicroRNAs as quantitative biomarkers for diagnosis and prognosis of uveal melanoma. *Front. Oncol.* 12, 854253. doi:10.3389/fonc.2022.854253

Zhu, Y., Wen, X., and Zhao, P. (2018). MicroRNA-365 inhibits cell growth and promotes apoptosis in melanoma by targeting BCL2 and cyclin D1 (CCND1). *Med. Sci. Monit.* 24, 3679–3692. doi:10.12659/msm.909633

Ziwei Li, Z., Tian, P., Huang, T., and Huang, J. (2021). Noncoding-RNA-mediated regulation in response to macronutrient stress in plants. *Int. J. Mol. Sci.* 22 (20), 11205. doi:10.3390/ijms222011205

Zou, Y., Wang, S. S., Wang, J., Su, H. L., and Xu, J. H. (2019). CircRNA_0016418 expedites the progression of human skin melanoma via miR-625/YY1 axis. *Eur. Rev. Med. Pharmacol. Sci.* 23 (24), 10918–10930. doi:10.26355/eurrev_201912_19795

Zou, S., Gao, Y., and Zhang, S. (2021). lncRNA HCP5 acts as a ceRNA to regulate EZH2 by sponging miR-138-5p in cutaneous squamous cell carcinoma. *Int. J. Oncol.* 59 (2), 56. doi:10.3892/ijo.2021.5236

Glossary

BCC basal cell carcinoma

CSCC cutaneous squamous cell carcinoma

CM cutaneous melanoma

miRNAs microRNAs

lncRNAs long non-coding RNAs

circRNAs circular RNAs

BRAF B-Raf proto-oncogeneserine/threonine kinase

ncRNA non-coding RNA

mRNA messenger ribonucleic acid

ciRNA circular intronic RNA

eiciRNA exon-intron circular RNA

RBP RNA binding protein

TBX1 T-box transcription factor 1

NFIB nuclear factor I B

LZTS1 leucine zipper tumor suppressor 1

CDK6 cyclin dependent kinase 6

SRCIN1 SRC kinase signaling inhibitor 1

CDH1 cadherin 1

LAMC1 laminin subunit gamma 1

RETREG1 reticulophagy regulator 1

PDCD5 programmed cell death 5

ATG2B autophagy related 2B

FLOT2 flotillin 2

LIMK1 LIM domain kinase 1

STAT3 signal transducer and activator of transcription 3

PTPN11 protein tyrosine phosphatase non-receptor type 11

FAM114A2 family with sequence similarity 114 member A2

KPNA4 karyopherin subunit alpha 4

FOXG1 forkhead box G1

TPX2TPX2 TPX2 microtubule nucleation factor

PDPK1 3-phosphoinositide dependent protein kinase 1

REV3L REV3 like, DNA directed polymerase zeta catalytic subunit

TAF15 TATA-box binding protein associated factor 15

HOTAIR HOX transcript antisense RNA

PRAF2PRAF2 PRA1 domain family member 2

SCARNA2 small Cajal body-specific RNA 2

MAP3K2 mitogen-activated protein kinase kinase kinase 2

PURPL p53 level regulator

HOXB7 homeobox B7

PRAF2PRAF2 PRA1 domain family member 2

TPX2TPX2 TPX2 microtubule nucleation factor

DUSP5 dual specificity phosphatase 5

TGFBR1 transforming growth factor beta receptor 1

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 24 August 2022
DOI 10.3389/fphar.2022.970280

Check for updates

OPEN ACCESS

EDITED BY
Yongye Huang,
Northeastern University, China

REVIEWED BY
Qingyu Zhou,
University of South Florida,
United States
Mithun Rudrapal,
Rasiklal M. Dhariwal Institute of
Pharmaceutical Education and
Research, India
Ángeles Carlos Reyes,
Instituto Nacional de Enfermedades
Respiratorias-México (INER), Mexico

*CORRESPONDENCE
Anait S. Levenson,
anait.levenson@liu.edu

SPECIALTY SECTION
This article was submitted to
Pharmacology of Anti-Cancer Drugs,
a section of the journal
Frontiers in Pharmacology

RECEIVED 15 June 2022
ACCEPTED 20 July 2022
PUBLISHED 24 August 2022

CITATION
Levenson AS (2022), Dietary stilbenes as
modulators of specific miRNAs in
prostate cancer.
Front. Pharmacol. 13:970280.
doi: 10.3389/fphar.2022.970280

COPYRIGHT
© 2022 Levenson. This is an open-
access article distributed under the
terms of the Creative Commons
Attribution License (CC BY). The use,
distribution or reproduction in other
forums is permitted, provided the
original author(s) and the copyright
owner(s) are credited and that the
original publication in this journal is
cited, in accordance with accepted
academic practice. No use, distribution
or reproduction is permitted which does
not comply with these terms.

Dietary stilbenes as modulators of specific miRNAs in prostate cancer

Anait S. Levenson*

College of Veterinary Medicine, Long Island University, Brookville, NY, United States

Accumulated experimental data have suggested that natural plant products may be effective miRNA-modulating chemopreventive and therapeutic agents. Dietary polyphenols such as flavonoids, stilbenes, and lignans, among others, have been intensively studied for their miRNA-mediated cardioprotective, antioxidant, anti-inflammatory and anticancer properties. The aim of this review is to outline known stilbene-regulated miRNAs in cancer, with a special focus on the interplay between various miRNAs and MTA1 signaling in prostate cancer. MTA1 is an epigenetic reader and an oncogenic transcription factor that is overexpressed in advanced prostate cancer and metastasis. Not surprisingly, miRNAs that are linked to MTA1 affect cancer progression and the metastatic potential of cells. Studies led to the identification of MTA1-associated pro-oncogenic miRNAs, which are regulated by stilbenes such as resveratrol and pterostilbene. Specifically, it has been shown that inhibition of the activity of the MTA1 regulated oncogenic miR-17 family of miRNAs, miR-22, and miR-34a by stilbenes leads to inhibition of prostatic hyperplasia and tumor progression in mice and reduction of proliferation, survival and invasion of prostate cancer cells *in vitro*. Taken together, these findings implicate the use of resveratrol and its analogs as an attractive miRNA-mediated chemopreventive and therapeutic strategy in prostate cancer and the use of circulating miRNAs as potential predictive biomarkers for clinical development.

KEYWORDS

stilbenes, miRNAs, MTA1, chemoprevention, interception, biomarkers, active surveillance, prostate cancer

Introduction

Countless efforts are aimed towards the development of cancer chemopreventive and therapeutic strategies with the use of natural bioactive polyphenols such as a large group of flavonoids, stilbenes, lignans, phenolic acids, and others (Asensi et al., 2011; Kang N.J et al., 2011; Miyata et al., 2019; Forni et al., 2021; Rudrapal et al., 2022). Polyphenols act on multiple targets in signaling pathways related to inflammation, oxidative stress and DNA damage, carcinogenesis, tumor cell proliferation, angiogenesis, and metastasis (Asensi et al., 2011; Zhao et al., 2020; Khan et al., 2021; Rudrapal et al., 2022). Studies over the past two decades have led to the development of the concept of "epigenetic prevention and therapy" for cancer, which includes regulation of genes by non-coding RNAs (ncRNAs)

(Shukla et al., 2019; Levenson, 2021). Non-coding RNAs regulate chromatin architecture (Taft et al., 2009) and control gene expression by negatively regulating gene expression through a number of mechanisms (Filipowicz et al., 2008). Among ncRNA, microRNAs (miRNAs, miRs) are small, single-stranded, sequence-specific RNAs that induce degradation or inhibit translation of their target mRNAs (Ventura and Jacks, 2009). MiRNAs modulations have been shown to be involved in many pathologies including inflammation and cancer. In cancer, miRNAs are designated as oncogenic (oncomiRs), which downregulate tumor suppressor (TS) or other genes involved in cell differentiation thereby contributing to tumor formation, or oncosuppressor miRs, which downregulate proteins with oncogenic activity (Shenouda and Alahari, 2009). MiRNAs expressed in a tissue-specific manner have a multi-target mode of action (Link et al., 2010). MiRNAs are gaining increasing attention as potential noninvasive diagnostic, prognostic, and predictive biomarkers in cancer because they are stable, highly sensitive, and easily detectable in extracellular fluids (Quirico and Orso, 2020; Wang J et al., 2020).

Numerous studies have investigated miRNA profiles and their regulation by dietary phytochemicals, and the accumulated data implicate natural polyphenols as attractive miRNA-mediated chemopreventive and therapeutic strategy options in solid tumors and hematological cancers (Dhar et al., 2011; Vanden Berghe, 2012; Kumar et al., 2016; Levenson and Kumar, 2020; Levenson, 2021). Prostate cancer is ideal disease for nutritional chemoprevention in the general population of elderly men due to its slow progression in and dependence on diet (Peisch et al., 2017; Matsushita et al., 2020). Unfortunately, the incidence of low-risk prostate cancer has increased in the last two decades. Active surveillance is accepted as a management option for favorable-risk prostate cancer. However, due to the heterogeneity of this population, the lack of personalized risk assessments and the absence of treatment, the long-term outcome of active surveillance is not satisfying for approximately 30% of patients (Dhawan et al., 2016; Moschini et al., 2017; Overland et al., 2019; Pastor-Navarro et al., 2021). Since active surveillance patients are diagnosed with either prostatic intraepithelial neoplasia (PIN) or early-stage cancer (Gleason <6), it is apparent that various carcinogenic signaling pathways are already activated and can lead to pathological or clinical progression. Thus, the active surveillance subpopulation of patients with varying cancer risks may benefit from more active, clinical-based nutritional chemopreventive, i.e, interceptive strategies. Natural bioactive compounds known for their anti-inflammatory, antioxidant and anticancer effects may represent risk-reducing agents recommended for cancer interception (Blackburn, 2011). Nutritional interception in intermediate- and high-risk active surveillance patients should target specific molecular pathways. Particularly, our group has shown that natural dietary stilbenes exhibit targeted chemoprotective, interceptive and therapeutic effects against prostate cancer *in vitro* and *in vivo* (Kai et al., 2010; Li et al., 2013; Dhar et al., 2016; Butt et al., 2017; Kumar et al., 2019; Gadkari et al., 2020; Levenson, 2020; Hemani et al., 2022). This review will focus on the potential of natural stilbenes to protect against prostate cancer through the modulation of specific miRNAs that can conceivably be detected in bloodstream and urine and serve as prognostic and predictive biomarkers for certain populations of prostate cancer patients in the future.

Stilbenes, miRNAs and cancer

Dietary stilbenes

Stilbenes are a class of polyphenols naturally found in a wide variety of a small and heterogeneous group of plants, including *Vitis vinifera*, *Vaccinium, Cissus adnata*, *Fallopia japonica*, *Polygonum cuspidatum*, and *Picea sitchensis* and *abies*, to mention some (Rimando et al., 2004; Riviere et al., 2012). More than 400 natural stilbenoids have been identified in plants (Shen et al., 2009) where they are produced in response to biotic and abiotic environmental stresses (Bavaresco, 2003). Family members of the stilbenoids have a C6-C2-C6 basic skeleton and consist of two or more phenolic rings linked by an ethane double bond. Natural stilbenes are composed of resveratrol derivatives and concentrated in dietary sources such as grapes, red wine, grape juice, peanuts, berries, passion fruit and some medicinal plants. There are several well-known dietary stilbenes such as resveratrol, piseid, resveratrol dimers pallidol, viniferins, and Gnetin C, pterostilbene, piceatannol, and astringin (Figure 1). Resveratrol ($C_{14}H_{12}O_3$) is the most widely studied stilbene compound, found mostly in grapes and red wine but also in cranberries, pistachios, and chocolate, has multiple and diverse pharmacological properties including antioxidant, anti-inflammatory and anticancer properties (Rauf et al., 2018; Galiniak et al., 2019; Kataria and Khatkar, 2019; Rudrapal, 2022). Pterostilbene ($C_{16}H_{16}O_3$) is a naturally occurring methoxylated analog of resveratrol, found in blueberries and grapes, with improved pharmacokinetic efficacy and more potent biological efficacy over resveratrol (Rimando et al., 2002; Rimando et al., 2004; Rimando et al., 2005; Kapetanovic et al., 2011; Hagiwara et al., 2012; Dhar et al., 2015b). Piceatannol ($C_{14}H_{12}O_4$) is another naturally occurring resveratrol metabolite, found in red wine, grapes, white tea or passion fruit, s recognized as a strong tyrosine kinase inhibitor in different types of cancer (Su and David, 2000; Vo et al., 2010; Kang C.H et al., 2011). Piceid, a major resveratrol glucoside in grape juices, demonstrated antiproliferative effects in epithelial and cancer cells (Su et al., 2013; Storniolo et al., 2014; Zhang T. et al., 2019). Piceatannol's glucoside, astringin ($C_{20}H_{22}O_9$), especially

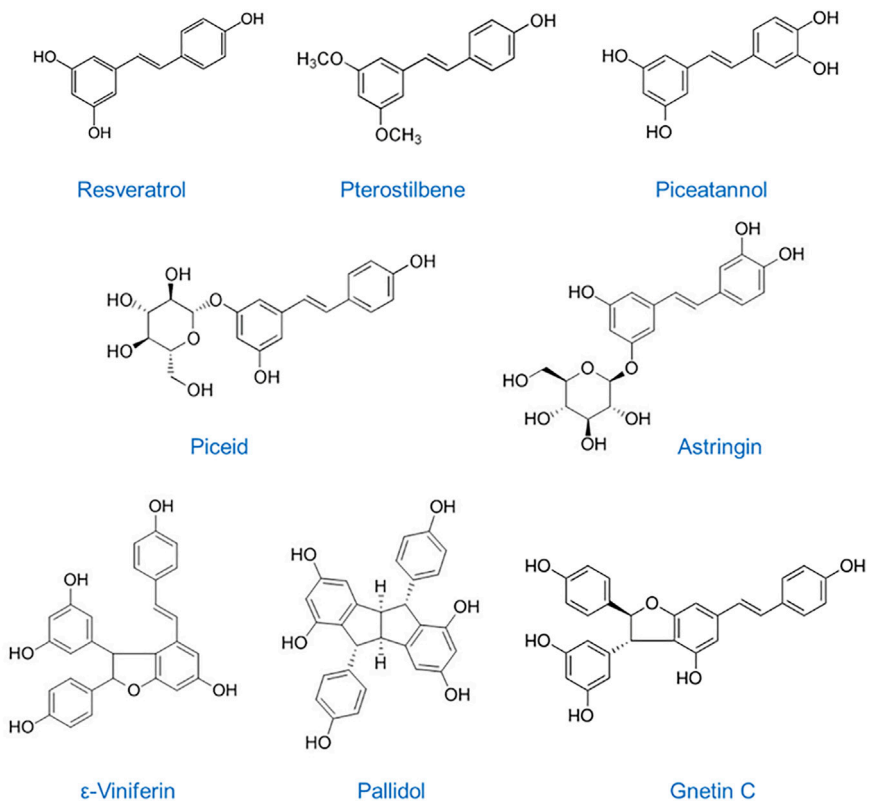


FIGURE 1
Chemical structures of dietary stilbenes: Resveratrol, *trans*-3, 4′, 5-trihydroxystilbene, MW: 228.24 g/mol; Pterostilbene, *trans*-3,5-dimethoxy-4′-hydroxystilbene, MW: 256.30 g/mol; Piceatannol, *trans*-3,5,3′4′-tetrahydroxystilbene, MW: 244.24 g/mol; Piceid, *trans*-resveratrol-3-*O*-glucoside, MW: 390.39 g/mol; Astringin, *trans*-piceatannol-3-β-D-glucuronide, MW: 406.38 g/mol; ε-Viniferin, *trans*-epsilon-resveratrol dimer, MW: 454.50 g/mol; Pallidol, tetracyclic homodimer resveratrol, MW: 454.48 g/mol; Gnetin C, dimer resveratrol, MW: 454.48 g/mol.

found in red wine, has been shown to function as potential cancer-chemopreventive agent by a mechanism different from that of resveratrol (Waffo-Teguo et al., 2001). Moreover, a recent report demonstrated potent anti-angiogenic effect of astringin as well as of resveratrol dimers ε-viniferin, ω-viniferin, and pallidol ($C_{28}H_{22}O_6$), also concentrated in grapes and red wine (Fernandez-Cruz et al., 2019). Gnetin C ($C_{28}H_{22}O_6$), a resveratrol dimer found in melinjo (*Gnetum gnemon*) seed extracts that are commonly used in Indonesian cuisine, possesses a broad spectrum of the same pharmacological activities as other stilbenes with the advantage of superior bioavailability and biological efficacy (Narayanan et al., 2015; Kumar et al., 2019; Gadkari et al., 2020). Chemopreventive effects of dietary stilbenes are mainly due to their anti-inflammatory and antioxidant activity but also direct targeting of multiple signaling pathways involved in carcinogenesis, cancer progression and metastasis. Importantly, accumulated data suggest that the beneficial effects of dietary stilbenes may be in part attributed to their epigenetic properties, including regulation of DNA methylation, histone modifications, and, notably, modulation of the expression of miRNAs/miRs (Kumar et al., 2015; Kumar et al., 2016; Kumar and Levenson, 2018; Levenson, 2021).

Stilbene-regulated miRNAs in cancer

In a high-throughput analysis of miRNA expression in response to stilbenes, modulated miRs were identified as essential for controlling cancer growth, survival, metabolism, motility, apoptosis, angiogenesis, and metastasis (Tili and Michaille, 2011). In general, studies have shown that stilbenes inhibit oncomiRs and upregulate oncosuppressor miRs, For example, resveratrol has been shown to upregulate miR-21, miR-129, miR-2014, and miR-489 in rodent mammary tumors tissues (Qin et al., 2014) and a number of other oncosuppressor miRs, causing cell cycle arrest and cell death in breast cancer cells (Venkatadri et al., 2016). In chemoresistant breast cancer cells, resveratrol restored cell chemosensitivity by upregulating miR-122-5p (Zhang W. et al., 2019). Resveratrol and pterostilbene reduced the cancer stem-like cells population

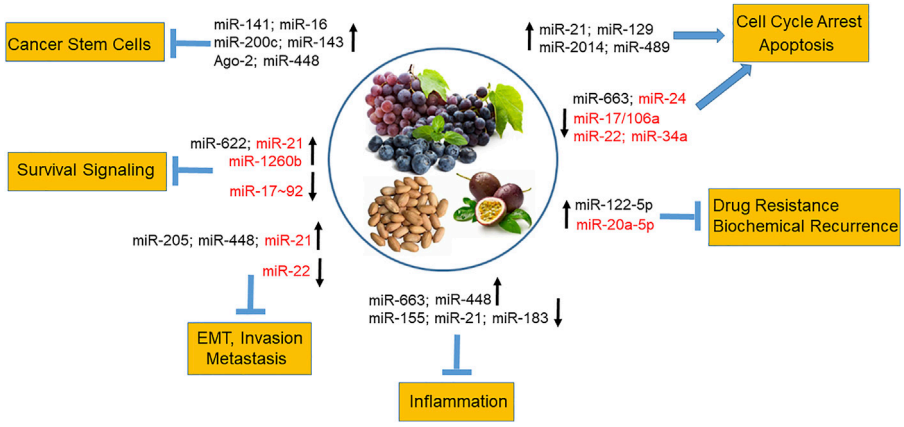


FIGURE 2
MiR-mediated biological effects of dietary stilbenes in cancer. Stilbenes from dietary sources such as grapes, blueberries, peanuts, and passion fruits modulate expression of miRNAs involved in promoting cell cycle arrest and apoptosis and inhibiting inflammation, survival pathways, cancer drug resistance and recurrence, invasion and metastasis as well as reducing cancer stem cell population. The stilbene-regulated miRNAs in prostate cancer are shown in red.

in mammary tumor formation *in vivo* by upregulating the expression of oncosuppressor miR-141, miR-16, miR-200c, miR-143 and Ago-2, a key regulator of miRNA homeostasis and biogenesis (Hagiwara et al., 2012). Seventy-one miRNAs were regulated in human lung cancer cells by resveratrol (Bae et al., 2011) including TS miR-622, which targets K-ras (Han et al., 2012). The miRNA-mediated link between inflammation and cancer under resveratrol treatment was demonstrated by revealing upregulation of anti-inflammatory and oncosuppressor miRNAs, such as miR-663, and downregulation of pro-inflammatory and oncogenic miRs, such as miR-155 and miR-21 (Tili et al., 2010a; Tili et al., 2011; Latruffe et al., 2015). Importantly, studies have reported subsequent mechanistic experiments, in which the identification of mRNA target transcripts whose levels were modified by stilbenoids, were validated on mRNA and/or protein levels. For example, resveratrol anti-inflammatory activity occurred *via* targeting the miR-663-mediated AP-1 signaling pathway (Tili et al., 2010a) and through modulating miRs involved in TGFβ signaling in human colorectal cancer cells (Tili et al., 2010b). Pterostilbene inhibited tumor growth and metastasis in breast cancer xenografts *via* induction of miR-205 expression, which targeted the Src/Fak signaling (Su et al., 2015) and suppressed glioblastoma through the miR-205/GRP78 axis (Huynh et al., 2015). A different study found that pterostilbene–induced modulation of miR-448/NF-κB axis resulted in suppression of the generation of breast cancer stem cells and metastatic potential (Mak et al., 2013). Further, pterostilbene downregulation of oncogenic miR-663, whose expression is correlated with poor prognosis in endometrial cancer patients, led to induction of pro-apoptotic BCL2L14 in endometrial cancer cells *in vitro* (Wang et al., 2017). Another natural analog of resveratrol, piceatannol, exhibited inhibition of colorectal cancer cell growth and induction of apoptosis by inducing miR-129-mediated downregulation of BCL-2 (Zhang et al., 2014). Furthermore, piceatannol inhibited Sp-1-mediated ADAM17 expression and the TNFα/NF-kB pathway in human leukemia cells by downregulating Akt/Foxo3-mediated miR-183 expression (Liu and Chang, 2012). Figure 2 summarizes reported miR-mediated biological effects of stilbenes in various cancers including prostate cancer (see below).

Stilbene-regulated miRNAs in prostate cancer

In prostate cancer, oncomiRs expression correlates with high Gleason score and clinical recurrence (Ambs et al., 2008; Brase et al., 2011) and can be easily detected in serum, plasma and urine (Ambs et al., 2008; Selth et al., 2012; Sapre and Selth, 2013; Jeon et al., 2020; Hasanoglu et al., 2021), which signifies future utilization of miR-regulated pathways as potential targets as well as prognostic and predictive biomarkers.

Differential miRNA expression profiling in LNCaP prostate cancer cells treated with resveratrol revealed considerable modulation of a set of 51 miRNAs, from which 23 miRNAs (putative oncomiRs) were significantly downregulated and 28 miRNAs (putative oncosuppressor miRs) were significantly upregulated (Dhar et al., 2011).

The downregulated by resveratrol miRs included miR-7, miR-24, miR-1260 and miR-17~92 (miR-17, miR-20a, miR-20b, miR-92b) and miR-106ab clusters. Subsequently, a growing body of evidence has revealed that many of these miRNAs act as oncogenes in prostate cancer. For instance,

miR-7 has been found to be upregulated in castrate-resistant prostate cancer (CRPC) clinical samples (Xie and Jiang, 2015). Downregulation of miR-24 induced apoptosis in DU145 prostate cancer cells by upregulating its target pro-apoptotic FAF1 protein (Qin et al., 2010). Multiple miRNAs-TGFβ checkpoints that control TGFβ/SMAD signaling in progression of prostate cancer were identified (Javed et al., 2020). For instance, overexpression of oncomiR-1260b resulted in reduced levels of tumor suppressor SMAD4 leading to prostate cancer progression. Interestingly, miR-1260b was also significantly downregulated by another natural polyphenol known for its anticancer properties, i.e. genistein that promoted SMAD4-mediated apoptosis in prostate cancer cells (Hirata et al., 2014). Overexpression of miR-20a has been detected in tumor tissue samples of prostate cancer patients with a Gleason score of 7–10 (Pesta et al., 2010). Moreover, a high miR-20a-5p expression in prostate tumor tissues was identified as one of the five miRNAs that may, as a panel, be used as a potential diagnostic biomarker (Damodaran et al., 2021) and as an independent predictor for biochemical recurrence (Stoen et al., 2021). The oncogenic role of miR-20a/CX43 (Li et al., 2012) and miR-20a/miR-17/ATG7 (Guo et al., 2016) in prostate cancer was also reported. The miR-17~92 and paralogs miR-106a~363 and miR-106b~25 are commonly described as oncogenic in many cancers (Kolenda et al., 2020) including prostate cancer, in which they have been validated clinically as significantly upregulated compared to normal samples (Taylor et al., 2010). Importantly, miR-17~92 and miR-106ab have been directly linked to the tumor suppressor PTEN (Olive et al., 2010; Poliseno et al., 2010), one of the frequently defective genes in primary and metastatic prostate cancer (Li et al., 1997). In consequent studies, using functional luciferase reporter assays, it was demonstrated that ectopically expressed miR-17, miR-20a and miR-106b directly target *Pten* 3′UTR to reduce its expression in DU145 and 22Rv1 prostate cancer cells. Notably, these effects were rescued upon treatment with resveratrol and pterostilbene (Dhar et al., 2015b). Moreover, pterostilbene treatment diminished the miR-17/106a-promoted tumor growth in DU145-Luc prostate cancer xenografts through miR-mediated upregulation of PTEN mRNA and protein levels in tumor tissues, causing apoptosis (Dhar et al., 2015b). A different report demonstrated resveratrol-induced reduction of prostate cancer growth and metastasis through Akt/miR-21/PDCD4 pathway (Sheth et al., 2012).

The upregulated by resveratrol miRs in prostate cancer included miR-1469, miR-612, miR-149, miR-638, miR-654-5p, miR-1908, miR-1915, miR-1231, miR-939, miR-671-5p (Dhar et al., 2011), many of which are currently documented as oncosuppressor miRs in several types of cancer (Jin et al., 2020; Li et al., 2021; Liu et al., 2020a, b; Tan et al., 2016). For example, a recent study demonstrated that miR-149 could inhibit AR expression and reduce the activity of PI3K/Akt1 signaling in castrate-resistant cells (Zhao et al., 2021) and also regulate RGS17-mediated oncogenic effects (Ma et al., 2021) revealing its tumor suppressor nature in prostate cancer. The tumor suppressive role of miR-1231 targeting EGFR in prostate cancer and reducing cell proliferation, migration, and invasion was recently demonstrated (Wang Y et al., 2020). The authors propose diminished miR-1231 levels as a prognostic biomarker for advanced prostate cancer. MiR-654-5p was among fifteen AR downregulating miRNAs that decreased androgen-induced proliferation of prostate cancer cells (Ostling et al., 2011). Another miR, upregulated by resveratrol (Dhar et al., 2011), namely miR-939-3p, was decreased in prostate cancer tissues and cell lines compared to normal adjacent tissues and normal epithelial cell line. MiR-939-3p acted as an oncosuppressor miR through the long non-coding RNA (lncRNA) brain cytoplasmic RNA1 BCYRN1/HDAC11 oncogenic axis in prostate cancer (Huo et al., 2020). Interesting results were reported on the overexpression of spermidine synthase (SRM) in prostate cancer tissues and miR-1908-mediated regulation of SRM, which controls the secretion of extracellular vesicles (EV) in prostate cancer (Urabe et al., 2020). Finally, upregulation of EV-associated miR-1915-3p was concomitant with improved survival time along with two other miRs, but only miR-1915-3p was associated with longer recurrence-free survival as an independent prognostic marker in prostate cancer patients with low and high Gleason scores and of various races (Ali et al., 2021). It is important to notice that detailed experimental mechanistic studies to prove biological consequences of stilbene modulated oncosuppressive miRs in prostate cancer are lucking. Of note, miR-1469 have been reported to be induced by genistein resulting in promotion of apoptosis *via* inhibition of the Mcl-1 pathway in laryngeal cancer cells (Ma et al., 2018).

The role of some miRs in cancer is complex and ambiguous: these miRs have been reported as both oncogenic and oncosuppressors. However, while this controversy can be attributed to the cell specificity, organ tissue microenvironment and various targets for a given miR, a disagreement about the behavior of a given miR in the same type of cancer is less understandable. For example, although recognized as a tumor suppressor in some tumors (Li et al., 2019; Xin et al., 2019; Wang et al., 2021) the unexpected oncogenic role of miR-671-5p associated with targeting tumor suppressor SOX6 in prostate cancer has been recently reported (Yu et al., 2018). Moreover, oncosuppressor miR-149 was found to be overexpressed in CRPC samples (Zhu et al., 2015) and has been seen as an oncogenic miR associated with syndecan-1 and inhibiting SOX2, NANOG, and Oct4 tumor suppressors (Fujii et al.,

TABLE 1 Confirmed Oncogenic miRNAs in Prostate Cancer[a].

Stilbene	miRNA	Identified Target	Associated information or Event	References
No treatment	miR-7		Upregulated in CRPC clinical samples	Xie and Jiang, (2015)
	miR-24	FAF1	Apoptosis in DU145 cells	Qin et al. (2010)
"Genistein"	miR-1260b	SMAD4	Apoptosis in PC3 cells	Hirata et al. (2014)
No treatment	miR-20		Overexpressed in aggressive prostate cancer	Pesta et al. (2010)
			Potential diagnostic marker	Damodaran et al. (2021)
			Predictor for biochemical recurrence	Stoen et al. (2021)
No treatment	miR- 20	CX43	PCa-2b cell proliferation, tumor growth	Li. et al. (2012)
No treatment	AR/miR-20a/miR-17	ATG7	Autophagy	Guo et al. (2016)
No treatment	miR-17~92		Upregulated in clinical samples	Taylor et al. (2010)
Resveratrol Pterostilbene	miR-17~92	PTEN	Reduced cell proliferation and xenograft tumor growth	Dhar et al. (2015b)
Resveratrol Pterostilbene	miR-106a~363	PTEN	Reduced cell proliferation and xenograft tumor growth	Dhar et al. (2015b)
Resveratrol Pterostilbene	miR-106b~25	PTEN	Reduced cell proliferation and xenograft tumor growth	Dhar et al. (2015b)
Resveratrol	Akt/miR-21	PDCD4	Cancer growth and metastasis	Sheth et al. (2012)
Grape extract-Diet	MTA1/c-miR-34a	p53	Reduced PIN in *Pten*$^{+/f}$; *Cre*$^{+}$ mice	Joshi et al. (2020)
	MTA1/c-miR-22	p21		
Pterostilbene-Diet	MTA1/c-miR-34a		Reduced hgPIN in *R26*^{MTA1}; *Pten*$^{+/f}$; *Cre*$^{+}$ mice	Hemani et al. (2022)
	MTA1/c-miR-22			
No treatment	MTA1/miR-22	E-cadherin	Invasion in RWPE1 & LNCaP cells	Dhar et al. (2017)

[a]These miRNAs, are putative stilbene-regulated onco-miRs previously identified by Dhar et al. (2011); c-miR, circulating miR detected in serum.

TABLE 2 Confirmed Oncosuppressor miRNAs in Prostate Cancer[a].

Stilbene	miRNA	Identified Target	Associated information or Events	References
No treatment	miR-654-5p	AR	PSA, LNCaP cell proliferation	Ostling et al. (2011)
No treatment	miR-149	AR P13K/Akt1	CRCP 22-Rv1 cells	Zhao et al. (2021)
No treatment	miR-149-5p	RGS17	Viability, proliferation, migration of 22Rv1 and C4-2 cells , high in prostate cancer tissues	Ma et al. (2021)
No treatment	miR-1231	EGFR	Cell proliferation, migration, invasion in DU145, 22Rv1, PC3, and VCaP, high in prostate cancer tissues	Wang Y et al. (2020)
No treatment	miR-939-3p	BCYRN1/HDAC11	Cell proliferation, Decreased in prostate cancer tissues vs. normal	Huo et al. (2020)
No treatment	miR-1908	SRM	22Rv1 cells, high in prostate cancer tissues	Urabe et al. (2020)
No treatment	miR-1915-3p		Upregulation was linked to recurrence-free survival Independent prognostic marker	Ali et al. (2021)

[a]These miRNAs are putative stilbene-regulated oncosuppressor miRs previously identified by Dhar et al., 2011.

2015). Tables 1, 2 summarize confirmed oncogenic (Table 1) and oncosuppressor miRs (Table 2) in prostate cancer earlier identified as resveratrol-regulated (Dhar et al., 2011). The data regarding the functions and exact roles of identified stilbene-regulated miRNAs in prostate cancer are incomplete and require further studies.

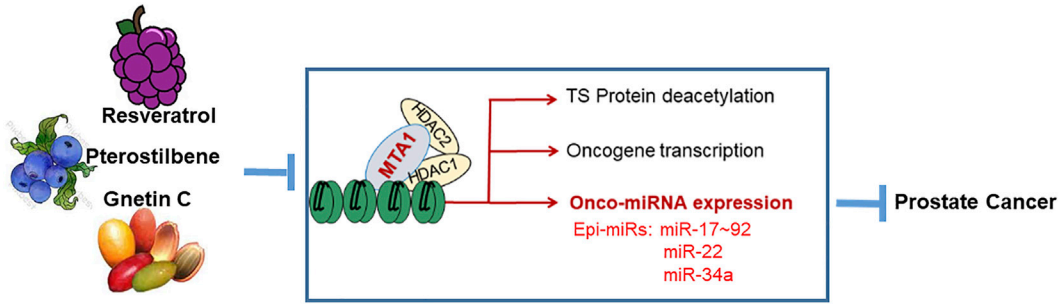


FIGURE 3
Effects of dietary stilbenes on the MTA1 signaling pathways including miRNAs. The MTA1/HDAC1,2 signaling is triggered by prostate cancer progression and involves deacetylation of some tumor suppressor (TS) proteins (p53 and PTEN), transcription of oncogenes, and activation of oncogenic miRNAs. These cascades can be inhibited by the dietary stilbenes accumulated in grapes, blueberries and melinjo berries.

Stilbenes as inhibitors of MTA1 signaling and associated miRNAs in prostate cancer

Understanding the specific molecular mechanisms of premalignancy and cancer progression opens opportunities for developing targeted interceptive measures. It is largely acknowledged that natural polyphenols, particularly stilbenes have pleiotropic anti-inflammatory and anticancer effects acting through various signal transduction pathways such as NF-kB, AP-1, and MAPK signaling among others.

In prostate cancer, metastasis associated protein 1 (MTA1) signaling is aberrantly activated due to overexpression of MTA1 and activation of associated pathways. Increased expression of MTA1 is associated with high Gleason score, recurrence, and metastasis in prostate cancer (Hofer et al., 2004; Dias et al., 2013). MTA1 plays a critical role in different stages of prostate cancer, including chronic inflammation, tumor growth, epithelial-to-mesenchymal transition (EMT), invasion, migration, angiogenesis and metastasis (Levenson et al., 2014). Studies have demonstrated the MTA1-mediated chemopreventive and therapeutic effects of natural stilbenes in prostate cancer (Levenson, 2020). Both resveratrol and pterostilbene inhibited survival pathways and induced apoptosis in prostate cancer through downregulation of the MTA1/HDAC1, 2 units of the NuRD complex, which resulted in the promotion of acetylation and reactivation of tumor suppressors p53 and PTEN (Kai et al., 2010; Dhar et al., 2015b). The inhibitory effects of resveratrol and pterostilbene on MTA1-mediated angiogenesis involving HIF-1a, VEGF, and IL-1β were also consistently reported (Kai et al., 2011; Li et al., 2013; Dhar et al., 2016; Butt et al., 2017; Hemani et al., 2022). We have also shown the MTA1-mediated and MTA1 independent chemopreventive and therapeutic value of Gnetin C in prostate cancer *in vitro* and *in vivo* (Kumar et al., 2019; Gadkari et al., 2020). Importantly, stilbenes can inhibit MTA1-associated oncogenic miRNAs (Figure 3).

MTA1, an epigenetic reader and "master co-regulator" and transcription factor, plays a role in direct or indirect transcriptional activation/suppression of specific genes including miRNAs (Manavathi and Kumar, 2007). MiRNAs that are regulated by epigenetic factors are called Epi-miRs (Valeri et al., 2009; Dhar et al., 2017; Levenson, 2020). MTA1-associated stilbene-regulated Epi-miRNAs have been identified by a systematic analysis and review of the results from the following three high-throughput analyses in prostate cancer: the identification of resveratrol responsive miRNAs using miRNA microarrays in LNCaP cells (Dhar et al., 2011), the identification of MTA1-associated miRNAs by differential miRNA microarrays profiling in LNCaP MTA1 knockdown cells (Dhar et al., 2017), and the identification of prostate tissues transcriptional targets of MTA1 by ChIP-Seq from prostate-specific *Pten*-deficient mice fed with pterostilbene-diet (Dhar et al., 2016).

Our special interest in oncomiR/PTEN axis came from previous studies, in which we and others showed upregulation of PTEN protein levels by resveratrol in prostate cancer cells (Wang et al., 2010; Dhar et al., 2011), one mechanism of which was the inhibition of MTA1-mediated deacetylation and inactivation of PTEN (Dhar et al., 2015a). In parallel, resveratrol downregulated miR-17-92, miR-106a~363, and miR-106b~25 clusters in prostate cancer cells (Dhar et al., 2011). Further, miR-17, miR-20a, and miR-106b directly targeted the 3′UTR of Pten, an event that was reversed by resveratrol and pterostilbene in prostate cancer cells (Dhar et al., 2015b). Moreover, the reduced levels of circulating miR-17 and miR-106a in the sera from mice treated with pterostilbene - led to reduced tumor growth and revealed the potential of stilbene-responsive miRs as chemopreventive and predictive biomarkers in prostate cancer (Dhar et al., 2015b). In addition, miRNA profiling of MTA1-knockdown cells revealed direct regulation of miR-92b by MTA1, among others (Dhar et al., 2017).

Other candidate MTA1-associated stilbene-regulated miRs are miR-22 and miR-34a (Dhar et al., 2017). According to publicly available prediction algorithms, oncomiRs miR-22 and miR-34a target tumor suppressors p21 and p53, respectively. An inverse relationship between MTA1/miR-22 and p21 and MTA1/miR-34a and p53 was demonstrated in MTA1 knockdown prostate cancer cells (Joshi et al., 2020). Notably, MTA1-associated miR-22 and miR-34a were regulated by low-fat and high-fat diets supplemented with grape powder fed to mice prone to developing PIN (*Pten*$^{+/f}$, *Pb-Cre*$^{+}$). These two PIN-derived circulating oncomiRs further were detected in murine serum, in which they showed statistically significant reduced levels in mice fed with diets supplemented with grape powder containing not only stilbenes but other polyphenols (Joshi et al., 2020). In a different set of experiments using a high-risk premalignant prostate cancer mouse model (*R26*^{MTA1}; *Pten*$^{+/f}$; *Pb-Cre*$^{+}$) fed a diet supplemented with pterostilbene (100 mg/kg diet), we registered MTA1-targeted chemoprevention along with reduced circulating miR-22 and miR-34a levels in response to pterostilbene treatment (Hemani et al., 2022). As the same miR can target multiple mRNAs, studies have reported miR-22 direct targeting of PTEN in prostate cancer (Poliseno et al., 2010) and downregulation of PTEN protein levels in RWPE1 prostate cancer cells ectopically expressing miR-22 (Dhar et al., 2017). Another valuable MTA1-associated miRNA/target axis was identified from miRNA profiling studies and was functionally validated in prostate cancer: the miR-22/E-cadherin axis (Dhar et al., 2017). E-cadherin is a valid adhesion factor that plays an essential role against EMT leading to invasion and metastasis (Frixen et al., 1991; Perl et al., 1998; Onder et al., 2008). In addition to correlative observation of aggressive DU145 and PC3M prostate cancer cells with high MTA1/miR-22 and low E-cadherin expression compared to "good" RWPE1 and LNCaP cells, meta-analysis of patient tumor samples indicated a positive correlation between MTA1 and miR-22 and a negative correlation between MTA1 and E-cadherin, supporting their inhibitory effect on E-cadherin expression. MTA1-induced drastic downregulation of E-cadherin was further shown in the prostate tissues of prostate-specific transgenic mice overexpressing MTA1 (*R26*^{MTA1}; *Pb-Cre*$^{+}$). Mechanistically, MTA1-induced overexpression of miR-22 reduced expression of E-cadherin resulting in increased cell invasiveness and migration of prostate cancer cells and the link between miR-22 and its putative target E-cadherin mRNA was demonstrated using reporter constructs of the 3′-UTR of E-cadherin. MTA1-promoted miR-22-regulation of this adhesion factor makes the MTA1/miR-22/E-cadherin axis critical for promoting tumor invasiveness in prostate cancer cells (Dhar et al., 2017). Bearing in mind that miR-22 is a confirmed regulator of EMT (Song et al., 2013), its role as a prognostic and predictive biomarker in advanced prostate cancer and a potential therapeutic target becomes essential.

Conclusion

Identifying miRNAs linked to specific signaling pathways that are critical for prostate tumor progression and metastasis might provide novel targeted chemoprevention and therapeutic opportunities. Pharmacological modulation of miRNA activities, specifically by dietary stilbenes may have tremendous impact in interceptive approaches for different stages of prostate cancer. The results from our studies suggest that miR regulation is conserved among stilbene family members and that the identified MTA1-miRNA network regulated by stilbenes plays a significant role in prostate cancer progression. These miRNAs are particularly attractive because they can be detected in serum or urine as "liquid biopsy" biomarkers essential for diagnosis, prediction of interception/therapy response, and prognosis in prostate cancer. Studies have also reported beneficial miR-mediated effects of stilbenes in combination with other natural polyphenols in various cancers. Due to their unique chemical structure, different classes of polyphenols may produce specific miR-mediated gene regulation, which may culminate in synergistic beneficial effects. Further studies have the potential of improving the goals of personalized medicine, specifically concerning personalized interception using miRNA-modulating natural products with potential chemopreventive and therapeutic benefits in prostate cancer.

Author contributions

Manuscript is written and approved by ASL.

Funding

This work was supported by the National Cancer Institute of the National Institutes of Health under Award Number R15CA216070 to ASL. The content is solely the responsibility of the author and does not necessarily represent the official views of the National Institutes of Health.

Acknowledgments

The author is grateful to Elena V. Levenson for editing the manuscript.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Ali, H. E. A., Gaballah, M. S. A., Gaballa, R., Mahgoub, S., Hassan, Z. A., Toraih, E. A., et al. (2021). Small extracellular vesicle-derived microRNAs stratify prostate cancer patients according to Gleason score, race and associate with survival of african American and caucasian men. *Cancers (Basel)* 13, 5236. doi:10.3390/cancers13205236

Ambs, S., Prueitt, R. L., Yi, M., Hudson, R. S., Howe, T. M., Petrocca, F., et al. (2008). Genomic profiling of microRNA and messenger RNA reveals deregulated microRNA expression in prostate cancer. *Cancer Res.* 68, 6162–6170. doi:10.1158/0008-5472.CAN-08-0144

Asensi, M., Ortega, A., Mena, S., Feddi, F., and Estrela, J. M. (2011). Natural polyphenols in cancer therapy. *Crit. Rev. Clin. Lab. Sci.* 48, 197–216. doi:10.3109/10408363.2011.631268

Bae, S., Lee, E. M., Cha, H. J., Kim, K., Yoon, Y., Lee, H., et al. (2011). Resveratrol alters microRNA expression profiles in A549 human non-small cell lung cancer cells. *Mol. Cells* 32, 243–249. doi:10.1007/s10059-011-1037-z

Bavaresco, L. (2003). Role of viticultural factors on stilbene concentrations of grapes and wine. *Drugs Exp. Clin. Res.* 29, 181–187.

Blackburn, E. H. (2011). Cancer interception. *Cancer Prev. Res.* 4, 787–792. doi:10.1158/1940-6207.CAPR-11-0195

Brase, J. C., Johannes, M., Schlomm, T., Falth, M., Haese, A., Steuber, T., et al. (2011). Circulating miRNAs are correlated with tumor progression in prostate cancer. *Int. J. Cancer* 128, 608–616. doi:10.1002/ijc.25376

Butt, N. A., Kumar, A., Dhar, S., Rimando, A. M., Akhtar, I., Hancock, J. C., et al. (2017). Targeting MTA1/HIF-1α signaling by pterostilbene in combination with histone deacetylase inhibitor attenuates prostate cancer progression. *Cancer Med.* 6, 2673–2685. doi:10.1002/cam4.1209

Damodaran, M., Chinambedu, D. M., SimonDuraiRaj, SandhyaSundaram, VenkatRamanan, S., Ramachandran, I., et al. (2021). Differentially expressed miR-20, miR-21, miR-100, miR-125a and miR-146a as a potential biomarker for prostate cancer. *Mol. Biol. Rep.* 48, 3349–3356. doi:10.1007/s11033-021-06384-z

Dhar, S., Hicks, C., and Levenson, A. S. (2011). Resveratrol and prostate cancer: Promising role for microRNAs. *Mol. Nutr. Food Res.* 55, 1219–1229. doi:10.1002/mnfr.201100141

Dhar, S., Kumar, A., Gomez, C. R., Akhtar, I., Hancock, J. C., Lage, J. M., et al. (2017). MTA1-activated Epi-microRNA-22 regulates E-cadherin and prostate cancer invasiveness. *FEBS Lett.* 591, 924–933. doi:10.1002/1873-3468.12603

Dhar, S., Kumar, A., Li, K., Tzivion, G., and Levenson, A. S. (2015a). Resveratrol regulates PTEN/Akt pathway through inhibition of MTA1/HDAC unit of the NuRD complex in prostate cancer. *Biochim. Biophys. Acta* 1853, 265–275. doi:10.1016/j.bbamcr.2014.11.004

Dhar, S., Kumar, A., Rimando, A. M., Zhang, X., and Levenson, A. S. (2015b). Resveratrol and pterostilbene epigenetically restore PTEN expression by targeting oncomiRs of the miR-17 family in prostate cancer. *Oncotarget* 6, 27214–27226. doi:10.18632/oncotarget.4877

Dhar, S., Kumar, A., Zhang, L., Rimando, A. M., Lage, J. M., Lewin, J. R., et al. (2016). Dietary pterostilbene is a novel MTA1-targeted chemopreventive and therapeutic agent in prostate cancer. *Oncotarget* 7, 18469–18484. doi:10.18632/oncotarget.7841

Dhawan, A., Graham, T. A., and Fletcher, A. G. (2016). A computational modeling approach for deriving biomarkers to predict cancer risk in premalignant disease. *Cancer Prev. Res.* 9, 283–295. doi:10.1158/1940-6207.CAPR-15-0248

Dias, S. J., Zhou, X., Ivanovic, M., Gailey, M. P., Dhar, S., Zhang, L., et al. (2013). Nuclear MTA1 overexpression is associated with aggressive prostate cancer, recurrence and metastasis in African Americans. *Sci. Rep.* 3, 2331–2341. doi:10.1038/srep02331

Fernandez-Cruz, E., Cerezo, A. B., Cantos-Villar, E., Richard, T., Troncoso, A. M., and Garcia-Parrilla, M. C. (2019). Inhibition of VEGFR-2 phosphorylation and effects on downstream signaling pathways in cultivated human endothelial cells by stilbenes from vitis spp. *J. Agric. Food Chem.* 67, 3909–3918. doi:10.1021/acs.jafc.9b00282

Filipowicz, W., Bhattacharyya, S. N., and Sonenberg, N. (2008). Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat. Rev. Genet.* 9, 102–114. doi:10.1038/nrg2290

Forni, C., Rossi, M., Borromeo, I., Feriotto, G., Platamone, G., Tabolacci, C., et al. (2021). Flavonoids: A myth or a reality for cancer therapy? *Molecules* 26, 3583. doi:10.3390/molecules26123583

Frixen, U. H., Behrens, J., Sachs, M., Eberle, G., Voss, B., Warda, A., et al. (1991). E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. *J. Cell Biol.* 113, 173–185. doi:10.1083/jcb.113.1.173

Fujii, T., Shimada, K., Tatsumi, Y., Fujimoto, K., and Konishi, N. (2015). Syndecan-1 responsive microRNA-126 and 149 regulate cell proliferation in prostate cancer. *Biochem. Biophys. Res. Commun.* 456, 183–189. doi:10.1016/j.bbrc.2014.11.056

Gadkari, K., Kolhatkar, U., Hemani, R., Campanelli, G., Cai, Q., Kumar, A., et al. (2020). Therapeutic potential of Gnetin C in prostate cancer: A pre-clinical study. *Nutrients* 12, 3631–3642. doi:10.3390/nu12123631

Galiniak, S., Aebisher, D., and Bartusik-Aebisher, D. (2019). Health benefits of resveratrol administration. *Acta Biochim. Pol.* 66, 13–21. doi:10.18388/abp.2018_2749

Guo, J., Mei, Y., Li, K., Huang, X., and Yang, H. (2016). Downregulation of miR-17-92a cluster promotes autophagy induction in response to celastrol treatment in prostate cancer cells. *Biochem. Biophys. Res. Commun.* 478, 804–810. doi:10.1016/j.bbrc.2016.08.029

Hagiwara, K., Kosaka, N., Yoshioka, Y., Takahashi, R. U., Takeshita, F., and Ochiya, T. (2012). Stilbene derivatives promote Ago2-dependent tumour-suppressive microRNA activity. *Sci. Rep.* 2, 314. doi:10.1038/srep00314

Han, Z., Yang, Q., Liu, B., Wu, J., Li, Y., Yang, C., et al. (2012). MicroRNA-622 functions as a tumor suppressor by targeting K-Ras and enhancing the anticarcinogenic effect of resveratrol. *Carcinogenesis* 33, 131–139. doi:10.1093/carcin/bgr226

Hasanoglu, S., Goncu, B., Yucesan, E., Atasoy, S., Kayali, Y., and Ozten Kandas, N. (2021). Investigating differential miRNA expression profiling using serum and urine specimens for detecting potential biomarkers for early prostate cancer diagnosis. *Turk. J. Med. Sci.* 51, 1764–1774. doi:10.3906/sag-2010-183

Hemani, R., Patel, I., Inamdar, N., Campanelli, G., Donovan, V., Kumar, A., et al. (2022). Dietary pterostilbene for MTA1-targeted interception in high-risk premalignant prostate cancer. *Cancer Prev. Res.* 15, 87–100. doi:10.1158/1940-6207.CAPR-21-0242

Hirata, H., Hinoda, Y., Shahryari, V., Deng, G., Tanaka, Y., Tabatabai, Z. L., et al. (2014). Genistein downregulates onco-miR-1260b and upregulates sFRP1 and Smad4 *via* demethylation and histone modification in prostate cancer cells. *Br. J. Cancer* 110, 1645–1654. doi:10.1038/bjc.2014.48

Hofer, M. D., Kuefer, R., Varambally, S., Li, H., Ma, J., Shapiro, G. I., et al. (2004). The role of metastasis-associated protein 1 in prostate cancer progression. *Cancer Res.* 64, 825–829. doi:10.1158/0008-5472.can-03-2755

Huo, W., Qi, F., and Wang, K. (2020). Long noncoding RNA BCYRN1 promotes prostate cancer progression *via* elevation of HDAC11. *Oncol. Rep.* 44, 1233–1245. doi:10.3892/or.2020.7680

Huynh, T. T., Lin, C. M., Lee, W. H., Wu, A. T., Lin, Y. K., Lin, Y. F., et al. (2015). Pterostilbene suppressed irradiation-resistant glioma stem cells by modulating GRP78/miR-205 axis. *J. Nutr. Biochem.* 26, 466–475. doi:10.1016/j.jnutbio.2014.11.015

Javed, Z., Khan, K., Rasheed, A., Sadia, H., Raza, S., Salehi, B., et al. (2020). MicroRNAs and natural compounds mediated regulation of TGF signaling in prostate cancer. *Front. Pharmacol.* 11, 613464. doi:10.3389/fphar.2020.613464

Jeon, J., Olkhov-Mitsel, E., Xie, H., Yao, C. Q., Zhao, F., Jahangiri, S., et al. (2020). Temporal stability and prognostic biomarker potential of the prostate cancer urine miRNA transcriptome. *J. Natl. Cancer Inst.* 112, 247–255. doi:10.1093/jnci/djz112

Jin, Y., Zhou, X., Yao, X., Zhang, Z., Cui, M., and Lin, Y. (2020). MicroRNA-612 inhibits cervical cancer progression by targeting NOB1. *J. Cell. Mol. Med.* 24, 3149–3156. doi:10.1111/jcmm.14985

Joshi, T., Patel, I., Kumar, A., Donovan, V., and Levenson, A. S. (2020). Grape powder supplementation attenuates prostate neoplasia associated with pten haploinsufficiency in mice fed high-fat diet. *Mol. Nutr. Food Res.* 64, e2000326. doi:10.1002/mnfr.202000326

Kai, L., Samuel, S. K., and Levenson, A. S. (2010). Resveratrol enhances p53 acetylation and apoptosis in prostate cancer by inhibiting MTA1/NuRD complex. *Int. J. Cancer* 126, 1538–1548. doi:10.1002/ijc.24928

Kai, L., Wang, J., Ivanovic, M., Chung, Y. T., Laskin, W. B., Schulze-Hoepfner, F., et al. (2011). Targeting prostate cancer angiogenesis through metastasis-associated protein 1 (MTA1). *Prostate* 71, 268–280. doi:10.1002/pros.21240

Kang, C. H., Moon, D. O., Choi, Y. H., Choi, I. W., Moon, S. K., Kim, W. J., et al. (2011). Piceatannol enhances TRAIL-induced apoptosis in human leukemia THP-1 cells through Sp1- and ERK-dependent DR5 up-regulation. *Toxicol. Vitro.* 25, 605–612. doi:10.1016/j.tiv.2010.12.006

Kang, N. J., Shin, S. H., Lee, H. J., and Lee, K. W. (2011). Polyphenols as small molecular inhibitors of signaling cascades in carcinogenesis. *Pharmacol. Ther.* 130, 310–324. doi:10.1016/j.pharmthera.2011.02.004

Kapetanovic, I. M., Muzzio, M., Huang, Z., Thompson, T. N., and McCormick, D. L. (2011). Pharmacokinetics, oral bioavailability, and metabolic profile of resveratrol and its dimethylether analog, pterostilbene, in rats. *Cancer Chemother. Pharmacol.* 68, 593–601. doi:10.1007/s00280-010-1525-4

Kataria, R., and Khatkar, A. (2019). Resveratrol in various pockets: A review. *Curr. Top. Med. Chem.* 19, 116–122. doi:10.2174/1568026619666190301173958

Khan, J., Deb, P. K., Priya, S., Medina, K. D., Devi, R., Walode, S. G., et al. (2021). Dietary flavonoids: Cardioprotective potential with antioxidant effects and their pharmacokinetic, toxicological and therapeutic concerns. *Molecules* 26, 4021. doi:10.3390/molecules26134021

Kolenda, T., Guglas, K., Kopczynska, M., Sobocinska, J., Teresiak, A., Blizniak, R., et al. (2020). Good or not good: Role of miR-18a in cancer biology. *Rep. Pract. Oncol. Radiother.* 25, 808–819. doi:10.1016/j.rpor.2020.07.006

Kumar, A., Butt, N. A., and Levenson, A. S. (2016). Natural epigenetic-modifying molecules in medical therapy. In *Medical epigenetics*, (United Kingdom: Elsevier), pp. pp.747–798.

Kumar, A., Dhar, S., Rimando, A. M., Lage, J. M., Lewin, J. R., Zhang, X., et al. (2015). Epigenetic potential of resveratrol and analogs in preclinical models of prostate cancer. *Ann. N. Y. Acad. Sci.* 1348, 1–9. doi:10.1111/nyas.12817

Kumar, A., Dholakia, K., Sikorska, G., Martinez, L. A., and Levenson, A. S. (2019). MTA1-Dependent anticancer activity of Gnetin C in prostate cancer. *Nutrients* 11, E2096. doi:10.3390/nu11092096

Kumar, A. L., and Levenson, A. S. (2018). "Epigenetic mechanisms of resveratrol and its analogs in cancer prevention and treatment," in *Epigenetics of cancer prevention*. Editors A. Bishayee and D. Bhatia (United Kingdom: Elsevier), 169–186.

Latruffe, N., Lancon, A., Frazzi, R., Aires, V., Delmas, D., Michaille, J. J., et al. (2015). Exploring new ways of regulation by resveratrol involving miRNAs, with emphasis on inflammation. *Ann. N. Y. Acad. Sci.* 1348, 97–106. doi:10.1111/nyas.12819

Levenson, A. S., and Kumar, A. (2020). "Pterostilbene as a potent chemopreventive agent in cancer," in *Natural products for chemoprevention: single compounds and combinations*. Editors J. M. Pezzuto and O. Vang (United Kingdom: Springer Nature), 49–108.

Levenson, A. S., Kumar, A., and Zhang, X. (2014). MTA family of proteins in prostate cancer: biology, significance, and therapeutic opportunities. *Cancer Metastasis Rev.* 33, 929–942. doi:10.1007/s10555-014-9519-z

Levenson, A. S. (2020). Metastasis-associated protein 1-mediated antitumor and anticancer activity of dietary stilbenes for prostate cancer chemoprevention and therapy. *Semin. Cancer Biol.* S1044-1579X (1020), 30045–30046.

Levenson, A. S. (2021). Nitrients and phytonutrients as promising epigenetic nutraceuticals. In *Medical epigenetics* (United Kingdom: Elsevier). 741–816.

Li, D., Zhang, Y., Li, Y., Wang, X., Wang, F., Du, J., et al. (2021). miR-149 suppresses the proliferation and metastasis of human gastric cancer cells by targeting FOXC1. *Biomed. Res. Int.* 2021, 1503403. doi:10.1155/2021/1503403

Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S. I., et al. (1997). PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 275, 1943–1947. doi:10.1126/science.275.5308.1943

Li, K., Dias, S. J., Rimando, A. M., Dhar, S., Mizuno, C. S., Penman, A. D., et al. (2013). Pterostilbene acts through metastasis-associated protein 1 to inhibit tumor growth, progression and metastasis in prostate cancer. *PLoS One* 8, e57542. doi:10.1371/journal.pone.0057542

Li, X., Nie, C., Tian, B., Tan, X., Han, W., Wang, J., et al. (2019). miR-671-5p blocks the progression of human esophageal squamous cell carcinoma by suppressing FGFR2. *Int. J. Biol. Sci.* 15, 1892–1904. doi:10.7150/ijbs.32429

Li, X., Pan, J. H., Song, B., Xiong, E. Q., Chen, Z. W., Zhou, Z. S., et al. (2012). Suppression of CX43 expression by miR-20a in the progression of human prostate cancer. *Cancer Biol. Ther.* 13, 890–898. doi:10.4161/cbt.20841

Link, A., Balaguer, F., and Goel, A. (2010). Cancer chemoprevention by dietary polyphenols: Promising role for epigenetics. *Biochem. Pharmacol.* 80, 1771–1792. doi:10.1016/j.bcp.2010.06.036

Liu, W. H., and Chang, L. S. (2012). Suppression of Akt/Foxp3-mediated miR-183 expression blocks Sp1-mediated ADAM17 expression and TNFα-mediated NFκB activation in piceatannol-treated human leukemia U937 cells. *Biochem. Pharmacol.* 84, 670–680. doi:10.1016/j.bcp.2012.06.007

Liu, Y., Lu, L. L., Wen, D., Liu, D. L., Dong, L. L., Gao, D. M., et al. (2020a). Correction to: MiR-612 regulates invadopodia of hepatocellular carcinoma by HADHA-mediated lipid reprogramming. *J. Hematol. Oncol.* 13, 44. doi:10.1186/s13045-020-00875-5

Liu, Y., Lu, L. L., Wen, D., Liu, D. L., Dong, L. L., Gao, D. M., et al. (2020b). MiR-612 regulates invadopodia of hepatocellular carcinoma by HADHA-mediated lipid reprogramming. *J. Hematol. Oncol.* 13, 12. doi:10.1186/s13045-019-0841-3

Ma, C. H., Zhang, Y. X., Tang, L. H., Yang, X. J., Cui, W. M., Han, C. C., et al. (2018). MicroRNA-1469, a p53-responsive microRNA promotes Genistein induced apoptosis by targeting Mcl1 in human laryngeal cancer cells. *Biomed. Pharmacother.* 106, 665–671. doi:10.1016/j.biopha.2018.07.005

Ma, J., Wei, H., Li, X., and Qu, X. (2021). Hsa-miR-149-5p suppresses prostate carcinoma malignancy by suppressing RGS17. *Cancer Manag. Res.* 13, 2773–2783. doi:10.2147/CMAR.S281968

Mak, K. K., Wu, A. T., Lee, W. H., Chang, T. C., Chiou, J. F., Wang, L. S., et al. (2013). Pterostilbene, a bioactive component of blueberries, suppresses the generation of breast cancer stem cells within tumor microenvironment and metastasis *via* modulating NF-κB/microRNA 448 circuit. *Mol. Nutr. Food Res.* 57, 1123–1134. doi:10.1002/mnfr.201200549

Manavathi, B., and Kumar, R. (2007). Metastasis tumor antigens, an emerging family of multifaceted master coregulators. *J. Biol. Chem.* 282, 1529–1533. doi:10.1074/jbc.R600029200

Matsushita, M., Fujita, K., and Nonomura, N. (2020). Influence of diet and nutrition on prostate cancer. *Int. J. Mol. Sci.* 21, 1447–1465. doi:10.3390/ijms21041447

Miyata, Y., Shida, Y., Hakariya, T., and Sakai, H. (2019). Anti-cancer effects of green tea polyphenols against prostate cancer. *Molecules* 24, E193. doi:10.3390/molecules24010193

Moschini, M., Carroll, P. R., Eggener, S. E., Epstein, J. I., Graefen, M., Montironi, R., et al. (2017). Low-risk prostate cancer: Identification, management, and outcomes. *Eur. Urol.* 72, 238–249. doi:10.1016/j.eururo.2017.03.009

Narayanan, N. K., Kunimasa, K., Yamori, Y., Mori, M., Mori, H., Nakamura, K., et al. (2015). Antitumor activity of melinjo (Gnetum gnemon L.) seed extract in human and murine tumor models *in vitro* and in a colon-26 tumor-bearing mouse model *in vivo*. *Cancer Med.* 4, 1767–1780. doi:10.1002/cam4.520

Olive, V., Jiang, I., and He, L. (2010). mir-17-92, a cluster of miRNAs in the midst of the cancer network. *Int. J. Biochem. Cell Biol.* 42, 1348–1354. doi:10.1016/j.biocel.2010.03.004

Onder, T. T., Gupta, P. B., Mani, S. A., Yang, J., Lander, E. S., and Weinberg, R. A. (2008). Loss of E-cadherin promotes metastasis *via* multiple downstream transcriptional pathways. *Cancer Res.* 68, 3645–3654. doi:10.1158/0008-5472.CAN-07-2938

Ostling, P., Leivonen, S. K., Aakula, A., Kohonen, P., Makela, R., Hagman, Z., et al. (2011). Systematic analysis of microRNAs targeting the androgen receptor in prostate cancer cells. *Cancer Res.* 71, 1956–1967. doi:10.1158/0008-5472.CAN-10-2421

Overland, M. R., Washington, S. L., 3rd, Carroll, P. R., Cooperberg, M. R., and Herlemann, A. (2019). Active surveillance for intermediate-risk prostate cancer: Yes, but for whom? *Curr. Opin. Urol.* 29, 605–611. doi:10.1097/MOU.0000000000000671

Pastor-Navarro, B., Rubio-Briones, J., Borque-Fernando, A., Esteban, L. M., Dominguez-Escrig, J. L., and Lopez-Guerrero, J. A. (2021). Active surveillance in prostate cancer: Role of available biomarkers in daily practice. *Int. J. Mol. Sci.* 22, 6266. doi:10.3390/ijms22126266

Peisch, S. F., Van Blarigan, E. L., Chan, J. M., Stampfer, M. J., and Kenfield, S. A. (2017). Prostate cancer progression and mortality: a review of diet and lifestyle factors. *World J. Urol.* 35, 867–874. doi:10.1007/s00345-016-1914-3

Perl, A. K., Wilgenbus, P., Dahl, U., Semb, H., and Christofori, G. (1998). A causal role for E-cadherin in the transition from adenoma to carcinoma. *Nature* 392, 190–193. doi:10.1038/32433

Pesta, M., Klecka, J., Kulda, V., Topolcan, O., Hora, M., Eret, V., et al. (2010). Importance of miR-20a expression in prostate cancer tissue. *Anticancer Res.* 30, 3579–3583.

Poliseno, L., Salmena, L., Riccardi, L., Fornari, A., Song, M. S., Hobbs, R. M., et al. (2010). Identification of the miR-106b~25 microRNA cluster as a proto-oncogenic PTEN-targeting intron that cooperates with its host gene MCM7 in transformation. *Sci. Signal.* 3, ra29. doi:10.1126/scisignal.2000594

Qin, W., Shi, Y., Zhao, B., Yao, C., Jin, L., Ma, J., et al. (2010). miR-24 regulates apoptosis by targeting the open reading frame (ORF) region of FAF1 in cancer cells. *PLoS One* 5, e9429. doi:10.1371/journal.pone.0009429

Qin, W., Zhang, K., Clarke, K., Weiland, T., and Sauter, E. R. (2014). Methylation and miRNA effects of resveratrol on mammary tumors vs. normal tissue. *Nutr. Cancer* 66, 270–277. doi:10.1080/01635581.2014.868910

Quirico, L., and Orso, F. (2020). The power of microRNAs as diagnostic and prognostic biomarkers in liquid biopsies. *Cancer Drug resist.* 3, 117–139. doi:10.20517/cdr.2019.103

Rauf, A., Imran, M., Butt, M. S., Nadeem, M., Peters, D. G., and Mubarak, M. S. (2018). Resveratrol as an anti-cancer agent: A review. *Crit. Rev. Food Sci. Nutr.* 58, 1428–1447. doi:10.1080/10408398.2016.1263597

Rimando, A. M., Cuendet, M., Desmarchelier, C., Mehta, R. G., Pezzuto, J. M., and Duke, S. O. (2002). Cancer chemopreventive and antioxidant activities of pterostilbene, a naturally occurring analogue of resveratrol. *J. Agric. Food Chem.* 50, 3453–3457. doi:10.1021/jf0116855

Rimando, A. M., Kalt, W., Magee, J. B., Dewey, J., and Ballington, J. R. (2004). Resveratrol, pterostilbene, and piceatannol in vaccinium berries. *J. Agric. Food Chem.* 52, 4713–4719. doi:10.1021/jf040095e

Rimando, A. M., Nagmani, R., Feller, D. R., and Yokoyama, W. (2005). Pterostilbene, a new agonist for the peroxisome proliferator-activated receptor alpha-isoform, lowers plasma lipoproteins and cholesterol in hypercholesterolemic hamsters. *J. Agric. Food Chem.* 53, 3403–3407. doi:10.1021/jf0580364

Riviere, C., Pawlus, A. D., and Merillon, J. M. (2012). Natural stilbenoids: distribution in the plant kingdom and chemotaxonomic interest in vitaceae. *Nat. Prod. Rep.* 29, 1317–1333. doi:10.1039/c2np20049j

Rudrapal, M., Khairnar, S. J., Khan, J., Dukhyil, A. B., Ansari, M. A., Alomary, M. N., et al. (2022). Dietary polyphenols and their role in oxidative stress-induced human diseases: Insights into protective effects, antioxidant potentials and mechanism(s) of action. *Front. Pharmacol.* 13, 806470. doi:10.3389/fphar.2022.806470

Rudrapal, M. (2022). *Phytoantioxidants and nanotherapeutics*. Hoboken, NJ: Wiley.

Sapre, N., and Selth, L. A. (2013). Circulating MicroRNAs as biomarkers of prostate cancer: The state of play. *Prostate Cancer* 2013, 539680. doi:10.1155/2013/539680

Selth, L. A., Townley, S., Gillis, J. L., Ochnik, A. M., Murti, K., Macfarlane, R. J., et al. (2012). Discovery of circulating microRNAs associated with human prostate cancer using a mouse model of disease. *Int. J. Cancer* 131, 652–661. doi:10.1002/ijc.26405

Shen, T., Wang, X. N., and Lou, H. X. (2009). Natural stilbenes: an overview. *Nat. Prod. Rep.* 26, 916–935. doi:10.1039/b905960a

Shenouda, S. K., and Alahari, S. K. (2009). MicroRNA function in cancer: oncogene or a tumor suppressor? *Cancer Metastasis Rev.* 28, 369–378. doi:10.1007/s10555-009-9188-5

Sheth, S., Jajoo, S., Kaur, T., Mukherjea, D., Sheehan, K., Rybak, L. P., et al. (2012). Resveratrol reduces prostate cancer growth and metastasis by inhibiting the Akt/MicroRNA-21 pathway. *PLoS One* 7, e51655. doi:10.1371/journal.pone.0051655

Shukla, S., Penta, D., Mondal, P., and Meeran, S. M. (2019). Epigenetics of breast cancer: Clinical status of epi-drugs and phytochemicals. *Adv. Exp. Med. Biol.* 1152, 293–310. doi:10.1007/978-3-030-20301-6_16

Song, S. J., Poliseno, L., Song, M. S., Ala, U., Webster, K., Ng, C., et al. (2013). MicroRNA-antagonism regulates breast cancer stemness and metastasis *via* TET-family-dependent chromatin remodeling. *Cell* 154, 311–324. doi:10.1016/j.cell.2013.06.026

Stoen, M. J., Andersen, S., Rakaee, M., Pedersen, M. I., Ingebriktsen, L. M., Donnem, T., et al. (2021). Overexpression of miR-20a-5p in tumor epithelium is an independent negative prognostic indicator in prostate cancer-A multi-institutional study. *Cancers (Basel)* 13, 4096. doi:10.3390/cancers13164096

Storniolo, C. E., Quifer-Rada, P., Lamuela-Raventos, R. M., and Moreno, J. J. (2014). Piceid presents antiproliferative effects in intestinal epithelial Caco-2 cells, effects unrelated to resveratrol release. *Food Funct.* 5, 2137–2144. doi:10.1039/c4fo00305e

Su, C. M., Lee, W. H., Wu, A. T., Lin, Y. K., Wang, L. S., Wu, C. H., et al. (2015). Pterostilbene inhibits triple-negative breast cancer metastasis *via* inducing microRNA-205 expression and negatively modulates epithelial-to-mesenchymal transition. *J. Nutr. Biochem.* 26, 675–685. doi:10.1016/j.jnutbio.2015.01.005

Su, D., Cheng, Y., Liu, M., Liu, D., Cui, H., Zhang, B., et al. (2013). Comparision of piceid and resveratrol in antioxidation and antiproliferation activities *in vitro*. *PLoS One* 8, e54505. doi:10.1371/journal.pone.0054505

Su, L., and David, M. (2000). Distinct mechanisms of STAT phosphorylation *via* the interferon-alpha/beta receptor. Selective inhibition of STAT3 and STAT5 by piceatannol. *J. Biol. Chem.* 275, 12661–12666. doi:10.1074/jbc.275.17.12661

Taft, R. J., Glazov, E. A., Cloonan, N., Simons, C., Stephen, S., Faulkner, G. J., et al. (2009). Tiny RNAs associated with transcription start sites in animals. *Nat. Genet.* 41, 572–578. doi:10.1038/ng.312

Tan, Y. Y., Xu, X. Y., Wang, J. F., Zhang, C. W., and Zhang, S. C. (2016). MiR-654-5p attenuates breast cancer progression by targeting EPSTI1. *Am. J. Cancer Res.* 6, 522–532.

Taylor, B. S., Schultz, N., Hieronymus, H., Gopalan, A., Xiao, Y., Carver, B. S., et al. (2010). Integrative genomic profiling of human prostate cancer. *Cancer Cell* 18, 11–22. doi:10.1016/j.ccr.2010.05.026

Tili, E., Michaille, J. J., Adair, B., Alder, H., Limagne, E., Taccioli, C., et al. (2010a). Resveratrol decreases the levels of miR-155 by upregulating miR-663, a microRNA targeting JunB and JunD. *Carcinogenesis* 31, 1561–1566. doi:10.1093/carcin/bgq143

Tili, E., Michaille, J. J., Alder, H., Volinia, S., Delmas, D., Latruffe, N., et al. (2010b). Resveratrol modulates the levels of microRNAs targeting genes encoding tumor-suppressors and effectors of TGFβ signaling pathway in SW480 cells. *Biochem. Pharmacol.* 80, 2057–2065. doi:10.1016/j.bcp.2010.07.003

Tili, E., and Michaille, J. J. (2011). Resveratrol, MicroRNAs, inflammation, and cancer. *J. Nucleic Acids* 2011, 102431. doi:10.4061/2011/102431

Tili, E., Michaille, J. J., Wernicke, D., Alder, H., Costinean, S., Volinia, S., et al. (2011). Mutator activity induced by microRNA-155 (miR-155) links inflammation and cancer. *Proc. Natl. Acad. Sci. U. S. A.* 108, 4908–4913. doi:10.1073/pnas.1101795108

Urabe, F., Kosaka, N., Sawa, Y., Ito, K., Kimura, T., Egawa, S., et al. (2020). The miR-1908/SRM regulatory axis contributes to extracellular vesicle secretion in prostate cancer. *Cancer Sci.* 111, 3258–3267. doi:10.1111/cas.14535

Valeri, N., Vannini, I., Fanini, F., Calore, F., Adair, B., and Fabbri, M. (2009). Epigenetics, miRNAs, and human cancer: a new chapter in human gene regulation. *Mamm. Genome* 20, 573–580. doi:10.1007/s00335-009-9206-5

Vanden Berghe, W. (2012). Epigenetic impact of dietary polyphenols in cancer chemoprevention: lifelong remodeling of our epigenomes. *Pharmacol. Res.* 65, 565–576. doi:10.1016/j.phrs.2012.03.007

Venkatadri, R., Muni, T., Iyer, A. K., Yakisich, J. S., and Azad, N. (2016). Role of apoptosis-related miRNAs in resveratrol-induced breast cancer cell death. *Cell Death Dis.* 7, e2104. doi:10.1038/cddis.2016.6

Ventura, A., and Jacks, T. (2009). MicroRNAs and cancer: short RNAs go a long way. *Cell* 136, 586–591. doi:10.1016/j.cell.2009.02.005

Vo, N. T., Madlener, S., Bago-Horvath, Z., Herbacek, I., Stark, N., Gridling, M., et al. (2010). Pro- and anticarcinogenic mechanisms of piceatannol are activated dose dependently in MCF-7 breast cancer cells. *Carcinogenesis* 31, 2074–2081. doi:10.1093/carcin/bgp199

Waffo-Teguo, P., Hawthorne, M. E., Cuendet, M., Merillon, J. M., Kinghorn, A. D., Pezzuto, J. M., et al. (2001). Potential cancer-chemopreventive activities of wine stilbenoids and flavans extracted from grape (Vitis vinifera) cell cultures. *Nutr. Cancer* 40, 173–179. doi:10.1207/S15327914NC402_14

Wang, J., Ni, J., Beretov, J., Thompson, J., Graham, P., and Li, Y. (2020). Exosomal microRNAs as liquid biopsy biomarkers in prostate cancer. *Crit. Rev. Oncol. Hematol.* 145, 102860. doi:10.1016/j.critrevonc.2019.102860

Wang, W. J., Yuan, Y., Zhang, D., Liu, P., and Liu, F. (2021). miR-671-5p repressed progression of papillary thyroid carcinoma *via* TRIM14. *Kaohsiung J. Med. Sci.* 37, 983–990. doi:10.1002/kjm2.12424

Wang, Y. L., Shen, Y., Xu, J. P., Han, K., Zhou, Y., Yang, S., et al. (2017). Pterostilbene suppresses human endometrial cancer cells *in vitro* by down-regulating miR-663b. *Acta Pharmacol. Sin.* 38, 1394–1400. doi:10.1038/aps.2017.60

Wang, Y., Romigh, T., He, X., Orloff, M. S., Silverman, R. H., Heston, W. D., et al. (2010). Resveratrol regulates the PTEN/AKT pathway through androgen receptor-dependent and -independent mechanisms in prostate cancer cell lines. *Hum. Mol. Genet.* 19, 4319–4329. doi:10.1093/hmg/ddq354

Wang, Y., Zhang, Q., Guo, B., Feng, J., and Zhao, D. (2020). miR-1231 is downregulated in prostate cancer with prognostic and functional implications. *Oncol. Res. Treat.* 43, 78–86. doi:10.1159/000504606

Xie, G. P., and Jiang, R. (2015). Non-coding RNAs in castration-resistant prostate cancer. *Zhonghua Nan Ke Xue* 21, 1014–1019.

Xin, C., Lu, S., Li, Y., Zhang, Y., Tian, J., Zhang, S., et al. (2019). miR-671-5p inhibits tumor proliferation by blocking cell cycle in osteosarcoma. *DNA Cell Biol.* 38, 996–1004. doi:10.1089/dna.2019.4870

Yu, Y., Wang, Z., Sun, D., Zhou, X., Wei, X., Hou, W., et al. (2018). miR-671 promotes prostate cancer cell proliferation by targeting tumor suppressor SOX6. *Eur. J. Pharmacol.* 823, 65–71. doi:10.1016/j.ejphar.2018.01.016

Zhang, H., Jia, R., Wang, C., Hu, T., and Wang, F. (2014). Piceatannol promotes apoptosis *via* up-regulation of microRNA-129 expression in colorectal cancer cell lines. *Biochem. Biophys. Res. Commun.* 452, 775–781. doi:10.1016/j.bbrc.2014.08.150

Zhang, T., Zhu, X., Wu, H., Jiang, K., Zhao, G., Shaukat, A., et al. (2019a). Targeting the ROS/PI3K/AKT/HIF-1α/HK2 axis of breast cancer cells: Combined administration of Polydatin and 2-Deoxy-d-glucose. *J. Cell. Mol. Med.* 23, 3711–3723. doi:10.1111/jcmm.14276

Zhang, W., Jiang, H., Chen, Y., and Ren, F. (2019b). Resveratrol chemosensitizes adriamycin-resistant breast cancer cells by modulating miR-122-5p. *J. Cell. Biochem.* 120, 16283–16292. doi:10.1002/jcb.28910

Zhao, J., Li, Q., Feng, B., Wei, D., Han, Y., Li, M., et al. (2021). MicroRNA149 inhibits cancer cell malignant phenotype by regulating Akt1 in C42 CRPC cell line. *Oncol. Rep.* 46, 258. doi:10.3892/or.2021.8209

Zhao, Y., Cheng, X., Wang, G., Liao, Y., and Qing, C. (2020). Linalool inhibits 22Rv1 prostate cancer cell proliferation and induces apoptosis. *Oncol. Lett.* 20, 289. doi:10.3892/ol.2020.12152

Zhu, J., Wang, S., Zhang, W., Qiu, J., Shan, Y., Yang, D., et al. (2015). Screening key microRNAs for castration-resistant prostate cancer based on miRNA/mRNA functional synergistic network. *Oncotarget* 6, 43819–43830. doi:10.18632/oncotarget.6102

Glossary

ADAM17 disintegrin and metallopeptidase domain-containing protein 17

Akt v-akt murine thymoma viral oncogene (protein kinase B)

AP1 jun proto-oncogene

AR androgen Receptor

ATG7 autophagy related 7

Ago-2 argonaute 2

BCL-2 B-cell lymphoma 2

BCL2L14 B-cell lymphoma like 14

BCYRN1 brain cytoplasmic RNA 1

ChIP-Seq chromatin immunoprecipitation sequencing

CRPC castrate resistant prostate cancer

CX43 connexin 43 (Gap junction alpha 1 protein, GJA1)

DU145 human prostate cancer cell line

EGFR epidermal growth factor receptor

EMT epithelial-to-mesenchymal transition

Epi-miRNA epigenetic miRNA (ribonucleic acid)

EV extracellular vehicle

FAF1 fas-associated factor 1

Fak focal adhesion kinase

Foxo3 forkhead box O3

GRP78 glucose-regulated protein 78

HDAC1/2 histone deacetylases 1 and 2

HDAC11 histone deacetylase 11

HIF-1α hypoxia inducing factor 1

IL-1β interleukin 1 Beta

K-ras kirsten rat sarcoma virus

LNCaP human prostate cancer cell line

lncRNA long non-coding ribonucleic acid

Luc luciferase

MAPK mitogen-activated protein kinase

Mcl-1 myeloid cell leukemia 1

miR microRNA (ribonucleic acid)

miRNA microRNA (ribonucleic acid)

mRNA messenger RNA (ribonucleic acid)

MTA1 metastasis-associated protein 1

mPIN mouse PIN

NANOG homeobox protein, a transcription factor in embryonic stem cells

ncRNA non-coding RNA

NF-κB nuclear factor kappa-light-chain-enhancer of activated B cells

NuRD nucleosome remodeling and deacetylase complex

Oct4 homeobox protein, a transcription factor in embryonic stem cells

OncomiR oncogenic miRNA

PC3 human prostate cancer cell line

PDCD4 programmed cell death 4

PI3K phosphatidylinositol 3-kinase/serine/threonine kinase PKB

PIN prostatic intraepithelial neoplasia

PTEN phosphatase and tensin homolog deleted in chromosome 10

Ptenf/f Pten gene, floxed

Pten+/f Pten heterozygous mice (Pb-Cre4+, Pten+/f)

RGS17 regulator of G protein signaling

R26MTA1 rosa26 locus; R26MTA1: MTA1 overexpressing mice

22Rv1 human prostate cancer cell line

RWPE1 human prostate cancer cell line

SMAD4 mothers against decapentaplegic homolog 4

SOX2 SRY (sex determining region Y)-box 2

SOX6 SRY (sex determining region Y)-box 6

Sp-1 specificity protein 1

Src proto-oncogene tyrosine-protein kinase

SRM spermidine synthase

TGFβ1 transforming growth factor β1

TNFα tumor necrosis factor alpha

TS tumor suppressor

3′UTR 3 prime untranslated region

VEGF vascular endothelial growth factor

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 12 September 2022
DOI 10.3389/fphar.2022.1008222

Check for updates

OPEN ACCESS

EDITED BY
Peng Qu,
National Institutes of Health (NIH),
United States

REVIEWED BY
Dennis Mans,
Anton de Kom University of Suriname,
Suriname
Xuejun Jin,
Yanbian University, China
Ming Zhang,
Shanghai Jiao Tong University, China

*CORRESPONDENCE
Wenyi Gao,
gaowy@ccucm.edu.cn
Donglu Wu,
wudl1020@163.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

RECEIVED 31 July 2022
ACCEPTED 24 August 2022
PUBLISHED 12 September 2022

CITATION
Lu M, Lan X, Wu X, Fang X, Zhang Y, Luo H, Gao W and Wu D (2022), Salvia miltiorrhiza in cancer: Potential role in regulating MicroRNAs and epigenetic enzymes.
Front. Pharmacol. 13:1008222.
doi: 10.3389/fphar.2022.1008222

COPYRIGHT
© 2022 Lu, Lan, Wu, Fang, Zhang, Luo, Gao and Wu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Salvia miltiorrhiza in cancer: Potential role in regulating MicroRNAs and epigenetic enzymes

Meng Lu[1†], Xintian Lan[1†], Xi Wu[1], Xiaoxue Fang[1], Yegang Zhang[1], Haoming Luo[1,2], Wenyi Gao[1]* and Donglu Wu[2,3]*

[1]School of Pharmacy, Changchun University of Chinese Medicine, Changchun, China, [2]Key Laboratory of Effective Components of Traditional Chinese Medicine, Changchun, China, [3]School of Clinical Medical, Changchun University of Chinese Medicine, Changchun, China

MicroRNAs are small non-coding RNAs that play important roles in gene regulation by influencing the translation and longevity of various target mRNAs and the expression of various target genes as well as by modifying histones and DNA methylation of promoter sites. Consequently, when dysregulated, microRNAs are involved in the development and progression of a variety of diseases, including cancer, by affecting cell growth, proliferation, differentiation, migration, and apoptosis. Preparations from the dried root and rhizome of *Salvia miltiorrhiza Bge* (Lamiaceae), also known as red sage or danshen, are widely used for treating cardiovascular diseases. Accumulating data suggest that certain bioactive constituents of this plant, particularly tanshinones, have broad antitumor effects by interfering with microRNAs and epigenetic enzymes. This paper reviews the evidence for the antineoplastic activities of *S. miltiorrhiza* constituents by causing or promoting cell cycle arrest, apoptosis, autophagy, epithelial-mesenchymal transition, angiogenesis, and epigenetic changes to provide an outlook on their future roles in the treatment of cancer, both alone and in combination with other modalities.

KEYWORDS

Salvia miltiorrhiza, microRNA, apoptosis, histone modifications, combination medication, cancer

Introduction

Salvia miltiorrhiza is a common herbal beverage in Traditional Chinese Medicine (TCM). *S. milthiorrhiza* belongs to the Lamiaceae family, and its medicinal parts are the roots or rhizomes (Zhang et al., 2022). *S. milthiorrhiza* has been widely used in TCM for the treatment of cardiovascular disease (CVD) (Li et al., 2018; Ren et al., 2019). For instance, Danshen-Honghua is a commonly used herb pair for promoting blood circulation and removing blood stasis, and *S. milthiorrhiza* often appears in some TCM compounds used for cardiovascular and cerebrovascular diseases, such as Danhong Huayu Oral Liquid, Danhong Injection, and Xinning Tablets (Wang et al., 2019b).

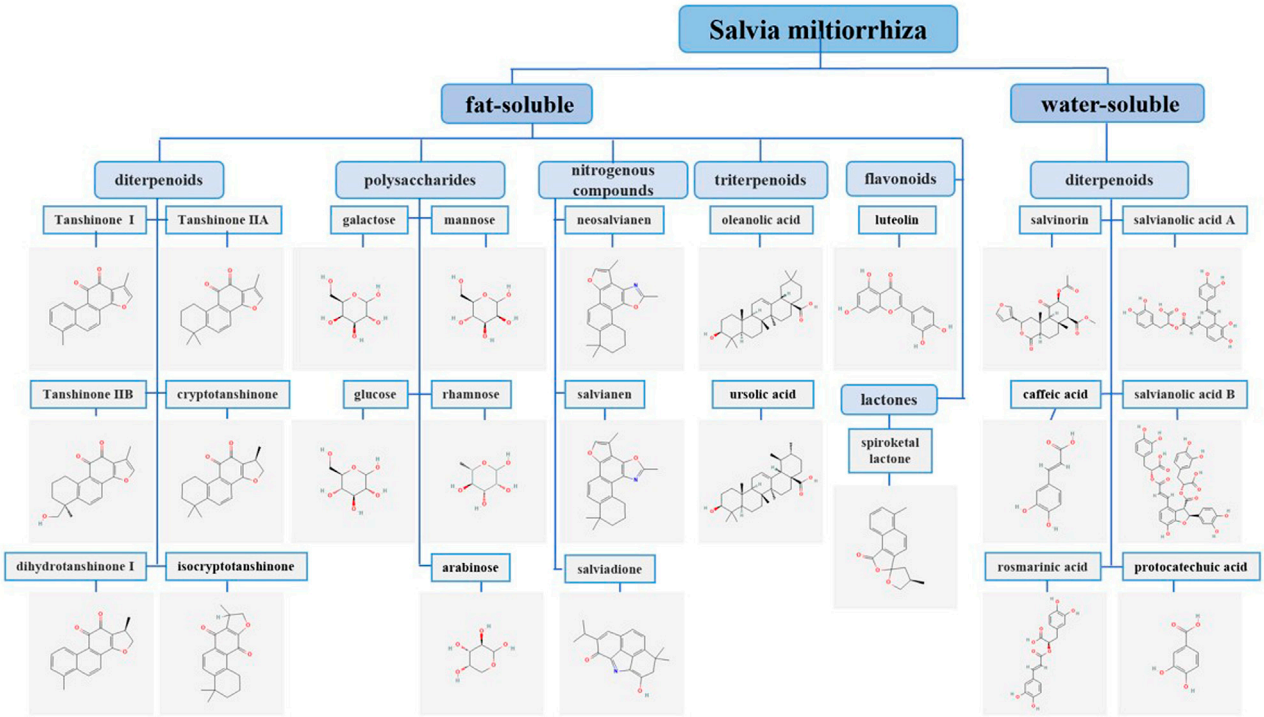


FIGURE 1
Classification and chemical structures of *S. miltiorrhiza* bioactive compounds. The chemical structures of the *S. miltiorrhiza* constituents included in this publication were obtained from PubChem and drawn with the ChemDraw program.

S. milthiorrhiza improves microcirculation, protects the nervous system, and dilates blood vessels, and it also has antitumor properties (Tao et al., 2018). Research has shown that the compounds in *S. milthiorrhiza* are mainly divided into two categories as follows: fat-soluble, which mainly include diterpenoids, triterpenoids, flavonoids, nitrogenous compounds, lactones, and polysaccharides; and water-soluble, which mainly include phenolic acids (Su et al., 2015). Diterpenoids mainly include tanshinones, such as tanshinone I (tan I), tanshinone IIA (tan IIA), tanshinone IIB (tan IIB), cryptotanshinone (CPT), dihydrotanshinone I (DHT I), and isocryptotanshinone (Tian et al., 2021). The water-soluble compounds in *S. milthiorrhiza* are primarily salvianolic acid A (Sal A), salvianolic acid B (Sal B), salvinorin, caffeic acid, rosmarinic acid, and protocatechuic acid (Xin et al., 2020). Most of the triterpenoids are derivatives of oleanolic acid (OA) and ursolic acid (UA) (Tung et al., 2017). The flavonoids in *S. milthiorrhiza* are mainly luteolin (LUT) and its derivatives (Salinas-Arellano et al., 2020). The polysaccharides in *S. milthiorrhiza* are mainly composed of mannose, rhamnose, arabinose, glucose, and galactose (Han et al., 2019). The nitrogen-containing compounds that have been isolated from *S. milthiorrhiza* include neosalvianen, salvianen, and salviadione. In addition, *S. milthiorrhiza* also contains lactone compounds, such as Dan shen spiroketal lactone (Liang et al., 2020; Wang et al., 2020) (Figure 1). Cancer is a disease that seriously endangers human health. According to the latest data released by the World Health Organization's International Agency for Research on Cancer (IARC), the number of new cancer cases worldwide in 2020 exceeded 19.3 million, and China ranks 68th in the world in cancer incidence rate (Sung et al., 2021). Many studies have reported that the chemical components in *S. milthiorrhiza,* such as tan IIA, have good antitumor activity. In addition to inhibiting the proliferation, blocking the cell cycle, inducing apoptosis, and inducing autophagic death of various types of tumor cells, tan IIA also significantly inhibits cell migration and invasion (Cao et al., 2018; Fang et al., 2020). Tan I and LUT also exert antitumor effects through mediating apoptosis and cell cycle arrest (Anson et al., 2018; Li et al., 2018a).

Antitumor activities of *S. miltiorrhiza*

Inhibition of proliferation

S. miltiorrhiza bioactive compounds, such as tan IIA and CPT, effectively inhibit tumor growth (Chen et al., 2014) and suppress tumor cell proliferation *in vitro* and *in vivo* (Lin et al.,

2015; Zhang et al., 2019c). For example, tan IIA inhibits tumor growth in acute promyelocytic leukemia NB4 cell xenograft mice (Zhang et al., 2016c). Liu et al. (2020) reported that CPT inhibits the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt) pathway by inducing the expression of phosphatase and tensin homologue (PTEN), which represses tumor growth in 5637 cell xenograft mice. Furthermore, tanshinol represses U2-OS tumor growth in zebrafish larvae in a dose-dependent manner (Yu et al., 2022). In addition, previous studies have demonstrated that tan IIA suppresses the proliferation of different types of tumor cells in a time- and dose-dependent manner *in vitro*, including gastric cancer (GC; SNU-638, MKN1 and AGS cells) (Zhang et al., 2018) and non-small cell lung cancer (NSCLC; A549 and H292 cells) (Qi et al., 2022). Additionally, it has been reported that tan IIA reduces the phosphorylation of insulin-like growth factor 1 receptor (IGF-1R) and its downstream PI3K/Akt and extracellular signal-regulated kinase 1/2 (ERK1/2) pathways, resulting in the suppression of pheochromocytoma growth (PC12 cells) (Wang et al., 2018a). Other tanshinones also inhibit the development of tumors. For example, tan I suppresses colorectal cancer (CRC) cell proliferation by decreasing aurora A-mediated upregulation of p53 or downregulation of survivin expression to induce apoptosis in HCT116 and SW480 cells (Lu et al., 2016). In addition, CPT enhances the cytotoxicity of $CD4^+$ T cells and prevents the development of H446 cells by upregulating the levels of phosphorylated JAK kinase 2 (p-JAK2) and phosphorylated signal transducer and activator of transcription 4 (p-STAT4) (Man et al., 2016). In addition, other components of *S. miltiorrhiza* have been reported to have antitumor properties. For example, LUT suppresses the viability of RPMI-8226 multiple myeloma cells by enhancing the expression of cleaved caspase 3 and microtubule-associated protein light chain 3 (LC3)-II/I (Chen et al., 2018). LUT also inhibits xenograft tumor growth by reducing Ki-67, p-LIMK, and p-cofilin expression (Zhang et al., 2021). Furthermore, salvianolic acid inhibits tumor cell proliferation. Sal A reduces the viability of NSCLC cells (Jin et al., 2021), and Sal B suppresses cell proliferation *via* regulating the Akt/mTOR pathway in DDP-resistant GC cells (Wang et al., 2021a).

The cell cycle is the foundation of cell proliferation, and blockade of the cell cycle prevents tumor development. It has been reported that tan IIA inhibits the proliferation of ovarian cancer (Zhou et al., 2020), cervical cancer (Pan et al., 2010), and GC (Chen et al., 2012) cells by arresting the cell cycle in the G2/M phase. In contrast, tan IIA arrests the cell cycle in lung cancer at the G0/G1 phase *via* upregulating the levels of p53 and p21 as well as downregulating the levels of cyclin D1, cyclin E1, and cyclin-dependent kinase 2 (CDK2) (Lee et al., 2016). Furthermore, tan IIA arrests the cell cycle in prostate cancer in the G0/G1 phase through increasing the expression of p21, p26, and p27 as well as decreasing the expression of cyclin D1, CDK2, and p-Rb (Chiu et al., 2013). Therefore, we propose that the different effects of tan IIA on the cell cycle may be related to cell types. Studies have indicated that other components of *S. miltiorrhiza* also block the cell cycle at different phases in various tumors cells. For example, LUT inhibits cell proliferation of bladder cancer and lung cancer through arresting the cell cycle in the G2/M phase and G1 phase, respectively (Iida et al., 2020; Zhang et al., 2021). LUT blocks the cell cycle in the G2/M phase through upregulating $p21^{Waf1/Cip1}$ and $p27^{Kip1}$ as well as downregulating cyclin A and D1 in bladder cancer T24 cells (Iida et al., 2020). In addition, LUT reduces the expression of cyclin D1 and cyclin D3, thereby inducing cycle arrest of lung cancer cells in the G1 phase (NCI-H1975 and NCI-H1650 cells) (Zhang et al., 2021). Moreover, OA reduces the levels of cyclin D1, CDK1, CDK2, CDK4, and CDK7 but increases the expression of p27, which in turn blocks the cell cycle of HeLa cells in the sub-G1 phase, thereby inhibiting cervical cancer cell growth (Edathara et al., 2021). Moreover, Sal B arrests the cell cycle of A549 cells in the G0/G1 phase by regulating cyclin B1 and p21, thereby repressing A549 cell proliferation (Han et al., 2022). Thus, these findings demonstrate that *S. miltiorrhiza* bioactive compounds suppress the proliferation of various cancers by driving cell cycle arrest, indicating the potential antitumor usage of *S. miltiorrhiza* bioactive compounds in the clinic.

Promotion of apoptosis

Apoptosis is an autonomous and orderly cell death process controlled by genes that occurs after cells are stimulated by physiological or pathological signals, and tumors have the ability to resist apoptosis *via* multiple strategies (Pistritto et al., 2016; Huang et al., 2021a). The antitumor effect of *S. miltiorrhiza* bioactive compounds has been linked to the induction of apoptosis in multiple studies (Sun et al., 2021). For example, tan IIA induces apoptosis of ovarian cancer A2780 cells by regulating the PI3K/Akt/c-Jun N-terminal kinase (JNK) signaling pathway, which increases the expression of caspase 3, caspase 8, and caspase 9 but decreases the expression of B cell lymphoma 2 (Bcl-2) family proteins (Bcl-w and Bcl-1L) (Zhang et al., 2019a). Tan IIA has also been shown to promote tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-induced apoptosis in glioblastoma by upregulating death receptor 5 (DR5) and blocking STAT3-mediated survivin downregulation (Zhou et al., 2021). Moreover, tan I induces leukemia cell apoptosis *via* inactivation of the PI3K/Akt/survivin signaling pathway, which disrupts the membrane potential, increases Bcl-2-related X (Bax) expression, and activates caspase 3 (Liu et al., 2010). Tanshinone analog 2-((Glycine methyl ester) methyl)-naphtho (TC7) induces cell apoptosis by regulating apoptosis-associated genes, such as p53, ERK1, Bax, p38, Bcl-2, caspase 8, cleaved caspase 8, and poly (ADP-ribose) polymerase 1 (PARP1),

as well as the phosphorylation levels of ERK1 and p38 (Wang et al., 2019). Additionally, LUT has been reported to promote apoptosis in a variety of tumor cells, including ovarian teratomas (Liu et al., 2019) and melanomas (Yao et al., 2019), by decreasing Bcl-2 expression, increasing Bax expression, and inhibiting the PI3K/Akt signaling pathway. Moreover, Sal B induces osteosarcoma MG63 cell apoptosis by increasing the expression of cleaved caspase 3, phosphorylated-p38 mitogen-activated protein kinase (p-p38 MAPK), and phosphorylated-p53 (p-p53) (Zeng et al., 2018).

S. miltiorrhiza bioactive compounds induce apoptosis of various tumor cells through the mitochondrial pathway (Ye et al., 2017). Tan IIA regulates mitochondrial fission through the JNK/mitochondrial fission factor (MFF) axis, which in turn upregulates mitochondrial pro-apoptotic proteins (Bax and Bad), activates caspase 9, and leads to colon cancer SW837 cell apoptosis (Jieensinue et al., 2018). Reactive oxygen species (ROS) interact with membrane receptors and transcription factors/repressors to activate endogenous and exogenous apoptotic signaling pathways, thereby inducing apoptosis (Su et al., 2019). DHT provokes mitochondrial dysfunction in the early stage by decreasing mitochondrial membrane permeability (MMP) and ROS levels, leading to cell death in colon cancer cells (Wang et al., 2013). Moreover, DHT I stimulates the release of both cytochrome c and apoptosis inducing factor (AIF) in HCT116 cells, which downregulates Bcl-xl and upregulates Bax, thereby inducing apoptosis (Wang et al., 2015). Another *S. miltiorrhiza* component, LUT, promotes apoptosis in canine osteosarcoma cells by generating ROS, increasing loss of MMP, and reducing cytosolic Ca^{2+} concentration (Ryu et al., 2019). In addition, CPT upregulates Bax and cleaved caspase3 but downregulates the anti-apoptotic protein, Bcl-2, which reduces the level of ROS, thereby inducing apoptosis in human melanoma A375 cells (Ye et al., 2016). Furthermore, Sal B induces mitochondria-mediated apoptosis in human colon cancer cells by reducing the mitochondrial potential and increasing mitochondrial cytochrome c release (Gong et al., 2016). Collectively, inducing mitochondria-dependent apoptosis is one of the important ways for *S. miltiorrhiza* bioactive compounds to exert an antitumor effect.

Inhibition of migration and invasion

Metastasis and invasion are the main manifestations of tumor malignancy, and they are key factors affecting cancer prognosis (Suhail et al., 2019). Tumor cell invasion and metastasis are complicated biological processes that involve the interplay of many growth factors, cytokines, enzyme systems, matrix metalloproteinases, signaling pathways, and epithelial-mesenchymal transition (EMT) (Patriarca et al., 2020). Many studies have reported that numerous *S. miltiorrhiza* bioactive compounds affect tumor metastasis and invasion *via* regulating angiogenesis, regulating tumor cell EMT, and blocking matrix metalloproteinases from hydrolyzing the basement membrane (Zhang et al., 2012). For instance, tan IIA blocks the nuclear factor kappa-B (NF-κB) signaling pathway to inhibit the activity of MMP2 and MMP9, thereby preventing the metastasis and invasion of human hepatoma cells and hepatocellular carcinoma (HCC) cells (Shan et al., 2009; Yuxian et al., 2009; Zhang et al., 2016d).

New blood vessels in tumor tissue provide a source of nutrients for cell proliferation and are also a channel for tumor cell migration (Jiang et al., 2020). Thus, inhibiting tumor angiogenesis has become one of the current antitumor strategies (Fu et al., 2020). Rapid tumor cell proliferation generates hypoxia in local tumor tissue, which activates and upregulates hypoxia-inducible factor 1α (HIF-1α), resulting in the upregulation of the expression of pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), to promote angiogenesis (Keeley et al., 2010). VEGF is a potent angiogenic factor that increases vascular permeability, enabling endothelial cell migration and inducing angiogenesis (Melincovici et al., 2018). Many components of *S. miltiorrhiza* have been demonstrated to suppress tumor cell invasion and metastasis *via* regulating HIF-1α- and VEGF- mediated signaling pathways (Macklin et al., 2017). Tan IIA, tan I, and LUT inhibit the secretion of VEGF under hypoxia by downregulating HIF-1α expression, further inhibiting the growth of new blood vessels and the metastasis and invasion of HCC cells, CRC cells, breast cancer cells, human endothelial progenitor cells, and human umbilical vein endothelial cells (Nizamutdinova et al., 2008; Li et al., 2015; Lee et al., 2017; Sui et al., 2017; Fang et al., 2018; Li et al., 2019; Xue et al., 2019; Zhou et al., 2020c).

During EMT, cells transition from an epithelial state to a mesenchymal state, enabling tumor cells to invade and metastasize (Pastushenko and Blanpain 2019). In the EMT process, epithelial proteins are up- or downregulated, and these proteins are identified as EMT markers. The occurrence of EMT is indicated by decreased E-cadherin and keratin as well increased mesenchymal markers, including vimentin, fibronectin, N-cadherin, and α-SMA (Zeisberg and Neilson 2009). Salvinorin and DHT prevent EMT in colon cancer cells and triple-negative breast cancer (TNBC) cells by increasing E-cadherin levels and decreasing N-cadherin and vimentin levels (Kashyap et al., 2021; Cao et al., 2022).

Numerous studies have shown that *S. miltiorrhiza* bioactive compounds reverse the levels of EMT markers through a complex pathway, including transcription factors, which inhibit the EMT process in tumor cells (Pastushenko and Blanpain 2019). TGF-β is a key factor in inducing EMT in the process of tumor cell proliferation, and elevated levels of TGF-β regulate the expression of EMT markers, which in turn induces EMT (Ali et al., 2015). It has been reported that Sal B and Sal A inhibit the EMT of renal tubular epithelial cells and endothelial cells, respectively, by inhibiting the expression of TGF-β and

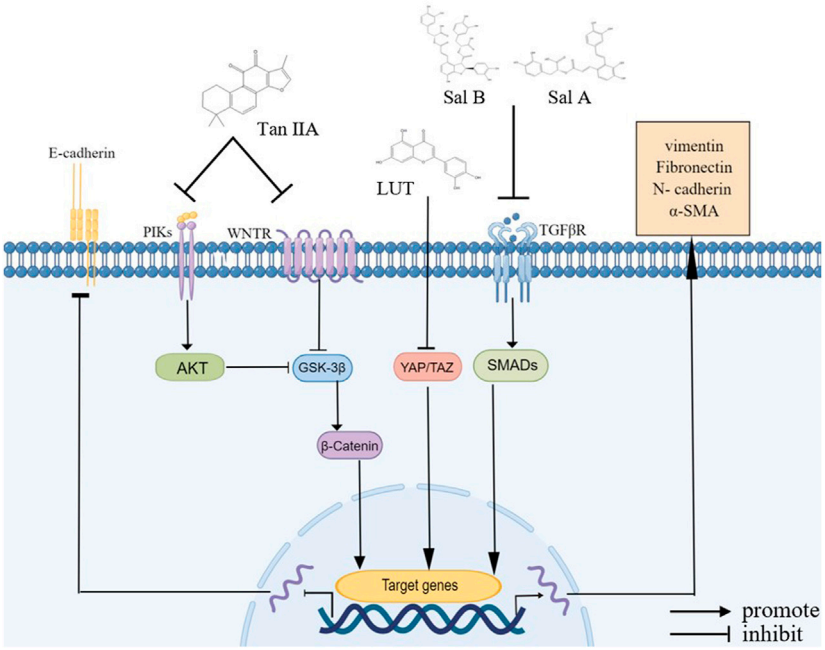


FIGURE 2
Bioactive compounds of *S. miltiorrhiza* involved in EMT regulation. Tan IIA, LUT, Sal A, and Sal B modulate various signaling pathways, including the NF-κB signaling pathway, VEGF signaling pathway, β-catenin signaling pathway, and PI3K/Akt signaling pathway, and they upregulate E-cadherin and downregulate N-cadherin, α-SMA, vimentin, and fibronectin.

reversing the expression of EMT markers (Wang et al., 2010; Zhao et al., 2017). YAP/TAZ, a co-activating transcription factor, translocates into the nucleus and binds to transcription factors, such as Twist and Snail, to promote gene transcription (Noguchi et al., 2018; Ma et al., 2019). LUT inhibits the EMT in TNBC cells by inhibiting the transcription activity of YAP/TAZ as well as preventing it from entering the nucleus, further upregulating the protein levels of E-cadherin and downregulating the protein levels of fibronectin, N-cadherin, and vimentin (Cao et al., 2020).

In addition, β-catenin, a cell adhesion regulator, combines with E-cadherin protein on the cell membrane to attach it to the actin cytoskeletal protein, thereby increasing cell adhesion (Shang et al., 2017). GSK-3β is an evolutionarily conserved serine/threonine (S/T) kinase that phosphorylates and degrades cytoplasmic β-catenin, which is regulated by β-arrestin1 and the PI3K/Akt pathway (Yu et al., 2021). Studies have shown that tan IIA increases the expression of GSK-3β by inhibiting the β-arrestin1 and PI3K/Akt signaling pathways, further reducing the nuclear localization of β-catenin and blocking the EMT in CRC and HCC cells (Zhang et al., 2019; Song et al., 2020). Moreover, adhesion factors, such as CCL2, are critical in the EMT process, and the reduction of adhesion factor expression leads to reduced adhesion between transitional cells and adjacent epithelial cells, which causes epithelial cells to lose apical basal cell polarity, thereby promoting the EMT process (Jin 2020; Zou et al., 2020). Studies have shown that tan IIA suppresses the EMT in bladder cancer cells by downregulating CCL2 *via* blocking the STAT3 pathway (Huang et al., 2017) (Figure 2).

In conclusion, *S. miltiorrhiza* bioactive compounds inhibit tumor cell metastasis and invasion through different pathways, including inhibiting the activity of MMP proteins, tumor angiogenesis, and the EMT process.

Regulation of autophagy

Autophagy is a type of programmed cell death, and it is a target in cancer treatment (Rodrigues et al., 2020). Investigations have revealed that *S. miltiorrhiza* bioactive compounds, such as tan IIA, tan I, CPT, DHT, and LUT, induce autophagy, thereby inhibiting cancer cell proliferation (Fu et al., 2020a).

One of the critical pathways to induce autophagy is the conversion of the soluble form of microtubule-associated protein light chain 3-I (LC3-I) to lipid-soluble LC3-II, which is involved in the formation of autophagosomes, thereby inducing autophagy (Wu et al., 2015). *In vitro* experiments have shown that CPT and DHT induce autophagy by increasing the accumulation of LC3-II in CRC, which induces tumor cell death (Hu et al., 2015).

In addition, the coordinated action of the unc-51-like kinase 1 (ULK1) complex and Beclin 1 functions in driving autophagy

(Russell et al., 2013). Beclin 1 is one of the key regulators of autophagy, which participates in the initiation of autophagosome formation, thereby promoting autophagy (Funderburk et al., 2010). For example, LUT induces autophagy in lung cancer cells, HCC cells, and skin squamous cell carcinoma cells by upregulating Beclin 1 expression, subsequently inhibiting tumor cell growth (Verschooten et al., 2012; Park et al., 2013; Potočnjak et al., 2020). Moreover, the ULK1/2 complex is activated in the early stage of autophagy and cooperates with Beclin 1 to initiate autophagosome formation and induce autophagy (Parzych and Klionsky 2014). Furthermore, tan I activates the ULK1 complex and upregulates the expression level of Beclin 1, which subsequently induces autophagy and prevents the growth of breast and liver cancer cells (Zheng et al., 2020).

Mechanistic target of rapamycin (mTOR) is a S/T kinase, which exists in two distinct complexes called mTOR complex 1 (mTORC1) and 2 (mTORC2), and mTORC1 is a key regulator of autophagy (Hua et al., 2019). The activity of mTORC1 reflects the nutritional status of the cell (Kim et al., 2011). Under nutrient-rich conditions, mTORC1 inhibits the autophagy-promoting kinase activity of the ULK1 complex by mediating specific site phosphorylation of ULK1 (Ser637 and Ser757) (Karabiyik et al., 2021). During periods of starvation and cellular stress, mTORC1 activity is inhibited and dissociates from ULK1, resulting in dephosphorylation of ULK1 (Zachari and Ganley 2017). At the same time, the ULK1 complex becomes active through autophosphorylation at Thr180, initiating autophagy (Zachari and Ganley 2017). Studies have shown that tan I inhibits the activity of mTORC1 under starvation conditions, subsequently increasing the activity of the ULK1 complex and promoting the occurrence of autophagy in breast and liver cancer cells (Zheng et al., 2020).

The mTOR pathway is regulated by multiple signaling pathways in autophagy, such as the PI3K/Akt, MAPK/MEK/ERK1/2, and AMPK pathways (Querfurth and Lee 2021). The PI3K/Akt and MAPK/MEK/ERK1/2 signaling pathways have a positive regulatory effect on mTOR, and blocking the above pathways causes autophagy, eventually leading to tumor cell death (Xu et al., 2020a). It has been reported that tan I and tan IIA induce autophagy by inhibiting the PI3K/Akt/mTOR pathway, further inhibiting the proliferation of tumor cells (including ovarian cancer cells, glioma cells, acute promyelocytic leukemia NB4 cells, acute mononuclear leukemia cells, oral squamous cell carcinoma (OSCC) cells, and melanoma cells) (Ding et al., 2017; Li et al., 2017; Qiu et al., 2018; Zhang et al., 2019c; Zhou et al., 2020b; Pan et al., 2021). In addition, tan IIA also drives autophagy through inhibiting the MEK/ERK/mTOR signaling pathway, thereby suppressing the proliferation of colon cancer cells (Qian et al., 2020).

In contrast, the AMPK pathway negatively regulates mTOR. Phosphorylated AMPK inhibits the activity of mTORC1, which indirectly increases the activity of the ULK1 complex, thereby promoting the occurrence of autophagy (Li and Chen 2019). Tan IIA activates AMPK phosphorylation to inhibit mTOR phosphorylation, thereby inducing autophagic death of leukemia cells (Yun et al., 2014). Moreover, under starvation conditions, AMPK activation directly catalyzes the phosphorylation of ULK1 to promote autophagy (Karabiyik et al., 2021). According to a previous study, tan I activates the AMPK signaling pathway to active the ULK1 complex, which subsequently induces autophagy and prevents the growth of breast and liver cancer cells (Zheng et al., 2020).

In summary, tan IIA inhibits the mTOR pathway through different mechanisms and increases the expression of autophagy-related proteins, such as LC3-II and Beclin 1, thereby inducing autophagy in tumor cells (Figure 3).

Targeting microRNAs (miRNAs)

MiRNAs are a class of non-coding small RNAs with a length of 21–25 nucleotides, and they are widely present in animal and plant cells (Mohr and Mott 2015). MiRNAs bind to the 3′-untranslated region (UTR) of messenger RNA (mRNA) to inhibit mRNA translation or induce mRNA degradation, thereby silencing gene expression at the post-transcriptional level (Lee and Dutta 2009; Wu et al., 2021). In malignant tumors, aberrant amplification, deletion, mutation, and epigenetic silencing of miRNAs have been identified to exert oncogenic or tumor-suppressive effects depending on the target (Rupaimoole and Slack 2017). As an epigenetic modification, miRNAs up- or downregulate the expression of downstream genes through targeting the promoter region of the target gene, thereby mediating the progression of indicated tumors (Ali Syeda et al., 2020). Thus, miRNAs may be used as diagnostic, prognostic, and predictive biomarkers of tumors, and targeting microRNAs may be a potential therapeutic approach for cancers (Iorio and Croce 2012). Studies have demonstrated that *S. miltiorrhiza* bioactive compounds mediate tumorigenesis by regulating the biological behaviors of miRNAs in cell proliferation, apoptosis, invasion, and metastasis.

Targeting miRNAs to inhibit proliferation

It has been reported that miRNAs target proliferation-related genes and proteins, such as PKM, Akt, and MEF2D, to regulate tumor cell proliferation (Xu et al., 2020). Tan IIA suppresses the proliferation of AML cells (HL-60 and THP-1), glioma cells, esophageal cancer cells (EC109), breast cancer cells, and NSCLC cells through targeting miR-497-5p/Akt3, miR-122/PKM2, miR-16-5p/TLN1, miR-125b/STAR13, and let-7a-5p/aurora kinase A (AURKA), respectively (Zhang et al., 2016; Liu et al., 2020a; Liu et al., 2020; Nie et al., 2020; You et al., 2020; Li et al., 2022). Moreover, tan I, CPT, and tan IIA share common targets,

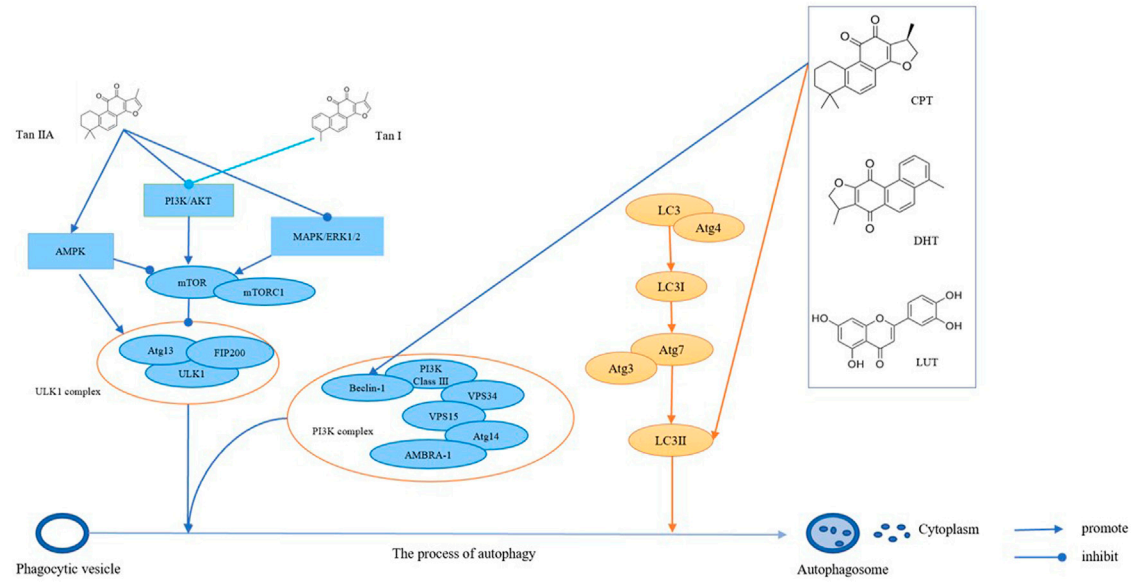


FIGURE 3
The bioactive compounds of *S. miltiorrhiza* promote autophagy in cancers. Tan IIA and Tan I regulate AMPK signaling, PI3K/AkT signaling, and MAPK/ERK1/2 signaling, thereby regulating mTOR and ULK1 complexes, which promotes the formation of autophagosomes. CPT, DHT, and LUT regulate the PI3K complex and LC3, which in turn promotes autophagosome formation.

including miR-137 and let-7a-5p, which function in inhibiting the proliferation of NSCLC by regulating the expression of ULK2/IBTK and AURKA, respectively; these common targets may be a result of highly similar chemical structures (Zhang et al., 2016; Liu et al., 2020a). It has been reported that tan I and CPT show inhibitory effects in NSCLC through targeting AURKA mediated by miR-32 (Ma et al., 2015). In addition, studies have reported that CPT inhibits the proliferation of NSCLC cells through targeting miR-146a-5p/EGFR (Qi et al., 2019). Furthermore, UA downregulates miR-499a-5/secreted frizzled related protein 4 (sFRP4) and miR-149-5p/myeloid differentiation primary response 88 (MyD88), thereby inhibiting the proliferation of NSCLC cells (Chen et al., 2020; Mandal et al., 2021). Another active ingredient of salvia, OA, suppresses the proliferation of A549 and GC cells through increasing the expression of miR-122/CCNG1/MEF2D and miR-98-5p/IL-6, respectively (Zhao et al., 2015; Xu et al., 2021). Additionally, studies have shown that LUT regulates the levels of miR-203/Ras/Raf/MEK/ERK, miR-8080/AR-V7 and miR-6809-5p/flotillin 1, which in turn inhibits the proliferation of breast cancer, castration-resistant prostate cancer (CRPC), and HCC, respectively (Gao et al., 2019; Yang et al., 2019; Naiki-Ito et al., 2020).

Researchers have also indicated that abnormal miRNAs promote tumor cell proliferation by regulating the expression of cell cycle-related proteins (Mens and Ghanbari 2018). Studies have shown that UA inhibits the proliferation of BGC-823 cells by upregulating miR-133a and inhibiting Akt1 expression, thereby promoting cell cycle arrest in the G phase (Xiang et al., 2014). Zhang et al. (2016b) reported that tan IIA induces the upregulation of miR-122 expression and inhibits the expression of pyruvate kinase M2 (PKM2) in human EC109 cells, which induces human EC109 tumor cells to arrest in S phase, thereby exerting an anticancer effect (Zhang et al., 2016).

Targeting microRNAs to promote apoptosis

Specific miRNAs directly or indirectly participate in the regulation of multiple apoptotic pathways by regulating the level of downstream genes, and they can play an oncogenic or tumor suppressor role (Bejarano et al., 2021). Tan IIA targets miR-125b/gasdermin D (GSDMD) and miR-145/caspase 1, which induces apoptosis of nasopharyngeal carcinoma (NPC) cells (HK1 cells) and cervical carcinoma cells (HeLa cells), respectively (Tong et al., 2020; Wang et al., 2021c). In addition, tan IIA targets miR30b, further upregulating p53 levels, which induces apoptosis and death of HCC cells (Ren et al., 2017). Studies have found that tan I and UA target miR135a-3p/DR5 (prostate cancer), miR-21 (glioma), and let7b (malignant mesothelioma), which mediates the activation of caspase 3, thereby inducing tumor cell apoptosis and proliferation (Wang et al., 2012; Shin et al., 2014; Sohn et al., 2016). It has been reported that UA increases the level of miR-

TABLE 1 Regulation of microRNAs by *S. miltiorrhiza* bioactive compounds in cancer progression inhibition.

Bioactive compounds of *S. miltiorrhiza*	Physiological effects	Cancer types	miRNAs	MiRNA targets	References
Regulation of microRNA by active ingredients of tanshinones					
Tan IIA	Inhibit proliferation	Esophageal cancer cells (EC109)	miR-122↑	PKM2↓	Zhang et al. (2016b)
		AML cells (HL-60 and THP-1)	miR-497-5p↑	AKT3↓	Nie et al. (2020)
		Glioma cells	miR-16-5p↑	TLN1↓	You et al. (2020)
Tan IIA	Inhibit proliferation	Breast cancer cells	miR-125b↓	STARD13↓	Li et al. (2022a)
		NSCLC	miR-137↑	ULK2 and IBTK↓	Zhang et al. (2016a), Liu et al. (2020)
		NSCLC	Let-7a-5p↑	AURKA↓	Zhang et al. (2016a), Liu et al. (2020)
	Induce apoptosis	Nasopharyngeal carcinoma (NPC)	miR-125b↓	caspase 1 ↑	Wang et al. (2021c)
		HepG2 cells	miR30b↓	p53↑	Ren et al. (2017)
		HeLa cells	miR-145↑	GSDMD↑	Tong et al. (2020)
Tan I	Inhibit proliferation	NSCLC	miR-137↑	ULK2 and IBTK↓	Zhang et al. (2016a), Liu et al. (2020)
		NSCLC	Let-7a-5p↑	AURKA↓	Zhang et al. (2016a), Liu et al. (2020)
Tan I	Inhibit proliferation	NSCLC	miR-32↑	AURKA↓	Ma et al. (2015)
	Induce apoptosis	Prostate cancer cells	miR135a-3p↑	DR5↑	Shin et al. (2014)
CPT	Inhibit proliferation	NSCLC	miR-137↑	AURKA↓	Zhang et al. (2016a), Liu et al. (2020)
		NSCLC	Let-7a-5p↑	AURKA↓	Zhang et al. (2016a), Liu et al. (2020)
		NSCLC	miR-32↑	AURKA↓	Ma et al. (2015)
		NSCLC	miR-146a-5p↑	EGFR↓	Qi et al. (2019)
Regulation of microRNA by active ingredients of triterpenoids					
OA	Inhibit proliferation	GC	miR-98-5p↑	IL-6↓	Xu et al. (2021)
		A549	miR-122↑	CCNG1 and MEF2D↓	Zhao et al. (2015)
UA	Inhibit proliferation	NSCLC	miR-499a-5↓	sFRP4↓	Mandal et al. (2021)
		NSCLC	miR-149-5p↓	MyD88↓	Chen et al. (2020)
		BGC-823 cells	miR-133a↑	Akt1↓	Xiang et al. (2014)
	Induce apoptosis	glioma cells	miR-21↓	caspase-3↑	Wang et al. (2012)
		CRC cells	miR-4500↑	STAT3↓	Kim et al. (2018)
		Malignant mesothelioma	let7b↑	caspase 3↑	Sohn et al. (2016)
	Inhibit invasion and metastasis	Malignant mesothelioma	let7b↑	AKT, β-catenin and Twist ↓	Sohn et al. (2016)
Regulation of microRNA by active ingredients of flavonoids					
LUT	Inhibit proliferation	Breast cancer	miR-203↑	Ras/Raf/MEK/ERK↓	Gao et al. (2019)
		Castration-resistant prostate cancer (CRPC)	miR-8080↑	AR-V7↓	Naiki-Ito et al. (2020)
LUT	Inhibit proliferation	Hepatocellular carcinoma (HCC)	miR-6809-5p↓	flotillin 1↓	Yang et al. (2019)
	Inhibit invasion and metastasis	Breast cancer	miR-203↑	Ras/Raf/MEK/ERK↓	Gao et al. (2019)
		CRC cells	miR-384↑	PTN↓	Yao et al. (2019a)
		Osteosarcoma (OS) cell	miR-384↑	PTN↓	Qin et al. (2022)
		NSCLC	miR-133a-3p↑	PURB↓	Pan et al. (2022)

4500, which suppresses the expression of p-STAT3 and cleaved PARP, thereby inducing CRC cell apoptosis and inhibiting CRC growth (Kim et al., 2018).

Targeting microRNAs to inhibit migration and invasion

Studies have found that miRNAs play an important role in tumor invasion and metastasis by affecting indicated targets and pathways (Wang and Chen 2019). Numerous studies have demonstrated that *S. miltiorrhiza* bioactive compounds regulate the levels of miRNAs, thereby inhibiting metastasis and invasion of tumor cells. LUT upregulates miR-203/Ras/Raf/MEK/ERK, which upregulates E-cadherin and downregulates N-cadherin, thereby inhibiting the progression of EMT and preventing tumor cell invasion and metastasis of breast cancer (Gao et al., 2019). Furthermore, LUT upregulates miR-384 and subsequently downregulates the expression of multivitamin trophic factor (PTN), which inhibits CRC and osteosarcoma (OS) cell migration and invasion (Yao et al., 2019; Qin et al., 2022). In addition, LUT inhibits migration- and invasion-related factors, including VEGF, MMP2, and MMP9, by upregulating miR-133a-3p levels, thereby suppressing invasion and metastasis of NSCLC (Pan et al., 2022). In addition, UA targets let 7b, which inhibits the expression of p-Akt, β-catenin, and Twist, thereby repressing the EMT process (Sohn et al., 2016).

In summary, *S. miltiorrhiza* bioactive compounds inhibit tumor cell proliferation, migration, and invasion as well as induce apoptosis by regulating the expression of miRNAs, thereby exerting antitumor effects (Table 1).

Regulation of epigenetic enzymes

Epigenetic mechanisms refer to heritable changes in gene expression without altering the DNA sequence, and they include DNA methylation, chromatin remodeling, histone modifications, and non-coding RNAs (Kanwal et al., 2015; Huang et al., 2021b). Abnormal epigenetic alterations are often involved in tumor initiation, progression, and metastasis (Esteller 2008). *S. miltiorrhiza* bioactive compounds have been reported to inhibit tumorigenesis and progression by modulating indicated epigenetic mechanisms, including DNA methylation, histone acetylation, ubiquitination, and DNA topoisomerase (Tian et al., 2018; Bhattacharjee and Dashwood 2020). For example, DHT promotes Keap1-mediated degradation of nuclear factor erythroid 2-related factor 2 (Nrf2) ubiquitination, and it increases the accumulation of ROS, which in turn downregulates Bcl-2, cyclin B1, and Cdc2 levels as well as upregulates Bax levels in ovarian cancer cells *in vivo* and *in vitro* (Sun et al., 2022).

DNA methylation

In normal cells, DNA methylation occurs mainly in repetitive genomic regions and is responsible for silencing gene expression and maintaining genome stability (Sandoval et al., 2011). However, special DNA regions, such as promoters, especially CpG islands (CpGs), are often unmethylated (Bird 1986). Hypomethylation in tumor cells is mainly due to methylation deficiency in repetitive genomic regions, leading to genomic instability and activation of transposon promoters, whereas hypermethylation is the ab initio methylation of CpGs, which can lead to transcriptional silencing of growth regulatory genes (Lakshminarasimhan and Liang 2016). Cancer cells display genome-wide hypomethylation and site-specific CpG promoter hypermethylation (Rodríguez-Paredes and Esteller 2011). Abnormal increased DNA methylation leads to transcriptional repression and decreased gene expression (Pan et al., 2018). Studies have reported that *S. miltiorrhiza* bioactive compounds regulate the level of methylation in tumor cells and inhibit tumor development (Kim et al., 2016; Li et al., 2022). For example, tan IIA, LUT, and UA target the abnormal hypermethylation at the CpG methylation region in the Nrf2 promoter by reducing the expression of DNA methyltransferases (DNMTs), including DNMT1, DNMT3a, and DNMT3b, thereby inhibiting cell proliferation of skin and colon cancers (Wang et al., 2014; Kim et al., 2016; Zuo et al., 2018). Moreover, it has been reported that the inhibition of NRF2 promoter methylation is mediated by tan IIA *via* ten-eleven translocation (TET)-2 activation (Yang et al., 2020). In addition, OA decreases the promoter DNA demethylation of programmed death-ligand 1 (PD-L1) by inhibiting the expression of TET-3, which downregulates PD-L1, ultimately enhancing T cell-mediated killing of MKN-45 cells (Lu et al., 2021). Moreover, it has been reported that Sal B induces DNMT1-mediated demethylation of the PTCH1 gene, which in turn inhibits hepatic stellate cell (HSC) EMT *in vivo* and *in vitro* (Yu et al., 2015).

Histone methylation

Histone methylation is a major player in the regulation of gene expression and genetic stability, and dysfunction of histone methylation contributes to the development of various cancers (Rotili and Mai 2011). Methylation occurs primarily on lysine or arginine residues of histones H3 and H4, which is controlled by histone methyltransferases (HMTs) and histone demethylases (HDMs) (Janardhan et al., 2018). Polycomb repressive complex 2 (PRC2) is a complex that mediates gene silencing by regulating chromatin structure (Chan and Morey 2019). Enhancer of zeste homolog 2 (EZH2), a polycomb group (PcG) protein, is an enzymatic catalytic subunit of PRC2 that regulates gene expression through catalyzing primarily tri-methylation of

histone H3 Lys-27 (H3K27), and it functions in the development and progression of a variety of cancers, such as breast cancer (Bachmann et al., 2006), GC (Gan et al., 2018), and NPC (Duan et al., 2020). It has been reported that tanshindiol C inhibits EZH2 activity, which in turn downregulates H3K27me3 levels, thereby inhibiting the proliferation of the Pfeiffer cell line, a type of B cell lymphoma (Woo et al., 2014). Furthermore, tan I directly binds to EZH2, which inhibits PRC2 activity, resulting in a decrease in tri-methylated modification of H3K27, and this decrease, in turn, upregulates MMP9 and ABCG2, ultimately inhibiting the proliferation of human leukemia cells *in vitro* and *in vitro* (Huang et al., 2021). Moreover, tan I reduces the expression of CCAAT-enhancer-binding protein β (C/EBPβ), which inhibits the mRNA levels of the JMJD2b histone H3K9 demethylase as well as cell cycle-related genes, further regulating the mitotic clonal expansion (MCE) process (Jung et al., 2020). Lysine specific demethylase 1 (LSD1) is a flavin adenine dinucleotide (FAD)-dependent amine oxidase (AO) that catalyzes the demethylation of mono- and di-methyl on H3K4 and H3K9, triggering transcriptional repression and activation, respectively (Karakaidos et al., 2019). Studies have demonstrated that CPT blocks the interaction of LSD1 and androgen receptor (AR) at the promoter of the AR target gene, prostate-specific antigen (PSA), which demethylates H3K9me1/2, thereby inhibiting the transcriptional activity of PSA and suppressing the proliferation of AR-positive prostate cancer cells (Wu et al., 2012). Moreover, tan IIA upregulates the level of H3K27me1 and H3K4m2 on the *TP53* promoter by inhibiting LSD1, thereby activating the transcriptional activity of p53 (Zheng et al., 2018). It has been reported that the mRNA and protein levels of p53 are downregulated in several types of tumors, such as prostate cancer and colon cancer (Bossi and Sacchi 2007). Therefore, reactivating p53 is an important strategy for tumor therapy. Taken together, these findings suggest that tan IIA may increase the level of H3K27me1 and H3K4m2 on the p53 promoter by targeting LSD1, which activates p53, thereby promoting tumor cell cycle arrest and apoptosis.

Histone acetylation

Histone acetylation is a reversible and homeostatic process regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) (Attoub et al., 2011). Studies have reported abnormal acetylation levels in certain tumors (Zhao and Shilatifard 2019). Histone lysine residues are hyperacetylated in drug-resistant prostate cancer, acute myeloid leukemia, gastrointestinal stromal tumors, lymphomas, and TNBC (He et al., 2021), whereas acetylation levels are decreased in ovarian cancer, colon cancer, liver cancer, and skin cancer (Wu et al., 2020). Overexpression of HDAC is present in tumors with low acetylation levels, indicating that targeting HDAC may be a strategy for treatment of these tumors (Li and Seto 2016). To date, numerous HDAC inhibitors are in various stages of clinical research; vorinostat (SAHA), beristatin (PXD101), and bilestat (LBH589) are approved for the clinical treatment of cutaneous T cell lymphoma, peripheral T cell lymphoma and multiple myeloma, respectively (Ho et al., 2020), and other HDAC inhibitors have been investigated in clinical trials for the treatment of various cancers (Ono et al., 2018; Xie et al., 2018). *S. miltiorrhiza* bioactive compounds have been found to target histone deacetyltransferases (HDACs) and identified as potential inhibitors of HDACs. Tan IIA suppresses the expression and enzymatic activity of HDAC1, HDAC3, HDAC4, and HDAC8, which increases histone 3 acetylation and RNA polymerase II recruitment to the Nrf2 transcription start site, thereby inhibiting TPA-induced JB6 P+ cell neoplastic transformation (Wang et al., 2014). In contrast, LUT increases the enrichment of H3K27ac in the promoter region of Nrf2 by downregulating HDAC1, HDAC2, HDAC3, HDAC6, and HDAC7 expression and enzymatic activity, further promoting the transcriptional activity of the Nrf2 gene, which in turn inhibits the cellular activity of HCT116 (Zuo et al., 2018).

The level of acetylation is closely related to tumor stage, metastasis, and invasion as well as prognosis (He et al., 2021). Aurora A is a mitotic S/T kinase involved in the process of cell division, and the expression of aurora A is elevated in various tumors (Meraldi et al., 2004). Tan I reduces the level of histone acetylation at the aurora A DNA promoter region, which downregulates aurora A and ultimately inhibits the growth of breast cancer cells (Gong et al., 2012). Furthermore, LUT reduces the level of H3K27ac and H3K56ac at the MMP9 promoter region by inactivating the Akt/mTOR signaling pathway, thereby inhibiting metastasis of AR-positive TNBC (Wu et al., 2021).

Ubiquitination

Ubiquitination is an important post-translational modification mediated by ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3) (Cockram et al., 2021). Abnormal ubiquitination alters intracellular physiological functions, leading to the initiation and development of cancer (Pellegrino et al., 2022). For example, overexpression of the E3 ligase, mouse double minute 2 (MDM2), mediates ubiquitination of p53 and promotes proteasomal degradation of p53, which inhibits p53 function, leading to tumorigenesis and cancer cell progression (Wang et al., 2017). It has been reported that tan IIA induces ubiquitous degradation of the anti-apoptotic protein, Mcl-1, through targeting the EGFR-Akt signaling pathway, which induces NSCC cell apoptosis (Gao et al., 2020). Tan IIA promotes the FBW7 E3 ligase-mediated ubiquitination degradation of c-Myc, which induces OSCC cell apoptosis (Chen et al., 2020a; Li et al., 2020). Moreover, tan I and DHT activate the Nrf2 pathway by reducing Nrf2 ubiquitination,

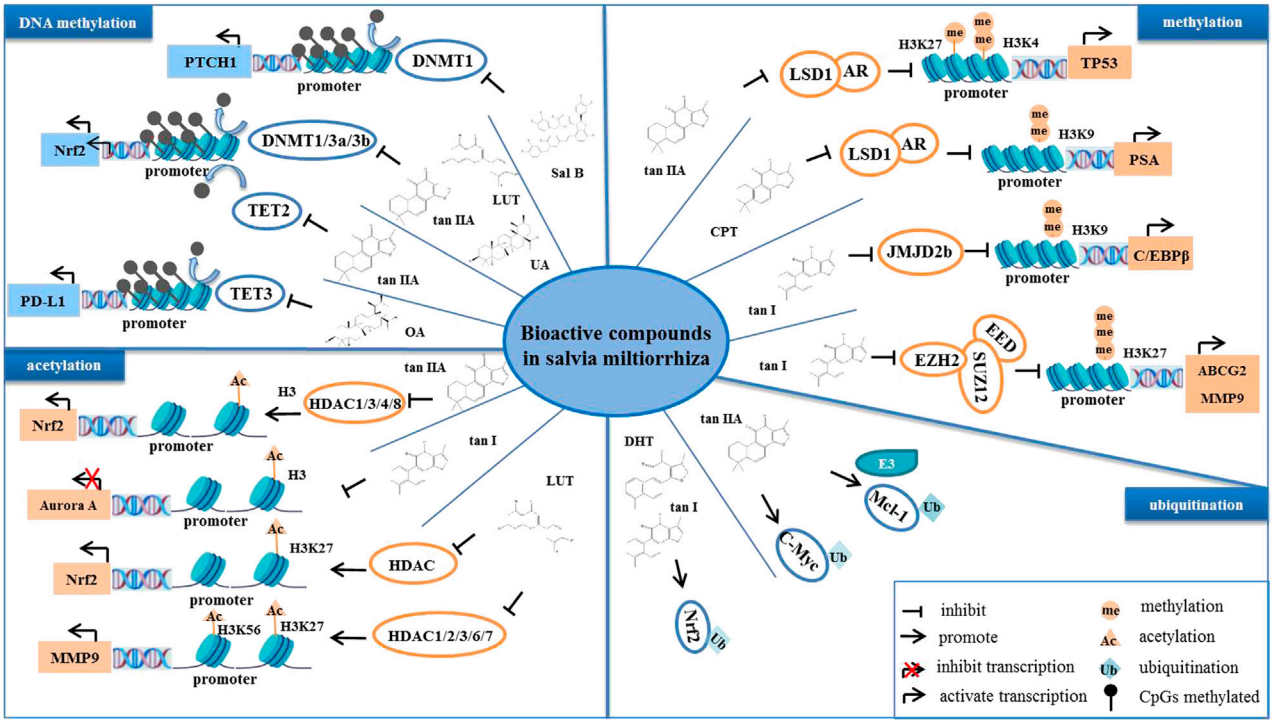


FIGURE 4
The bioactive compounds of *S. miltiorrhiza* regulate epigenetic modifications. *S. miltiorrhiza* bioactive compounds suppress the transcriptional activity and enzymatic activity of their target genes by regulating epigenetic modification-related enzymes.

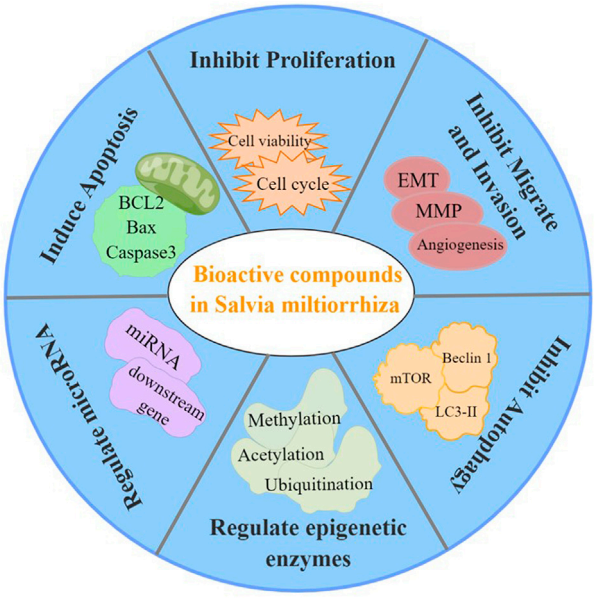


FIGURE 5
Pharmacological activities of *S. miltiorrhiza* bioactive compounds resulting in antitumor effects.

thereby maintaining redox homeostasis of skin fibroblasts and HBE cells (Tao et al., 2013a; Tao et al., 2013).

In summary, *S. miltiorrhiza* bioactive compounds suppress tumor growth and development by regulating DNA methylation, histone methylation, acetylation, and ubiquitination to silence tumor suppressors or promote oncogenes (Figure 4).

Future prospects

Currently, chemotherapy and radiotherapy are the main methods for the clinical treatment of tumors, but these methods have serious shortcomings, such as resistance and toxic side effects. Thus, combination therapy may be important to improve tumor sensitivity. We previously demonstrated that the bioactive compounds of *S. miltiorrhiza* inhibit cancer cell proliferation, induce apoptosis, suppress metastasis, and suppress invasion. Thus, we speculate that *S. miltiorrhiza* bioactive compounds may be used as adjuvant drugs to improve the sensitivity of drug-resistant tumors.

At present, drug resistance is one of the major barriers to cancer treatment, which limits the efficacy of chemotherapy drugs, leading to tumor recurrence, metastasis, and cell death

TABLE 2 Effect of *S. miltiorrhiza* bioactive compounds combined with chemotherapy and radiotherapy.

Chemical composition	Combination drugs	Mechanism	Resistant cells	Physiological effect	References
Bioactive compounds of S. miltiorrhiza improves sensitivity of drug-resistant tumors					
Tan IIA	Oxaliplatin	Regulate Akt/ERK pathway, decrease the levels of Bcl-2, p-Akt and p-ERK, and increase the levels of Bax and active caspase 3	Oxaliplatin-resistant colon cancer cell lines (SW480/OXA and HT29/OXA)	Anti-proliferation	Zhang et al. (2019b)
Tan IIA	Doxorubicin	Inhibit β-catenin nuclear translocation, decrease the expressions of c-Myc, E-cadherin, MMP-2 and MMP-9	Doxorubicin-resistant breast cancer cell (MCF-7/dox)	Suppress proliferation and migration	Li et al. (2021a)
	Doxorubicin	Downregulate MRP1expression, arrest cell cycle at G2/M phase, down-regulate BCL2 and up-regulate BAX and p53	Doxorubicin-resistant gastric cancer cell lines (SNU-719R and SNU-620)	Arrest cell cycle induce apoptosis	Xu et al. (2018)
	Doxorubicin	Downregulate the expression of P-gp, BCRP and MRP1 Upregulate PTEN, activate AKT	Doxorubicin-resistant breast cancer cell (MCF7/dox)	Inhibit proliferation, induce apoptosis	Li and Lai (2017), Li et al. (2019a)
	Gefitinib	Regulate VEGFR2/Akt pathway	Gefitinib-resistant cell lines (HCC827/gefitinib and PC-9/gefitinib)	Suppress proliferation, migration and invasion	Wang et al. (2019a)
	Taxol	Reduce the expression of microtubule-associated protein (Tau)	Taxol tolerant MCF-7 cells (MCF/Taxol)	Inhibit cell viability	Lin et al. (2018)
CPT	Doxorubicin and irinotecan	Reduce the level of P-gp mRNA and protein, inhibit P-gp ATPase activity	Doxorubicin and irinotecan- resistant cells (SW620 Ad300)	Inhibit cell viability	Hu et al. (2014)
DHT	Doxorubicin and irinotecan	Reduce the level of P-gp mRNA and protein, inhibit P-gp ATPase activity	Doxorubicin and irinotecan- resistant cells (SW620 Ad300)	Inhibit cell viability	Hu et al. (2014)
DHT I	Caclitaxel	Decrease ABCB1 and NF-κB expression, reduce NF-κB activity	Paclitaxel-resistant anaplastic thyroid cancer cells (SW1736 and 8505C)	Inhibit proliferation	Allegri et al. (2021)
LUT	Cisplatin (DDP)	Downregulate BCL2 expression	Cisplatin-resistant ovarian cancer cells (CAOV3/DDP)	Suppress proliferation, migration and invasion, reduce apoptosis	Wang et al. (2018)
Synergistic therapy with bioactive compounds of S. miltiorrhiza and chemotherapeutic drugs					
Tan IIA	5-FU	Repress the activation of NF-κB	HCT1116 and COLO205 cells	Inhibit cell proliferation	Bai et al. (2016)
	Adriamycin	Decrease the activity of VEGF/PI3K/Akt signaling pathway	A549 and PC9 cells	Suppress proliferation, metastasis and invasion, promote apoptosis	Xie et al. (2016)
Tan IIA	5-FU	Downregulate P-gp, LC3-II, VEGF, MMP-7 and NF-κB p65 protein expression	Colo205 cells	Repress tumor growth	Su (2012)
	Paclitaxel	Upregulate the expression of γ-H2AX, p21, p16 and Bax, downregulate the expression of BCL2	Ovarian cancer cells (A2780 and ID-8)	Inhibits proliferation and metastasis	Zhou et al. (2020a)
	Nutlin-3	Regulate MDM2-P53 and AKT/mTOR pathway, induce cell cycle arrest at S and G2 phase	ALL cell lines (SUP-B15, NALM-6, HL-60 and MV4-11)	Suppress cell viability reduce apoptosis and autophagy	Guo et al. (2019)
	Doxorubicin	Increase LDH leakage, and the level of ROS and NADPH oxidase 4 activity, meantime decrease the expression of SOD1 Upregulate the expression of caspase3	HepG2 cells	Repress cell viability	Kan et al. (2014)
	Nutlin-3 and imatinib	Inhibit AKT/mTOR pathway and reactivation of p53 pathway arrest cell cycle at S phase	ALL cell lines	Inhibit cell viability	Guo et al. (2017)
Tan IIA	Cisplatin		FaDu cells	Suppress proliferation	

(Continued on following page)

TABLE 2 (*Continued*) Effect of *S. miltiorrhiza* bioactive compounds combined with chemotherapy and radiotherapy.

Chemical composition	Combination drugs	Mechanism	Resistant cells	Physiological effect	References
		Increase the expression of cleaved caspase 3 and cleaved PARP, decrease the expression of survivin			Zhao et al. (2017a)
	As2O3	Arrest cell cycle at G0/G1 phase, reduces the expression of Pgp	NB4 cells, MR2 cells	Inhibit proliferation	Li et al. (2009), Zhang et al. (2010)
DHT	Temozolomide	Reduce NF-kB activity Arrest cell cycle at G0/G1 phase	Glioblastoma cells (U87-MG (MGMT-) and T98G (MGMT+))	Educe proliferation	Kumar et al. (2019)
Tanshinone	Carboplatin	Increase the percentage of CD4þ and CD8þ subsets and the activity of NK and CTL	B16 cells	Inhibit tumor growth	Wu et al. (2016)
LUT	Indole-3-Carbinol	Regulate SIRT1/ERα pathway Arrest cell cycle at G1phase	ER^+ breast cancer MCF7 and T47D cells	Repress proliferation	Wang et al. (2021b)
	Paclitaxel	Inhibit the expression of Nrf2, HO-1, Sirt3 and Cripto-1	MDA-MB-231 cells	Inhibit cell viability	Tsai et al. (2021)
	Vaccinia virus (VV) that harbors IL-24 (VV-IL-24)	Upregulate IL-24 gene expression	Liver cancer cell lines (MHCC97-H, HepG2, PLC/PRF/5, Hep3B and HEK293)	Inhibit the development of tumor induce apoptosis	Wang et al. (2021)
LUT	Lapatinib	Upregulate the expression of FOXO3a and NQO1 and their downstream target genes Bim, GADD45, P21, and decrease the phosphorylation level of FOXO3a protein	Breast cancer cell lines (SKBR-3、ZR-75-1 and BT-474)	Suppress tumor growth, migration and invasion	Zhang et al. (2020)
	Myo-inositol (MI)	Decrease the expression of p-PDK1, p-Akt Increase cyclin D1 levels, and arrest cell cycle at G0/G1 phase	A549	Inhibit proliferation and migration	Wang et al. (2018b)
	OXA	Upregulate the expression levels of Cyt c, cleaved caspase-3 and Bax, downregulate Bcl-2 and pro-caspase-3	SGC-7901 cells	Inhibit proliferation Induce apoptosis	Ren et al. (2020)
Bioactive compounds of S. miltiorrhiza improves radiosensitivity					
Tan IIA	Ray(4Gy)	Reduce histone H3 (S10) phosphorylation, induce DNA damage	Ionizing-radiation-acquired resistance cell lines (CAL27-IR and SCC25-IR)	Promote apoptosis, inhibit tumor growth	Li et al. (2021)
	Ray(2Gy)	Increase ROS production, upregulate the protein levels of Beclin 1, Atg5 and LC3-II	Oral squamous cell carcinoma SCC090 cells	Promote autophagy	Ding et al. (2016)
Tan I	Ray(6Gy)	Own-regulate the expression of PPAT	Radiation-resistant lung cancer cells (H358-IR and H157-IR)	Inhibit cell proliferation	Yan et al. (2018)

resistance (Haider et al., 2020). Doxorubicin (Dox) and paclitaxel are first-line treatments for patients with metastatic and early-stage breast cancer (Abu Samaan, Samec et al., 2019; Jamialahmadi et al., 2021). However, it has been reported that patients develop resistance to these drugs during the treatment of breast cancer, greatly affecting the efficacy of the drugs (Rivera and Gomez 2010). Moreover, platinum drugs, such as cisplatin and oxaliplatin (OXA), induce DNA damage in tumor cells; they are mainly used in the treatment of ovarian cancer, but their efficacy is severely limited due to drug resistance (Pujade-Lauraine et al., 2019). Studies have found that the bioactive compounds of *S. miltiorrhiza* have potential roles in reversing resistance and enhancing chemotherapeutic drug sensitivity. For example, tan IIA increases the sensitivity of OXA-resistant CRC cells to OXA by regulating the Akt/ERK signaling pathway, thereby enhancing the anti-proliferation effect and inducing apoptosis in SW480/OXA and HT29/OXA cells (Zhang et al., 2019b). Furthermore, CPT/DHT reduces the P-glycoprotein (P-gp) mRNA and protein levels as well as inhibits P-gp ATPase activity of SW620 Ad300 cells (Hu et al., 2014).

Moreover, tan IIA reduces the accumulation of β-catenin in the nucleus by repressing β-catenin nuclear translocation, which enhances Dox inhibition of MCF-7/Dox cell proliferation and migration (Li et al., 2021). Adenosine triphosphate (ATP)-binding cassette (ABC) transporters, such as P-gp, breast cancer resistance protein (BCRP), and multidrug resistance-related protein 1 (MRP1), catalyze the transduction of structurally diverse compounds across cell membranes *via* ATP-dependent transport (Zahra et al., 2020). Because ABC transporters are overexpressed in tumor cells, they pump out the chemotherapeutic drugs, thereby inducing tumor cells to acquire drug resistance (Choi and Yu 2014). It has been reported that tan IIA downregulates the expression of P-gp, BCRP, and MRP1, further promoting intracellular accumulation of Dox, which induces apoptosis and suppresses proliferation of MCF7/Dox cells, thereby alleviating the inhibitory effect of Dox on the hematopoietic function and Dox-induced cardiotoxicity and nephrotoxicity (Li et al., 2019).

S. miltiorrhiza bioactive compounds not only increase the sensitivity of drug-resistant cells to chemotherapeutic drugs but also synergistically inhibit tumor development. Bai et al. reported that tan IIA combined with 5-FU represses the activation of NF-κB and inhibits CRC cell proliferation (Bai et al., 2016). Furthermore, tan IIA and Dox cotreatment decreases the activity of the VEGF/PI3K/Akt signaling pathway, which promotes apoptosis and induces cell cycle arrest, thereby suppressing proliferation, metastasis, and invasion of A549 cells (Xie et al., 2016). Moreover, tan I and paclitaxel cooperate to promote apoptosis and accelerate cell senescence, thereby inhibiting proliferation and metastasis of ovarian cancer cells (A2780 and ID-8 cells) (Zhou et al., 2020). In addition, the combination of LUT and OXA induces apoptosis and cell cycle arrest as well as inhibits proliferation of SGC-7901 gastric cancer cells (Ren et al., 2020).

Radiotherapy is an effective method of cancer treatment, but the resistance and side effects of radiation therapy affect its efficacy. Therefore, sensitizers that kill tumor cells improve the efficacy of radiotherapy (Chevalier 2020). Tan IIA combined with irradiation reduces histone H3 (S10) phosphorylation in ionizing radiation-resistant cell lines (CAL27-IR and SCC25-IR cells), and this combination enhances irradiation-induced DNA damage and promotes apoptosis, thereby inhibiting tumor growth *in vivo* and *in vitro* (Li et al., 2021). Moreover, tan IIA increases radiation-induced ROS production and induces autophagy, which in turn increases the sensitivity of OSCC cells (SCC090 cells) to radiation (Ding et al., 2016). Pro-oncogenic protein phosphoribosyl pyrophosphate aminotransferase is an essential enzyme that catalyzes the first step in purine biosynthesis and promotes the increase in purine metabolism in cancer cells, and its overexpression promotes cancer cell proliferation and invasion. Tan I downregulates the expression of PPAT, which in turn enhances the radiosensitivity of radiation-resistant lung cancer cells (H358-IR and H157-IR cells), thereby inhibiting cell proliferation (Yan et al., 2018).

In summary, the bioactive compounds of *S. miltiorrhiza* target miRNAs and epigenetic enzymes as well as function to inhibit cancer cell proliferation, induce cell cycle arrest, induce apoptosis, trigger autophagy, suppress metastasis, and suppress invasion (Figure 5). Moreover, *S. miltiorrhiza* bioactive compounds have multiple roles as adjuvant drugs in chemotherapy and radiotherapy as follows: 1) improve the sensitivity of drug-resistant cells to chemotherapeutic drugs; 2) work together with chemotherapeutic drugs to enhance the therapeutic effect; and 3) combine with radiotherapy to improve the effect of radiotherapy (Table 2). Thus, *S. miltiorrhiza* bioactive compounds have an antitumor effect and potential application prospects in tumor treatment and adjuvant medicine. In addition, one critical mechanism of *S. miltiorrhiza* bioactive compounds in suppressing cancer initiation and progression is through targeting miRNAs, including miR-125b, miRNA-122, and miRNA-384, which are present in various cancer cells, suggesting that these miRNAs may play important roles in cancer progression. The pharmacological activity and mechanism of *S. miltiorrhiza* bioactive compounds are continually being elucidated, thereby supporting their potential application in future clinical cancer therapies.

Author contributions

WG and DW designed the review; XL, ML, XW, XF, YZ, and HL contributed to the writing of the manuscirpt.

Funding

This work was supported by National Natural Science Foundation of China (Grant No. 81903876), Jilin Provincial Department of Education (JJKH20210982KJ), Jilin Province Youth Science and Technology Talent Support Project (2020025).

Acknowledgments

We would like to thank the Charlesworth Author Services (https://www.cwauthors.com.cn) for English language editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Abu Samaan, T. M., Samec, M., Liskova, A., Kubatka, P., and Büsselberg, D. (2019). Paclitaxel's mechanistic and clinical effects on breast cancer. *Biomolecules* 9 (12), E789. doi:10.3390/biom9120789

Ali, A., Zhang, P., Liangfang, Y., Wenshe, S., Wang, H., Lin, X., et al. (2015). KLF17 empowers TGF-β/Smad signaling by targeting Smad3-dependent pathway to suppress tumor growth and metastasis during cancer progression. *Cell Death Dis.* 6 (3), e1681. doi:10.1038/cddis.2015.48

Ali Syeda, Z., Langden, S. S. S., Munkhzul, C., Lee, M., and Song, S. J. (2020). Regulatory mechanism of MicroRNA expression in cancer. *Int. J. Mol. Sci.* 21 (5), E1723. doi:10.3390/ijms21051723

Allegri, L., Capriglione, F., Maggisano, V., Damante, G., and Baldan, F. (2021). Effects of dihydrotanshinone I on proliferation and invasiveness of paclitaxel-resistant anaplastic thyroid cancer cells. *Int. J. Mol. Sci.* 22 (15), 8083. doi:10.3390/ijms22158083

Anson, D. M., Wilcox, R. M., Huseman, E. D., Stump, T. A., Paris, R. L., Darkwah, B. O., et al. (2018). Luteolin decreases epidermal growth factor receptor-mediated cell proliferation and induces apoptosis in glioblastoma cell lines. *Basic Clin. Pharmacol. Toxicol.* 123 (6), 678–686. doi:10.1111/bcpt.13077

Attoub, S., Hassan, A. H., Vanhoecke, B., Iratni, R., Takahashi, T., Gaben, A. M., et al. (2011). Inhibition of cell survival, invasion, tumor growth and histone deacetylase activity by the dietary flavonoid luteolin in human epithelioid cancer cells. *Eur. J. Pharmacol.* 651 (1-3), 18–25. doi:10.1016/j.ejphar.2010.10.063

Bachmann, I. M., Halvorsen, O. J., Collett, K., Stefansson, I. M., Straume, O., Haukaas, S. A., et al. (2006). EZH2 expression is associated with high proliferation rate and aggressive tumor subgroups in cutaneous melanoma and cancers of the endometrium, prostate, and breast. *J. Clin. Oncol.* 24 (2), 268–273. doi:10.1200/JCO.2005.01.5180

Bai, Y., Zhang, L., Fang, X., and Yang, Y. (2016). Tanshinone IIA enhances chemosensitivity of colon cancer cells by suppressing nuclear factor-κB. *Exp. Ther. Med.* 11 (3), 1085–1089. doi:10.3892/etm.2016.2984

Bejarano, F., Chang, C. H., Sun, K., Hagen, J. W., Deng, W. M., and Lai, E. C. (2021). A comprehensive *in vivo* screen for anti-apoptotic miRNAs indicates broad capacities for oncogenic synergy. *Dev. Biol.* 475, 10–20. doi:10.1016/j.ydbio.2021.02.010

Bhattacharjee, S., and Dashwood, R. H. (2020). Epigenetic regulation of NRF2/KEAP1 by phytochemicals. *Antioxidants (Basel)* 9 (9), E865. doi:10.3390/antiox9090865

Bird, A. P. (1986). CpG-rich islands and the function of DNA methylation. *Nature* 321 (6067), 209–213. doi:10.1038/321209a0

Bossi, G., and Sacchi, A. (2007). Restoration of wild-type p53 function in human cancer: Relevance for tumor therapy. *Head. Neck* 29 (3), 272–284. doi:10.1002/hed.20529

Cao, D., Zhu, G. Y., Lu, Y., Yang, A., Chen, D., Huang, H. J., et al. (2020). Luteolin suppresses epithelial-mesenchymal transition and migration of triple-negative breast cancer cells by inhibiting YAP/TAZ activity. *Biomed. Pharmacother.* 129, 110462. doi:10.1016/j.biopha.2020.110462

Cao, Y. F., Wang, S. F., Li, X., Zhang, Y. L., and Qiao, Y. J. (2018). The anticancer mechanism investigation of Tanshinone II(A) by pharmacological clustering in protein network. *BMC Syst. Biol.* 12 (1), 90. doi:10.1186/s12918-018-0606-6

Cao, Y., Lu, K., Xia, Y., Wang, Y., Wang, A., and Zhao, Y. (2022). Danshensu attenuated epithelial-mesenchymal transformation and chemoresistance of colon cancer cells induced by platelets. *Front. Biosci.* 27 (5), 160. doi:10.31083/j.fbl2705160

Chan, H. L., and Morey, L. (2019). Emerging roles for polycomb-group proteins in stem cells and cancer. *Trends biochem. Sci.* 44 (8), 688–700. doi:10.1016/j.tibs.2019.04.005

Chen, J., Ding, C., Chen, Y., Hu, W., Lu, Y., Wu, W., et al. (2020). ACSL4 promotes hepatocellular carcinoma progression via c-Myc stability mediated by ERK/FBW7/c-Myc axis. *Oncogenesis* 9 (4), 42. doi:10.1038/s41389-020-0226-z

Chen, J., Shi, D. Y., Liu, S. L., and Zhong, L. (2012). Tanshinone IIA induces growth inhibition and apoptosis in gastric cancer *in vitro* and *in vivo*. *Oncol. Rep.* 27 (2), 523–528. doi:10.3892/or.2011.1524

Chen, Q., Luo, J., Wu, C., Lu, H., Cai, S., Bao, C., et al. (2020a). The miRNA-149-5p/MyD88 axis is responsible for ursolic acid-mediated attenuation of the stemness and chemoresistance of non-small cell lung cancer cells. *Environ. Toxicol.* 35 (5), 561–569. doi:10.1002/tox.22891

Chen, T., Li, X. F., Wang, J. F., Zhou, S., and Fang, F. (2018). Effects of luteolin on proliferation and programmed cell death of human multiple myeloma cell RPMI-8226. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 26 (5), 1425–1429. doi:10.7534/j.issn.1009-2137.2018.05.028

Chen, X., Guo, J., Bao, J., Lu, J., and Wang, Y. (2014). The anticancer properties of salvia miltiorrhiza bunge (danshen): A systematic review. *Med. Res. Rev.* 34 (4), 768–794. doi:10.1002/med.21304

Chevalier, F. (2020). Counteracting radio-resistance using the optimization of radiotherapy. *Int. J. Mol. Sci.* 21 (5), E1767. doi:10.3390/ijms21051767

Chiu, S. C., Huang, S. Y., Chen, S. P., Su, C. C., Chiu, T. L., and Pang, C. Y. (2013). Tanshinone IIA inhibits human prostate cancer cells growth by induction of endoplasmic reticulum stress *in vitro* and *in vivo*. *Prostate Cancer Prostatic Dis.* 16 (4), 315–322. doi:10.1038/pcan.2013.38

Choi, Y. H., and Yu, A. M. (2014). ABC transporters in multidrug resistance and pharmacokinetics, and strategies for drug development. *Curr. Pharm. Des.* 20 (5), 793–807. doi:10.2174/13816128200514021416521 2

Cockram, P. E., Kist, M., Prakash, S., Chen, S. H., Wertz, I. E., and Vucic, D. (2021). Ubiquitination in the regulation of inflammatory cell death and cancer. *Cell Death Differ.* 28 (2), 591–605. doi:10.1038/s41418-020-00708-5

Ding, L., Ding, L., Wang, S., Wang, S., Wang, W., Wang, W., et al. (2017). Tanshinone IIA affects autophagy and apoptosis of glioma cells by inhibiting phosphatidylinositol 3-kinase/akt/mammalian target of rapamycin signaling pathway. *Pharmacology* 99 (3-4), 188–195. doi:10.1159/000452340

Ding, L., Wang, S., Qu, X., and Wang, J. (2016). Tanshinone IIA sensitizes oral squamous cell carcinoma to radiation due to an enhanced autophagy. *Environ. Toxicol. Pharmacol.* 46, 264–269. doi:10.1016/j.etap.2016.07.021

Duan, R., Du, W., and Guo, W. (2020). EZH2: A novel target for cancer treatment. *J. Hematol. Oncol.* 13 (1), 104. doi:10.1186/s13045-020-00937-8

Edathara, P. M., Chintalapally, S., Makani, V. K. K., Pant, C., Yerramsetty, S., D, R. M., et al. (2021). Inhibitory role of oleanolic acid and esculetin in HeLa cells involve multiple signaling pathways. *Gene* 771, 145370. doi:10.1016/j.gene.2020.145370

Esteller, M. (2008). Epigenetics in cancer. *N. Engl. J. Med.* 358 (11), 1148–1159. doi:10.1056/NEJMra072067

Fang, B., Chen, X., Wu, M., Kong, H., Chu, G., Zhou, Z., et al. (2018). Luteolin inhibits angiogenesis of the M2-like TAMs via the downregulation of hypoxia inducible factor-1α and the STAT3 signalling pathway under hypoxia. *Mol. Med. Rep.* 18 (3), 2914–2922. doi:10.3892/mmr.2018.9250

Fang, Z. Y., Zhang, M., Liu, J. N., Zhao, X., Zhang, Y. Q., and Fang, L. (2020). Tanshinone IIA: A review of its anticancer effects. *Front. Pharmacol.* 11, 611087. doi:10.3389/fphar.2020.611087

Fu, L., Han, B., Zhou, Y., Ren, J., Cao, W., Patel, G., et al. (2020). The anticancer properties of tanshinones and the pharmacological effects of their active ingredients. *Front. Pharmacol.* 11, 193. doi:10.3389/fphar.2020.00193

Fu, L. Q., Du, W. L., Cai, M. H., Yao, J. Y., Zhao, Y. Y., and Mou, X. Z. (2020a). The roles of tumor-associated macrophages in tumor angiogenesis and metastasis. *Cell. Immunol.* 353, 104119. doi:10.1016/j.cellimm.2020.104119

Funderburk, S. F., Wang, Q. J., and Yue, Z. (2010). The Beclin 1-VPS34 complex--at the crossroads of autophagy and beyond. *Trends Cell Biol.* 20 (6), 355–362. doi:10.1016/j.tcb.2010.03.002

Gan, L., Xu, M., Hua, R., Tan, C., Zhang, J., Gong, Y., et al. (2018). The polycomb group protein EZH2 induces epithelial-mesenchymal transition and pluripotent phenotype of gastric cancer cells by binding to PTEN promoter. *J. Hematol. Oncol.* 11 (1), 9. doi:10.1186/s13045-017-0547-3

Gao, F., Li, M., Liu, W., and Li, W. (2020). Inhibition of EGFR signaling and activation of mitochondrial apoptosis contribute to tanshinone IIA-mediated

tumor suppression in non-small cell lung cancer cells. *Onco. Targets. Ther.* 13, 2757–2769. doi:10.2147/OTT.S246606

Gao, G., Ge, R., Li, Y., and Liu, S. (2019). Luteolin exhibits anti-breast cancer property through up-regulating miR-203. *Artif. Cells Nanomed. Biotechnol.* 47 (1), 3265–3271. doi:10.1080/21691401.2019.1646749

Gong, L., Di, C., Xia, X., Wang, J., Chen, G., Shi, J., et al. (2016). AKT/mTOR signaling pathway is involved in salvianolic acid B-induced autophagy and apoptosis in hepatocellular carcinoma cells. *Int. J. Oncol.* 49 (6), 2538–2548. doi:10.3892/ijo.2016.3748

Gong, Y., Li, Y., Abdolmaleky, H. M., Li, L., and Zhou, J. R. (2012). Tanshinones inhibit the growth of breast cancer cells through epigenetic modification of Aurora A expression and function. *PLoS One* 7 (4), e33656. doi:10.1371/journal.pone.0033656

Guo, Y., Li, Y., Wang, F. F., Xiang, B., Huang, X. O., Ma, H. B., et al. (2019). The combination of Nutlin-3 and Tanshinone IIA promotes synergistic cytotoxicity in acute leukemic cells expressing wild-type p53 by co-regulating MDM2-P53 and the AKT/mTOR pathway. *Int. J. Biochem. Cell Biol.* 106, 8–20. doi:10.1016/j.biocel.2018.10.008

Guo, Y., Li, Y., Xiang, B., Huang, X. O., Ma, H. B., Wang, F. F., et al. (2017). Nutlin-3 plus tanshinone IIA exhibits synergetic anti-leukemia effect with imatinib by reactivating p53 and inhibiting the AKT/mTOR pathway in Ph+ ALL. *Biochem. J.* 474 (24), 4153–4170. doi:10.1042/BCJ20170386

Haider, T., Pandey, V., Banjare, N., Gupta, P. N., and Soni, V. (2020). Drug resistance in cancer: Mechanisms and tackling strategies. *Pharmacol. Rep.* 72 (5), 1125–1151. doi:10.1007/s43440-020-00138-7

Han, C., Wei, Y., Wang, X., Ba, C., and Shi, W. (2019). Protective effect of Salvia miltiorrhiza polysaccharides on liver injury in chickens. *Poult. Sci.* 98 (9), 3496–3503. doi:10.3382/ps/pez153

Han, G., Wang, Y., Liu, T., Gao, J., Duan, F., Chen, M., et al. (2022). Salvianolic acid B acts against non-small cell lung cancer A549 cells via inactivation of the MAPK and Smad2/3 signaling pathways. *Mol. Med. Rep.* 25 (5), 184. doi:10.3892/mmr.2022.12700

He, Z. X., Wei, B. F., Zhang, X., Gong, Y. P., Ma, L. Y., and Zhao, W. (2021). Current development of CBP/p300 inhibitors in the last decade. *Eur. J. Med. Chem.* 209, 112861. doi:10.1016/j.ejmech.2020.112861

Ho, T. C. S., Chan, A. H. Y., and Ganesan, A. (2020). Thirty years of HDAC inhibitors: 2020 insight and hindsight. *J. Med. Chem.* 63 (21), 12460–12484. doi:10.1021/acs.jmedchem.0c00830

Hu, T., To, K. K., Wang, L., Zhang, L., Lu, L., Shen, J., et al. (2014). Reversal of P-glycoprotein (P-gp) mediated multidrug resistance in colon cancer cells by cryptotanshinone and dihydrotanshinone of Salvia miltiorrhiza. *Phytomedicine* 21 (11), 1264–1272. doi:10.1016/j.phymed.2014.06.013

Hu, T., Wang, L., Zhang, L., Lu, L., Shen, J., Chan, R. L., et al. (2015). Sensitivity of apoptosis-resistant colon cancer cells to tanshinones is mediated by autophagic cell death and p53-independent cytotoxicity. *Phytomedicine* 22 (5), 536–544. doi:10.1016/j.phymed.2015.03.010

Hua, H., Kong, Q., Zhang, H., Wang, J., Luo, T., and Jiang, Y. (2019). Targeting mTOR for cancer therapy. *J. Hematol. Oncol.* 12 (1), 71. doi:10.1186/s13045-019-0754-1

Huang, S. Y., Chang, S. F., Liao, K. F., and Chiu, S. C. (2017). Tanshinone IIA inhibits epithelial-mesenchymal transition in bladder cancer cells via modulation of STAT3-CCL2 signaling. *Int. J. Mol. Sci.* 18 (8), E1616. doi:10.3390/ijms18081616

Huang, Y., Yu, S. H., Zhen, W. X., Cheng, T., Wang, D., Lin, J. B., et al. (2021). Tanshinone I, a new EZH2 inhibitor restricts normal and malignant hematopoiesis through upregulation of MMP9 and ABCG2. *Theranostics* 11 (14), 6891–6904. doi:10.7150/thno.53170

Huang, Y., Yuan, K., Tang, M., Yue, J., Bao, L., Wu, S., et al. (2021a). Melatonin inhibiting the survival of human gastric cancer cells under ER stress involving autophagy and Ras-Raf-MAPK signalling. *J. Cell. Mol. Med.* 25 (3), 1480–1492. doi:10.1111/jcmm.16237

Huang, Y., Zhou, Z., Zhang, J., Hao, Z., He, Y., Wu, Z., et al. (2021b). lncRNA MALAT1 participates in metformin inhibiting the proliferation of breast cancer cell. *J. Cell. Mol. Med.* 25 (15), 7135–7145. doi:10.1111/jcmm.16742

Iida, K., Naiki, T., Naiki-Ito, A., Suzuki, S., Kato, H., Nozaki, S., et al. (2020). Luteolin suppresses bladder cancer growth via regulation of mechanistic target of rapamycin pathway. *Cancer Sci.* 111 (4), 1165–1179. doi:10.1111/cas.14334

Iorio, M. V., and Croce, C. M. (2012). MicroRNA dysregulation in cancer: Diagnostics, monitoring and therapeutics. A comprehensive review. *EMBO Mol. Med.* 4 (3), 143–159. doi:10.1002/emmm.201100209

Jamialahmadi, K., Zahedipour, F., and Karimi, G. (2021). The role of microRNAs on doxorubicin drug resistance in breast cancer. *J. Pharm. Pharmacol.* 73 (8), 997–1006. doi:10.1093/jpp/rgaa031

Janardhan, A., Kathera, C., Darsi, A., Ali, W., He, L., Yang, Y., et al. (2018). Prominent role of histone lysine demethylases in cancer epigenetics and therapy. *Oncotarget* 9 (76), 34429–34448. doi:10.18632/oncotarget.24319

Jiang, X., Wang, J., Deng, X., Xiong, F., Zhang, S., Gong, Z., et al. (2020). The role of microenvironment in tumor angiogenesis. *J. Exp. Clin. Cancer Res.* 39 (1), 204. doi:10.1186/s13046-020-01709-5

Jieensinue, S., Zhu, H., Li, G., Dong, K., Liang, M., and Li, Y. (2018). Tanshinone IIA reduces SW837 colorectal cancer cell viability via the promotion of mitochondrial fission by activating JNK-Mff signaling pathways. *BMC Cell Biol.* 19 (1), 21. doi:10.1186/s12860-018-0174-z

Jin, L., Chen, C., Huang, L., Bu, L., Zhang, L., and Yang, Q. (2021). Salvianolic acid A blocks vasculogenic mimicry formation in human non-small cell lung cancer via PI3K/Akt/mTOR signalling. *Clin. Exp. Pharmacol. Physiol.* 48 (4), 508–514. doi:10.1111/1440-1681.13464

Jin, W. (2020). Role of JAK/STAT3 signaling in the regulation of metastasis, the transition of cancer stem cells, and chemoresistance of cancer by epithelial-mesenchymal transition. *Cells* 9 (1), E217. doi:10.3390/cells9010217

Jung, D. Y., Kim, J. H., and Jung, M. H. (2020). Anti-obesity effects of tanshinone I from salvia miltiorrhiza bunge in mice fed a high-fat diet through inhibition of early adipogenesis. *Nutrients* 12 (5), E1242. doi:10.3390/nu12051242

Kan, S., Cheung, W. M., Zhou, Y., and Ho, W. S. (2014). Enhancement of doxorubicin cytotoxicity by tanshinone IIA in HepG2 human hepatoma cells. *Planta Med.* 80 (1), 70–76. doi:10.1055/s-0033-1360126

Kanwal, R., Gupta, K., and Gupta, S. (2015). Cancer epigenetics: An introduction. *Methods Mol. Biol.* 1238, 3–25. doi:10.1007/978-1-4939-1804-1_1

Karabiyik, C., Vicinanza, M., Son, S. M., and Rubinsztein, D. C. (2021). Glucose starvation induces autophagy via ULK1-mediated activation of PIKfyve in an AMPK-dependent manner. *Dev. Cell* 56 (13), 1961–1975.e5. doi:10.1016/j.devcel.2021.05.010

Karakaidos, P., Verigos, J., and Magklara, A. (2019). LSD1/KDM1A, a gate-keeper of cancer stemness and a promising therapeutic target. *Cancers (Basel)* 11 (12), E1821. doi:10.3390/cancers11121821

Kashyap, A., Umar, S. M., Dev, J. R. A., and Prasad, C. P. (2021). Dihydrotanshinone-I modulates epithelial mesenchymal transition (EMT) thereby impairing migration and clonogenicity of triple negative breast cancer cells. *Asian pac. J. Cancer Prev.* 22 (7), 2177–2184. doi:10.31557/APJCP.2021.22.7.2177

Keeley, E. C., Mehrad, B., and Strieter, R. M. (2010). CXC chemokines in cancer angiogenesis and metastases. *Adv. Cancer Res.* 106, 91–111. doi:10.1016/S0065-230X(10)06003-3

Kim, H., Ramirez, C. N., Su, Z. Y., and Kong, A. N. (2016). Epigenetic modifications of triterpenoid ursolic acid in activating Nrf2 and blocking cellular transformation of mouse epidermal cells. *J. Nutr. Biochem.* 33, 54–62. doi:10.1016/j.jnutbio.2015.09.014

Kim, J., Kundu, M., Viollet, B., and Guan, K. L. (2011). AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat. Cell Biol.* 13 (2), 132–141. doi:10.1038/ncb2152

Kim, K., Shin, E. A., Jung, J. H., Park, J. E., Kim, D. S., Shim, B. S., et al. (2018). Ursolic acid induces apoptosis in colorectal cancer cells partially via upregulation of MicroRNA-4500 and inhibition of JAK2/STAT3 phosphorylation. *Int. J. Mol. Sci.* 20 (1), E114. doi:10.3390/ijms20010114

Kumar, V., Radin, D., and Leonardi, D. (2019). Studies examining the synergy between Dihydrotanshinone and Temozolomide against MGMT+ glioblastoma cells *in vitro*: Predicting interactions with the blood-brain barrier. *Biomed. Pharmacother.* 109, 386–390. doi:10.1016/j.biopha.2018.10.069

Lakshminarasimhan, R., and Liang, G. (2016). The role of DNA methylation in cancer. *Adv. Exp. Med. Biol.* 945, 151–172. doi:10.1007/978-3-319-43624-1_7

Lee, H. P., Liu, Y. C., Chen, P. C., Tai, H. C., Li, T. M., Fong, Y. C., et al. (2017). Tanshinone IIA inhibits angiogenesis in human endothelial progenitor cells *in vitro* and *in vivo*. *Oncotarget* 8 (65), 109217–109227. doi:10.18632/oncotarget.22649

Lee, W. D., Liang, Y. J., and Chen, B. H. (2016). Effects of tanshinone nanoemulsion and extract on inhibition of lung cancer cells A549. *Nanotechnology* 27 (49), 495101. doi:10.1088/0957-4484/27/49/495101

Lee, Y. S., and Dutta, A. (2009). MicroRNAs in cancer. *Annu. Rev. Pathol.* 4, 199–227. doi:10.1146/annurev.pathol.4.110807.092222

Li, C., Wang, Q., Shen, S., Wei, X., and Li, G. (2019). HIF-1α/VEGF signaling-mediated epithelial-mesenchymal transition and angiogenesis is critically involved in anti-metastasis effect of luteolin in melanoma cells. *Phytother. Res.* 33 (3), 798–807. doi:10.1002/ptr.6273

Li, G., Shan, C., Liu, L., Zhou, T., Zhou, J., Hu, X., et al. (2015). Tanshinone IIA inhibits HIF-1α and VEGF expression in breast cancer cells via mTOR/p70S6K/

RPS6/4E-BP1 signaling pathway. *PLoS One* 10 (2), e0117440. doi:10.1371/journal.pone.0117440

Li, K., and Lai, H. (2017). TanshinoneIIA enhances the chemosensitivity of breast cancer cells to doxorubicin through down-regulating the expression of MDR-related ABC transporters. *Biomed. Pharmacother.* 96, 371–377. doi:10.1016/j.biopha.2017.10.016

Li, K., Liu, W., Zhao, Q., Wu, C., Fan, C., Lai, H., et al. (2019a). Combination of tanshinone IIA and doxorubicin possesses synergism and attenuation effects on doxorubicin in the treatment of breast cancer. *Phytother. Res.* 33 (6), 1658–1669. doi:10.1002/ptr.6353

Li, L., Zhang, Z. H., and Zhao, W. D. (2009). Apoptosis of MR2 cells induced by Tanshinone II A combined with arsenic trioxide. *Sichuan Da Xue Xue Bao Yi Xue Ban.* 40 (5), 812–816.

Li, M., Gao, F., Zhao, Q., Zuo, H., Liu, W., and Li, W. (2020). Tanshinone IIA inhibits oral squamous cell carcinoma via reducing Akt-c-Myc signaling-mediated aerobic glycolysis. *Cell Death Dis.* 11 (5), 381. doi:10.1038/s41419-020-2579-9

Li, M., Liu, H., Zhao, Q., Han, S., Zhou, L., Liu, W., et al. (2021). Targeting Aurora B kinase with Tanshinone IIA suppresses tumor growth and overcomes radioresistance. *Cell Death Dis.* 12 (2), 152. doi:10.1038/s41419-021-03434-z

Li, Q., Zhang, J., Liang, Y., Mu, W., Hou, X., Ma, X., et al. (2018). Tanshinone l exhibits anticancer effects in human endometrial carcinoma HEC-1-A cells via mitochondrial mediated apoptosis, cell cycle arrest and inhibition of JAK/STAT signalling pathway. *J. buon* 23 (4), 1092–1096.

Li, S., Wu, C., Fan, C., Zhang, P., Yu, G., and Li, K. (2021a). Tanshinone II A improves the chemosensitivity of breast cancer cells to doxorubicin by inhibiting β-catenin nuclear translocation. *J. Biochem. Mol. Toxicol.* 35 (1), e22620. doi:10.1002/jbt.22620

Li, S., Wu, R., Wang, L., Dina Kuo, H. C., Sargsyan, D., Zheng, X., et al. (2022). Triterpenoid ursolic acid drives metabolic rewiring and epigenetic reprogramming in treatment/prevention of human prostate cancer. *Mol. Carcinog.* 61 (1), 111–121. doi:10.1002/mc.23365

Li, X., Jia, Q., Zhou, Y., Jiang, X., Song, L., Wu, Y., et al. (2022a). Tanshinone IIA attenuates the stemness of breast cancer cells via targeting the miR-125b/STARD13 axis. *Exp. Hematol. Oncol.* 11 (1), 2. doi:10.1186/s40164-022-00255-4

Li, X., Li, Z., Li, X., Liu, B., and Liu, Z. (2017). Mechanisms of Tanshinone II a inhibits malignant melanoma development through blocking autophagy signal transduction in A375 cell. *BMC Cancer* 17 (1), 357. doi:10.1186/s12885-017-3329-y

Li, Y., and Chen, Y. (2019). AMPK and autophagy. *Adv. Exp. Med. Biol.* 1206, 85–108. doi:10.1007/978-981-15-0602-4_4

Li, Y., and Seto, E. (2016). HDACs and HDAC inhibitors in cancer development and therapy. *Cold Spring Harb. Perspect. Med.* 6 (10), a026831. doi:10.1101/cshperspect.a026831

Li, Z. M., Xu, S. W., and Liu, P. Q. (2018a). Salvia miltiorrhizaBurge (danshen): A golden herbal medicine in cardiovascular therapeutics. *Acta Pharmacol. Sin.* 39 (5), 802–824. doi:10.1038/aps.2017.193

Liang, Y. Y., Wan, X. H., Niu, F. J., Xie, S. M., Guo, H., Yang, Y. Y., et al. (2020). Salvia plebeia R. Br, an overview about its traditional uses, chemical constituents, pharmacology and modern applications. *Biomed. Pharmacother.* 121, 109589. doi:10.1016/j.biopha.2019.109589

Lin, H., Zheng, L., Li, S., Xie, B., Cui, B., Xia, A., et al. (2018). Cytotoxicity of Tanshinone IIA combined with Taxol on drug-resist breast cancer cells MCF-7 through inhibition of Tau. *Phytother. Res.* 32 (4), 667–671. doi:10.1002/ptr.6014

Lin, L. L., Hsia, C. R., Hsu, C. L., Huang, H. C., and Juan, H. F. (2015). Integrating transcriptomics and proteomics to show that tanshinone IIA suppresses cell growth by blocking glucose metabolism in gastric cancer cells. *BMC Genomics* 16 (1), 41. doi:10.1186/s12864-015-1230-0

Liu, J. J., Liu, W. D., Yang, H. Z., Zhang, Y., Fang, Z. G., Liu, P. Q., et al. (2010). Inactivation of PI3k/Akt signaling pathway and activation of caspase-3 are involved in tanshinone I-induced apoptosis in myeloid leukemia cells *in vitro*. *Ann. Hematol.* 89 (11), 1089–1097. doi:10.1007/s00277-010-0996-z

Liu, T., Xu, J., Yan, H. L., Cheng, F. C., and Liu, X. J. (2019). Luteolin suppresses teratoma cell growth and induces cell apoptosis via inhibiting bcl-2. *Oncol. Res.* 27 (7), 773–778. doi:10.3727/096504018X15208986577685

Liu, X., Zou, H., Zhao, Y., Chen, H., Liu, T., Wu, Z., et al. (2020). Tanshinone inhibits NSCLC by downregulating AURKA through let-7a-5p. *Front. Genet.* 11, 838. doi:10.3389/fgene.2020.00838

Liu, Y., Lin, F., Chen, Y., Wang, R., Liu, J., Jin, Y., et al. (2020a). Cryptotanshinone inhibites bladder cancer cell proliferation and promotes apoptosis via the PTEN/PI3K/AKT pathway. *J. Cancer* 11 (2), 488–499. doi:10.7150/jca.31422

Lu, M., Wang, C., and Wang, J. (2016). Tanshinone I induces human colorectal cancer cell apoptosis: The potential roles of Aurora A-p53 and survivin-mediated signaling pathways. *Int. J. Oncol.* 49 (2), 603–610. doi:10.3892/ijo.2016.3565

Lu, X., Li, Y., Yang, W., Tao, M., Dai, Y., Xu, J., et al. (2021). Inhibition of NF-κB is required for oleanolic acid to downregulate PD-L1 by promoting DNA demethylation in gastric cancer cells. *J. Biochem. Mol. Toxicol.* 35 (1), e22621. doi:10.1002/jbt.22621

Ma, S., Meng, Z., Chen, R., and Guan, K. L. (2019). The hippo pathway: Biology and pathophysiology. *Annu. Rev. Biochem.* 88, 577–604. doi:10.1146/annurev-biochem-013118-111829

Ma, Z. L., Zhang, B. J., Wang, D. T., Li, X., Wei, J. L., Zhao, B. T., et al. (2015). Tanshinones suppress AURKA through up-regulation of miR-32 expression in non-small cell lung cancer. *Oncotarget* 6 (24), 20111–20120. doi:10.18632/oncotarget.3933

Macklin, P. S., McAuliffe, J., Pugh, C. W., and Yamamoto, A. (2017). Hypoxia and HIF pathway in cancer and the placenta. *Placenta* 56, 8–13. doi:10.1016/j.placenta.2017.03.010

Man, Y., Yang, L., Zhang, D., and Bi, Y. (2016). Cryptotanshinone inhibits lung tumor growth by increasing CD4(+) T cell cytotoxicity through activation of the JAK2/STAT4 pathway. *Oncol. Lett.* 12 (5), 4094–4098. doi:10.3892/ol.2016.5123

Mandal, S., Gamit, N., Varier, L., Dharmarajan, A., and Warrier, S. (2021). Inhibition of breast cancer stem-like cells by a triterpenoid, ursolic acid, via activation of Wnt antagonist, sFRP4 and suppression of miRNA-499a-5p. *Life Sci.* 265, 118854. doi:10.1016/j.lfs.2020.118854

Melincovici, C. S., Boşca, A. B., Şuşman, S., Mărginean, M., Mihu, C., Istrate, M., et al. (2018). Vascular endothelial growth factor (VEGF) - key factor in normal and pathological angiogenesis. *Rom. J. Morphol. Embryol.* 59 (2), 455–467.

Mens, M. M. J., and Ghanbari, M. (2018). Cell cycle regulation of stem cells by MicroRNAs. *Stem Cell Rev. Rep.* 14 (3), 309–322. doi:10.1007/s12015-018-9808-y

Meraldi, P., Honda, R., and Nigg, E. A. (2004). Aurora kinases link chromosome segregation and cell division to cancer susceptibility. *Curr. Opin. Genet. Dev.* 14 (1), 29–36. doi:10.1016/j.gde.2003.11.006

Mohr, A. M., and Mott, J. L. (2015). Overview of microRNA biology. *Semin. Liver Dis.* 35 (1), 3–11. doi:10.1055/s-0034-1397344

Naiki-Ito, A., Naiki, T., Kato, H., Iida, K., Etani, T., Nagayasu, Y., et al. (2020). Recruitment of miR-8080 by luteolin inhibits androgen receptor splice variant 7 expression in castration-resistant prostate cancer. *Carcinogenesis* 41 (8), 1145–1157. doi:10.1093/carcin/bgz193

Nie, Z. Y., Zhao, M. H., Cheng, B. Q., Pan, R. F., Wang, T. R., Qin, Y., et al. (2020). Tanshinone IIA regulates human AML cell proliferation, cell cycle, and apoptosis through miR-497-5p/AKT3 axis. *Cancer Cell Int.* 20, 379. doi:10.1186/s12935-020-01468-5

Nizamutdinova, I. T., Lee, G. W., Lee, J. S., Cho, M. K., Son, K. H., Jeon, S. J., et al. (2008). Tanshinone I suppresses growth and invasion of human breast cancer cells, MDA-MB-231, through regulation of adhesion molecules. *Carcinogenesis* 29 (10), 1885–1892. doi:10.1093/carcin/bgn151

Noguchi, S., Saito, A., and Nagase, T. (2018). YAP/TAZ signaling as a molecular link between fibrosis and cancer. *Int. J. Mol. Sci.* 19 (11), E3674. doi:10.3390/ijms19113674

Ono, H., Sowa, Y., Horinaka, M., Iizumi, Y., Watanabe, M., Morita, M., et al. (2018). The histone deacetylase inhibitor OBP-801 and eribulin synergistically inhibit the growth of triple-negative breast cancer cells with the suppression of survivin, Bcl-xL, and the MAPK pathway. *Breast Cancer Res. Treat.* 171 (1), 43–52. doi:10.1007/s10549-018-4815-x

Pan, J., Cai, X., Zheng, X., Zhu, X., Feng, J., and Wang, X. (2022). Luteolin inhibits viability, migration, angiogenesis and invasion of non-small cell lung cancer vascular endothelial cells via miR-133a-3p/purine rich element binding protein B-mediated MAPK and PI3K/Akt signaling pathways. *Tissue Cell* 75, 101740. doi:10.1016/j.tice.2022.101740

Pan, T. L., Hung, Y. C., Wang, P. W., Chen, S. T., Hsu, T. K., Sintupisut, N., et al. (2010). Functional proteomic and structural insights into molecular targets related to the growth inhibitory effect of tanshinone IIA on HeLa cells. *Proteomics* 10 (5), 914–929. doi:10.1002/pmic.200900178

Pan, Y., Chen, L., Li, R., Liu, Y., Nan, M., and Hou, L. (2021). Tanshinone IIa induces autophagy and apoptosis via PI3K/Akt/mTOR Axis in acute promyelocytic leukemia NB4 cells. *Evid. Based. Complement. Altern. Med.* 2021, 3372403. doi:10.1155/2021/3372403

Pan, Y., Liu, G., Zhou, F., Su, B., and Li, Y. (2018). DNA methylation profiles in cancer diagnosis and therapeutics. *Clin. Exp. Med.* 18 (1), 1–14. doi:10.1007/s10238-017-0467-0

Park, S. H., Park, H. S., Lee, J. H., Chi, G. Y., Kim, G. Y., Moon, S. K., et al. (2013). Induction of endoplasmic reticulum stress-mediated apoptosis and non-canonical autophagy by luteolin in NCI-H460 lung carcinoma cells. *Food Chem. Toxicol.* 56, 100–109. doi:10.1016/j.fct.2013.02.022

Parzych, K. R., and Klionsky, D. J. (2014). An overview of autophagy: Morphology, mechanism, and regulation. *Antioxid. Redox Signal.* 20 (3), 460–473. doi:10.1089/ars.2013.5371

Pastushenko, I., and Blanpain, C. (2019). EMT transition states during tumor progression and metastasis. *Trends Cell Biol.* 29 (3), 212–226. doi:10.1016/j.tcb.2018.12.001

Patriarca, C., Pini, G. M., and Conti, G. (2020). Invasion and metastasis: A historical perspective. *Pathologica* 112 (4), 229–233. doi:10.32074/1591-951X-111

Pellegrino, N. E., Guven, A., Gray, K., Shah, P., Kasture, G., Nastke, M. D., et al. (2022). The next frontier: Translational development of ubiquitination, SUMOylation, and NEDDylation in cancer. *Int. J. Mol. Sci.* 23 (7), 3480. doi:10.3390/ijms23073480

Pistritto, G., Trisciuoglio, D., Ceci, C., Garufi, A., and D'Orazi, G. (2016). Apoptosis as anticancer mechanism: Function and dysfunction of its modulators and targeted therapeutic strategies. *Aging (Albany NY)* 8 (4), 603–619. doi:10.18632/aging.100934

Potočnjak, I., Šimić, L., Gobin, I., Vukelić, I., and Domitrović, R. (2020). Antitumor activity of luteolin in human colon cancer SW620 cells is mediated by the ERK/FOXO3a signaling pathway. *Toxicol. Vitro* 66, 104852. doi:10.1016/j.tiv.2020.104852

Pujade-Lauraine, E., Banerjee, S., and Pignata, S. (2019). Management of platinum-resistant, relapsed epithelial ovarian cancer and new drug perspectives. *J. Clin. Oncol.* 37 (27), 2437–2448. doi:10.1200/JCO.19.00194

Qi, H., Chen, Z., Qin, Y., Wang, X., Zhang, Z., and Li, Y. (2022). Tanshinone IIA inhibits cell growth by suppressing SIX1-induced aerobic glycolysis in non-small cell lung cancer cells. *Oncol. Lett.* 23 (6), 184. doi:10.3892/ol.2022.13304

Qi, P., Li, Y., Liu, X., Jafari, F. A., Zhang, X., Sun, Q., et al. (2019). Cryptotanshinone suppresses non-small cell lung cancer via microRNA-146a-5p/EGFR Axis. *Int. J. Biol. Sci.* 15 (5), 1072–1079. doi:10.7150/ijbs.31277

Qian, J., Cao, Y., Zhang, J., Li, L., Wu, J., Wei, G., et al. (2020). Tanshinone IIA induces autophagy in colon cancer cells through MEK/ERK/mTOR pathway. *Transl. Cancer Res.* 9 (11), 6919–6928. doi:10.21037/tcr-20-1963

Qin, T., Zhu, W., Kan, X., Li, L., and Wu, D. (2022). Luteolin attenuates the chemoresistance of osteosarcoma through inhibiting the PTN/β-catenin/MDR1 signaling axis by upregulating miR-384. *J. Bone Oncol.* 34, 100429. doi:10.1016/j.jbo.2022.100429

Qiu, Y., Li, C., Wang, Q., Zeng, X., and Ji, P. (2018). Tanshinone IIA induces cell death via Beclin-1-dependent autophagy in oral squamous cell carcinoma SCC-9 cell line. *Cancer Med.* 7 (2), 397–407. doi:10.1002/cam4.1281

Querfurth, H., and Lee, H. K. (2021). Mammalian/mechanistic target of rapamycin (mTOR) complexes in neurodegeneration. *Mol. Neurodegener.* 16 (1), 44. doi:10.1186/s13024-021-00428-5

Ren, J., Fu, L., Nile, S. H., Zhang, J., and Kai, G. (2019). Salvia miltiorrhiza in treating cardiovascular diseases: A review on its pharmacological and clinical applications. *Front. Pharmacol.* 10, 753. doi:10.3389/fphar.2019.00753

Ren, L. Q., Li, Q., and Zhang, Y. (2020). Luteolin suppresses the proliferation of gastric cancer cells and acts in synergy with oxaliplatin. *Biomed. Res. Int.* 2020, 9396512. doi:10.1155/2020/9396512

Ren, X., Wang, C., Xie, B., Hu, L., Chai, H., Ding, L., et al. (2017). Tanshinone IIA induced cell death via miR30b-p53-PTPN11/SHP2 signaling pathway in human hepatocellular carcinoma cells. *Eur. J. Pharmacol.* 796, 233–241. doi:10.1016/j.ejphar.2016.11.046

Rivera, E., and Gomez, H. (2010). Chemotherapy resistance in metastatic breast cancer: The evolving role of ixabepilone. *Breast Cancer Res.* 12 (2), S2. doi:10.1186/bcr2573

Rodrigues, C., B, L., Lallo, M. A., and Perez, E. C. (2020). The controversial role of autophagy in tumor development: A systematic review. *Immunol. Invest.* 49 (4), 386–396. doi:10.1080/08820139.2019.1682600

Rodríguez-Paredes, M., and Esteller, M. (2011). Cancer epigenetics reaches mainstream oncology. *Nat. Med.* 17 (3), 330–339. doi:10.1038/nm.2305

Rotili, D., and Mai, A. (2011). Targeting histone demethylases: A new avenue for the fight against cancer. *Genes Cancer* 2 (6), 663–679. doi:10.1177/1947601911417976

Rupaimoole, R., and Slack, F. J. (2017). MicroRNA therapeutics: Towards a new era for the management of cancer and other diseases. *Nat. Rev. Drug Discov.* 16 (3), 203–222. doi:10.1038/nrd.2016.246

Russell, R. C., Tian, Y., Yuan, H., Park, H. W., Chang, Y. Y., Kim, J., et al. (2013). ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. *Nat. Cell Biol.* 15 (7), 741–750. doi:10.1038/ncb2757

Ryu, S., Park, S., Lim, W., and Song, G. (2019). Effects of luteolin on canine osteosarcoma: Suppression of cell proliferation and synergy with cisplatin. *J. Cell. Physiol.* 234 (6), 9504–9514. doi:10.1002/jcp.27638

Salinas-Arellano, E., Pérez-Vásquez, A., Rivero-Cruz, I., Torres-Colin, R., González-Andrade, M., Rangel-Grimaldo, M., et al. (2020). Flavonoids and terpenoids with PTP-1B inhibitory properties from the infusion of salvia amarissima ortega. *Molecules* 25 (15), E3530. doi:10.3390/molecules25153530

Sandoval, J., Heyn, H., Moran, S., Serra-Musach, J., Pujana, M. A., Bibikova, M., et al. (2011). Validation of a DNA methylation microarray for 450, 000 CpG sites in the human genome. *Epigenetics* 6 (6), 692–702. doi:10.4161/epi.6.6.16196

Shan, Y. F., Shen, X., Xie, Y. K., Chen, J. C., Shi, H. Q., Yu, Z. P., et al. (2009). Inhibitory effects of tanshinone II-A on invasion and metastasis of human colon carcinoma cells. *Acta Pharmacol. Sin.* 30 (11), 1537–1542. doi:10.1038/aps.2009.139

Shang, S., Hua, F., and Hu, Z. W. (2017). The regulation of β-catenin activity and function in cancer: Therapeutic opportunities. *Oncotarget* 8 (20), 33972–33989. doi:10.18632/oncotarget.15687

Shin, E. A., Sohn, E. J., Won, G., Choi, J. U., Jeong, M., Kim, B., et al. (2014). Upregulation of microRNA135a-3p and death receptor 5 plays a critical role in Tanshinone I sensitized prostate cancer cells to TRAIL induced apoptosis. *Oncotarget* 5 (14), 5624–5636. doi:10.18632/oncotarget.2152

Sohn, E. J., Won, G., Lee, J., Yoon, S. W., Lee, I., Kim, H. J., et al. (2016). Blockage of epithelial to mesenchymal transition and upregulation of let 7b are critically involved in ursolic acid induced apoptosis in malignant mesothelioma cell. *Int. J. Biol. Sci.* 12 (11), 1279–1288. doi:10.7150/ijbs.13453

Song, Q., Yang, L., Han, Z., Wu, X., Li, R., Zhou, L., et al. (2020). Tanshinone IIA inhibits epithelial-to-mesenchymal transition through hindering β-arrestin1 mediated β-catenin signaling pathway in colorectal cancer. *Front. Pharmacol.* 11, 586616. doi:10.3389/fphar.2020.586616

Su, C. C. (2012). Tanshinone IIA potentiates the efficacy of 5-FU in Colo205 colon cancer cells *in vivo* through downregulation of P-gp and LC3-II. *Exp. Ther. Med.* 3 (3), 555–559. doi:10.3892/etm.2011.441

Su, C. Y., Ming, Q. L., Rahman, K., Han, T., and Qin, L. P. (2015). Salvia miltiorrhiza: Traditional medicinal uses, chemistry, and pharmacology. *Chin. J. Nat. Med.* 13 (3), 163–182. doi:10.1016/S1875-5364(15)30002-9

Su, L. J., Zhang, J. H., Gomez, H., Murugan, R., Hong, X., Xu, D., et al. (2019). Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and ferroptosis. *Oxid. Med. Cell. Longev.* 2019, 5080843. doi:10.1155/2019/5080843

Suhail, Y., Cain, M. P., Vanaja, K., Kurywchak, P. A., Levchenko, A., Kalluri, R., et al. (2019). Systems biology of cancer metastasis. *Cell Syst.* 9 (2), 109–127. doi:10.1016/j.cels.2019.07.003

Sui, H., Zhao, J., Zhou, L., Wen, H., Deng, W., Li, C., et al. (2017). Tanshinone IIA inhibits β-catenin/VEGF-mediated angiogenesis by targeting TGF-β1 in normoxic and HIF-1α in hypoxic microenvironments in human colorectal cancer. *Cancer Lett.* 403, 86–97. doi:10.1016/j.canlet.2017.05.013

Sun, C., Han, B., Zhai, Y., Zhao, H., Li, X., Qian, J., et al. (2022). Dihydrotanshinone I inhibits ovarian tumor growth by activating oxidative stress through Keap1-mediated Nrf2 ubiquitination degradation. *Free Radic. Biol. Med.* 180, 220–235. doi:10.1016/j.freeradbiomed.2022.01.015

Sun, S., Zhu, L., Lai, M., Cheng, R., and Ge, Y. (2021). Tanshinone I inhibited growth of human chronic myeloid leukemia cells via JNK/ERK mediated apoptotic pathways. *Braz J. Med. Biol. Res.* 54 (8), e10685. doi:10.1590/1414-431X2020e10685

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Ca. Cancer J. Clin.* 71 (3), 209–249. doi:10.3322/caac.21660

Tao, L., Xu, M., Dai, X., Ni, T., Li, D., Jin, F., et al. (2018). Polypharmacological profiles underlying the antitumor property of salvia miltiorrhiza root (danshen) interfering with NOX-dependent neutrophil extracellular traps. *Oxid. Med. Cell. Longev.* 2018, 4908328. doi:10.1155/2018/4908328

Tao, S., Justiniano, R., Zhang, D. D., and Wondrak, G. T. (2013). The Nrf2-inducers tanshinone I and dihydrotanshinone protect human skin cells and reconstructed human skin against solar simulated UV. *Redox Biol.* 1 (1), 532–541. doi:10.1016/j.redox.2013.10.004

Tao, S., Zheng, Y., Lau, A., Jaramillo, M. C., Chau, B. T., Lantz, R. C., et al. (2013a). Tanshinone I activates the Nrf2-dependent antioxidant response and protects against As(III)-induced lung inflammation *in vitro* and *in vivo*. *Antioxid. Redox Signal.* 19 (14), 1647–1661. doi:10.1089/ars.2012.5117

Tian, H., Li, Y., Mei, J., Cao, L., Yin, J., Liu, Z., et al. (2021). Effects of Salvia miltiorrhiza extract on lung adenocarcinoma. *Exp. Ther. Med.* 22 (2), 794. doi:10.3892/etm.2021.10226

Tian, Q. T., Ding, C. Y., Song, S. S., Wang, Y. Q., Zhang, A., and Miao, Z. H. (2018). New tanshinone I derivatives S222 and S439 similarly inhibit topoisomerase I/II but reveal different p53-dependency in inducing G2/M arrest and apoptosis. *Biochem. Pharmacol.* 154, 255–264. doi:10.1016/j.bcp.2018.05.006

Tong, W., Guo, J., and Yang, C. (2020). Tanshinone II A enhances pyroptosis and represses cell proliferation of HeLa cells by regulating miR-145/GSDMD signaling pathway. *Biosci. Rep.* 40 (4), BSR20200259. doi:10.1042/BSR20200259

Tsai, K. J., Tsai, H. Y., Tsai, C. C., Chen, T. Y., Hsieh, T. H., Chen, C. L., et al. (2021). Luteolin inhibits breast cancer stemness and enhances chemosensitivity through the nrf2-mediated pathway. *Molecules* 26 (21), 6452. doi:10.3390/molecules26216452

Tung, N. H., Nakajima, K., Uto, T., Hai, N. T., Long, D. D., Ohta, T., et al. (2017). Bioactive triterpenes from the root of salvia miltiorrhiza bunge. *Phytother. Res.* 31 (9), 1457–1460. doi:10.1002/ptr.5877

Verschooten, L., Barrette, K., Van Kelst, S., Rubio Romero, N., Proby, C., De Vos, R., et al. (2012). Autophagy inhibitor chloroquine enhanced the cell death inducing effect of the flavonoid luteolin in metastatic squamous cell carcinoma cells. *PLoS One* 7 (10), e48264. doi:10.1371/journal.pone.0048264

Wang, C., Li, Q., Xiao, B., Fang, H., Huang, B., Huang, F., et al. (2021). Luteolin enhances the antitumor efficacy of oncolytic vaccinia virus that harbors IL-24 gene in liver cancer cells. *J. Clin. Lab. Anal.* 35 (3), e23677. doi:10.1002/jcla.23677

Wang, H., Luo, Y., Qiao, T., Wu, Z., and Huang, Z. (2018). Luteolin sensitizes the antitumor effect of cisplatin in drug-resistant ovarian cancer via induction of apoptosis and inhibition of cell migration and invasion. *J. Ovarian Res.* 11 (1), 93. doi:10.1186/s13048-018-0468-y

Wang, H., Su, X., Fang, J., Xin, X., Zhao, X., Gaur, U., et al. (2018a). Tanshinone IIA attenuates insulin like growth factor 1 -induced cell proliferation in PC12 cells through the PI3K/Akt and MEK/ERK pathways. *Int. J. Mol. Sci.* 19 (9), E2719. doi:10.3390/ijms19092719

Wang, J., Li, Y., Wang, X., and Jiang, C. (2012). Ursolic acid inhibits proliferation and induces apoptosis in human glioblastoma cell lines U251 by suppressing TGF-β1/miR-21/PDCD4 pathway. *Basic Clin. Pharmacol. Toxicol.* 111 (2), 106–112. doi:10.1111/j.1742-7843.2012.00870.x

Wang, J., Ma, Y., Guo, M., Yang, H., and Guan, X. (2021a). Salvianolic acid B suppresses EMT and apoptosis to lessen drug resistance through AKT/mTOR in gastric cancer cells. *Cytotechnology* 73 (1), 49–61. doi:10.1007/s10616-020-00441-4

Wang, J. Y., and Chen, L. J. (2019). The role of miRNAs in the invasion and metastasis of cervical cancer. *Biosci. Rep.* 39 (3), BSR20181377. doi:10.1042/BSR20181377

Wang, L., Hu, T., Shen, J., Zhang, L., Chan, R. L., Lu, L., et al. (2015). Dihydrotanshinone I induced apoptosis and autophagy through caspase dependent pathway in colon cancer. *Phytomedicine* 22 (12), 1079–1087. doi:10.1016/j.phymed.2015.08.009

Wang, L., Yeung, J. H., Hu, T., Lee, W. Y., Lu, L., Zhang, L., et al. (2013). Dihydrotanshinone induces p53-independent but ROS-dependent apoptosis in colon cancer cells. *Life Sci.* 93 (8), 344–351. doi:10.1016/j.lfs.2013.07.007

Wang, L., Zhang, C., Guo, Y., Su, Z. Y., Yang, Y., Shu, L., et al. (2014). Blocking of JB6 cell transformation by tanshinone IIA: Epigenetic reactivation of Nrf2 antioxidative stress pathway. *Aaps J.* 16 (6), 1214–1225. doi:10.1208/s12248-014-9666-8

Wang, M., Zeng, X., Li, S., Sun, Z., Yu, J., Chen, C., et al. (2019). A novel tanshinone analog exerts anti-cancer effects in prostate cancer by inducing cell apoptosis, arresting cell cycle at G2 phase and blocking metastatic ability. *Int. J. Mol. Sci.* 20 (18), E4459. doi:10.3390/ijms20184459

Wang, Q. L., Tao, Y. Y., Yuan, J. L., Shen, L., and Liu, C. H. (2010). Salvianolic acid B prevents epithelial-to-mesenchymal transition through the TGF-beta1 signal transduction pathway *in vivo* and *in vitro*. *BMC Cell Biol.* 11, 31. doi:10.1186/1471-2121-11-31

Wang, R., Luo, Z., Zhang, H., and Wang, T. (2019a). Tanshinone IIA reverses gefitinib-resistance in human non-small-cell lung cancer via regulation of VEGFR/Akt pathway. *Onco. Targets. Ther.* 12, 9355–9365. doi:10.2147/OTT.S221228

Wang, S., Zhao, Y., Aguilar, A., Bernard, D., and Yang, C. Y. (2017). Targeting the MDM2-p53 protein-protein interaction for new cancer therapy: Progress and challenges. *Cold Spring Harb. Perspect. Med.* 7 (5), a026245. doi:10.1101/cshperspect.a026245

Wang, X. P., Wang, P. F., Bai, J. Q., Gao, S., Wang, Y. H., Quan, L. N., et al. (2019b). Investigating the effects and possible mechanisms of danshen- honghua herb pair on acute myocardial ischemia induced by isoproterenol in rats. *Biomed. Pharmacother.* 118, 109268. doi:10.1016/j.biopha.2019.109268

Wang, X., Yang, Y., Liu, X., and Gao, X. (2020). Pharmacological properties of tanshinones, the natural products from Salvia miltiorrhiza. *Adv. Pharmacol.* 87, 43–70. doi:10.1016/bs.apha.2019.10.001

Wang, X., Zhang, L., Dai, Q., Si, H., Zhang, L., Eltom, S. E., et al. (2021b). Combined luteolin and indole-3-carbinol synergistically constrains erα-positive breast cancer by dual inhibiting estrogen receptor alpha and cyclin-dependent kinase 4/6 pathway in cultured cells and xenograft mice. *Cancers (Basel)* 13 (9), 2116. doi:10.3390/cancers13092116

Wang, Y., Jin, W., and Wang, J. (2021c). Tanshinone IIA regulates microRNA-125b/foxp3/caspase-1 signaling and inhibits cell viability of nasopharyngeal carcinoma. *Mol. Med. Rep.* 23 (5), 371. doi:10.3892/mmr.2021.12010

Wang, Y., Zhang, Y., Chen, X., Hong, Y., and Wu, Z. (2018b). Combined treatment with myo-inositol and luteolin selectively suppresses growth of human lung cancer A549 cells possibly by suppressing activation of PDK1 and Akt. *Nan Fang. Yi Ke Da Xue Xue Bao* 38 (11), 1378–1383. doi:10.12122/j.issn.1673-4254.2018.11.17

Woo, J., Kim, H. Y., Byun, B. J., Chae, C. H., Lee, J. Y., Ryu, S. Y., et al. (2014). Biological evaluation of tanshindiols as EZH2 histone methyltransferase inhibitors. *Bioorg. Med. Chem. Lett.* 24 (11), 2486–2492. doi:10.1016/j.bmcl.2014.04.010

Wu, C. Y., Hsieh, C. Y., Huang, K. E., Chang, C., and Kang, H. Y. (2012). Cryptotanshinone down-regulates androgen receptor signaling by modulating lysine-specific demethylase 1 function. *Int. J. Cancer* 131 (6), 1423–1434. doi:10.1002/ijc.27343

Wu, D., Qiu, Y., Jiao, Y., Qiu, Z., and Liu, D. (2020). Small molecules targeting HATs, HDACs, and BRDs in cancer therapy. *Front. Oncol.* 10, 560487. doi:10.3389/fonc.2020.560487

Wu, H. T., Lin, J., Liu, Y. E., Chen, H. F., Hsu, K. W., Lin, S. H., et al. (2021). Luteolin suppresses androgen receptor-positive triple-negative breast cancer cell proliferation and metastasis by epigenetic regulation of MMP9 expression via the AKT/mTOR signaling pathway. *Phytomedicine.* 81, 153437. doi:10.1016/j.phymed.2020.153437

Wu, J., Xu, H., Zhang, L., and Zhang, X. (2016). Radix astragali and tanshinone help carboplatin inhibit B16 tumor cell growth. *Technol. Cancer Res. Treat.* 15 (4), 583–588. doi:10.1177/1533034615588682

Wu, S., Sun, C., Tian, D., Li, Y., Gao, X., He, S., et al. (2015). Expression and clinical significances of Beclin1, LC3 and mTOR in colorectal cancer. *Int. J. Clin. Exp. Pathol.* 8 (4), 3882–3891.

Xiang, F., Pan, C., Kong, Q., Wu, R., Jiang, J., Zhan, Y., et al. (2014). Ursolic acid inhibits the proliferation of gastric cancer cells by targeting miR-133a. *Oncol. Res.* 22 (5-6), 267–273. doi:10.3727/096504015X14410238486685

Xie, J., Liu, J. H., Liu, H., Liao, X. Z., Chen, Y., Lin, M. G., et al. (2016). Tanshinone IIA combined with adriamycin inhibited malignant biological behaviors of NSCLC A549 cell line in a synergistic way. *BMC Cancer* 16 (1), 899. doi:10.1186/s12885-016-2921-x

Xie, R., Li, Y., Tang, P., and Yuan, Q. (2018). Design, synthesis and biological evaluation of novel 2-aminobenzamides containing dithiocarbamate moiety as histone deacetylase inhibitors and potent antitumor agents. *Eur. J. Med. Chem.* 143, 320–333. doi:10.1016/j.ejmech.2017.08.041

Xin, M., Hao, Y., Huang, G., Wang, X., Liang, Z., Miao, J., et al. (2020). The efficacy and safety of salvianolic acids on acute cerebral infarction treatment: A protocol for systematic review and meta analysis. *Med. Baltim.* 99 (23), e20059. doi:10.1097/MD.0000000000020059

Xu, P., Wu, Q., Lu, D., Yu, J., Rao, Y., Kou, Z., et al. (2020). A systematic study of critical miRNAs on cells proliferation and apoptosis by the shortest path. *BMC Bioinforma.* 21 (1), 396. doi:10.1186/s12859-020-03732-x

Xu, Q. F., Peng, H. P., Lu, X. R., Hu, Y., Xu, Z. H., and Xu, J. K. (2021). Oleanolic acid regulates the Treg/Th17 imbalance in gastric cancer by targeting IL-6 with miR-98-5p. *Cytokine* 148, 155656. doi:10.1016/j.cyto.2021.155656

Xu, Z., Chen, L., Xiao, Z., Zhu, Y., Jiang, H., Jin, Y., et al. (2018). Potentiation of the anticancer effect of doxorubicinin drug-resistant gastric cancer cells by tanshinone IIA. *Phytomedicine* 51, 58–67. doi:10.1016/j.phymed.2018.05.012

Xu, Z., Han, X., Ou, D., Liu, T., Li, Z., Jiang, G., et al. (2020a). Targeting PI3K/AKT/mTOR-mediated autophagy for tumor therapy. *Appl. Microbiol. Biotechnol.* 104 (2), 575–587. doi:10.1007/s00253-019-10257-8

Xue, J., Jin, X., Wan, X., Yin, X., Fang, M., Liu, T., et al. (2019). Effects and mechanism of tanshinone II A in proliferation, apoptosis, and migration of human colon cancer cells. *Med. Sci. Monit.* 25, 4793–4800. doi:10.12659/MSM.914446

Yan, Y., Su, W., Zeng, S., Qian, L., Chen, X., Wei, J., et al. (2018). Effect and mechanism of tanshinone I on the radiosensitivity of lung cancer cells. *Mol. Pharm.* 15 (11), 4843–4853. doi:10.1021/acs.molpharmaceut.8b00489

Yang, P. W., Lu, Z. Y., Pan, Q., Chen, T. T., Feng, X. J., Wang, S. M., et al. (2019). MicroRNA-6809-5p mediates luteolin-induced anticancer effects against hepatoma by targeting flotillin 1. *Phytomedicine* 57, 18–29. doi:10.1016/j.phymed.2018.10.027

Yang, Y., Liu, L., Zhang, X., Jiang, X., and Wang, L. (2020). Tanshinone IIA prevents rifampicin-induced liver injury by regulating BSEP/NTCP expression via epigenetic activation of NRF2. *Liver Int.* 40 (1), 141–154. doi:10.1111/liv.14262

Yao, X., Jiang, W., Yu, D., and Yan, Z. (2019). Luteolin inhibits proliferation and induces apoptosis of human melanoma cells *in vivo* and *in vitro* by suppressing MMP-2 and MMP-9 through the PI3K/AKT pathway. *Food Funct.* 10 (2), 703–712. doi:10.1039/c8fo02013b

Yao, Y., Rao, C., Zheng, G., and Wang, S. (2019a). Luteolin suppresses colorectal cancer cell metastasis via regulation of the miR-384/pleiotrophin axis. *Oncol. Rep.* 42 (1), 131–141. doi:10.3892/or.2019.7136

Ye, T., Zhu, S., Zhu, Y., Feng, Q., He, B., Xiong, Y., et al. (2016). Cryptotanshinone induces melanoma cancer cells apoptosis via ROS-mitochondrial apoptotic pathway and impairs cell migration and invasion. *Biomed. Pharmacother.* 82, 319–326. doi:10.1016/j.biopha.2016.05.015

Ye, Y. T., Zhong, W., Sun, P., Wang, D., Wang, C., Hu, L. M., et al. (2017). Apoptosis induced by the methanol extract of Salvia miltiorrhiza Bunge in non-small cell lung cancer through PTEN-mediated inhibition of PI3K/Akt pathway. *J. Ethnopharmacol.* 200, 107–116. doi:10.1016/j.jep.2016.12.051

You, S., He, X., Wang, M., Mao, L., and Zhang, L. (2020). Tanshinone IIA suppresses glioma cell proliferation, migration and invasion both *in vitro* and *in vivo* partially through miR-16-5p/talin-1 (TLN1) Axis. *Cancer Manag. Res.* 12, 11309–11320. doi:10.2147/CMAR.S256347

Yu, F., Lu, Z., Chen, B., Wu, X., Dong, P., and Zheng, J. (2015). Salvianolic acid B-induced microRNA-152 inhibits liver fibrosis by attenuating DNMT1-mediated Patched1 methylation. *J. Cell. Mol. Med.* 19 (11), 2617–2632. doi:10.1111/jcmm.12655

Yu, F., Yu, C., Li, F., Zuo, Y., Wang, Y., Yao, L., et al. (2021). Wnt/β-catenin signaling in cancers and targeted therapies. *Signal Transduct. Target. Ther.* 6 (1), 307. doi:10.1038/s41392-021-00701-5

Yu, S., Guo, L., Yan, B., Yuan, Q., Shan, L., Zhou, L., et al. (2022). Tanshinol suppresses osteosarcoma by specifically inducing apoptosis of U2-OS cells through p53-mediated mechanism. *J. Ethnopharmacol.* 292, 115214. doi:10.1016/j.jep.2022.115214

Yun, S. M., Jung, J. H., Jeong, S. J., Sohn, E. J., Kim, B., and Kim, S. H. (2014). Tanshinone IIA induces autophagic cell death via activation of AMPK and ERK and inhibition of mTOR and p70 S6K in KBM-5 leukemia cells. *Phytother. Res.* 28 (3), 458–464. doi:10.1002/ptr.5015

Yuxian, X., Feng, T., Ren, L., and Zhengcai, L. (2009). Tanshinone II-A inhibits invasion and metastasis of human hepatocellular carcinoma cells *in vitro* and *in vivo*. *Tumori* 95 (6), 789–795. doi:10.1177/030089160909500623

Zachari, M., and Ganley, I. G. (2017). The mammalian ULK1 complex and autophagy initiation. *Essays Biochem.* 61 (6), 585–596. doi:10.1042/EBC20170021

Zahra, R., Furqan, M., Ullah, R., Mithani, A., Saleem, R. S. Z., and Faisal, A. (2020). A cell-based high-throughput screen identifies inhibitors that overcome P-glycoprotein (Pgp)-mediated multidrug resistance. *PLoS One* 15 (6), e0233993. doi:10.1371/journal.pone.0233993

Zeisberg, M., and Neilson, E. G. (2009). Biomarkers for epithelial-mesenchymal transitions. *J. Clin. Invest.* 119 (6), 1429–1437. doi:10.1172/JCI36183

Zeng, Z., Zhang, H., Wang, X., Liu, K., Li, T., Sun, S., et al. (2018). Salvianolic acid B suppresses cell proliferation and induces apoptosis in osteosarcoma through p38-mediated reactive oxygen species generation. *Oncol. Lett.* 15 (2), 2679–2685. doi:10.3892/ol.2017.7609

Zhang, B., Ma, Z., Li, X., Zhang, C., Shao, Y., Liu, Z., et al. (2016a). Tanshinones suppress non-small cell lung cancer through up-regulating miR-137. *Acta Biochim. Biophys. Sin.* 48 (8), 768–770. doi:10.1093/abbs/gmw053

Zhang, G., Yang, Y. M., Meng, W. T., and Zhou, J. (2010). Apoptosis of NB4 cells induced by Tanshinone II A combined with arsenic trioxide. *Sichuan Da Xue Xue Bao Yi Xue Ban.* 41 (1), 57–61.

Zhang, H. S., Zhang, F. J., Li, H., Liu, Y., Du, G. Y., and Huang, Y. H. (2016b). Tanshinone ⅡA inhibits human esophageal cancer cell growth through miR-122-mediated PKM2 down-regulation. *Arch. Biochem. Biophys.* 598, 50–56. doi:10.1016/j.abb.2016.03.031

Zhang, K., Li, J., Meng, W., Xing, H., and Yang, Y. (2016c). Tanshinone IIA inhibits acute promyelocytic leukemia cell proliferation and induces their apoptosis *in vivo*. *Blood Cells Mol. Dis.* 56 (1), 46–52. doi:10.1016/j.bcmd.2015.10.007

Zhang, L., Lin, W., Chen, X., Wei, G., Zhu, H., and Xing, S. (2019). Tanshinone IIA reverses EGF- and TGF-β1-mediated epithelial-mesenchymal transition in HepG2 cells via the PI3K/Akt/ERK signaling pathway. *Oncol. Lett.* 18 (6), 6554–6562. doi:10.3892/ol.2019.11032

Zhang, L., Liu, Q., Huang, L., Yang, F., Liu, A., and Zhang, J. (2020). Combination of lapatinib and luteolin enhances the therapeutic efficacy of lapatinib on human breast cancer through the FOXO3a/NQO1 pathway. *Biochem. Biophys. Res. Commun.* 531 (3), 364–371. doi:10.1016/j.bbrc.2020.07.049

Zhang, M., Wang, R., Tian, J., Song, M., Zhao, R., Liu, K., et al. (2021). Targeting LIMK1 with luteolin inhibits the growth of lung cancer *in vitro* and *in vivo*. *J. Cell. Mol. Med.* 25 (12), 5560–5571. doi:10.1111/jcmm.16568

Zhang, R. W., Liu, Z. G., Xie, Y., Wang, L. X., Li, M. C., and Sun, X. (2016d). *In vitro* inhibition of invasion and metastasis in colon cancer cells by TanIIA. *Genet. Mol. Res.* 15 (3). doi:10.4238/gmr.15039008

Zhang, W., Liu, C., Li, J., Lu, Y., Li, H., Zhuang, J., et al. (2022). Tanshinone IIA: New perspective on the anti-tumor mechanism of A traditional natural medicine. *Am. J. Chin. Med.* 50 (1), 209–239. doi:10.1142/S0192415X22500070

Zhang, X., Zhou, Y., and Gu, Y. E. (2019a). Tanshinone IIA induces apoptosis of ovarian cancer cells *in vitro* and *in vivo* through attenuation of PI3K/AKT/JNK signaling pathways. *Oncol. Lett.* 17 (2), 1896–1902. doi:10.3892/ol.2018.9744

Zhang, Y., Ge, T., Xiang, P., Zhou, J., Tang, S., Mao, H., et al. (2019b). Tanshinone IIA reverses oxaliplatin resistance in human colorectal cancer via inhibition of ERK/Akt signaling pathway. *Onco. Targets. Ther.* 12, 9725–9734. doi:10.2147/OTT.S217914

Zhang, Y., Geng, Y., He, J., Wu, D., Zhang, T., Xue, L., et al. (2019c). Tanshinone IIA induces apoptosis and autophagy in acute monocytic leukemia via downregulation of PI3K/Akt pathway. *Am. J. Transl. Res.* 11 (5), 2995–3006.

Zhang, Y., Guo, S., Fang, J., Peng, B., Zhang, Y., and Cao, T. (2018). Tanshinone IIA inhibits cell proliferation and tumor growth by downregulating STAT3 in human gastric cancer. *Exp. Ther. Med.* 16 (4), 2931–2937. doi:10.3892/etm.2018.6562

Zhang, Y., Jiang, P., Ye, M., Kim, S. H., Jiang, C., and Lü, J. (2012). Tanshinones: Sources, pharmacokinetics and anti-cancer activities. *Int. J. Mol. Sci.* 13 (10), 13621–13666. doi:10.3390/ijms131013621

Zhao, W., Feng, H., Guo, S., Han, Y., and Chen, X. (2017). Danshenol A inhibits TNF-α-induced expression of intercellular adhesion molecule-1 (ICAM-1) mediated by NOX4 in endothelial cells. *Sci. Rep.* 7 (1), 12953. doi:10.1038/s41598-017-13072-1

Zhao, X., Liu, M., and Li, D. (2015). Oleanolic acid suppresses the proliferation of lung carcinoma cells by miR-122/Cyclin G1/MEF2D axis. *Mol. Cell. Biochem.* 400 (1-2), 1–7. doi:10.1007/s11010-014-2228-7

Zhao, Y. X., Luo, D., Zhang, Y. H., Shen, B., Wang, B. X., and Sun, Z. F. (2017a). The effect of tanshinone ⅡA potentiates the effects of Cisplatin in Fadu cells *in vitro* through downregulation of survivin. *Lin. Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi* 31 (10), 781–784. doi:10.13201/j.issn.1001-1781.2017.10.011

Zhao, Z., and Shilatifard, A. (2019). Epigenetic modifications of histones in cancer. *Genome Biol.* 20 (1), 245. doi:10.1186/s13059-019-1870-5

Zheng, L., Guan, Z. J., Pan, W. T., Du, T. F., Zhai, Y. J., and Guo, J. (2018). Tanshinone suppresses arecoline-induced epithelial-mesenchymal transition in oral submucous fibrosis by epigenetically reactivating the p53 pathway. *Oncol. Res.* 26 (3), 483–494. doi:10.3727/096504017X14941825760362

Zheng, L., Zhang, Y., Liu, G., Cheng, S., Zhang, G., An, C., et al. (2020). Tanshinone I regulates autophagic signaling via the activation of AMP-activated protein kinase in cancer cells. *Anticancer. Drugs* 31 (6), 601–608. doi:10.1097/CAD.0000000000000908

Zhou, J., Jiang, Y. Y., Chen, H., Wu, Y. C., and Zhang, L. (2020). Tanshinone I attenuates the malignant biological properties of ovarian cancer by inducing apoptosis and autophagy via the inactivation of PI3K/AKT/mTOR pathway. *Cell Prolif.* 53 (2), e12739. doi:10.1111/cpr.12739

Zhou, J., Jiang, Y. Y., Wang, H. P., Chen, H., Wu, Y. C., Wang, L., et al. (2020a). Natural compound Tan-I enhances the efficacy of Paclitaxel chemotherapy in ovarian cancer. *Ann. Transl. Med.* 8 (12), 752. doi:10.21037/atm-20-4072

Zhou, J., Jiang, Y. Y., Wang, X. X., Wang, H. P., Chen, H., Wu, Y. C., et al. (2020b). Tanshinone IIA suppresses ovarian cancer growth through inhibiting malignant properties and angiogenesis. *Ann. Transl. Med.* 8 (20), 1295. doi:10.21037/atm-20-5741

Zhou, L., Sui, H., Wang, T., Jia, R., Zhang, Z., Fu, J., et al. (2020c). Tanshinone IIA reduces secretion of pro-angiogenic factors and inhibits angiogenesis in human colorectal cancer. *Oncol. Rep.* 43 (4), 1159–1168. doi:10.3892/or.2020.7498

Zhou, X., Lv, L., Tan, Y., Zhang, Z., Wei, S., and Xiao, S. (2021). Tanshinone IIA sensitizes TRAIL-induced apoptosis in glioblastoma through inducing the expression of death receptors (and suppressing STAT3 activation). *Brain Res.* 1766, 147515. doi:10.1016/j.brainres.2021.147515

Zou, S., Tong, Q., Liu, B., Huang, W., Tian, Y., and Fu, X. (2020). Targeting STAT3 in cancer immunotherapy. *Mol. Cancer* 19 (1), 145. doi:10.1186/s12943-020-01258-7

Zuo, Q., Wu, R., Xiao, X., Yang, C., Yang, Y., Wang, C., et al. (2018). The dietary flavone luteolin epigenetically activates the Nrf2 pathway and blocks cell transformation in human colorectal cancer HCT116 cells. *J. Cell. Biochem.* 119 (11), 9573–9582. doi:10.1002/jcb.27275

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 20 September 2022
DOI 10.3389/fonc.2022.1006114

Check for updates

OPEN ACCESS

EDITED BY
Yue Hou,
Northeastern University, China

REVIEWED BY
Guanyu Chen,
Sun Yat-sen University, China
Chenhui Wang,
Chongqing University, China

*CORRESPONDENCE
Zhidong Qiu
qzdcczy@163.com
Da Liu
liuda_1986@163.com

SPECIALTY SECTION
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Oncology

RECEIVED 29 July 2022
ACCEPTED 05 September 2022
PUBLISHED 20 September 2022

CITATION
Hu S, Liu Y, Guan S, Qiu Z and Liu D (2022) Natural products exert anti-tumor effects by regulating exosomal ncRNA.
Front. Oncol. 12:1006114.
doi: 10.3389/fonc.2022.1006114

COPYRIGHT
© 2022 Hu, Liu, Guan, Qiu and Liu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Natural products exert anti-tumor effects by regulating exosomal ncRNA

Shuang Hu, Yi Liu, Shuguang Guan, Zhidong Qiu* and Da Liu*

School of Pharmacy, Changchun University of Chinese Medicine, Changchun, China

Currently, more than 60% of the approved anti-cancer drugs come from or are related to natural products. Natural products and exosomal non-coding RNAs (ncRNAs) exert anti-cancer effects through various regulatory mechanisms, which are of great research significance. Exosomes are a form of intercellular communication and contain ncRNAs that can act as intercellular signaling molecules involved in the metabolism of tumor cells. This review exemplifies some examples of natural products whose active ingredients can play a role in cancer prevention and treatment by regulating exosomal ncRNAs, with the aim of illustrating the mechanism of action of exosomal ncRNAs in cancer prevention and treatment. Meanwhile, the application of exosomes as natural drug delivery systems and predictive disease biomarkers in cancer prevention and treatment is introduced, providing research ideas for the development of novel anti-tumor drugs.

KEYWORDS

natural products, exosomes ncRNAs, cancer treatment, biomarkers, exosomes vector

Introduction

Cancer has become a major disease that threatens human health and safety. The American Cancer Society (ACS) released the Cancer Statistics 2022 report stating that prostate, breast, lung, and colorectal cancers are the most prevalent cancers. However, the number of cancer deaths in the U.S. dropped 32% compared to 1991 (1), and the use of innovative drugs has directly impacted the survival rate of cancer patients (1–3).

Natural products include components and metabolites of plants, animals, insects, marine organisms and microorganisms (4), and many endogenous chemicals in humans and animals can also be used as natural products (5, 6). Many of them have been shown to have the ability of anti-inflammatory, anti-cancer, antioxidant, antibacterial and antiviral (7). Moreover, there exist studies showing that the natural products can promote the apoptosis, inhibit the migration and proliferation to exert anti-cancer effects (4). And the natural products have the advantages of easy to apply, low cost, easy

to obtain and acceptable therapeutic method with minimal cytotoxicity (8). Therefore, it is of great significance to study the therapeutic effect of natural products on cancer.

Exosomes are endogenous components of organisms released by a variety of cells that contain ncRNAs that are genetically linked and involved in cancer development and progression (9, 10). Moreover, it can serve as a delivery system to deliver a variety of stuff such as drugs to cells (11). Nowadays, there are a growing number of researches detailing the mechanism of action of natural products through the regulation of exosomal ncRNAs to produce anticancer efficacy (12). In this review, the pharmacological effects of natural products and the anti-tumor mechanism of exosomal ncRNAs induced by natural products are clarified.

Pharmacological effects of natural products against cancer

Research on natural products began in the 1990s. In recent years, with the development of scientific research technology, countries are paying more and more attention to the research and development of new natural products, and the research and development of natural products has shown a rapid growth trend (13). According to relevant information, natural products research hotspots focus on plants, animals and microorganisms on land and in the sea, involving a variety of components including proteins, peptides, amino acids, alkaloids and antibiotics (14, 15).

Natural products have a wide range of promising applications in the field of antitumor research. Traditional antitumor drugs act by affecting DNA synthesis and cell mitosis. Therefore, compared with natural antitumor drugs, traditional antitumor drugs have higher toxicity and side effects and are less selective (16). The development of natural antitumor drugs has not only ensured the efficacy of resulting in many innovative drugs with higher targeting for clinical practice (17).

For cancer treatment, drugs that directly inhibit the growth of tumor cells or promote their apoptosis are the most direct ways to fight cancer. Natural products can kill tumor cells through targeted aggregation, and also take advantage of qualitative differences between tumor cells and normal cells and molecular biological differences to target tumors, thereby inhibiting tumor cell proliferation (18). The biological processes involved in this process include signal transduction, cell fusion, and multiple types of metabolic pathways. In addition, natural products can reduce platelet aggregation, prevent cancer cell retention and inhibit cancer cell metastasis (19). It has been reported that the natural products may improve the local hypoxia of solid tumors by affecting microcirculation and increasing vascular permeability, which improves the sensitivity of treatment (20). Besides, natural products can produce some series of biological effects to exert anti-cancer pharmacological effects, such as promoting active ingredients, immune cells and cytotoxins to reach the tumor site (21). Studies have shown that natural products improve cellular immune function by increasing the body's complement level, and reduce treatment-induced tissue fibrosis by inhibiting the formation of rough fibroblasts. Natural products can regulate the immune status of the body by promoting the phagocytosis of the reticuloendothelial system and enhance the resistance of the body to external malignant stimuli (22, 23).

Moreover, the natural products can also help to improve the drug resistance of tumors. Methods to reduce drug resistance of tumor cells are also the current focus of researchers (24). Cellular drug resistance is caused by the increase of target enzymes or the change of the affinity of target enzymes to anticancer drugs during the treatment of anticancer drugs, resulting in weakened drug activity, accelerated drug inactivation and rapid DNA repair, which affect the absorption or excretion of anticancer drugs (25). Research approaches reported in recent years to address drug resistance in tumor cells include combining anti-cancer drugs, eliminating the reversibility of anti-cancer drugs for cancer treatment, and affecting the tumor microenvironment (TME) (26–28). In addition, drug combination application and personalized medication guidance are also significant to eliminate tumor drug resistance (29, 30). Natural products may also enhance the effects of radiotherapy through mechanisms such as enhancing hormonal regulation, promoting pituitary-adrenocortical function, and increasing the relative value of cyclic adenosine monophosphate (31).

Exosomes ncRNAs regulate cancer progression

Exosomes are tiny vesicles secreted by functionally normal or abnormal cells in the form of outgrowths and are a means of

Abbreviations: ncRNA, non-coding RNA; lncRNA, long non-coding RNA; miRNA, microRNA; siRNA, small interfering RNA; rRNA, ribosomal RNA; tRNA, transfer RNA; snRNA, small nuclear RNA; EMT, epithelial-mesenchymal transition; ceRNA, competing endogenous RNA; circRNA, circular RNA; ACS, American Cancer Society; UTR, untranslated region; RISCs, RNA-induced gene silencing complexes; TME, tumor microenvironment; CAFs, cancer-associated fibroblasts; IGF1R, insulin-like growth factor 1 receptor; EMT, epithelial mesenchymal transition; HSC, hepatic stellate cells; NK, natural killer; TAM, tumor-associated macrophages; DCIS, ductal carcinoma in situ; ALDH1, aldehyde dehydrogenase 1; EPC, endothelial progenitor cells; DHA, Docosahexaenoic acid; DRβ-H, d Rhamnose β-hederin; LPS, Lipopolysaccharide; MDR, multidrug resistance; EZH2, zeste homolog 2; DKK3, dickkopf 3; NTECs, normal tongue epithelial cells; EPC-EXS, Endothelial progenitor cell exosomes; DUSP1, dual protein phosphatase 1; ACACA, acetyl coenzyme a carboxylase 1; CML, chronic myeloid leukemia; ILD, interstitial lung disease; MDA5, melanoma differentiation-associated protein 5; ccRCC, renal cell carcinoma; TBI, traumatic brain injury; EVs, extracellular vesicles.

communication between cells. There are many substances included in the exosomes, such as the DNA, miRNA, lncRNA and proteins (32). Exosomes have a lipid bilayer structure and their production is regulated by multiple mechanisms involving the intra- and extracellular and microenvironment. Researchers have detected the presence of exosomes in various body fluids such as blood, saliva and urine. The characteristic structural proteins of exosomes, CD9, CD63, CD81, TSG101 and HSP70, are involved in the structural composition of exosomes. In addition, annexin, RAB protein family and flocculins are involved in biosynthesis and fusion (33, 34). The production process of exosomes is: the cell membrane is invaginated and the endosomes are formed then forming multivesicular bodies (MVB), and finally secreted to the outside of the cell to become exosomes (11). The structure and secretion mode of exosomes are shown in Figure 1. The contents of exosomes are different due to their different functions, and the origin of exosomes can be identified according to their contents (35). Exosomes are enriched with a variety of ncRNAs with biological functions, including lncRNAs, circRNAs, snRNAs, miRNAs, etc., which are important for the treatment of diseases. With the development of biology and other research fields, genomic ncRNAs are receiving more and more attention (36). A PubMed search shows a large number of scientific studies involving ncRNAs in the last decade, as shown in Figure 2. NcRNAs are RNA molecules that are transcribed from DNA, but not translated into proteins. NcRNAs used to be thought to be nonfunctional, but in recent years, functional ncRNAs have played important roles in higher order chromosome dynamics, embryonic stem cell differentiation, telomere biology, and subcellular structure. The potential properties of ncRNAs as a diagnostic and healing biomarker or therapeutic target for cancer have also been extensively studied (37).

Tumor cells secrete exosomal ncRNAs that form RNA-induced gene silencing complexes (RISCs), which effectively silence mRNAs at high rates in recipient cells and reprogram the transcriptome (38). Among the exosome-encapsulated ncRNAs, miRNAs is a universal non-coding single-stranded RNA that controls post-transcriptional gene expression by binding to complementary sequences in the 3 'untranslated region (UTR) of the target messenger RNA (mRNA) (39). Specific miRNAs expression and interference can be used to study the role of miRNAs in cancer development and progression (40). MiRNAs is closely related to cell development, proliferation, apoptosis, aging, carcinogenesis, lipid metabolism and viral infection (41). During tumor formation, overexpressed miRNAs can act as oncogenes, and conversely, low-expressed miRNAs are considered as tumor suppressor genes. It is worth mentioning that miRNAs are not randomly present among exosomes, but are directed to specific exosomes by a specific sorting mechanism dominated by parental cells. Therefore, there is a significant difference in exosomal miRNAs expression between normal and tumor tissues during cancer diagnosis, making miRNAs specific biomarkers designed for application in targeted cancer therapy (42). Exosomal lncRNAs can also serve as biomarkers of tumors to provide help for tumor therapy. Exosomal AFAP1-AS1 could induce trastuzumab resistance by binding to AUF1 and promoting ERBB2 translation. Therefore the level of AFAP1-AS1 may be used to predict trastuzumab resistance and breast cancer treatment (43). Consequently, the study of ncRNAs in tumor cell exosomes is of great significance for the diagnosis and treatment of tumors.

Natural drugs regulate tumor genesis and development by regulating exosomal ncRNAs

Tumor development is inseparable from the TME (44, 45). Altering the TME and inhibiting angiogenesis directly affects the migration and invasion of tumor cells (46, 47). In addition,

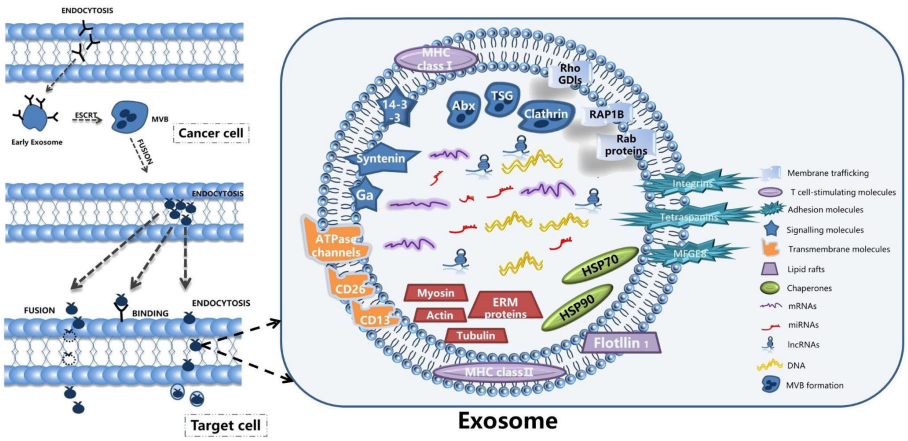


FIGURE 1
The structure and secretion mode of exosomes.

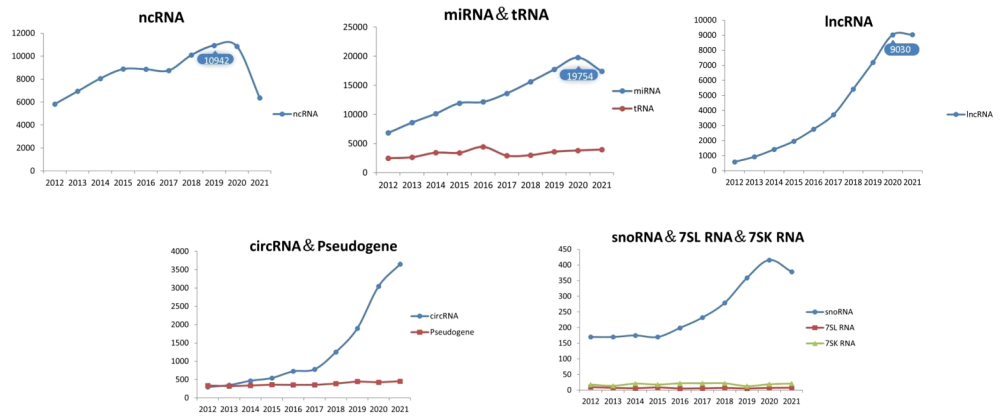


FIGURE 2
Number of articles related to ncRNAs.

tumor cells are often accompanied by abnormal mechanisms of apoptosis, and selective induction of apoptosis using modern research techniques is also a fundamental strategy for cancer treatment. Exosomal ncRNAs, as outstanding antitumor factors, have been well documented to interfere with cancer progression and many other aspects (48). Table 1 lists detailed examples of natural products that regulate exosomal ncRNAs to influence cancer progression.

Natural drugs improve the TME by regulating exosomal ncRNAs

Tumor cells and their microenvironment are a functional whole and both interact with each other to promote tumorigenesis (71). TME plays an important role in tumor growth, metabolism and metastasis, and even influences the therapeutic effect of anti-cancer drugs (72). In recent years, an increasing number of natural products exert anti-cancer effects by interfering with the TME, as shown in detail in Figure 3.

Cancer-associated fibroblasts (CAFs) are considered to be one of the key stromal cells of tumors. It has been reported that Matrine inhibit CRC tumorigenesis by blocking the release of CAFs exosome circSLC7A6, and chemokine receptor CXCR5 is a key effector of circSLC7A6 in regulating tumor formation (49).

In vitro experiments showed that Indian currant extract (Emblica officinalis, AE) upregulated miR-375 and adhesion protein e-cadherin, and downregulated insulin-like growth factor 1 receptor (IGF1R) and epithelial mesenchymal transition (EMT) factor SNAIL1. AE targets IGF1R and SNAIL1 by activating miR-375 in osteoblasts (50).

Activation of hepatic stellate cells (HSC) is an important driver of liver fibrosis and is closely associated with the formation of hepatocellular carcinoma. Exosomes from natural killer (NK) cells attenuate TGF-β 1-induced stellate cell activation. The secondary metabolite rapamycin, secreted by Streptomyces soil, inhibits miR-223 expression in natural killer exosomes (NK-EXO) (51).

Tumor-associated macrophages (TAM) play an important role in the TME. In particular, NF-κB expression affects M2 macrophages and promotes tumor progression. Tumor-derived exosomes can regulate the TME by transferring miRNAs to immune cells. Gallate (EGCG) upregulates exosomal miR-16 secreted by tumor cells, prevents its metastasis to TAM, and inhibits TAM infiltration and M2 polarization (52).

Basal-like ductal carcinoma *in situ* (DCIS) contains cancer stem cell-like cells (CSCs) with a high migration potential. Sulforaphane targets DCIS stem cell-like cells, decreases aldehyde dehydrogenase 1 (ALDH1) expression, and reduces mammary and progenitor cell colony formation. Differentially expressed miR-140 in exosomes secreted by DCIS affects signal transduction in nearby breast cancer cells (53).

Docetaxel increased the expression of miR-9-5p, miR-195-5p, and miR-203A-3p in circulating extracellular vesicles (EVs), decreased ONECUT2 expression, and increased the levels of related genes in xenograft mammary tumor-bearing mice (53). It has also been reported that resveratrol-mediated exosomal lncRNAs SNHG29 inhibit HCC progression by suppressing autophagy and Wnt/β-catenin pathway activation (54).

Natural drugs regulate exosomal ncRNAs to inhibit angiogenesis

Angiogenesis is critical to the development of cancer and is the primary means of nutrient acquisition for tumors. Without vascular supply, tumors will not exceed 2 mm in diameter, which is the result of poor metabolism caused by inadequate nutrient and oxygen supply (46). Research has demonstrated that inhibition of

TABLE 1 Detailed example of natural products affecting tumor exosomal ncRNAs.

Natural Product	Types of cancer/ Associated with cancer	ncRNA	Target Genes	Related Hallmark	Reference
Matrine	Colorectal Cancer	circSLC7A6	Matrine	Inhibits tumorigenesis	49
Emblica officinalis	Ovarian Cancer	miR-375	IGF1R/ SNAIL1	Regulation of TME	50
Rapamycin	Liver Fibrosis	miR-223	TGF-β	Suppressing autophagy	51
Epigallocatechin gallate	Cancer	miR-16	NF-κB	Inti-TAM/Inti-M2 polarization	52
Sulforaphane	Ductal Carcinoma In Situ	miR-140	ALDH1	Reduce cell colonies	53
Docetaxel	Breast Cancer	miR-9-5p,miR-195-5p,miR-203a-3p	ONECUT2	Dormant cells	53
Resveratrol	Liver Cancer	lncRNA SNHG29	Wnt/β-catenin	Anti-autophagy	54
G-rg1	Cancer blood vessels	miR-126-5P, miR-146a-5P,miR-210, miR-214-5P	VEGF	Regulate endothelial angiogenesis	55
Docosahexaenoic acid	Lymphoma	miR-34a,miR-125b,miR-221,miR-222/miR-9,miR-17-5p,miR-19,miR-126,miR-130,miR-132,miR-296,miR-378	VEGF	Anti-angiogenesis	56
Astragaloside IV	Cancer	miRNA-126	Exosome	Improve secretory exosomes	57
D Rhamnose β-hederin	Breast Cancer	miR-130a,miR-425	Exosome	Anti-proliferation	58
Shikonin	Breast Cancer	miR-128	Bax	Anti-proliferation	59
Lipopolysaccharide	Colorectal Cancer	miR-200c-3p	ZEB-1	Anti-migration/invasion/Anti-proliferation	60
Propofol	Liver Cancer	lncRNA H19,miR-520a-3p	LIMK1	Promoted the proliferation/ Migration and invasion/Inhibited the apoptosis	61
Tanshinone II A	Laryngocarcinoma	miR-656-3p	apoptin	Anti-proliferation and invasion	62
Olive oil polyphenol hydroxytyrosol	Chronic Inflammation	miR-155-5p,miR-34a-5p,let-7c-5p	NF-κB	Anti- recruitment	63
Tazemetostat	Lymphoma	miR-378a-3p,miR-378d	Dickkopf 3/NUMB	Regulatory resistance	64
Docetaxel	Tongue Squamous Cell Aarcinoma	miR-200c	TUBB3/ PPP2R1B	Regulatory resistance/Migration and invasion	65
Taxane	Ovarian Cancer	miR-200c	TUBB3	Regulatory resistance	65
Docetaxel	Ovarian Cancer	miR-146a	LAMC2	Regulatory resistance	66b
Docetaxel	Prostate Cancer	circ-XIAP,miR-1182	TPD52	Regulatory resistance	67
Docetaxel	Prostate Cancer	miR-27a	P53	Regulatory resistance	27
D Rhamnose β-hederin	Breast Cancer	miR-16,miR-23a,miR-24, miR-26a,miR-27a	D/exo	Regulatory resistance	68
β-elemene	Breast Cancer	miR-34a,miR-452	PTEN/Pgp	Regulatory resistance	69
Adriamycin	Breast Cancer	H19	miR-152/ DNMT1	Decrease cell viability/Induction of apoptosis/Regulatory resistance	70

angiogenesis can inhibit tumor growth and metastasis (73). Thus angiogenesis has emerged as a target for cancer therapy (74).

Xiong et al. proposed the effects of ginsenoside RG-1 and astragaloside on angiogenesis. Among them, ginsenoside RG-1 has the property of mediating miRNA-126-5P, miRNA-146A-5P, miRNA-210 and miRNA-214-5P to regulate endothelial angiogenesis (55), while Astragaloside IV increased human endothelial progenitor cells (EPC) secretion of exosomal miRNA-126-3p and miRNA-126-5p expression and promote angiogenesis (57).

Docosahexaenoic acid (DHA, ω 3:22-6) is a known omega-3 fatty acid derived from animal (e.g., fish oil) and plant sources (e.g., flaxseed oil) that plays an important role in influencing angiogenesis. DHA treatment of cells increased the expression levels of tumor suppressors' miR-101, miR-199 and miR-342 and decreased the expression levels of miR-382 and miR-21. The secretion of exosomes was significantly reduced by DHA treatment under either normal or hypoxic conditions (75). The exosomal let-7a, miR -23b, miR -27a/b, miR -21, let-7 and miR -320b have anti-cancer or anti-angiogenic activity. DHA inhibits angiogenesis by altering exosome secretion

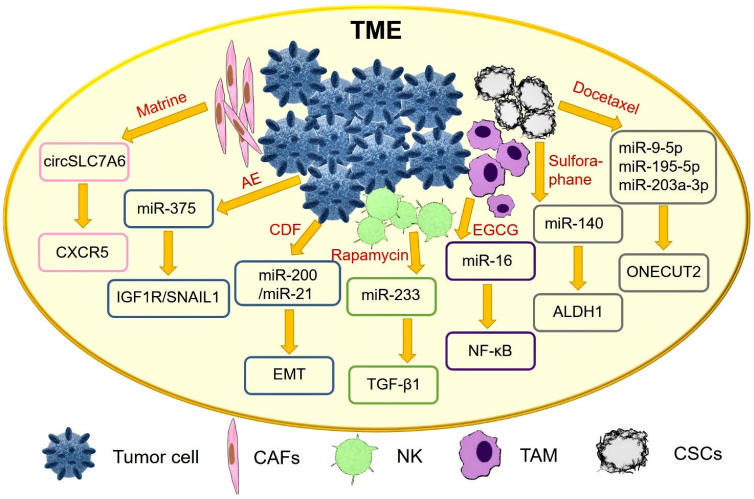


FIGURE 3
Natural drugs improve the TME by regulating exosomal ncRNAs.

and miRNA content in breast cancer cell lines (MDA-MB-231, ZR751 and BT20). After DHA treatment of MCF7 cells, their exosomes were applied directly to endothelial cell culture, and the expression of exosomal miRNAs in endothelial cells was increased (76). DHA treatment has been reported to lead to a significant reduction in VEGF expression and secretion in BC cells. The expression of anti-angiogenic miRNAs (miR-34a, miR-125b, miR-221 and miR-222) was increased and the expression of pro-angiogenic miRNAs (i.e. miR-9, miR-17-5p, miR-19a, miR-126, miR-130a, miR -132, miR-296 and miR-378) was decreased in TDE (DHA+) exosomes of DHA-treated BC cells. DHA reverses the therapeutic efficacy of these miRNAs from promoting angiogenesis to inhibiting angiogenesis by upregulating the content of exosomal miRNAs to achieve the purpose of modifying angiogenesis (56). All the above studies indicate that natural drugs have more complex forms of action, and people need to conduct deeper research on their pharmacological effects in order to achieve targeted treatment of diseases.

Natural drugs regulate exosomes ncRNA to promote tumor cell apoptosis or inhibit proliferation

Inhibition of apoptosis is an important basis for tumorigenesis, which disrupts the balance between cell proliferation and apoptosis in normal tissues (77). Since the body cannot normally carry out the process of cell proliferation and apoptosis, the number of cells continues to increase, resulting in the formation of tumors. For tumors with the same proliferative capacity, the decrease in apoptosis rate also increases the net growth rate of tumor cells (78).

Natural products inhibit tumor cell proliferation by regulating exosomal ncRNAs as shown in detail in Figure 4. The active ingredient d Rhamnose β-hederin (DRβ-H) showed anti-proliferative and pro-apoptotic activities in human breast cancer cells (McF-7/S), and DRβ-H inhibited the growth of human breast cancer cells by suppressing the secretion of exosomal miR-130a and miR-425 (58).

Shikonin is a naphthoquinone isolated from the traditional Chinese medicine Lithospermum, inhibits the proliferation of McF-7 breast cancer cells by reducing the tumor-derived exosomal miR-128, which has the property of negatively regulating Bax levels in McF-7 receptor cells (59).

Lipopolysaccharide (LPS) is an abundant component of the gut microbiota and has now been shown to be involved in CRC progression and metastasis by regulating exosomal miRNAs composition of CRC origin. In the presence of LPS, exosomal miR-200c-3p negatively regulates the migration and invasive ability of HCT-116 cells and promotes their apoptosis (52).

During the development of hepatocellular carcinoma, circulating H19 promotes proliferation, migration and invasion and inhibits apoptosis in isoproterenol-treated hepatocellular carcinoma cells by sponging miR-520a-3p upregulating LIMK1 (61). Tanshinone IIA also upregulated the expression level of miR-656-3p in the exosomes of CAFs and inhibited the proliferation and invasion of laryngeal cancer cells (62).

We know that chronic inflammation can induce a range of inflammatory diseases, including malignancies (79). A review of the literature revealed that natural products affect the inflammatory response by regulating exosomal ncRNAs. For example, olive oil polyphenol hydroxytyrosol (HT) significantly abolished the expression of miR-155-5p, miR-34a-5p and let-7c-

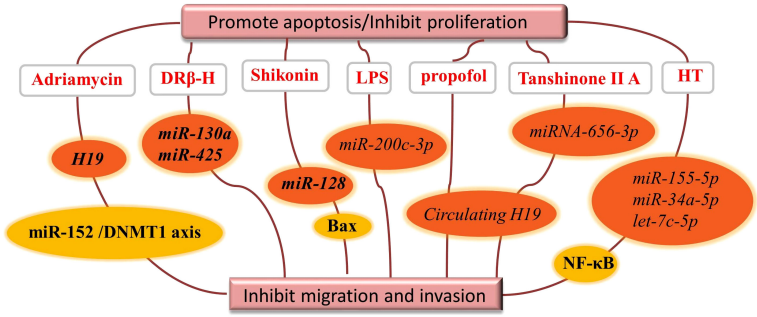


FIGURE 4
Natural drugs regulate exosomal ncRNAs to promote tumor cell apoptosis or inhibit.

5p in cells and exosomes and inhibited NF-κB activation and reactive oxygen species production. HT inhibited macrophage aggregation and improved chronic inflammatory inflammation in adipose tissue. MiR-155 was found to be an important factor in hepatocyte and macrophage in autophagy and exosome production of alcohol-related mediators (63).

Nowadays, the extensively studied natural fluorescent anthracycline antibiotic Adriamycin is a broad-spectrum antitumor antibiotic produced by the actinomycete Caesius. Increased expression of long-stranded ncRNA H19 in adriamycin-resistant breast cancer cells compared to the corresponding parental cells reduced cell viability and colony formation capacity and induced apoptosis. Another study pointed out that exosomal H19 promotes proliferation and invasion of breast cancer using miR-152/DNMT1 axis, providing a new mechanism for breast cancer development (70, 80).

Altogether, extensive evidence suggests that the pro-apoptotic and anti-proliferative properties of natural drugs are at least partially derived from exosomal ncRNAs.

Natural drugs regulate exosomal ncRNAs to regulate drug resistance in tumor cells

Compared to normal patients, cancer patients secrete different levels of ncRNAs, which are ideal biomarkers for predicting early disease progression and drug resistance in cancer (81). Researchers have found that many ncRNAs expression profiles associated with drug resistance in tumor cells are concentrated in exosomes, and that exosomal ncRNAs can alter chemosensitivity, in part due to the successful transfer of multidrug resistance (MDR)-specific miRNAs between cells (82).

Docetaxel (DTX) is a semisynthetic product of precursors extracted from the needles of redbud (t. b accata L. Taxus). It has been reported that breast cancer cells resistant to DTX can pass on drug resistance by transferring specific miRNAs contained in exosomes to alter gene expression in sensitive cells (83). Exosomal miR-200c secreted by normal tongue epithelial cells (NTECs) is transported to HSC-3DR, making HSC-3DR more sensitive to DTX by binding TUBB3 and PPP2R1B. MiR-200c regulates HSC-3DR sensitivity to DTX by targeting TUBB3 and PPP2R1B. The researchers concluded that exosome-mediated miR-200c delivery may be an effective and promising strategy for the treatment of tongue squamous cell chemoresistance (84). In ovarian cancer, miR-200c sensitizes tumor cells to paclitaxel by binding TUBB3. Overexpression of miR-200c reversed cell resistance to DTX-mediated migration and invasion (65).In addition, exosomal miRNA-146a from mesenchymal stem cells (MSCs) increased the sensitivity of ovarian cancer cells to DTX and paclitaxel *via* the lamcc2-mediated PI3K/Akt axis (66). Exosomal CIRC-XIAP promotes DTX resistance of PCa by regulating miR-1182/TPD52 axis, providing a promising therapeutic target for PCa chemotherapy (67). It was also reported that treatment of SCS -27 cells with DTX resulted in a significant increase in the level of exosomal miR-27a expression and inhibition of P53 gene expression, which contributed to the enhancement of chemoresistance in PCa (27).Several abundant miRNAs (miR-16, miR-23a, miR-24, miR-26a and miR-27a) were transported by exosomes secreted by breast cancer cells McF-7. DRβ-H extracted from the traditional Chinese medicinal plant Clematis ganpiniana, was able to reduce the expression of miR-16, miR-23a, miR-24, miR-26a and miR-27a transported by D/exo and reverse the DTX resistance (68).

New studies have shown that exosomal ncRNAs are mediators of intercellular communication between heterogeneous tumor cell populations, and that some exosomal ncRNAs promote tumor cell resistance to natural products during the communication process, and that only inhibition of constant shuttling of ncRNAs can improve tumor cell drug resistance (85). In the treatment of breast cancer, β-elemene mediates MDR-related miR-34a and miR-452 in cells and regulates the expression of target genes PTEN and PGP,

reducing chemoresistance transmission through exosomes and reversing drug resistance in breast cancer cells (86). Zeste homolog 2 (EZH2)-enhanced STAT3 binds to the promoter regions of miR-378a-3p and miR-378d, thereby increasing their expression in exosomes. Exosomes produced by BC cells after stimulation with DOX or PTX deliver miR-378a-3p and miR-378d to neighboring cells to activate the WNT and NOTCH stemness pathways and induce drug resistance by targeting Dickkopf 3 (DKK3) and NUMB. Tazemetostat, an EZH2 inhibitor, is an epigenetic drug whose combination with chemotherapeutic agents can reverse chemotherapy-induced resistance (64). Additionally, exosomal SNHG7 activated the phosphatidylinositol 3-kinase (PI3K)/AKT pathway to promote M2 polarization in macrophages *via* recruiting cullin 4A (CUL4A) to induce ubiquitination and degradation of phosphatase and tensin homolog (PTEN). Silencing of SNHG7 enhances the potency of doxorubicin and inhibits proliferation and autophagy in LUAD cells (84). Perhaps exosome-mediated resistance reversal is one of the mechanisms by which it acts as a vehicle to enhance the efficacy of drugs.

Exosomes of natural origin for cancer prevention and treatment

Exosomes can act as natural products to exert pharmacological activity, that is, to target lesions. In existing studies, there are numerous reports of exosomes carrying ncRNAs that regulate relevant metabolic pathways to influence disease.

Exosomal miR-133b may inhibit tumor growth *in vivo* through upregulation of dual protein phosphatase 1 (DUSP1). Increased expression of exosomal miR-133b leads to inhibition of bladder cancer cell viability and increased apoptosis (87). MiR-139-5p is a mesenchymal stem cell-derived exosomal ncRNA that inhibits bladder cancer development *in vitro* and *in vivo* (88).Exosomal miR-499 not only significantly inhibited endometrial cancer cell proliferation and endothelial cell tube formation *in vitro*, but also tumor growth and angiogenesis *in vivo* (89). In addition, in exosomes derived from tumor-associated macrophages, miR-192-5p overexpression effectively inhibited EC progression by regulating EC apoptosis and EMT and inhibiting the IRAK1/NF-κB signaling pathway (90). CAF-secreted exosomal miR-320a was directly transferred to EC cells, inhibiting their proliferation and leading to downregulation of HIF1α and decreased VEGFA expression *in vitro* (91). For breast, lung, and oral squamous cell carcinomas, the normal cell-secreted exosomal PTENP1 mediates intercellular communication by promoting apoptosis in breast cancer cells as well as inhibiting invasion and migration (92).

Exosome-mediated intercellular communication influences several features of cancer, including regulation of the immune response, reprogramming of stromal cells, remodeling of the structure of the extracellular matrix, and even conferring characteristics of drug resistance to cancer cells (93, 94). Uptake of exophytic vesicles by tumor cells can alter gene expression and reduce cancer-associated phenotypes (95). Similar to animal exosomes, plant exosomal vesicles contain specific lipids, proteins, nucleic acids, and other components, each of which may play a role in the development of disease by performing the corresponding biological functions through specific mechanisms. Similar exosomal nanoparticles isolated from citrus limoncello juice inhibit lipid metabolism, leading to a significant downregulation of acetyl coenzyme a carboxylase 1 (ACACA), thereby inhibiting tumor cell growth *in vitro* and *in vivo* (96). Mango Nano Capsules inhibit chronic myeloid leukemia (CML) tumor growth *in vivo* by specifically reaching the tumor site and activating TRAIL-mediated apoptotic cell processes (97). Berry-derived anthocyanins significantly enhance their proliferative activity against ovarian cancer cell growth and inhibit tumor growth more effectively (98). Natural plant exosomes are rich in miRNAs, and research on miRNAs in common plants is expanding and intensifying. A growing number of studies suggest that exosomal miRNAs may interact with mammalian cancer-related systems as novel bioactive components. Ginger-derived nanoparticles are a novel and effective drug that blocks the assembly and activation of pyrane-structured domain NLRP3 inflammatory vesicles (99). Exosomal MDO-miR7267-3p ameliorates colitis in mice through an il-22-dependent mechanism (100), and regulate AhR expression by inducing miR-375 and VAMP7 to prevent high-fat diet-induced insulin resistance and obesity (101). In addition, ginger-derived nanoparticles also inhibited LPS-induced inflammatory responses through downregulation of NF-κβ, IL-6, IL-8 and TNF-α (102). In conclusion, numerous natural exosomes have been found to be used for cancer prevention and treatment, which provides new ideas for the clinical treatment of cancer.

Exosomes ncRNAs as predictive biomarkers

Exosomes have potential as predictive biomarkers and play an important role in intercellular communication in cancer cells. MiRNAs inhibits translation of oncogenes and is involved in cancer development by regulating cell proliferation and differentiation (103). In some cases, dysregulated exosomal miRNAs can be used as "tumor markers" for disease diagnosis (103, 104). Table 2 shows the expression of exosomal ncRNAs in high-incidence cancers.

The expression of miRNAs in exosomes changed with altered physiological conditions. For example, when DNA damage was induced by the application of bleomycin, RNA molecules with relatively low expression levels (lincRNAs-p21, HOTAIR, ncRNAs-

TABLE 2 Detailed information on exosomal ncRNAs as biomarkers.

Types of cancer/Associated with cancer	ncRNA	Expression	Potential clinical value	Type of biomarker	Reference
DNA damage	lincRNA-p21, HOTAIR, ncRNA-CCND1	increased	Biomarker	Diagnosis	105
large B-cell lymphoma	miR-99a-5p, miR-125b-5p	increased	Biomarker	Diagnosis	106
large B-cell lymphoma	miR-483-3p, miR-451a	decreased	Biomarker	Diagnosis	107
large B-cell lymphoma	miR-379-5p, miR-135a-3p, miR-4476	increased	Biomarker	Diagnosis	107
Anaplastic large cell lymphoma Burkitt lymphoma Hodgkin lymphoma mature B-cell acute lymphoblastic leukemia	miR-191-5p	increased	Biomarker	Diagnosis	108
esophagus cancer	miR-21	increased	Biomarker	Diagnosis	69
glioblastoma	miR-21	increased	Biomarker	Diagnosis	109
colorectal cancer	let-7a, miR-1229, miR-1246, miR-150 miR-21, miR-223, miR-23a	increased	Biomarker	Diagnosis	110
Metastatic colorectal cancer	miR-25-3p	increased	Biomarker	Diagnosis	111
prostatic cancer	miR-1290, miR-375	increased	Biomarker	Prognosis	112
prostatic cancer	miR-99a-5p	decreased	Biomarker	Diagnosis	113
prostatic cancer	miR-21-5p	increased	Biomarker	Diagnosis	114
prostatic cancer	miR-200c-3p	decreased	Biomarker	Diagnosis	114
prostatic cancer	miR-96-5p, miR-183-5p	increased	Biomarker	Diagnosis	115
endometrial cancer	miR-15a-5p	increased	Biomarker	Diagnosis	116a
ovarian cancer	miR-21, miR141	increased	Biomarker	Diagnosis	117
melanoma	miR-494	increased	Biomarker	Diagnosis	118
melanoma	miR-1180-3p	decreased	Biomarker	Diagnosis	119
melanoma	miR-143, 221	increased	Biomarker	Diagnosis	120
DM-ILD-MDA5 Ab(+)	hsa-miR-1228-5p	increased	Biomarker	Diagnosis	121
DM-ILD-MDA6 Ab(+)	hsa-miR-4488	increased	Biomarker	Diagnosis	
renal cell carcinoma	miR-30c-5p	increased	Biomarker	Diagnosis	122
oral squamous cell carcinoma	miR-365	increased	Biomarker	Diagnosis	123
gastric carcinoma	miR-let-7	increased	Biomarker	Diagnosis	124
gastric carcinoma	miR-328-3p, miR-339-5p	increased	Biomarker	Prognosis	125
gastric carcinoma	miR-1-3p, miR-151a-3p, miR-184 miR-202-5p, miR-34c-5p, miR-3470a miR-3470b, miR-466i-5p	decreased	Biomarker	Prognosis	125
non-small cell lung cancer	let-7f, miR-20b, miR-30e-3p	decreased	Biomarker	Diagnosis	126
non-small cell lung cancer	hsa-miR-320d, hsa-miR-320c hsa-miR-320b	increased	Biomarker	Diagnosis	127
non-small cell lung cancer	hsa-miR-125b-5p	increased	Biomarker	Diagnosis	127
liver cancer	miRNA-21, lncRNA-ATB	increased	Biomarker	Prognosis	128
liver cancer	miR-34a	decreased	Therapeutic target	Prognosis	129

CCND1) were highly enriched in exosomes, reflecting changes in the expression levels of ncRNAs after cells are exposed to the drug (105). For diffuse large B-cell lymphoma, exosomal miR-99a-5p and miR-125b-5p can be used as predictive biomarkers of chemotherapy resistance (106). In contrast, the exosomal miR-483-3p and miR-451a serve as potential biomarkers for monitoring patient response to treatment (107). In lymphoma, exosomal miR-191-5p expression was higher (108). For colorectal cancer, a group of exosomal miRNAs including let-7a, miR-1229, miR-1246, miR-150, miR-21, miR-223 and miR-23a can be used as diagnostic biomarkers for colorectal cancer (110). And the expression level of exosomal miR-25-3p was also significantly correlated with colorectal cancer cell metastasis (111). Exosomal miR-21 is a potential marker for patients with esophageal cancer (69). Similarly, its level in glioblastoma is higher than normal (109). Urinary miR-21-5p and miR-200c-3p can be used as potential non-invasive biomarkers for prostate cancer patients (114). Another group of miR-1290 and miR-375 could be used as prognostic

markers for trend resistant prostate cancer (112). Meanwhile, miR-99a-5p was downregulated in prostate tumor tissues (113). In breast cancer patients, urinary exosomal miR-96-5p and miR-183-5p expression levels were higher (115). Plasma-derived exosomal miR-15a-5p has been reported to be a promising biomarker for the diagnosis of endometrial cancer (116), and the expression levels of miR-21 and miR141 in benign tumor exosomes were different from those in ovarian cancer (117). For melanoma cells, increased expression of exosomal miR-494 and downregulated expression of Rab27A inhibited tumor growth and metastasis (118). Besides, the level of exosomal miR-1180-3p was negatively correlated with the proliferation, migration and invasion of melanoma cells, and reduced the high expression of ST3GAL4 in melanoma cells (119). In addition, miR-143 and miR-221 were significantly increased in plasma exosomes of patients with metastatic melanoma (120). In patients with dermatomyositis (DM)-associated interstitial lung disease (ILD) combined with anti-melanoma differentiation-associated protein 5 antibody (MDA5), compared with myositis-specific antibody-negative patients without ILD (DM-nonILD - msa16(-)) and normal human controls (HC), hsa- miR-4488 was significantly upregulated and hsa-miR-1228-5P was upregulated in DM-ILD-MDA5 Ab(+) and downregulated in DM-nonILD-MSA16(-) compared to HC (121). Song et al. reported that urinary exosomal miR-30C-5p overexpression inhibited the progression of renal cell carcinoma (ccRCC). MiR-30c-5p prevented the depletion of heat shock protein 5 and reversed the growth-promoting effect of ccRCC (122). Elevated miR-365 levels in oral squamous cell carcinoma exosomes can be used as a potential biomarker for oral squamous cell carcinoma exosomes and EVs (123). In studies related to gastric cancer, members of the exosomal miR-let-7 family were most abundantly expressed in AZ-P7A cells (124). Others explored the changes in the expression of exosomes miR-328-3p, miR-339-5p, miR-1-3p, miR-151a-3p, miR-184, miR-202-5p, miR-34c-5p, miR-3470a, miR-3470b, and miR-466i-5p in gastric cancer cells (125). Hsa-miR-320d, hsa-miR-320c and hsa-miR-320b are considered potential biomarkers for predicting the efficacy of immunotherapy in advanced NSCLC, and the levels of let-7f, miR-20b and miR-30e-3p are lower in plasma vesicles of NSCLC patients than in normal controls (126), when the T-cell suppressor hsa-miR-125b-5p was downregulated during treatment, patients had increased T-cell function and responded well to immunotherapy (127). Circulating exosomal miRNA-21 and lncRNAs-ATB are new prognostic markers and therapeutic targets for HCC (128). In addition, exosomal circRNAs secreted by adipocytes promoted HCC tumor growth and reduced DNA damage by inhibiting miR-34a and activating the USP7/Cyclin A2 signaling pathway (129).

Exosomes carry a wealth of bioinformatics molecules and are actively involved in mediating a variety of biological functions. A growing number of studies have shown that the use of exosomes as therapeutic targets for cancer can improve the efficiency of cancer treatment. For example, metastasis of exosomal MDR-1/P-GP excreted by prostate cancer cells resulted in docetaxel resistance to DU145, 22Rv1 and LNCap cells. Serum exosomes induced increased cell proliferation and invasion in prostate cancer patients compared to controls (130). Signaling between preadipocytes and breast cancer cells has been found to promote breast tumor formation and metastasis. Exosomes secreted by preadipocytes are an important component of the tumor stem cell ecotone and regulate differentiation and migration in the TME through the critical miR-140/SOX2/SOX9 axis. Targeting exosome-related signaling may help to block tumor progression (131). And cd147-positive exosomes from epithelial ovarian cancer cells promote endothelial angiogenesis *in vitro* (132). In summary, we speculate that exosomal ncRNAs will develop in two directions in the future: firstly, as a new clinical malignancy marker to help timely detection and diagnosis of cancer, and secondly, as a tumor therapeutic target to help alleviate clinical disease symptoms.

Discussion

Natural products have biomolecules and drug targets that have natural affinities and natural possibilities to participate in various physiological processes in living organisms, which will contribute to the discovery of additional and deeper drug mechanisms.

For tumor treatment, the development of new drug delivery strategies to accurately eliminate cancer cells has become the focus of conquering tumors. At present, the carriers of natural drugs such as curcumin and vogonin in the treatment of cancer include liposomes (133, 134), phospholipid complexes, emulsion systems (135) and metastases (136, 137). However, these drug delivery systems have problems such as low utilization rate and incomplete efficacy. Exosomes have the advantages of low immunogenicity, good biocompatibility, high drug-carrying capacity and long life span, and tumor cell-derived exosomes have great potential as drug carriers for targeting parental tumors (138–143). One investigator used DTX to select effective exosome loads by electroporation to deliver small molecules and siRNAs to tumor sites. (144).

In addition to acting as a vector for drugs to increase efficacy, exosomes themselves can be involved in the diagnosis and treatment of cancer as markers or anti-cancer drugs. In the TME, exosomes selectively wrap ncRNAs and are involved in cell-cell information exchange. Exosomes promote cancer progression and migration through this cell-to-cell communication. For instance, exosome MicroRNA-103 can promote proliferation and invasion of liver cancer cells (145). Numerous studies have shown that abnormalities in the secretion of exosomes occur during the development of cancer,

providing a theoretical basis for the use of exosomes in early cancer screening (146). Recent studies have found that exosomes derived from bone marrow MSCs carry ncRNAs that can suppress cancer, including lncRNA PTENP1, which can suppress bladder cancer, and microRNA-551b-3p, which can suppress breast cancer. (147, 148).

Natural products as drugs have advantages such as low toxicity and multiple targets. Drugs found in natural products such as paclitaxel and pergolide have excellent therapeutic effects, but their mechanisms of action are not fully understood, and many natural products and traditional herbs are believed to have good anti-tumor effects. The vast majority of studies have discussed the role of natural products in inhibiting cell migration and value addition during tumorigenesis, and also discussed the role of natural products in tumor microenvironment and immune regulation, but their specific anti-cancer mechanisms have not been elucidated. Natural products are complex in composition and have multiple targets in cancer. Great progress has been made in recent years in the research of anti-tumor mechanisms of various monomeric components, such as curcumin, berberine, resveratrol, etc. The combination of these drugs with clinical anticancer drugs can delay the survival time of patients, reduce chemotherapy drug dosing, reverse drug resistance, and alleviate adverse effects. Relevant technologies regarding new drug development are constantly being improved, the level of drug research is increasing, and potential antitumor natural products are being explored. It is crucial to further elucidate the anticancer pharmacological effects of natural products and to give full play to their anticancer advantages. Exosomal ncRNAs are involved in the physiological and pathological processes of cancer by directly or indirectly regulating the expression of target genes, and play a key role in transcription and translation. The development of targeted anti-cancer drugs using exosomes not only can break the blood-brain barrier, but also avoid immune rejection by the human body (149).

Exosomes have a lipid bilayer structure that protects ncRNAs from degradation by ribonucleases, thus preserving their functional activity and facilitating specimen collection and storage. The enrichment of specific signaling molecules within the exosome membrane is more conducive to detection, as well as deep involvement in tumor events and closer to tumor nature, which is clinically important for achieving minimally invasive detection and early diagnosis and treatment of cancer. Compared to tumor exosome-specific proteins and lipids, exosomal ncRNAs have higher sensitivity and specificity and can be repeatedly sampled for large-scale assays.

Exosomal ncRNAs are a foothold for the development of new cancer diagnostic and therapeutic models, and it is important to identify and isolate exosomes secreted by tumor cells. However, at present, the technology of exosome isolation and purification is not mature enough to ensure the purity and activity of exosomes, and the establishment of a stable method for isolation and storage of exosomes is still an urgent problem. In addition, exosomes are secreted by a variety of cells, and it remains to be studied how to determine their source and the amount of ncRNA in exosomes is limited, and the technique to allow exosomes to precisely reach the tumor site needs to be improved. After literature survey, we found that the regulatory mechanisms of natural products using exosomal ncRNAs against cancer still need to be exhaustively demonstrated, and the specificity of exosomal ncRNAs as molecular markers for cancer diagnosis cannot be determined yet, and it is still difficult to fully resolve their regulatory networks. With the resolution of these unknown issues, the therapeutic application of exosomal ncRNAs in clinical practice for disease treatment will be realized soon.

Author contributions

SH was responsible for the writing of the manuscript, YL for the editing of the graphs and charts, SG for the editing and recording of the tables, and ZQ and DL for the revision and final review of the manuscript. All authors were involved in the creation and were responsible for the content of the work.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No. 81973712, 82003985). Jilin Province Science and Technology Development Project in China (Grant No. 20210204013YY). Jilin Province Science and Technology Development Plan Project (Grant No. 20200708081YY).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistic. *CA Cancer J Clin* (2022) 72(1):7–33. doi: 10.3322/caac.21708

2. Dembic Z. Antitumor drugs and their targets. *Molecules* (2020) 25(23):5776. doi: 10.3390/molecules25235776

3. Hoey R, Sharpe E, Kukula A, Workman P. Enhancing access to innovative cancer drugs: Cross-sector consensus on a way forward to benefit patients. *Drug Discovery Today* (2022) 27(4):946–50. doi: 10.1016/j.drudis.2021.12.015

4. Ma L, Zhang M, Zhao R, Wang D, Ma Y, Li A. Plant natural products: Promising resources for cancer chemoprevention. *Molecules* (2021) 26(4):933. doi: 10.3390/molecules26040933

5. Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. *J Nat Prod* (2016) 79(3):629–61. doi: 10.1021/acs.jnatprod.5b01055

6. Devadasan V, Raman P, Dasararaju G. Anti-cancer compounds from terrestrial and marine resources -in silico and experimental studies. *Curr Comput Aided Drug Des* (2021) 17(7):865–80. doi: 10.2174/1573409916666200910140801

7. Vo TS. Natural products targeting FcepsilonRI receptor for anti-allergic therapeutics. *J Food Biochem* (2020) 44(8):e13335. doi: 10.1111/jfbc.13335

8. Dutta S, Mahalanobish S, Saha S, Ghosh S, Sil PC. Natural products: An upcoming therapeutic approach to cancer. *Food Chem Toxicol* (2019) 128:240–55. doi: 10.1016/j.fct.2019.04.012

9. Akyurekli C, Le Y, Richardson RB, Fergusson D, Tay J, Allan DS. A systematic review of preclinical studies on the therapeutic potential of mesenchymal stromal cell-derived microvesicles. *Stem Cell Rev Rep* (2015) 11 (1):150–60. doi: 10.1007/s12015-014-9545-9

10. Wang M, Zhang B. The immunomodulation potential of exosomes in tumor microenvironment. *J Immunol Res* (2021) 2021:3710372. doi: 10.1155/2021/3710372

11. Shao J, Zaro J, Shen Y. Advances in exosome-based drug delivery and tumor targeting: From tissue distribution to intracellular fate. *Int J Nanomed* (2020) 15:9355–71. doi: 10.2147/IJN.S281890

12. Yin J, Li Q, Sun LD, Yang Q, Zhao Z, Ran QS, et al. [Research advancement in natural anti-cancer product]. *Zhongguo Zhong Yao Za Zhi* (2019) 44(1):19–27. doi: 10.19540/j.cnki.cjcmm.20181107.001

13. Paine MF. Natural products: Experimental approaches to elucidate disposition mechanisms and predict pharmacokinetic drug interactions. *Drug Metab Dispos* (2020) 48(10):956–62. doi: 10.1124/dmd.120.000182

14. Calixto JB. The role of natural products in modern drug discovery. *Acad Bras Cienc* (2019) 91 Suppl 3:e20190105. doi: 10.1590/0001-3765201920190105

15. Yang Y, He PY, Zhang Y, Li N. Natural products targeting the mitochondria in cancers. *Molecules* (2020) 26(1):92. doi: 10.3390/molecules26010092

16. Sauter ER. Cancer prevention and treatment using combination therapy with natural compounds. *Expert Rev Clin Pharmacol* (2020) 13(3):265–85. doi: 10.1080/17512433.2020.1738218

17. Schneider P, Walters WP, Plowright AT, Sieroka N, Listgarten J, Goodnow RAJr., et al. Rethinking drug design in the artificial intelligence era. *Nat Rev Drug Discovery* (2020) 19(5):353–64. doi: 10.1038/s41573-019-0050-3

18. Fulda S. Inhibitor of apoptosis proteins as targets for anticancer therapy. *Expert Rev Anticancer Ther* (2007) 7(9):1255–64. doi: 10.1586/14737140.7.9.1255

19. Chen X, Li Q, Kan XX, Wang YJ, Li YJ, Yang Q, et al. Extract of caulis spatholobi, a novel blocker targeting tumor cell–induced platelet aggregation, inhibits breast cancer metastasis. *Oncol Rep* (2016) 36(6):3215–24. doi: 10.3892/or.2016.5184

20. Kaul R, Risinger AL, Mooberry SL. Microtubule-targeting drugs: More than antimitotics. *J Nat Prod* (2019) 82(3):680–5. doi: 10.1021/acs.jnatprod.9b00105

21. Bailly C. Cepharanthine: An update of its mode of action, pharmacological properties and medical applications. *Phytomedicine* (2019) 62:152956. doi: 10.1016/j.phymed.2019.152956

22. Lovelace ES, Polyak SJ. Natural products as tools for defining how cellular metabolism influences cellular immune and inflammatory function during chronic infection. *Viruses* (2015) 7(12):6218–32. doi: 10.3390/v7122933

23. Raeber ME, Rosalia RA, Schmid D, Karakus U, Boyman O. Interleukin-2 signals converge in a lymphoid-dendritic cell pathway that promotes anticancer immunity. *Sci Transl Med* (2020) 12(561):eaba5464. doi: 10.1126/scitranslmed.aba5464

24. Mashouri L, Yousefi H, Aref AR, Ahadi AM, Molaei F, Alahari SK. Exosomes: composition, biogenesis, and mechanisms in cancer metastasis and drug resistance. *Mol Cancer* (2019) 18(1):75. doi: 10.1186/s12943-019-0991-5

25. Bukowski K, Kciuk M, Kontek R. Mechanisms of multidrug resistance in cancer chemotherapy. *Int J Mol Sci* (2020) 21(9):3233. doi: 10.3390/ijms21093233

26. Seca AML, Pinto DCGA. Plant secondary metabolites as anticancer agents: Successes in clinical trials and therapeutic application. *Int J Mol Sci* (2018) 19 (1):263. doi: 10.3390/ijms19010263

27. Cao Z, Xu L, Zhao S. Exosome-derived miR-27a produced by PSC-27 cells contributes to prostate cancer chemoresistance through p53. *Biochem Biophys Res Commun* (2019) 515(2):345–51. doi: 10.1016/j.bbrc.2019.05.120

28. Xiang Y, Guo Z, Zhu P, Chen J, Huang Y. Traditional Chinese medicine as a cancer treatment: Modern perspectives of ancient but advanced science. *Cancer Med* (2019) 8(5):1958–75. doi: 10.1002/cam4.2108

29. Li YJ, Lei YH, Yao N, Wang CR, Hu N, Ye WC, et al. Autophagy and multidrug resistance in cancer. *Chin J Cancer* (2017) 36(1):52. doi: 10.1186/s40880-017-0219-2

30. To KKW, Cho WCS. Flavonoids overcome drug resistance to cancer chemotherapy by epigenetically modulating multiple mechanisms. *Curr Cancer Drug Targets* (2021) 21(4):289–305. doi: 10.2174/1568009621666210203111220

31. Deng LJ, Qi M, Li N, Lei YH, Zhang DM, Chen JX. Natural products and their derivatives: Promising modulators of tumor immunotherapy. *J Leukoc Biol* (2020) 108(2):493–508. doi: 10.1002/jlb.3mr0320-444r

32. Tang XH, Guo T, Gao XY, Wu XL, Xing XF, Ji JF, et al. Exosome-derived noncoding RNAs in gastric cancer: Functions and clinical applications. *Mol Cancer* (2021) 20(1):99. doi: 10.1186/s12943-021-01396-6

33. He C, Zheng S, Luo Y, Wang B. Exosome theranostics: Biology and translational medicine. *Theranostics* (2018) 8(1):237–55. doi: 10.7150/thno.21945

34. Mathieu M, Névo N, Jouve M, Valenzuela JI, Maurin M, Verweij FJ, et al. Specificities of exosome versus small ectosome secretion revealed by live intracellular tracking of CD63 and CD9. *Nat Commun* (2021) 12(1):1–18. doi: 10.1038/s41467-021-24384-2

35. Farooqi AA, Desai NN, Qureshi MZ, Librelotto DRN, Gasparri ML, Bishayee A, et al. Exosome biogenesis, bioactivities and functions as new delivery systems of natural compounds. *Biotechnol Adv* (2018) 36(1):328–34. doi: 10.1016/j.biotechadv.2017.12.010

36. Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *J Nat Prod* (2020) 83(3):770–803. doi: 10.1021/acs.jnatprod.9b01285

37. Lai Y, Feng B, Abudoureyimu M, Zhi Y, Zhou H, Wang T, et al. Non-coding RNAs: Emerging regulators of sorafenib resistance in hepatocellular carcinoma. *Front Oncol* (2019) 9:1156. doi: 10.3389/fonc.2019.01156

38. Dutta A. Exosomes-based cell-free cancer therapy: A novel strategy for targeted therapy. *Immunol Med* (2021) 44(2):116–23. doi: 10.1080/25785826.2020.1818482

39. Hausser J, Zavolan M. Identification and consequences of miRNA-target interactions–beyond repression of gene expression. *Nat Rev Genet* (2014) 15 (9):599–612. doi: 10.1038/nrg3765

40. Satapathy S, Batra J, Jeet V, Thompson EW, Punyadeera C. MicroRNAs in HPV associated cancers: small players with big consequences. *Expert Rev Mol Diagn* (2017) 17(7):711–22. doi: 10.1080/14737159.2017.1339603

41. Hill M, Tran N. miRNA interplay: Mechanisms and consequences in cancer. *Dis Model Mech* (2021) 14(4):dmm047662. doi: 10.1242/dmm.047662

42. Sun Z, Shi K, Yang S, Liu J, Zhou Q, Wang G, et al. Effect of exosomal miRNA on cancer biology and clinical applications. *Mol Cancer* (2018) 17(1):147. doi: 10.1186/s12943-018-0897-7

43. Han M, Gu Y, Lu P, Li J, Cao H, Li X, et al. Exosome-mediated lncRNA AFAP1-AS1 promotes trastuzumab resistance through binding with AUF1 and activating ERBB2 translation. *Mol Cancer* (2020) 19(1):26. doi: 10.1186/s12943-020-1145-5

44. Liao Z, Tan ZW, Zhu P, Tan NS. Cancer-associated fibroblasts in tumor microenvironment - accomplices in tumor malignancy. *Cell Immunol* (2019) 343:103729. doi: 10.1016/j.cellimm.2017.12.003

45. Maacha S, Bhat AA, Jimenez L, Raza A, Haris M, Uddin S, et al. Extracellular vesicles-mediated intercellular communication: Roles in the tumor microenvironment and anti-cancer drug resistance. *Mol Cancer* (2019) 18(1):55. doi: 10.1186/s12943-019-0965-7

46. Viallard C, Larrivée B. Tumor angiogenesis and vascular normalization: Alternative therapeutic targets. *Angiogenesis* (2017) 20(4):409–26. doi: 10.1007/s10456-017-9562-9

47. Li X, Wenes M, Romero P, Huang SC, Fendt SM, Ho PC. Navigating metabolic pathways to enhance antitumour immunity and immunotherapy. *Nat Rev Clin Oncol* (2019) 16(7):425–41. doi: 10.1038/s41571-019-0203-7

48. Mirzaei H, Masoudifar A, Sahebkar A, Zare N, Sadri Nahand J, Rashidi B, et al. MicroRNA: A novel target of curcumin in cancer therapy. *J Cell Physiol* (2018) 233(4):3004–15. doi: 10.1002/jcp.26055

49. Gu C, Lu H, Qian Z. Matrine reduces the secretion of exosomal circSLC7A6 from cancer-associated fibroblast to inhibit tumorigenesis of colorectal cancer by regulating CXCR5. *Biochem Biophys Res Commun* (2020) 527(3):638–45. doi: 10.1016/j.bbrc.2020.04.142

50. De A, Powers B, De A, Zhou J, Sharma S, Van Veldhuizen P, et al. Emblica officinalis extract downregulates pro-angiogenic molecules *via* upregulation of cellular and exosomal miR-375 in human ovarian cancer cells. *Oncotarget* (2016) 7 (21):31484–500. doi: 10.18632/oncotarget.8966

51. Soubani O, Ali AS, Logna F, Ali S, Philip PA, Sarkar FH. Re-expression of miR-200 by novel approaches regulates the expression of PTEN and MT1-MMP in pancreatic cancer. *Carcinogenesis* (2012) 33(8):1563–71. doi: 10.1093/carcin/bgs189

52. Jang JY, Lee JK, Jeon YK, Kim CW. Exosome derived from epigallocatechin gallate treated breast cancer cells suppresses tumor growth by inhibiting tumor-associated macrophage infiltration and M2 polarization. *BMC Cancer* (2013) 13:421. doi: 10.1186/1471-2407-13-421

53. Li Q, Eades G, Yao Y, Zhang Y, Zhou Q. Characterization of a stem-like subpopulation in basal-like ductal carcinoma *in situ* (DCIS) lesions. *J Biol Chem* (2014) 289(3):1303–12. doi: 10.1074/jbc.M113.502278

54. Tong K. *Mechanisms of resveratrol-mediated inhibition of hepatocellular carcinoma progression by exosomes*. Hubei University of Medicine (2021). doi: 10.27913/d.cnki.ghyby.2021.000032

55. Xiong W, Tan M, Zou X, Bai X, C. H, Liang Y, et al. Effects of ginsenoside Rg1 on exosome secretion and angiogenesis related miRNAs expression of endothelial progenitor cells. *Lishizhen Med Materia Med Res* (2022) 33(02):277–80. doi: 10.3969/j.issn.1008-0805.2022.02.06

56. Ghaffari-Makhmalbaf P, Sayyad M, Pakravan K, Razmara E, Bitaraf A, Bakhshinejad B, et al. Docosahexaenoic acid reverses the promoting effects of breast tumor cell-derived exosomes on endothelial cell migration and angiogenesis. *Life Sci* (2021) 264:118719. doi: 10.1016/j.lfs.2020.118719

57. Xiong W, Bai X, Xiao H, Lan HW, Zhu CH, Zhao SQ, et al. Effects of astragaloside on exosome secretion and its microRNA-126 expression in human endothelial progenitor cells. *Lishizhen Med Materia Med Res* (2020) 36(12):1183–90. doi: 10.3760/cma.j.cn501120-20191222-00466

58. Chen WX, Cheng L, Pan M, Qian Q, Zhu YL, Xu LY, et al. D rhamnose β-hederin against human breast cancer by reducing tumor-derived exosomes. *Oncol Lett* (2018) 16(4):5172–8. doi: 10.3892/ol.2018.9254

59. Wei Y, Li M, Cui S, Wang D, Zhang CY, Zen K, et al. Shikonin inhibits the proliferation of human breast cancer cells by reducing tumor-derived exosomes. *Molecules* (2016) 21(6):777. doi: 10.3390/molecules21060777

60. Jiang Y, Ji X, Liu K, Shi Y, Wang C, Li Y, et al. Exosomal miR-200c-3p negatively regulates the migraion and invasion of lipopolysaccharide (LPS)-stimulated colorectal cancer (CRC). *BMC Mol Cell Biol* (2020) 21(1):48. doi: 10.1186/s12860-020-00291-0

61. Wang D, Xing N, Yang T, Liu J, Zhao H, He J, et al. Exosomal lncRNA H19 promotes the progression of hepatocellular carcinoma treated with propofol *via* miR-520a-3p/LIMK1 axis. *Cancer Med* (2020) 9(19):7218–30. doi: 10.1002/cam4.3313

62. Wang M, Wu C, Gu Y, L. H, Hu R, Zhang J, et al. Regulation of cancer-associated fibroblasts-derived exosomal miR-656-3p on the proliferation and invasion of laryngeal cancer and the influence of tanshinone IIA on the regulation. *Chin J Ophthalmol Otorhinolaryngol* (2022) 22(01):66–71+77. doi: 10.14166/j.issn.1671-2420.2022.01.015

63. Babuta M, Furi I, Bala S, Bukong TN, Lowe P, Catalano D, et al. Dysregulated autophagy and lysosome function are linked to exosome production by micro-RNA 155 in alcoholic liver disease. *Hepatology* (2019) 70 (6):2123–41. doi: 10.1002/hep.30766

64. Yang Q, Zhao S, Shi Z, Cao L, Liu J, Pan T, et al. Chemotherapy-elicited exosomal miR-378a-3p and miR-378d promote breast cancer stemness and chemoresistance *via* the activation of EZH2/STAT3 signaling. *J Exp Clin Cancer Res* (2021) 40(1):120. doi: 10.1186/s13046-021-01901-1

65. Cui J, Wang H, Zhang X, Sun X, Zhang J, Ma J. Exosomal miR-200c suppresses chemoresistance of docetaxel in tongue squamous cell carcinoma by suppressing TUBB3 and PPP2R1B. *Aging (Albany NY)* (2020) 12(8):6756–73. doi: 10.18632/aging.103036

66. Qiu L, Wang J, Chen M, Chen F, Tu W. Exosomal microRNA−146a derived from mesenchymal stem cells increases the sensitivity of ovarian cancer cells to docetaxel and taxane *via* a LAMC2−mediated PI3K/Akt axis. *Int J Mol Med* (2020) 46(2):609–20. doi: 10.3892/ijmm.2020.4634

67. Zhang H, Li M, Zhang J, Shen Y, Gui Q. Exosomal circ-XIAP promotes docetaxel resistance in prostate cancer by regulating miR-1182/TPD52 axis. *Drug Des Devel Ther* (2021) 15:1835–49. doi: 10.2147/dddt.S300376

68. Chen WX, Xu LY, Qian Q, He X, Peng WT, Fan WQ, et al. D rhamnose β-hederin reverses chemoresistance of breast cancer cells by regulating exosome-mediated resistance transmission. *Biosci Rep* (2018) 38(5):BSR20180110. doi: 10.1042/bsr20180110

69. Zhang J, Li S, Li L, Li M, Guo C, Yao J, et al. Exosome and exosomal microRNA: trafficking, sorting, and function. *Genomics Proteomics Bioinf* (2015) 13(1):17–24. doi: 10.1016/j.gpb.2015.02.001

70. Li Z, Li Y, Li Y, Ren K, Li X, Han X, et al. Long non-coding RNA H19 promotes the proliferation and invasion of breast cancer through upregulating DNMT1 expression by sponging miR-152. *J Biochem Mol Toxicol* (2017) 31(9): e21933. doi: 10.1002/jbt.21933

71. Sionov RV. Leveling up the controversial role of neutrophils in cancer: When the complexity becomes entangled. *Cells* (2021) 10(9):2486. doi: 10.3390/cells10092486

72. Bader JE, Voss K, Rathmell JC. Targeting metabolism to improve the tumor microenvironment for cancer immunotherapy. *Mol Cell* (2020) 78(6):1019–33. doi: 10.1016/j.molcel.2020.05.034

73. Cao J, Liu X, Yang Y, Wei B, Li Q, Mao G, et al. Decylubiquinone suppresses breast cancer growth and metastasis by inhibiting angiogenesis *via* the ROS/p53/BAI1 signaling pathway. *Angiogenesis* (2020) 23(3):325–38. doi: 10.1007/s10456-020-09707-z

74. Zhang C, Zhao W. The efficacy and safety of angiogenesis inhibitors for recurrent ovarian cancer: A meta−analysis. *J Ovarian Res* (2022) 15(1):99. doi: 10.1186/s13048-022-01028-7

75. Aslan C, Maralbashi S, Kahroba H, Asadi M, Soltani-Zangbar MS, Javadian M, et al. Docosahexaenoic acid (DHA) inhibits pro-angiogenic effects of breast cancer cells *via* down-regulating cellular and exosomal expression of angiogenic genes and microRNAs. *Life Sci* (2020) 258:118094. doi: 10.1016/j.lfs.2020.118094

76. Hannafon BN, Carpenter KJ, Berry WL, Janknecht R, Dooley WC, Ding WQ. Exosome-mediated microRNA signaling from breast cancer cells is altered by the anti-angiogenesis agent docosahexaenoic acid (DHA). *Mol Cancer* (2015) 14:133. doi: 10.1186/s12943-015-0400-7

77. Sinha R, El-Bayoumy K. Apoptosis is a critical cellular event in cancer chemoprevention and chemotherapy by selenium compounds. *Curr Cancer Drug Targets* (2004) 4(1):13–28. doi: 10.2174/1568009043481614

78. Tornero D, Posadas I, Ceña V. Bcl-x(L) blocks a mitochondrial inner membrane channel and prevents Ca2+ overload-mediated cell death. *PloS One* (2011) 6(6):e20423. doi: 10.1371/journal.pone.0020423

79. Jarboe T, Tuli NY, Chakraborty S, Maniyar RR, DeSouza N, Xiu-Min L, et al. Inflammatory components of the thyroid cancer microenvironment: An avenue for identification of novel biomarkers. *Adv Exp Med Biol* (2021) 1350:1–31. doi: 10.1007/978-3-030-83282-7_1

80. Wang X, Pei X, Guo G, Qian X, Dou D, Zhang Z, et al. Exosome-mediated transfer of long noncoding RNA H19 induces doxorubicin resistance in breast cancer. *J Cell Physiol* (2020) 235(10):6896–904. doi: 10.1002/jcp.29585

81. Guo C, Liu J, Zhou Q, Song J, Zhang Z, Li Z, et al. Exosomal noncoding RNAs and tumor drug resistance. *Cancer Res* (2020) 80(20):4307–13. doi: 10.1158/0008-5472.Can-20-0032

82. Li C, Xu X. Biological functions and clinical applications of exosomal non-coding RNAs in hepatocellular carcinoma. *Cell Mol Life Sci* (2019) 76(21):4203–19. doi: 10.1007/s00018-019-03215-0

83. Chen WX, Cai YQ, Lv MM, Chen L, Zhong SL, Ma TF, et al. Exosomes from docetaxel-resistant breast cancer cells alter chemosensitivity by delivering microRNAs. *Tumour Biol* (2014) 35(10):9649–59. doi: 10.1007/s13277-014-2242-0

84. Zhang K, Chen J, Li C, Yuan Y, Fang S, Liu W, et al. Exosome-mediated transfer of SNHG7 enhances docetaxel resistance in lung adenocarcinoma. *Cancer Lett* (2022) 526:142–54. doi: 10.1016/j.canlet.2021.10.029

85. Nakano I, Garnier D, Minata M, Rak J. Extracellular vesicles in the biology of brain tumour stem cells–implications for inter-cellular communication, therapy and biomarker development. *Semin Cell Dev Biol* (2015) 40:17–26. doi: 10.1016/j.semcdb.2015.02.011

86. Zhang J, Zhang HD, Yao YF, Zhong SL, Zhao JH, Tang JH. β-elemene reverses chemoresistance of breast cancer cells by reducing resistance transmission *via* exosomes. *Cell Physiol Biochem* (2015) 36(6):2274–86. doi: 10.1159/000430191

87. Cai X, Qu L, Yang J, Xu J, Sun L, Wei X, et al. Exosome-transmitted microRNA-133b inhibited bladder cancer proliferation by upregulating dual-specificity protein phosphatase 1. *Cancer Med* (2020) 9(16):6009–19. doi: 10.1002/cam4.3263

88. Jia Y, Ding X, Zhou L, Zhang L, Yang X. Mesenchymal stem cells-derived exosomal microRNA-139-5p restrains tumorigenesis in bladder cancer by targeting PRC1. *Oncogene* (2021) 40(2):246–61. doi: 10.1038/s41388-020-01486-7

89. Nie H, Xie X, Zhang D, Zhou Y, Li B, Li F, et al. Use of lung-specific exosomes for miRNA-126 delivery in non-small cell lung cancer. *Nanoscale* (2020) 12(2):877–87. doi: 10.1039/c9nr09011h

90. Wang Y, Ma H, Li Y, Su R. MiR-192-5p-Modified tumor-associated macrophages-derived exosome suppressed endometrial cancer progression through targeting IRAK1/NF-κB signaling. *Reprod Sci* (2022) 29(2):436–47. doi: 10.1007/s43032-021-00789-8

91. Zhang N, Wang Y, Liu H, Shen W. Extracellular vesicle encapsulated microRNA-320a inhibits endometrial cancer by suppression of the HIF1α/VEGFA axis. *Exp Cell Res* (2020) 394(2):112113. doi: 10.1016/j.yexcr.2020.112113

92. Zheng R, Du M, Wang X, Xu W, Liang J, Wang W, et al. Exosome-transmitted long non-coding RNA PTENP1 suppresses bladder cancer progression. *Mol Cancer* (2018) 17(1):143. doi: 10.1186/s12943-018-0880-3

93. Mazumdar A, Urdinez J, Boro A, Arlt MJE, Egli FE, Niederöst B, et al. Exploring the role of osteosarcoma-derived extracellular vesicles in pre-metastatic niche formation and metastasis in the 143-b xenograft mouse osteosarcoma model. *Cancers (Basel)* (2020) 12(11):3457. doi: 10.3390/cancers12113457

94. Caballero D, Abreu CM, Lima AC, Neves NN, Reis RL, Kundu SC. Precision biomaterials in cancer theranostics and modelling. *Biomaterials* (2022) 280:121299. doi: 10.1016/j.biomaterials.2021.121299

95. Nakase I, Takatani-Nakase T. Exosomes: Breast cancer-derived extracellular vesicles; recent key findings and technologies in disease progression, diagnostics, and cancer targeting. *Drug Metab Pharmacokinet* (2022) 42:100435. doi: 10.1016/j.dmpk.2021.100435

96. Raimondo S, Saieva L, Cristaldi M, Monteleone F, Fontana S, Alessandro R. Label-free quantitative proteomic profiling of colon cancer cells identifies acetyl-CoA carboxylase alpha as antitumor target of citrus limon-derived nanovesicles. *J Proteomics* (2018) 173:1–11. doi: 10.1016/j.jprot.2017.11.017

97. Raimondo S, Naselli F, Fontana S, Monteleone F, Lo Dico A, Saieva L, et al. Citrus limon-derived nanovesicles inhibit cancer cell proliferation and suppress CML xenograft growth by inducing TRAIL-mediated cell death. *Oncotarget* (2015) 6(23):19514–27. doi: 10.18632/oncotarget.4004

98. Aqil F, Jeyabalan J, Agrawal AK, Kyakulaga AH, Munagala R, Parker L, et al. Exosomal delivery of berry anthocyanidins for the management of ovarian cancer. *Food Funct* (2017) 8(11):4100–7. doi: 10.1039/c7fo00882a

99. Chen X, Zhou Y, Yu J. Exosome-like nanoparticles from ginger rhizomes inhibited NLRP3 inflammasome activation. *Mol Pharm* (2019) 16(6):2690–9. doi: 10.1021/acs.molpharmaceut.9b00246

100. Teng Y, Ren Y, Sayed M, Hu X, Lei C, Kumar A, et al. Plant-derived exosomal MicroRNAs shape the gut microbiota. *Cell Host Microbe* (2018) 24 (5):637–652.e638. doi: 10.1016/j.chom.2018.10.001

101. Kumar A, Ren Y, Sundaram K, Mu J, Sriwastva MK, Dryden GW, et al. miR-375 prevents high-fat diet-induced insulin resistance and obesity by targeting the aryl hydrocarbon receptor and bacterial tryptophanase (tnaA) gene. *Theranostics* (2021) 11(9):4061–77. doi: 10.7150/thno.52558

102. Yin L, Yan L, Yu Q, Wang J, Liu C, Wang L, et al. Characterization of the MicroRNA profile of ginger exosome-like nanoparticles and their anti-inflammatory effects in intestinal caco-2 cells. *J Agric Food Chem* (2022) 70 (15):4725–34. doi: 10.1021/acs.jafc.1c07306

103. Lin YC, Chen TH, Huang YM, Wei PL, Lin JC. Involvement of microRNA in solid cancer: Role and regulatory mechanisms. *Biomedicines* (2021) 9(4):343. doi: 10.3390/biomedicines9040343

104. Amuran GG, Eyuboglu IP, Tinay I, Akkiprik M. New insights in bladder cancer diagnosis: Urinary miRNAs and proteins. *Med Sci (Basel)* (2018) 6(4):113. doi: 10.3390/medsci6040113

105. Gezer U, Özgür E, Cetinkaya M, Isin M, Dalay N. Long non-coding RNAs with low expression levels in cells are enriched in secreted exosomes. *Cell Biol Int* (2014) 38(9):1076–9. doi: 10.1002/cbin.10301

106. Feng Y, Zhong M, Zeng S, Wang L, Liu P, Xiao X, et al. Exosome-derived miRNAs as predictive biomarkers for diffuse large b-cell lymphoma chemotherapy resistance. *Epigenomics* (2019) 11(1):35–51. doi: 10.2217/epi-2018-0123

107. Cao D, Cao X, Jiang Y, Xu J, Zheng Y, Kang D, et al. Circulating exosomal microRNAs as diagnostic and prognostic biomarkers in patients with diffuse large b-cell lymphoma. *Hematol Oncol* (2022) 40(2):172–80. doi: 10.1002/hon.2956

108. Damanti CC, Gaffo E, Lovisa F, Garbin A, Di Battista P, Gallingani I, et al. MiR-26a-5p as a reference to normalize MicroRNA qRT-PCR levels in plasma exosomes of pediatric hematological malignancies. *Cells* (2021) 10(1):101. doi: 10.3390/cells10010101

109. Skog J, Würdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol* (2008) 10(12):1470–6. doi: 10.1038/ncb1800

110. Ogata-Kawata H, Izumiya M, Kurioka D, Honma Y, Yamada Y, Furuta K, et al. Circulating exosomal microRNAs as biomarkers of colon cancer. *PloS One* (2014) 9(4):e92921. doi: 10.1371/journal.pone.0092921

111. Zeng Z, Li Y, Pan Y, Lan X, Song F, Sun J, et al. Cancer-derived exosomal miR-25-3p promotes pre-metastatic niche formation by inducing vascular permeability and angiogenesis. *Nat Commun* (2018) 9(1):1–14. doi: 10.1038/s41467-018-07810-w

112. Huang X, Yuan T, Liang M, Du M, Xia S, Dittmar R, et al. Exosomal miR-1290 and miR-375 as prognostic markers in castration-resistant prostate cancer. *Eur Urol* (2015) 67(1):33–41. doi: 10.1016/j.eururo.2014.07.035

113. Huang X, Yuan T, Tschannen M, Sun Z, Jacob H, Du M, et al. Characterization of human plasma-derived exosomal RNAs by deep sequencing. *BMC Genomics* (2013) 14:319. doi: 10.1186/1471-2164-14-319

114. Danarto R, Astuti I, Umbas R, Haryana SM. Urine miR-21-5p and miR-200c-3p as potential non-invasive biomarkers in patients with prostate cancer. *Turk J Urol* (2020) 46(1):26–30. doi: 10.5152/tud.2019.19163

115. El-Shal AS, Shalaby SM, Abouhashem SE, Elbary EHA, Azazy S, Rashad NM, et al. Urinary exosomal microRNA-96-5p and microRNA-183-5p expression as potential biomarkers of bladder cancer. *Mol Biol Rep* (2021) 48(5):4361–71. doi: 10.1007/s11033-021-06451-5

116. Zhou L, Wang W, Wang F, Yang S, Hu J, Lu B, et al. Plasma-derived exosomal miR-15a-5p as a promising diagnostic biomarker for early detection of endometrial carcinoma. *Mol Cancer* (2021) 20(1):57. doi: 10.1186/s12943-021-01352-4

117. Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. *Gynecol Oncol* (2008) 110 (1):13–21. doi: 10.1016/j.ygyno.2008.04.033

118. Li S, Xu HX, Wu CT, Wang WQ, Jin W, Gao HL, et al. Angiogenesis in pancreatic cancer: current research status and clinical implications. *Angiogenesis* (2019) 22(1):15–36. doi: 10.1007/s10456-018-9645-2

119. Guo Y, Zhang X, Wang L, Li M, Shen M, Zhou Z, et al. The plasma exosomal miR-1180-3p serves as a novel potential diagnostic marker for cutaneous melanoma. *Cancer Cell Int* (2021) 21(1):487. doi: 10.1186/s12935-021-02164-8

120. Husna AA, Rahman MM, Lai YC, Chen HW, Hasan MN, Nakagawa T, et al. Identification of melanoma-specific exosomal miRNAs as the potential biomarker for canine oral melanoma. *Pigment Cell Melanoma Res* (2021) 34 (6):1062–73. doi: 10.1111/pcmr.13000

121. Zhong D, Wu C, Xu D, Bai J, Wang Q, Zeng X. Plasma-derived exosomal hsa-miR-4488 and hsa-miR-1228-5p: Novel biomarkers for dermatomyositis-associated interstitial lung disease with anti-melanoma differentiation-associated protein 5 antibody-positive subset. *BioMed Res Int* (2021) 2021:6676107. doi: 10.1155/2021/6676107

122. Song S, Long M, Yu G, Cheng Y, Yang Q, Liu J, et al. Urinary exosome miR-30c-5p as a biomarker of clear cell renal cell carcinoma that inhibits progression by targeting HSPA5. *J Cell Mol Med* (2019) 23(10):6755–65. doi: 10.1111/jcmm.14553

123. Coon J, Kingsley K, Howard KM. miR-365 (microRNA): Potential biomarker in oral squamous cell carcinoma exosomes and extracellular vesicles. *Int J Mol Sci* (2020) 21(15):5317. doi: 10.3390/ijms21155317

124. Ohshima K, Inoue K, Fujiwara A, Hatakeyama K, Kanto K, Watanabe Y, et al. Let-7 microRNA family is selectively secreted into the extracellular environment *via* exosomes in a metastatic gastric cancer cell line. *PloS One* (2010) 5(10):e13247. doi: 10.1371/journal.pone.0013247

125. Lei HJ. *Exploring the molecular mechanism of Si Jun Zi Tang based on exosomal RNA for the treatment of spleen and stomach qi deficiency evidence in gastric cancer*. Hunan University of Chinese Medicine (2021). doi: 10.27138/d.cnki.ghuzc.2021.000394

126. Silva J, García V, Zaballos >Á., Provencio M, Lombardía L, Almonacid L, et al. Vesicle-related microRNAs in plasma of nonsmall cell lung cancer patients and correlation with survival. *Eur Respir J* (2011) 37(3):617–23. doi: 10.1183/09031936.00029610

127. Peng XX, Yu R, Wu X, Wu SY, Pi C, Chen ZH, et al. Correlation of plasma exosomal microRNAs with the efficacy of immunotherapy in EGFR / ALK wild-type advanced non-small cell lung cancer. *J Immunother Cancer* (2020) 8(1): e000376. doi: 10.1136/jitc-2019-000376

128. Lee YR, Kim G, Tak WY, Jang SY, Kweon YO, Park JG, et al. Circulating exosomal noncoding RNAs as prognostic biomarkers in human hepatocellular carcinoma. *Int J Cancer* (2019) 144(6):1444–52. doi: 10.1002/ijc.31931

129. Zhang H, Deng T, Ge S, Liu Y, Bai M, Zhu K, et al. Exosome circRNA secreted from adipocytes promotes the growth of hepatocellular carcinoma by targeting deubiquitination-related USP7. *Oncogene* (2019) 38(15):2844–59. doi: 10.1038/s41388-018-0619-z

130. Corcoran C, Rani S, O'Brien K, O'Neill A, Prencipe M, Sheikh R, et al. Docetaxel-resistance in prostate cancer: evaluating associated phenotypic changes and potential for resistance transfer *via* exosomes. *PloS One* (2012) 7(12):e50999. doi: 10.1371/journal.pone.0050999

131. Gernapudi R, Yao Y, Zhang Y, Wolfson B, Roy S, Duru N, et al. Targeting exosomes from preadipocytes inhibits preadipocyte to cancer stem cell signaling in early-stage breast cancer. *Breast Cancer Res Treat* (2015) 150(3):685–95. doi: 10.1007/s10549-015-3326-2

132. Millimaggi D, Mari M, D'Ascenzo S, Carosa E, Jannini EA, Zucker S, et al. Tumor vesicle-associated CD147 modulates the angiogenic capability of endothelial cells. *Neoplasia* (2007) 9(4):349–57. doi: 10.1593/neo.07133

133. Chen Y, Lin X, Park H, Greever R. Study of artemisinin nanocapsules as anticancer drug delivery systems. *Nanomedicine* (2009) 5(3):316–22. doi: 10.1016/j.nano.2008.12.005

134. Li DC, Zhong XK, Zeng ZP, Jiang JG, Li L, Zhao MM, et al. Application of targeted drug delivery system in Chinese medicine. *J Control Release* (2009) 138 (2):103–12. doi: 10.1016/j.jconrel.2009.05.008

135. Cao F,H, Ouyang W,Q, Wang Y,P, Dong H,B. Study of preparation of matrin nanoemulsion and its anti-oxidation on mice. *J Northwest A&F Univ (Nat. Sci. Ed.)* (2007) 03):61–4. doi: 10.13207/j.cnki.jnwafu.2007.03.014

136. Zhaowu Z, Xiaoli W, Yangde Z, Nianfeng L. Preparation of matrine ethosome, its percutaneous permeation *in vitro* and anti-inflammatory activity *in vivo* in rats. *J Liposome Res* (2009) 19(2):155–62. doi: 10.1080/08982100902722381

137. Obeid MA, Al Qaraghuli MM, Alsaadi M, Alzahrani AR, Niwasabutra K, Ferro VA. Delivering natural products and biotherapeutics to improve drug efficacy. *Ther Delivery* (2017) 8(11):947–56. doi: 10.4155/tde-2017-0060

138. Tian X, Zhu M, Nie G. How can nanotechnology help membrane vesicle-based cancer immunotherapy development? *Hum Vaccin Immunother* (2013) 9 (1):222–5. doi: 10.4161/hv.22130

139. Pitt JM, André F, Amigorena S, Soria JC, Eggermont A, Kroemer G, et al. Dendritic cell-derived exosomes for cancer therapy. *J Clin Invest* (2016) 126 (4):1224–32. doi: 10.1172/jci81137

140. Srivastava A, Babu A, Filant J, Moxley KM, Ruskin R, Dhanasekaran D, et al. Exploitation of exosomes as nanocarriers for gene-, chemo-, and immune-therapy of cancer. *J BioMed Nanotechnol* (2016) 12(6):1159–73. doi: 10.1166/jbn.2016.2205

141. Wang J, Faict S, Maes K, De Bruyne E, Van Valckenborgh E, Schots R, et al. Extracellular vesicle cross-talk in the bone marrow microenvironment: implications in multiple myeloma. *Oncotarget* (2016) 7(25):38927–45. doi: 10.18632/oncotarget.7792

142. Yang L, Shi P, Zhao G, Xu J, Peng W, Zhang J, et al. Targeting cancer stem cell pathways for cancer therapy. *Signal Transduct Target Ther* (2020) 5(1):8. doi: 10.1038/s41392-020-0110-5

143. Del Pozo-Acebo L, Hazas MLL, Tomé-Carneiro J, Gil-Cabrerizo P, San-Cristobal R, Busto R, et al. Bovine milk-derived exosomes as a drug delivery vehicle for miRNA-based therapy. *Int J Mol Sci* (2021) 22(3):1105. doi: 10.3390/ijms22031105

144. Kim KU, Kim WH, Jeong CH, Yi DY, Min H. More than nutrition: Therapeutic potential of breast milk-derived exosomes in cancer. *Int J Mol Sci* (2020) 21(19):7327. doi: 10.3390/ijms21197327

145. Lin Y, Shi F, Yan J, Sui Z, Sun B, Xu G. The role of exosome MicroRNA-103 in the proliferation and invasion of hepatoma cells and its mechanism. *Cell Mol Biol (Noisy-le-grand)* (2022) 68(5):47–53. doi: 10.14715/cmb/2022.68.5.6

146. You Q, Zhuang L, Chang Z, Ge M, Mei Q, Yang L, et al. Hierarchical au nanoarrays functionalized 2D Ti(2)CT(x) MXene membranes for the detection of exosomes isolated from human lung carcinoma cells. *Biosens Bioelectron* (2022) 216:114647. doi: 10.1016/j.bios.2022.114647

147. Liu SC, Cao YH, Chen LB, Kang R, Huang ZX, Lu XS. BMSC-derived exosomal lncRNA PTENP1 suppresses the malignant phenotypes of bladder cancer by upregulating SCARA5 expression. *Cancer Biol Ther* (2022) 23(1):1–13. doi: 10.1080/15384047.2022.2102360

148. Yang Z, Xu B, Wu S, Yang W, Luo R, Geng S, et al. Exosomal microRNA-551b-3p from bone marrow-derived mesenchymal stromal cells inhibits breast cancer progression *via* regulating TRIM31/Akt signaling. *Hum Cell* (2022) 1–16. doi: 10.1007/s13577-022-00753-x

149. Beeraka NM, Doreswamy SH, Sadhu SP, Srinivasan A, Pragada RR, Madhunapantula SV, et al. The role of exosomes in stemness and neurodegenerative diseases-Chemoresistant-Cancer therapeutics and phytochemicals. *Int J Mol Sci* (2020) 21(18):6818. doi: 10.3390/ijms21186818

frontiers | Frontiers in Pharmacology

TYPE Mini Review
PUBLISHED 29 September 2022
DOI 10.3389/fphar.2022.1006299

Check for updates

OPEN ACCESS

EDITED BY
Yongye Huang,
Northeastern University, China

REVIEWED BY
Zhourui Xu,
Shenzhen University, China
Weina Ke,
University of North Carolina at Charlotte, United States
Lesheng Teng,
Jilin University, China

*CORRESPONDENCE
Yang-Yang Liu,
996439299@qq.com
Ye Jin,
jy_ccucm@163.com

SPECIALTY SECTION
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

RECEIVED 29 July 2022
ACCEPTED 13 September 2022
PUBLISHED 29 September 2022

CITATION
Tan Z-L, Li J-F, Luo H-M, Liu Y-Y and Jin Y (2022), Plant extracellular vesicles: A novel bioactive nanoparticle for tumor therapy.
Front. Pharmacol. 13:1006299.
doi: 10.3389/fphar.2022.1006299

COPYRIGHT
© 2022 Tan, Li, Luo, Liu and Jin. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Plant extracellular vesicles: A novel bioactive nanoparticle for tumor therapy

Zhao-Lin Tan, Jing-Fei Li, Hao-Ming Luo, Yang-Yang Liu* and Ye Jin*

School of Pharmacy, Changchun University of Traditional Chinese Medicine, Changchun, China

Extracellular vesicles are tiny lipid bilayer-enclosed membrane particles, including apoptotic bodies, micro vesicles, and exosomes. Organisms of all life forms can secrete extracellular vesicles into their surrounding environment, which serve as important communication tools between cells and between cells and the environment, and participate in a variety of physiological processes. According to new evidence, plant extracellular vesicles play an important role in the regulation of transboundary molecules with interacting organisms. In addition to carrying signaling molecules (nucleic acids, proteins, metabolic wastes, etc.) to mediate cellular communication, plant cells External vesicles themselves can also function as functional molecules in the cellular microenvironment across cell boundaries. This review introduces the source and extraction of plant extracellular vesicles, and attempts to clarify its anti-tumor mechanism by summarizing the current research on plant extracellular vesicles for disease treatment. We speculate that the continued development of plant extracellular vesicle-based therapeutic and drug delivery platforms will benefit their clinical applications.

KEYWORDS

plant extracellular vesicles, exosomes, anti-tumor, drug delivery, isolation, non-coding RNA

Introduction

Cancer is a complex disease, that is, facilitated by many factors, including genetic and environmental factors. Unfortunately, the latest cancer statistics show that cancer mortality and morbidity have been increasing, and cancer remains the leading cause of death in every country worldwide (Sung et al., 2021). Chemotherapy, as the main means of clinical treatment, has achieved good results in overcoming the problem of cancer (Galmarini et al., 2012). Chemotherapy, however, does not perfectly target tumors, and often fails to completely eliminate cancer cells, and can even damage more normal cells while killing tumor cells (Behranvand et al., 2022). A new therapeutic material with stability, good target specificity and biosafety is urgently needed (Liang et al., 2021). The combined application of extracellular vesicles and chemotherapeutic agents has brought new light to cancer patients, although they were initially considered cellular debris (Pan and Johnstone, 1983; Li et al., 2022). Extracellular vesicles are bilayer-membrane lipid

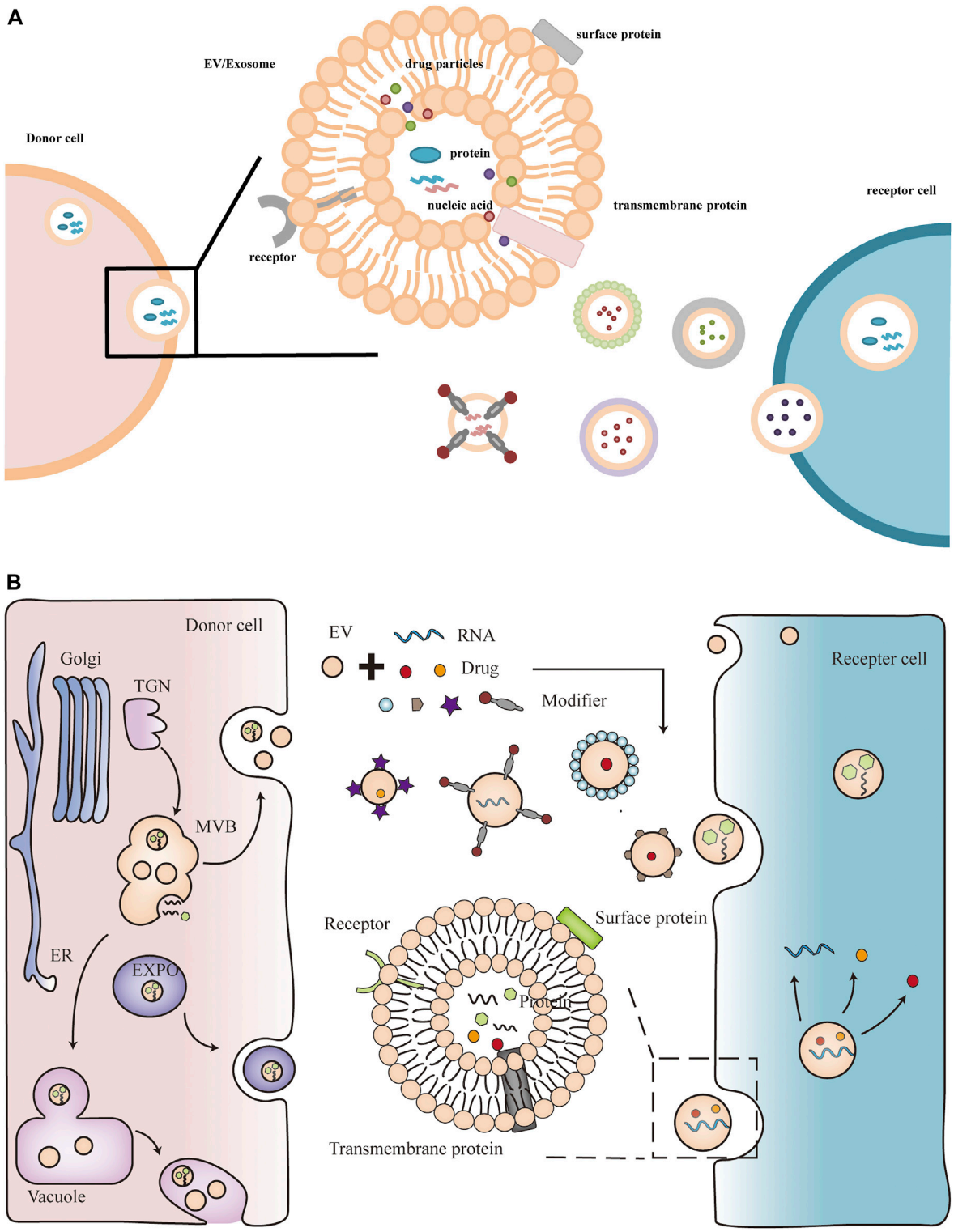


FIGURE 1
Plant extracellular vesicles contain active components such as nucleic acids and proteins. Plant extracellular vesicles can also be modified as delivery vehicles for therapeutics such as miRNAs and drugs.

spheres secreted by various organisms. Exosomes are a subset of extracellular vesicles that can carry biologically active molecules such as nucleic acids, proteins and lipids (Ratajczak et al., 2006; Chen et al., 2019; Doyle and Wang, 2019; Mathieu et al., 2019). Although various types of extracellular vesicles have been identified at the first International Society for Extracellular Vesicles (ISEV) meeting, since most of the examples in this review cannot distinguish their types, this paper uses extracellular vesicles (or exosomes) to refer to them (Witwer and Thery, 2019). In mammalian cells, both normal cells and cancer cells can secrete extracellular vesicles as a tool for cell-to-cell communication (Muralidharan-Chari et al., 2010; Kuriyama et al., 2020; Mittal et al., 2020; Schweiger and Tannous, 2020; Wu et al., 2021), Information transfer through binding of vesicle membrane proteins to receptor membrane proteins and through internalization of vesicle contents by receptor cells (Menard et al., 2018; Ko and Naora, 2020; Vu et al., 2020; Tang et al., 2022). Similarly, plant multi-vesicular bodies secrete exosome-like nanoparticles (An et al., 2007), which, in addition to being involved in the formation of the plant's own cell wall (Chukhchin et al., 2019) are also involved in plant-microbe interactions (Rutter and Innes, 2018), including plant defense and silencing fungi gene et al. (Regente et al., 2017; Cai et al., 2018; Bleackley et al., 2019; Cai et al., 2019). Transboundary regulation mediated by plant extracellular vesicles is therefore increasingly sought after by researchers. In fact, many experimental teams have successfully isolated these vesicles from plants and used them to overcome cancer as an emerging means of human disease treatment (Raimondo et al., 2015; Potesta et al., 2020; Yang et al., 2020).

Source of plant extracellular vesicles

In eukaryotic cells, protein secretion is usually divided into two pathways, the conventional protein secretion pathway and the unconventional protein secretion pathway (Chung and Zeng, 2017). Plant extracellular vesicles are produced mainly through unconventional protein secretion, including the alternative Golgi pathway and three pathways mediated by specific organelles: multivesicular bodies (MVB), exosome-positive organelles (EXPO), vacuolar bodies (Tse et al., 2004; Ding and Wang, 2017; Wang et al., 2018). The conventional protein secretion pathway is the transfer of proteins from the lumen of the endoplasmic reticulum (ER) to the Golgi apparatus, and then through the plasma membrane (PM), where they are released into the extracellular environment to secrete proteins. Most secreted proteins transported through the conventional protein secretion pathway have an N-terminal leader sequence, often with post-translational modifications, while the unconventional protein secretion pathway has no leader (Wang et al., 2018). In mammalian cells, EVs play an important role in mediating intercellular communication by delivering biomolecules, including proteins and RNAs, to recipient cells through the extracellular space (Menard et al., 2018). Similar to mammalian cells, there is growing evidence that plants also generate EVs that are involved in various functions. During fungal infection, ARA6 (a plant-specific Rab GTPase)-positive MVBs accumulated in plant cells and co-localized with TET8 (Tetraspanin-8) in the infected area (An et al., 2006). TET8 is structurally similar to the animal cell exosome marker CD63, therefore, TET8-labeled EVs can be regarded as plant exosomes (Kowal et al., 2016; Cai et al., 2019). This process is similar to the fusion of MVBs with the plasma membrane (PM) in animal cells to release extracellular vesicles. In addition, the SNARE (soluble N-ethylmaleimide-sensitive factor-associated protein receptor) protein SYNTAXIN121 (SYP121)/PENETRATION1 (PEN1) was also detected in plant extracellular vesicles (Meyer et al., 2009). These proteins lack typical signal peptides and may rely on unconventional protein secretion pathways to be transported out of cells, such as extracellular vesicles. EXPO is a plant-specific double-membrane structure, distinct from MVB, but also dependent on exocytosis. EXO70E2-labeled EXPO was found to fuse with PM and release internal vesicles outside the cell (Wang et al., 2010). During bacterial pathogen infection, plants initiate fusion of vacuoles with PM, releasing hydrolases and defense proteins to inhibit bacterial proliferation (Hatsugai et al., 2009). The source of plant extracellular vesicles and the way they function are shown in Figure 1.

Extraction of plant extracellular vesicles

In addition to plant extracellular vesicles for plant growth and development, defense responses, and plant-microbe symbiosis (Regente et al., 2017; Rutter and Innes, 2018; Chukhchin et al., 2019; Ivanov et al., 2019; Zhou et al., 2021), plant extracellular vesicles have attracted attention for their potential roles in human health and disease. For example, extracellular vesicles extracted from edible fruits and vegetables have antioxidant functions, and strawberry- and blueberry-derived exosome-like nanoparticles prevent oxidative stress in human mesenchymal stromal cells and endothelial cells (De Robertis et al., 2020; Logozzi et al., 2021; Perut et al., 2021). In order to study the cargo and function of plant extracellular vesicles, isolation and purification of plant extracellular vesicles are the primary tasks. Differential centrifugation is the most commonly used method for the isolation of extracellular vesicles, which separates and removes components other than extracellular vesicles from solution in a step-by-step fashion based on differences in vesicle density and size (Carnino et al., 2019; Akbar et al., 2022). Typically, low-speed centrifugation is used to separate cellular debris, and high-speed centrifugation is used to collect extracellular vesicles by

TABLE 1 Sources, pathways, extraction methods and applications of exosomes.

Source	Pathway	Method	Application	References
Ginseng	TLR4 MyD88	Differential centrifugation	Melanoma	Cao et al., (2019)
Dendropanax morbifera	MITF TYR TRP-1 TRP-2	Differential centrifugation	Melanoma	Lee et al., (2020)
Grapefruit	CCNB1 CCNB2 p21 ICAM-1	Differential centrifugation	Melanoma	Stanly et al., (2020)
Moringa oleifera	Bcl-2	Differential centrifugation	HeLa cell	Abdul Rahman et al., (2020)
Asparagus	Ki67 PCNA	Differential centrifugation	HCC	Zhang et al., (2021)
Lemon	Cas3 Cleaved caspase-3 TRAIL	Density gradient centrifugation	A549 SW480, SW480 LAMA84	Raimondo et al., (2015)
Corn	TNF-α	Sucrose cushion ultracentrifugation	CT26	Sasaki et al., (2021)
Garlic	p53 Bax Cas3 Cas9 Bcl-2 VEGF	Differential centrifugation	ATCC HTB-44 ATCC CCL-185	Ozkan et al., (2021)

density (Thery et al., 2006; Xu et al., 2015). For example, ginseng nanovesicles were isolated from ginseng root grinding liquid by differential centrifugation and sucrose density gradient method (Cho et al., 2021). In addition, density gradient centrifugation, which can also collect extracellular vesicles based on the buoyant density of the particles, is commonly used to isolate extracellular vesicles partially isolated by centrifugation (Carnino et al., 2019). Density gradient centrifugation utilizes two methods to form gradients, a continuous density gradient or a stepwise gradient, on a sucrose pad (Konoshenko et al., 2018). For example, citrus lemon nanovesicles obtained by ultra-fast separation were purified on a 30% sucrose gradient (Raimondo et al., 2015). Corn-derived nanovesicles were obtained by ultracentrifugation of corn homogenate using a sucrose pad (Sasaki et al., 2021). In addition, based on size, precipitation and immunoaffinity capture of exosomes, ultrafiltration (UF), size exclusion chromatography (SEC), polymer precipitation, and immunoprecipitation have also been developed for the isolation of extracellular vesicles (Konoshenko et al., 2018). The separation principle of ultrafiltration is the same as that of filter membrane, and compared with ultracentrifugation, ultrafiltration takes less time (Konoshenko et al., 2018; Liangsupree et al., 2021). In particular, ultrafiltration can be alternated with successive ultracentrifugation stages (Sasaki et al., 2021). Size exclusion chromatography is a separation method based on particle size. Larger vesicles are washed out earlier because they cannot penetrate into the cavity of the column, and smaller vesicles are retained in the cavity of the column. The benefit of this approach is that it preserves the integrity of the vesicle structure (Liangsupree et al., 2021; Akbar et al., 2022). The PEG precipitation method can precipitate extracellular vesicles from the sample solution according to the solubility difference of the particles in PEG (Liangsupree et al., 2021; Akbar et al., 2022). However, due to similar solubility, some non-extracellular vesicle proteins are also precipitated. Immunoprecipitation is based on surface proteins of extracellular vesicles, such as CD63 (Carnino et al., 2019; Akbar et al., 2022). The advantage of this method is that it can specifically identify a type of extracellular vesicles, and can also better protect the integrity of the vesicles. Although the isolation methods of plant extracellular vesicles are constantly improving, the rapid, efficient and reproducible isolation of these extracellular vesicles is still challenging. Above, we briefly listed the uses and isolation methods of some plant extracellular vesicles in clinical settings, and summarized them in Tables 1, 2.

Plant extracellular vesicles as therapeutic agents against tumors

Plant extracellular vesicles contain a large amount of proteins, lipids, and miRNAs, which can act as cell messengers to transfer these biologically active substances from *in vitro* to *in vivo*, then to the lesion tissue, and finally to cells (Yim and Choi, 2016; Xiao et al., 2018). Thus, plant extracellular vesicles can mediate specific transboundary cellular or tissue responses (Zhang et al., 2012; Chin et al., 2016). Among them, plant-derived miRNAs are not only used for plant growth, development and defense responses (Xu et al., 2022), such small non-coding RNAs have also recently been used as tumor suppressor RNAs to play a role in tumor progression (Potesta et al., 2020). For example, broccoli miR159 can inhibit the growth of breast cancer cells across borders (Chin et al., 2016); olive small RNA is functionally homologous to human miR34a, and transfection of it can reduce the protein expression of hsa-miR34a mRNA targets across borders and increase different tumors Cell apoptosis and reduced proliferation (Minutolo et al., 2018); some small RNAs from maize can significantly reduce HeLa cell proliferation (Luo et al., 2017). In addition to oral absorption, the way that these plant RNAs enter the human body may also be mediated through extracellular vesicles. The molecular structure of plant extracellular vesicles is similar to that of animal extracellular vesicles, which makes plant extracellular vesicles also used as a natural therapeutic agent for disease treatment (Akers et al., 2013; Rutter and Innes, 2018). For example, exosomes isolated from wheat, asparagus, and grapefruit act as antioxidants, enhance cell viability, and promote skin cell proliferation and migration during wound healing and skin regeneration (Sahin et al., 2019; Kim et al., 2021;

TABLE 2 Exosomes as drug carriers to deliver different drugs.

Source	Pathway	Compound	Method	Application	References
Bitter melon	NLRP3 MAP30	5-FU	Electrophoresis Dialysis	OSSC	Yang et al., (2021)
Grapefruit	MHCI	miR17	Sucrose gradient centrifugation	Brain tumor	Friedmann, (2016)
Grapefruit	HER2	Doxorubicin	Ultracentrifugation	Breast cancer	Tang et al., (2020)
Grapefruit	LFA-1 CXCR1 CXCR2	Doxorubicin	Density gradient centrifugation	CT26	Wang et al., (2015)
Lemon	P-gp	Doxorubicin	Density gradient centrifugation	Ovarian cancer	Xiao et al., (2022)

Savci et al., 2021). Food-derived plant exosomes (green tea, broccoli, lemon) can directly shape intestinal flora and maintain immune homeostasis through oral administration, thereby preventing and treating various intestinal diseases (Teng et al., 2018; Lei et al., 2020; Zu et al., 2021). Therefore, inspired researchers began to try to apply plant extracellular vesicles to tumor cells. It is gratifying that these vesicles also showed their good anticancer activities, Including inhibiting the proliferation and invasion of malignant tumors, promoting the apoptosis of cancer cells, inhibiting the cell cycle, and reducing the drug resistance of cancer cells (Friedmann, 2016; Cao et al., 2019; Potesta et al., 2020; Stanly et al., 2020; Yang et al., 2021; Zhang et al., 2021).

Melanoma, a deadly skin disease, is a major public health problem in many white countries (Jackett and Scolyer, 2019). UV radiation is often considered a trigger, and increased UV exposure increases the incidence of melanoma (Keim et al., 2021). Ginseng is a traditional Chinese medicine in Southeast Asian countries, in addition to ginsenosides exhibiting tumor-suppressive effects in the treatment of melanoma, Ginseng extracellular vesicles isolated from ginseng cell culture supernatants improve cellular melanosis induced by UV-B radiation by downregulating melanin-producing proteins (Zhou and Yang, 2015; Meng et al., 2019; Cho et al., 2021). Ginseng extracellular vesicles, dependent on TLR4 and MyD88 signaling, promote the transformation of tumor-associated macrophages from the M2 phenotype to the M1 phenotype and inhibit the growth of melanoma (Cao et al., 2019). Extracellular vesicles extracted from the leaves and stems of Dendrobium inhibited the expression of the melanin production-related gene MITF and the tyrosine-related proteins TYR, TRP-1 and TRP-2, inducing melanin reduction in melanoma cells (Lee et al., 2020). In addition to ginseng extracellular vesicles, grapefruit extracellular vesicles have been shown to reduce the expression levels of cyclins B1 and B2 and upregulate the expression of the cell cycle inhibitor P21, arrest the melanoma cell cycle at the G2/M point, and inhibit melanin Proliferation of tumor cells (Stanly et al., 2020). Cervical cancer caused by human papillomavirus has an extremely high fatality rate in less developed regions. Vesicles isolated from aqueous extracts of *Moringa oleifera* seeds were shown to induce decreased tumor cell growth and increased apoptosis in cultured hela cells *in vitro* by downregulating B-cell lymphoma 2 anti-apoptotic proteins (Abdul Rahman et al., 2020; Potesta et al., 2020). In the research of liver cancer treatment, the research of asparagus extracellular vesicles has made new achievements. The results of *in vivo* and *in vitro* experiments showed that asparagus extracellular vesicles inhibited the proliferation of cancer cells by reducing the expression levels of Ki67 and PCNA in liver cancer cells, and increase the protein levels of AIF, Bax and Bak to trigger the activation of caspase-9, leading to cleavage of key cellular proteins including the DNA repair enzyme PARP and ultimately cancer cell death (Zhang et al., 2021). The down-regulation of apoptosis-related protein caspase 3 and the up-regulation of cleaved caspase 3 in the experiments of lemon extracellular vesicles for cancer treatment indicated that lemon extracellular vesicles could induce apoptosis of gastric cancer cells. In addition, gastric cancer cells undergo S-phase cell cycle arrest, and cancer cell proliferation is also inhibited (Yang et al., 2020). In addition, citrus lemon exosomes have also been shown to inhibit different tumor cell lines by specifically reaching the tumor site and activating TRAIL-mediated apoptotic cell death: A549 (human lung cancer cell line), SW480 (human colorectal adenocarcinoma) cell line), LAMA84 (chronic myeloid leukemia cell line) cancer cell proliferation (Raimondo et al., 2015). In mouse colon tumor cells, the researchers found that maize extracellular vesicles produced TNF-α by inhibiting colon 26 cell proliferation (direct effect) and by activating macrophages and other immune cells to infiltrate the tumor (indirect effect) to inhibit tumor growth in mice. The synergistic or additive effects of the two actions resulted in a significant *in vivo* antitumor effect of maize extracellular vesicles (Sasaki et al., 2021). Garlic-derived extracellular vesicles have anticancer properties against A498 (human renal carcinoma cell line), with reduced cell proliferation and cell cycle arrest in S phase. The expression levels of pro-apoptotic genes such as p53, Bax, Cas3 and Cas9 in tumor cells treated with garlic extracellular vesicles were significantly increased, and the expression levels of Bcl-2 anti-apoptotic genes were significantly decreased. As a potent angiogenic factor, VEGF secretion was also significantly reduced, adversely affecting tumor angiogenesis in cancer cells (Ozkan et al., 2021). In conclusion, plant extracellular vesicles can inhibit the proliferation and invasion of cancer cells, promote cell

apoptosis, and inhibit cell cycle through immune pathways (macrophages and B lymphocytes) and direct effects. The detailed pathways of plant extracellular vesicles are shown in Table 1.

Plant extracellular vesicles as drug delivery vehicles for antitumor

To date, the application of mammalian extracellular vesicles in cancer therapy has covered various aspects such as targeted chemotherapy, gene therapy and vaccine development (Liang et al., 2021; Tarasov et al., 2021). However, plant-derived extracellular vesicles have a similar structural composition to animal extracellular vesicles (Baldrich et al., 2019; Pocsfalvi et al., 2019; Stanly et al., 2019), yet only a few studies have developed them as a drug delivery platform. Compared with animal extracellular vesicles, plant extracellular vesicles are superior to animal-derived extracellular vesicles by virtue of their natural origin and the absence of zoonotic or human pathogens (De Jong and Borm, 2008; Dad et al., 2021). Plant extracellular vesicles have been proved to be safe and reliable through three routes of administration: oral, transdermal, and blood transport (Kusuma et al., 2016; Munir et al., 2020; Kim et al., 2021). Recent studies have shown that plant extracellular vesicles play an important role in the pathogenesis of various diseases, including cancer. Ginger exosomes have miRNAs that inhibit the expression of Nsp12 in lung epithelial cells and prevent Nsp12+-mediated lung inflammation in the treatment of inflammation (Teng et al., 2021). Ginger exosomes can be used to deliver the anti-inflammatory drug methotrexate for colitis and as an immunotherapeutic agent to maintain intestinal macrophage homeostasis (Wang et al., 2014). It is also used in Alzheimer's disease and diabetes treatment by preventing abnormal activation of the NLRP3 (9). In the field of cancer treatment, new breakthroughs have also been made. It has been demonstrated that in combination with the chemotherapeutic drug 5-fluorouracil (5-FU), bitter melon exosomes enhance cytotoxicity and reduce 5-FU resistance during oral squamous cell carcinoma cell (OSCC) therapy by downregulating the expression of inflammasome NLRP3. Bitter melon extracellular vesicles induce apoptosis in oral squamous cell carcinoma cells by triggering ROS-mediated mitochondrial damage, a process possibly mediated by MAP30 protein (Yang et al., 2021). To Inhibit brain tumor progression, miR17 was encapsulated in grapefruit exosomes coated with folic acid and polyethylenimine, miR17 was rapidly delivered to the brain *via* the intranasal route and was selectively taken up by GL-26 cancer cells. Killed cancer cells by inhibiting MHC1 expression in GL-26 cancer cells and triggered the activation of NK cells (Friedmann, 2016). In addition to delivering miRNA therapeutics, some research groups have combined grapefruit extracellular vesicles with aptamer HA1 to load the chemotherapeutic drug azithromycin to target and kill HER2+ breast cancer cells (Tang et al., 2020). Coating grapefruit exosomes with membranes of activated leukocytes increases LFA-1 or CXCR1 and CXCR2 expression and improves the recruitment of grapefruit exosomes to cancer cells. Doxorubicin encapsulated in grapefruit exosomes was successfully delivered to the CT26 colon cancer and exerted tumoricidal effect (Wang et al., 2015). In addition, new research shows that in the treatment of ovarian cancer, functional heparin-cRGD (HR) was modified on the surface of lemon exosomes, and then the modified lemon exosomes were loaded with the drug doxorubicin through endocytosis into cancer cells. The expression of P-glycoprotein (P-gp) is inhibited, the production of ATP is reduced, the energy generated by ATP hydrolysis is reduced, and the excretion of drugs from cancer cells is reduced, thereby achieving the purpose of overcoming cancer drug resistance (Xiao et al., 2022). In summary, in practical applications, in addition to directly using the original plant extracellular vesicles as a drug delivery platform, in order to enhance its targeting, specific tumor-specific ability, and enhance tumor-related anti-tumor properties, researchers have also tried different approaches to redesign plant extracellular vesicles. The modification methods of each plant extracellular vesicle in this review are shown in Table 2.

Discussion

Cancer is increasingly important as the leading cause of premature human death globally and, based on current trends, may even surpass cardiovascular disease as the leading cause of premature death in most countries, especially developing countries (Jiang et al., 2021). Accurate diagnosis and effective treatment of cancer is undoubtedly a huge problem in clinical treatment. In addition to the commonly used treatments such as gene therapy, immunotherapy, and chemotherapy (Vatner et al., 2014; Dayakar et al., 2022), researchers have attempted to develop extracellular vesicles as biomarker molecules and delivery platforms for cancer diagnosis and treatment (Pullan et al., 2019; Zhou et al., 2020). More and more studies have demonstrated that extracellular vesicles participate in and play an important role in multiple processes of cancer development. In addition to being an integral part of the plant's own biological machinery and acting as a defense frontline during plant infection and growth and development, plant extracellular vesicles have also been developed as therapeutic agents to play a role in human health and disease (Rutter and Innes, 2018; Dad et al., 2021; Xu et al., 2022). The active substances such as proteins and nucleic acids contained in plant extracellular vesicles can maintain their physiological activities in recipient cells and affect the development of diseases (Baldrich et al., 2019; Chen et al., 2022). Plant extracellular vesicles can resist the

activity of digestive enzymes in the gastrointestinal tract without being broken down, can be absorbed in the intestine, and can also function through the blood route (Munir et al., 2020). In addition to plant extracellular vesicles themselves acting as therapeutic particles, plant extracellular vesicles are also often designed as drug carriers to deliver drugs. Compared with delivery vehicles such as liposomes, silver nanoparticles and animal extracellular vesicles, plant extracellular vesicles are regarded as a natural source, biocompatibility, non-immunogenicity and non-toxicity to normal cells, an ideal nanosphere to hold and transport cargo (De Jong and Borm, 2008). It is also relatively easy to obtain plant extracellular vesicles, which can be obtained directly from plant stems and leaves, plant seeds and fruit juices (Rutter and Innes, 2017; Potesta et al., 2020; Bruno et al., 2021; Karimi et al., 2022). Although the application of plant extracellular vesicles seems to be easier and faster than that of animal extracellular vesicles, many efforts are still required. In addition, extensive immunogenicity studies are required to assess *in vivo* safety and efficacy before large-scale production use. In the face of tumor cells that may metastasize at any time, how to effectively load drugs, target delivery and release of drugs, deeply penetrate tumors and increase drug accumulation in tumor cells still need further research by scholars. Finally, the lack of specific markers still hinders the characterization of plant extracellular vesicles, although the omics analyses associated with them are increasing. Whether the source of plant extracellular vesicles is contaminated or not deserves further investigation.

Conclusion

Plant extracellular vesicles play an important role in the treatment of diseases including cancer. Plant extracellular vesicles contain biologically active particles such as miRNA, proteins, and nucleic acids, which can interfere with the development of tumor cells. In addition, plant extracellular vesicles are also an ideal drug delivery vehicle, which can be modified for targeted delivery of anticancer drugs.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No. 81973712). Jilin Province Science and Technology Development Project in China (Grant No. 20210204013YY, 20200708081YY).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Abdul Rahman, S. F., Xiang Lian, B. S., and Mohana-Kumaran, N. (2020). Targeting the B-cell lymphoma 2 anti-apoptotic proteins for cervical cancer treatment. *Future Oncol.* 16 (28), 2235–2249. doi:10.2217/fon-2020-0389

Akbar, A., Malekian, F., Baghban, N., Kodam, S. P., and Ullah, M. (2022). Methodologies to isolate and purify clinical grade extracellular vesicles for medical applications. *Cells* 11 (2), 186. doi:10.3390/cells11020186

Akers, J. C., Gonda, D., Kim, R., Carter, B. S., and Chen, C. C. (2013). Biogenesis of extracellular vesicles (EV): Exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies. *J. Neurooncol.* 113 (1), 1–11. doi:10.1007/s11060-013-1084-8

An, Q., Ehlers, K., Kogel, K.-H., van Bel, A. J. E., and Hueckelhoven, R. (2006). Multivesicular compartments proliferate in susceptible and resistant MLA12-barley leaves in response to infection by the biotrophic powdery mildew fungus. *New Phytol.* 172 (3), 563–576. doi:10.1111/j.1469-8137.2006.01844.x

An, Q., van Bel, A. J., and Huckelhoven, R. (2007). Do plant cells secrete exosomes derived from multivesicular bodies? *Plant Signal. Behav.* 2 (1), 4–7. doi:10.4161/psb.2.1.3596

Baldrich, P., Rutter, B. D., Karimi, H. Z., Podicheti, R., Meyers, B. C., and Innes, R. W. (2019). Plant extracellular vesicles contain diverse small RNA species and are enriched in 10- to 17-nucleotide "tiny" RNAs. *Plant Cell* 31 (2), 315–324. doi:10.1105/tpc.18.00872

Behranvand, N., Nasri, F., Zolfaghari Emameh, R., Khani, P., Hosseini, A., Garssen, J., et al. (2022). Chemotherapy: A double-edged sword in cancer treatment. *Cancer Immunol. Immunother.* 71 (3), 507–526. doi:10.1007/s00262-021-03013-3

Bleackley, M. R., Samuel, M., Garcia-Ceron, D., McKenna, J. A., Lowe, R. G. T., Pathan, M., et al. (2019). Extracellular vesicles from the cotton pathogen Fusarium oxysporum f. sp. vasinfectum induce a phytotoxic response in plants. *Front. Plant Sci.* 10, 1610. doi:10.3389/fpls.2019.01610

Bruno, S. P., Paolini, A., D'Oria, V., Sarra, A., Sennato, S., Bordi, F., et al. (2021). Extracellular vesicles derived from citrus sinensis modulate inflammatory genes and tight junctions in a human model of intestinal epithelium. *Front. Nutr.* 8, 778998. doi:10.3389/fnut.2021.778998

Cai, Q., He, B., and Jin, H. (2019). A safe ride in extracellular vesicles – small RNA trafficking between plant hosts and pathogens. *Curr. Opin. Plant Biol.* 52, 140–148. doi:10.1016/j.pbi.2019.09.001

Cai, Q., Qiao, L., Wang, M., He, B., Lin, F. M., Palmquist, J., et al. (2018). Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes. *Science* 360 (6393), 1126–1129. doi:10.1126/science.aar4142

Cao, M., Yan, H., Han, X., Weng, L., Wei, Q., Sun, X., et al. (2019). Ginseng-derived nanoparticles alter macrophage polarization to inhibit melanoma growth. *J. Immunother. Cancer* 7 (1), 326. doi:10.1186/s40425-019-0817-4

Carnino, J. M., Lee, H., and Jin, Y. (2019). Isolation and characterization of extracellular vesicles from broncho-alveolar lavage fluid: A review and comparison of different methods. *Respir. Res.* 20 (1), 240. doi:10.1186/s12931-019-1210-z

Chen, C., Wang, J., Sun, M., Li, J., and Wang, H. D. (2022). Toward the next-generation phyto-nanomedicines: Cell-derived nanovesicles (CDNs) for natural product delivery. *Biomed. Pharmacother.* 145, 112416. doi:10.1016/j.biopha.2021.112416

Chen, X., Zhou, Y., and Yu, J. (2019). Exosome-like nanoparticles from ginger rhizomes inhibited NLRP3 inflammasome activation. *Mol. Pharm.* 16 (6), 2690–2699. doi:10.1021/acs.molpharmaceut.9b00246

Chin, A. R., Fong, M. Y., Somlo, G., Wu, J., Swiderski, P., Wu, X., et al. (2016). Cross-kingdom inhibition of breast cancer growth by plant miR159. *Cell Res.* 26 (2), 217–228. doi:10.1038/cr.2016.13

Cho, E. G., Choi, S. Y., Kim, H., Choi, E. J., Lee, E. J., Park, P. J., et al. (2021). Panax ginseng-derived extracellular vesicles facilitate anti-senescence effects in human skin cells: An eco-friendly and sustainable way to use ginseng substances. *Cells* 10 (3), 486. doi:10.3390/cells10030486

Chukhchin, D. G., Bolotova, K., Sinelnikov, I., Churilov, D., and Novozhilov, E. (2019). Exosomes in the phloem and xylem of woody plants. *Planta* 251 (1), 12. doi:10.1007/s00425-019-03315-y

Chung, K. P., and Zeng, Y. (2017). "An overview of protein secretion in plant cells," in *Plant protein secretion: Methods and protocols*. Editor L. Jiang (Charm: Springer), 19–32.

Dad, H. A., Gu, T. W., Zhu, A. Q., Huang, L. Q., and Peng, L. H. (2021). Plant exosome-like nanovesicles: Emerging therapeutics and drug delivery nanoplatforms. *Mol. Ther.* 29 (1), 13–31. doi:10.1016/j.ymthe.2020.11.030

Dayakar, A., Shanmukha, K. D., and Kalangi, S. K. (2022). Spectrum of microRNAs and their target genes in cancer: Intervention in diagnosis and therapy. *Mol. Biol. Rep.* 49, 6827–6846. doi:10.1007/s11033-021-07040-2

De Jong, W. H., and Borm, P. J. A. (2008). Drug delivery and nanoparticles: Applications and hazards. *Int. J. Nanomedicine* 3 (2), 133–149. doi:10.2147/ijn.s596

De Robertis, M., Sarra, A., D'Oria, V., Mura, F., Bordi, F., Postorino, P., et al. (2020). Blueberry-derived exosome-like nanoparticles counter the response to TNF-alpha-induced change on gene expression in EA.hy926 cells. *Biomolecules* 10 (5), E742. doi:10.3390/biom10050742

Ding, Y., and Wang, J. (2017). "Analysis of exocyst-positive organelle (EXPO)-Mediated unconventional protein secretion (UPS) in plant cells," in *Plant protein secretion: Methods and protocols*. Editor L. Jiang (Charm: Springer), 231–241.

Doyle, L. M., and Wang, M. Z. (2019). Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis. *Cells* 8 (7), E727. doi:10.3390/cells8070727

Friedmann, T. (2016). An ASGCT perspective on the national academies genome editing summit. *Mol. Ther.* 24 (1), 1–2. doi:10.1038/mt.2015.228

Galmarini, D., Galmarini, C. M., and Galmarini, F. C. (2012). Cancer chemotherapy: A critical analysis of its 60 years of history. *Crit. Rev. Oncol. Hematol.* 84 (2), 181–199. doi:10.1016/j.critrevonc.2012.03.002

Hatsugai, N., Iwasaki, S., Tamura, K., Kondo, M., Fuji, K., Ogasawara, K., et al. (2009). A novel membrane fusion-mediated plant immunity against bacterial pathogens. *Genes Dev.* 23 (21), 2496–2506. doi:10.1101/gad.1825209

Ivanov, S., Austin, J., II, Berg, R. H., and Harrison, M. J. (2019). Extensive membrane systems at the host-arbuscular mycorrhizal fungus interface. *Nat. Plants* 5(2), 194–203. doi:10.1038/s41477-019-0364-5

Jackett, L. A., and Scolyer, R. A. (2019). A review of key biological and molecular events underpinning transformation of melanocytes to primary and metastatic melanoma. *Cancers (Basel)* 11 (12), E2041. doi:10.3390/cancers11122041

Jiang, D., Zhang, L., Liu, W., Ding, Y., Yin, J., Ren, R., et al. (2021). Trends in cancer mortality in China from 2004 to 2018: A nationwide longitudinal study. *Cancer Commun.* 41 (10), 1024–1036. doi:10.1002/cac2.12195

Karimi, H. Z., Baldrich, P., Rutter, B. D., Borniego, L., Zajt, K. K., Meyers, B. C., et al. (2022). Arabidopsis apoplastic fluid contains sRNA- and circular RNA-protein complexes that are located outside extracellular vesicles. *Plant Cell* 34, 1863–1881. doi:10.1093/plcell/koac043

Keim, U., Gandini, S., Amaral, T., Katalinic, A., Holleczek, B., Flatz, L., et al. (2021). Cutaneous melanoma attributable to UVR exposure in Denmark and Germany. *Eur. J. Cancer* 159, 98–104. doi:10.1016/j.ejca.2021.09.044

Kim, M. K., Choi, Y. C., Cho, S. H., Choi, J. S., and Cho, Y. W. (2021). The antioxidant effect of small extracellular vesicles derived from aloe vera peels for wound healing. *Tissue Eng. Regen. Med.* 18 (4), 561–571. doi:10.1007/s13770-021-00367-8

Ko, S. Y., and Naora, H. (2020). Extracellular vesicle membrane-associated proteins: Emerging roles in tumor angiogenesis and anti-angiogenesis therapy resistance. *Int. J. Mol. Sci.* 21 (15), E5418. doi:10.3390/ijms21155418

Konoshenko, M. Y., Lekchnov, E. A., Vlassov, A. V., and Laktionov, P. P. (2018). Isolation of extracellular vesicles: General methodologies and latest trends. *Biomed. Res. Int.* 2018, 8545347. doi:10.1155/2018/8545347

Kowal, J., Arras, G., Colombo, M., Jouve, M., Morath, J. P., Primdal-Bengtson, B., et al. (2016). Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. *Proc. Natl. Acad. Sci. U. S. A.* 113 (8), E968–E977. doi:10.1073/pnas.1521230113

Kuriyama, N., Yoshioka, Y., Kikuchi, S., Azuma, N., and Ochiya, T. (2020). Extracellular vesicles are key regulators of tumor neovasculature. *Front. Cell Dev. Biol.* 8, 611039. doi:10.3389/fcell.2020.611039

Kusuma, R. J., Manca, S., Friemel, T., Sukreet, S., Nguyen, C., and Zempleni, J. (2016). Human vascular endothelial cells transport foreign exosomes from cow's milk by endocytosis. *Am. J. Physiol. Cell Physiol.* 310 (10), C800–C807. doi:10.1152/ajpcell.00169.2015

Lee, R., Ko, H. J., Kim, K., Sohn, Y., Min, S. Y., Kim, J. A., et al. (2020). Anti-melanogenic effects of extracellular vesicles derived from plant leaves and stems in mouse melanoma cells and human healthy skin. *J. Extracell. Vesicles* 9 (1), 1703480. doi:10.1080/20013078.2019.1703480

Lei, C., Mu, J., Teng, Y., He, L., Xu, F., Zhang, X., et al. (2020). Lemon exosome-like nanoparticles-manipulated probiotics protect mice from C. d iff infection. *iScience* 23 (10), 101571. doi:10.1016/j.isci.2020.101571

Li, H., Xu, W., Li, F., Zeng, R., Zhang, X., Wang, X., et al. (2022). Amplification of anticancer efficacy by co-delivery of doxorubicin and lonidamine with extracellular vesicles. *Drug Deliv.* 29 (1), 192–202. doi:10.1080/10717544.2021.2023697

Liang, Y., Duan, L., Lu, J., and Xia, J. (2021). Engineering exosomes for targeted drug delivery. *Theranostics* 11 (7), 3183–3195. doi:10.7150/thno.52570

Liangsupree, T., Multia, E., and Riekkola, M. L. (2021). Modern isolation and separation techniques for extracellular vesicles. *J. Chromatogr. A* 1636, 461773. doi:10.1016/j.chroma.2020.461773

Logozzi, M., Di Raimo, R., Mizzoni, D., and Fais, S. (2021). Nanovesicles from organic agriculture-derived fruits and vegetables: Characterization and functional antioxidant content. *Int. J. Mol. Sci.* 22 (15), 8170. doi:10.3390/ijms22158170

Luo, Y., Wang, P., Wang, X., Wang, Y., Mu, Z., Li, Q., et al. (2017). Detection of dietetically absorbed maize-derived microRNAs in pigs. *Sci. Rep.* 7 (1), 645. doi:10.1038/s41598-017-00488-y

Mathieu, M., Martin-Jaular, L., Lavieu, G., and Thery, C. (2019). Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. *Nat. Cell Biol.* 21 (1), 9–17. doi:10.1038/s41556-018-0250-9

Menard, J. A., Cerezo-Magana, M., and Belting, M. (2018). Functional role of extracellular vesicles and lipoproteins in the tumour microenvironment. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 373, 20160480. doi:10.1098/rstb.2016.0480

Meng, L., Ji, R., Dong, X., Xu, X., Xin, Y., and Jiang, X. (2019). Antitumor activity of ginsenoside Rg3 in melanoma through downregulation of the ERK and Akt pathways. *Int. J. Oncol.* 54 (6), 2069–2079. doi:10.3892/ijo.2019.4787

Meyer, D., Pajonk, S., Micali, C., O'Connell, R., and Schulze-Lefert, P. (2009). Extracellular transport and integration of plant secretory proteins into pathogen-induced cell wall compartments. *Plant J.* 57 (6), 986–999. doi:10.1111/j.1365-313X.2008.03743.x

Minutolo, A., Potesta, M., Gismondi, A., Pirro, S., Cirilli, M., Gattabria, F., et al. (2018). Olea europaea small RNA with functional homology to human miR34a in cross-kingdom interaction of anti-tumoral response. *Sci. Rep.* 8 (1), 12413. doi:10.1038/s41598-018-30718-w

Mittal, S., Gupta, P., Chaluvally-Raghavan, P., and Pradeep, S. (2020). Emerging role of extracellular vesicles in immune regulation and cancer progression. *Cancers (Basel)* 12 (12), E3563. doi:10.3390/cancers12123563

Munir, J., Lee, M., and Ryu, S. (2020). Exosomes in food: Health benefits and clinical relevance in diseases. *Adv. Nutr.* 11 (3), 687–696. doi:10.1093/advances/nmz123

Muralidharan-Chari, V., Clancy, J. W., Sedgwick, A., and D'Souza-Schorey, C. (2010). Microvesicles: Mediators of extracellular communication during cancer progression. *J. Cell Sci.* 123 (10), 1603–1611. doi:10.1242/jcs.064386

Ozkan, I., Kocak, P., Yildirim, M., Unsal, N., Yilmaz, H., Telci, D., et al. (2021). Garlic (Allium sativum)-derived SEVs inhibit cancer cell proliferation and induce caspase mediated apoptosis. *Sci. Rep.* 11 (1), 14773. doi:10.1038/s41598-021-93876-4

Pan, B. T., and Johnstone, R. M. (1983). Fate of the transferrin receptor during maturation of sheep reticulocytes *in vitro*: Selective externalization of the receptor. *Cell* 33 (3), 967–978. doi:10.1016/0092-8674(83)90040-5

Perut, F., Roncuzzi, L., Avnet, S., Massa, A., Zini, N., Sabbadini, S., et al. (2021). Strawberry-derived exosome-like nanoparticles prevent oxidative stress in human mesenchymal stromal cells. *Biomolecules* 11 (1), 87. doi:10.3390/biom11010087

Pocsfalvi, G., Turiak, L., Ambrosone, A., Del Gaudio, P., Puska, G., Fiume, I., et al. (2019). Physiochemical and protein datasets related to citrus juice sac cells-derived nanovesicles and microvesicles. *Data Brief.* 22, 251–254. doi:10.1016/j.dib.2018.12.036

Potesta, M., Roglia, V., Fanelli, M., Pietrobono, E., Gismondi, A., Vumbaca, S., et al. (2020). Effect of microvesicles from Moringa oleifera containing miRNA on proliferation and apoptosis in tumor cell lines. *Cell Death Discov.* 6, 43. doi:10.1038/s41420-020-0271-6

Pullan, J. E., Confeld, M. I., Osborn, J. K., Kim, J., Sarkar, K., and Mallik, S. (2019). Exosomes as drug carriers for cancer therapy. *Mol. Pharm.* 16 (5), 1789–1798. doi:10.1021/acs.molpharmaceut.9b00104

Raimondo, S., Naselli, F., Fontana, S., Monteleone, F., Lo Dico, A., Saieva, L., et al. (2015). Citrus limon-derived nanovesicles inhibit cancer cell proliferation and suppress CML xenograft growth by inducing TRAIL-mediated cell death. *Oncotarget* 6 (23), 19514–19527. doi:10.18632/oncotarget.4004

Ratajczak, J., Wysoczynski, M., Hayek, F., Janowska-Wieczorek, A., and Ratajczak, M. Z. (2006). Membrane-derived microvesicles: Important and underappreciated mediators of cell-to-cell communication. *Leukemia* 20 (9), 1487–1495. doi:10.1038/sj.leu.2404296

Regente, M., Pinedo, M., San Clemente, H., Balliau, T., Jamet, E., and de la Canal, L. (2017). Plant extracellular vesicles are incorporated by a fungal pathogen and inhibit its growth. *J. Exp. Bot.* 68 (20), 5485–5495. doi:10.1093/jxb/erx355

Rutter, B. D., and Innes, R. W. (2018). Extracellular vesicles as key mediators of plant-microbe interactions. *Curr. Opin. Plant Biol.* 44, 16–22. doi:10.1016/j.pbi.2018.01.008

Rutter, B. D., and Innes, R. W. (2017). Extracellular vesicles isolated from the leaf apoplast carry stress-response proteins. *Plant Physiol.* 173 (1), 728–741. doi:10.1104/pp.16.01253

Sahin, F., Kocak, P., Gunes, M. Y., Ozkan, I., Yildirim, E., and Kala, E. Y. (2019). *In vitro* wound healing activity of wheat-derived nanovesicles. *Appl. Biochem. Biotechnol.* 188 (2), 381–394. doi:10.1007/s12010-018-2913-1

Sasaki, D., Kusamori, K., Takayama, Y., Itakura, S., Todo, H., and Nishikawa, M. (2021). Development of nanoparticles derived from corn as mass producible bionanoparticles with anticancer activity. *Sci. Rep.* 11 (1), 22818. doi:10.1038/s41598-021-02241-y

Savci, Y., Kirbas, O. K., Bozkurt, B. T., Abdik, E. A., Tasli, P. N., Sahin, F., et al. (2021). Grapefruit-derived extracellular vesicles as a promising cell-free therapeutic tool for wound healing. *Food Funct.* 12 (11), 5144–5156. doi:10.1039/d0fo02953j

Schweiger, M. W., and Tannous, B. A. (2020). Small but fierce: Tracking the role of extracellular vesicles in glioblastoma progression and therapeutic resistance. *Adv. Biosyst.* 4 (12), e2000035. doi:10.1002/adbi.202000035

Stanly, C., Alfieri, M., Ambrosone, A., Leone, A., Fiume, I., and Pocsfalvi, G. (2020). Grapefruit-derived micro and nanovesicles show distinct metabolome profiles and anticancer activities in the A375 human melanoma cell line. *Cells* 9 (12), E2722. doi:10.3390/cells9122722

Stanly, C., Moubarak, M., Fiume, I., Turiak, L., and Pocsfalvi, G. (2019). Membrane transporters in citrus clementina fruit juice-derived nanovesicles. *Int. J. Mol. Sci.* 20 (24), E6205. doi:10.3390/ijms20246205

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Ca. Cancer J. Clin.* 71 (3), 209–249. doi:10.3322/caac.21660

Tang, D., Liu, S., Shen, H., Deng, G., and Zeng, S. (2022). Extracellular vesicles promote the formation of pre-metastasis niche in gastric cancer. *Front. Immunol.* 13, 813015. doi:10.3389/fimmu.2022.813015

Tang, Z., Jun, Y., Lv, Y., Li, Y., Zhang, Z., Tao, M., et al. (2020). Aptamer-conjugated and doxorubicin-loaded grapefruit-derived nanovectors for targeted therapy against HER2(+) breast cancer. *J. Drug Target.* 28 (2), 186–194. doi:10.1080/1061186X.2019.1624970

Tarasov, V. V., Svistunov, A. A., Chubarev, V. N., Dostdar, S. A., Sokolov, A. V., Brzecka, A., et al. (2021). Extracellular vesicles in cancer nanomedicine. *Semin. Cancer Biol.* 69, 212–225. doi:10.1016/j.semcancer.2019.08.017

Teng, Y., Ren, Y., Sayed, M., Hu, X., Lei, C., Kumar, A., et al. (2018). Plant-derived exosomal MicroRNAs shape the gut microbiota. *Cell Host Microbe* 24 (5), 637–652. doi:10.1016/j.chom.2018.10.001

Teng, Y., Xu, F., Zhang, X., Mu, J., Sayed, M., Hu, X., et al. (2021). Plant-derived exosomal microRNAs inhibit lung inflammation induced by exosomes SARS-CoV-2 Nsp12. *Mol. Ther.* 29 (8), 2424–2440. doi:10.1016/j.ymthe.2021.05.005

Thery, C., Amigorena, S., Raposo, G., and Clayton, A. (2006). Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Curr. Protoc. Cell Biol.* 3, 22. doi:10.1002/0471143030.cb0322s30

Tse, Y. C., Mo, B. X., Hillmer, S., Zhao, M., Lo, S. W., Robinson, D. G., et al. (2004). Identification of multivesicular bodies as prevacuolar compartments in Nicotiana tabacum BY-2 cells. *Plant Cell* 16 (3), 672–693. doi:10.1105/tpc.019703

Vatner, R. E., Cooper, B. T., Vanpouille-Box, C., Demaria, S., and Formenti, S. C. (2014). Combinations of immunotherapy and radiation in cancer therapy. *Front. Oncol.* 4, 325. doi:10.3389/fonc.2014.00325

Vu, L. T., Gong, J., Pham, T. T., Kim, Y., and Le, M. T. N. (2020). microRNA exchange via extracellular vesicles in cancer. *Cell Prolif.* 53 (11), e12877. doi:10.1111/cpr.12877

Wang, B., Zhuang, X., Deng, Z. B., Jiang, H., Mu, J., Wang, Q., et al. (2014). Targeted drug delivery to intestinal macrophages by bioactive nanovesicles released from grapefruit. *Mol. Ther.* 22 (3), 522–534. doi:10.1038/mt.2013.190

Wang, J., Ding, Y., Wang, J., Hillmer, S., Miao, Y., Lo, S. W., et al. (2010). EXPO, an exocyst-positive organelle distinct from multivesicular endosomes and autophagosomes, mediates cytosol to cell wall exocytosis in arabidopsis and tobacco cells. *Plant Cell* 22 (12), 4009–4030. doi:10.1105/tpc.110.080697

Wang, Q., Ren, Y., Mu, J., Egilmez, N. K., Zhuang, X., Deng, Z., et al. (2015). Grapefruit-derived nanovectors use an activated leukocyte trafficking pathway to deliver therapeutic agents to inflammatory tumor sites. *Cancer Res.* 75 (12), 2520–2529. doi:10.1158/0008-5472.CAN-14-3095

Wang, X., Chung, K. P., Lin, W., and Jiang, L. (2018). Protein secretion in plants: Conventional and unconventional pathways and new techniques. *J. Exp. Bot.* 69 (1), 21–37. doi:10.1093/jxb/erx262

Witwer, K. W., and Thery, C. (2019). Extracellular vesicles or exosomes? On primacy, precision, and popularity influencing a choice of nomenclature. *J. Extracell. Vesicles* 8 (1), 1648167. doi:10.1080/20013078.2019.1648167

Wu, F., Xie, M., Hun, M., She, Z., Li, C., Luo, S., et al. (2021). Natural killer cell-derived extracellular vesicles: Novel players in cancer immunotherapy. *Front. Immunol.* 12, 658698. doi:10.3389/fimmu.2021.658698

Xiao, J., Feng, S., Wang, X., Long, K., Luo, Y., Wang, Y., et al. (2018). Identification of exosome-like nanoparticle-derived microRNAs from 11 edible fruits and vegetables. *PeerJ* 6, e5186. doi:10.7717/peerj.5186

Xiao, Q., Zhao, W., Wu, C., Wang, X., Chen, J., Shi, X., et al. (2022). Lemon-derived extracellular vesicles nanodrugs enable to efficiently overcome cancer multidrug resistance by endocytosis-triggered energy dissipation and energy production reduction. *Adv. Sci.* 9, e2105274. doi:10.1002/advs.202105274

Xu, R., Greening, D. W., Rai, A., Ji, H., and Simpson, R. J. (2015). Highly-purified exosomes and shed microvesicles isolated from the human colon cancer cell line LIM1863 by sequential centrifugal ultrafiltration are biochemically and functionally distinct. *Methods* 87, 11–25. doi:10.1016/j.ymeth.2015.04.008

Xu, Z., Chen, Y., and Hu, F. (2022). Advances in the research of microRNA in Orchidaceae. *Sheng Wu Gong Cheng Xue Bao* 38 (1), 66–76. doi:10.13345/j.cjb.210118

Yang, M., Liu, X., Luo, Q., Xu, L., and Chen, F. (2020). An efficient method to isolate lemon derived extracellular vesicles for gastric cancer therapy. *J. Nanobiotechnology* 18 (1), 100. doi:10.1186/s12951-020-00656-9

Yang, M., Luo, Q., Chen, X., and Chen, F. (2021). Bitter melon derived extracellular vesicles enhance the therapeutic effects and reduce the drug resistance of 5-fluorouracil on oral squamous cell carcinoma. *J. Nanobiotechnology* 19 (1), 259. doi:10.1186/s12951-021-00995-1

Yim, N., and Choi, C. (2016). Extracellular vesicles as novel carriers for therapeutic molecules. *BMB Rep.* 49 (11), 585–586. doi:10.5483/bmbrep.2016.49.11.174

Zhang, L., He, F., Gao, L., Cong, M., Sun, J., Xu, J., et al. (2021). Engineering exosome-like nanovesicles derived from Asparagus cochinchinensis can inhibit the proliferation of hepatocellular carcinoma cells with better safety profile. *Int. J. Nanomedicine* 16, 1575–1586. doi:10.2147/IJN.S293067

Zhang, L., Hou, D., Chen, X., Li, D., Zhu, L., Zhang, Y., et al. (2012). Exogenous plant MIR168a specifically targets mammalian LDLRAP1: Evidence of cross-kingdom regulation by microRNA. *Cell Res.* 22 (1), 107–126. doi:10.1038/cr.2011.158

Zhou, Q.-L., and Yang, X.-W. (2015). Four new ginsenosides from red ginseng with inhibitory activity on melanogenesis in melanoma cells. *Bioorg. Med. Chem. Lett.* 25 (16), 3112–3116. doi:10.1016/j.bmcl.2015.06.017

Zhou, Q., Ma, K., Hu, H., Xing, X., Huang, X., and Gao, H. (2021). Extracellular vesicles: Their functions in plant-pathogen interactions. *Mol. Plant Pathol.* 23, 760–771. doi:10.1111/mpp.13170

Zhou, S., Hu, T., Han, G., Wu, Y., Hua, X., Su, J., et al. (2020). Accurate cancer diagnosis and stage monitoring enabled by comprehensive profiling of different types of exosomal biomarkers: Surface proteins and miRNAs. *Small* 16 (48), e2004492. doi:10.1002/smll.202004492

Zu, M., Xie, D., Canup, B. S. B., Chen, N., Wang, Y., Sun, R., et al. (2021). 'Green' nanotherapeutics from tea leaves for orally targeted prevention and alleviation of colon diseases. *Biomaterials* 279, 121178. doi:10.1016/j.biomaterials.2021.121178

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 05 October 2022
DOI 10.3389/fphar.2022.1033017

Check for updates

OPEN ACCESS

EDITED BY
Yue Hou,
Northeastern University, China

REVIEWED BY
Di Wang,
Jilin Agriculture University, China
Shi Qiu,
Shanghai University of Traditional Chinese Medicine, China

*CORRESPONDENCE
Haoming Luo,
luo.haoming@163.com
Donglu Wu,
wudl1020@163.com

SPECIALTY SECTION
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

RECEIVED 31 August 2022
ACCEPTED 20 September 2022
PUBLISHED 05 October 2022

CITATION
Fan M, Shan M, Lan X, Fang X, Song D, Luo H and Wu D (2022), Anti-cancer effect and potential microRNAs targets of ginsenosides against breast cancer. *Front. Pharmacol.* 13:1033017. doi: 10.3389/fphar.2022.1033017

COPYRIGHT
© 2022 Fan, Shan, Lan, Fang, Song, Luo and Wu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Anti-cancer effect and potential microRNAs targets of ginsenosides against breast cancer

Meiling Fan[1], Mengyao Shan[2,3], Xintian Lan[2,3], Xiaoxue Fang[2,3], Dimeng Song[2,3], Haoming Luo[2,3]* and Donglu Wu[3,4]*

[1]Changchun University of Chinese Medicine, Changchun, China, [2]School of Pharmacy, Changchun University of Chinese Medicine, Changchun, China, [3]Key Laboratory of Effective Components of Traditional Chinese Medicine, Changchun, China, [4]School of Clinical Medical, Changchun University of Chinese Medicine, Changchun, China

Breast cancer (BC) is one of the most common malignant tumor, the incidence of which has increased worldwide in recent years. Ginsenosides are the main active components of *Panax ginseng* C. A. Mey., *in vitro* and *in vivo* studies have confirmed that ginsenosides have significant anti-cancer activity, including BC. It is reported that ginsenosides can induce BC cells apoptosis, inhibit BC cells proliferation, migration, invasion, as well as autophagy and angiogenesis, thereby suppress the procession of BC. In this review, the therapeutic effects and the molecular mechanisms of ginsenosides on BC will be summarized. And the combination strategy of ginsenosides with other drugs on BC will also be discussed. In addition, epigenetic changes, especially microRNAs (miRNAs) targeted by ginsenosides in the treatment of BC are clarified.

KEYWORDS

ginsenosides, breast cancer, MicroRNAs, combination therapy, molecular mechanism

Introduction

Ginsenosides are the main active components of traditional Chinese herbal medicine *Panax ginseng* C. A. Mey. (Song et al., 2022). At present, nearly 200 ginsenosides have been isolated and identified from the roots, stems, leaves, flowers buds, and berries of *Panax ginseng* C. A. Mey. (Zhao A. et al., 2022). Ginsenosides can be divided into three classes according to different aglycone structures, protopanaxadiol (PPD), protopanaxatriol (PPT) and oleanolic acid. PPD types mainly includes ginsenosides Rb1, Rd, Rg3, Rh2, CK and F2, etc., PPT types mainly includes ginsenosides Re, Rf, Rg1, Rg2, Rh1 and F1, etc., and oleanolic acid types mainly includes ginsenosides Ro, etc. (Hou et al., 2021; Liu G. et al., 2022). Modern pharmacology has shown that ginsenosides have neuroprotective (Zarneshan et al., 2022), anti-aging (Meng et al., 2022), anti-oxidant (He et al., 2022), anti-inflammatory (Xu et al., 2022) and anti-cancer (Zhao L. et al., 2022) effects. Numerous ginsenosides has been reported have various anti-cancer activity. Such as, ginsenoside Rg3 can effectively inhibit prostate cancer, gastric cancer, gallblader cancer

and ovarian cancer, etc., while ginsenoside CK can function in suppress the proliferation and procession of liver cancer, lung cancer, colon cancer, and bladder cancer, etc. (Liu Z. et al., 2021; Liu J. et al., 2022).

Breast cancer (BC) is the most common cancer among female patients in the world (Zeng et al., 2020). According to the data of International Agency for Research on Cancer (IARC) (Hu et al., 2022), more than 2.26 million women in the world were diagnosed with BC and nearly 68.5 million women died of BC in 2020. While this number was estimated around more than 3 million new cases and 1 million deaths every year by 2040. BC is a disease with complex etiology and high heterogeneity, can be divided into three types, hormone receptor positive (estrogen and progesterone), human epidermal growth factor receptor 2 (HER2) positive and triple negative breast cancer (TNBC, accounting for 10%–20% of BC cases) (Karami Fath et al., 2022). In clinical, treatment of BC mainly include surgical resection, radiotherapy, hormone therapy and so on (Qiu et al., 2021). However, these treatments are accompanied by adverse conditions such as drug resistance (Hobbs et al., 2022).

Studies have shown that ginsenosides can mediating numerous processions of BC, including drive apoptosis and autophagy, regulate cell cycle and inhibit metastasis (Jin et al., 2020). At present, a variety of ginsenosides have been reported to inhibit the proliferation of BC, such as ginsenoside Rg3 and ginsenoside Rd can inhibit the metastasis of BC cells. While ginsenosides Rg2, Rg5 and CK can induce autophagy, apoptosis and cell cycle arrest of BC cells (Hong et al., 2021). Moreover, combination treatment of ginsenosides and other chemotherapeutic drugs have been reported. For example, combination of ginsenoside Rg3 with curcumin or endostar can improve BC cells radiosensitivity, and inhibit metastasis of BC cells, respectively (Zhang et al., 2016; Changizi et al., 2021). The relationship between microRNAs (miRNAs) and BC was first clarified in 2005, and subsequent studies revealed numerous miRNAs closely related to the development of BC (Iorio et al., 2005; Shekari et al., 2022). It has been reported that miRNAs can be used to determine the stage of BC, thereby predicting the survival rate of BC patients (Karami Fath et al., 2022). Moreover, miRNAs detected in body fluids serve as biomarkers for the diagnosis and prognosis of BC (Liu L. et al., 2022). Ginsenoside Rd inhibited the expression of miR-18a, which in turn inhibited the proliferation, metastasis and invasion of BC cells (Wang et al., 2016). While ginsenoside Rh2 inhibits proliferation and induces apoptosis of BC cells by inhibiting miR-4425 and miR-3614-3p expression, respectively (Park et al., 2021; Park et al., 2022). The present review is aim to summarize the pharmacological activities of ginsenosides in BC therapy, as well as indicated molecular mechanism. Further overview the combination therapies against BC and discuss the clinical prospects of ginsenosides. Moreover, miRNAs targeted by ginsenosides in BC treatment will also be demonstrated.

Anti-breast cancer activity of ginsenosides

PPD and PPT are both dammarane tetracyclic triterpene saponins, and the main difference in structure is whether there is a hydroxyl substitution on the carbon at position 6 (Pan et al., 2018). Moreover, in the anti-BC research of ginsenosides, it mainly revolves around PPD and PPT types ginsenosides, and in details, the structure is mainly different in groups on the carbon at positions 3, 6 and 20 (Figure 1). In the following section, the biological activity of ginsenosides against BC according to ginsenosides chemical structures will be investigated (Figure 2, Table 1).

Anti-breast cancer activity of PPD types

PPD has good biological activities of anti-oxidation, anti-inflammatory, as well as anti-cancer (Yang et al., 2019). At present, several studies have reported the anti-BC effects of PPD and related mechanisms. Such as, ginsenoside F2 inhibits the proliferation of breast cancer stem cells (CSCs) by regulating p53 to induce apoptosis and stimulate autophagy (Mai et al., 2012). Ginsenoside Rb1 can decrease tumor growth and tumor weight *in vivo*, and induce apoptosis of BC cells *in vitro* by binding to carbon nanotubes (Lahiani et al., 2017; Zuo et al., 2022). Moreover, ginsenoside Rb3 increases the anti-proliferative activity of cisplatin at higher concentrations (Aung et al., 2007). In this report, the berry extract of American ginseng has the effect of anti-proliferation of BC cells, and ginsenoside Rb3 is the active ingredient with the highest content. We speculate that ginsenoside Rb3 has the effect of anti-BC.

Ginsenoside Rd Ginsenoside Rd is one of the metabolites of ginsenoside Rb1 in the intestine (Zhang et al., 2021) and has a wide range of biological activities including neuroprotection, improved metabolism, as well as anti-cancer (Li J. et al., 2022). It is reported that ginsenoside Rd inhibits proliferation and drives apoptosis of BC cells (Kim 2013). Ginsenoside Rd can target transient receptor potential (TRP) melastatin 7 (TRPM7), a member of the TRP channel family and functions in cell cycle regulation, further mediate the proliferation and survival of BC cells (Liu H. et al., 2020). On the other hand, ginsenoside Rd shows the activity of suppressing angiogenesis and metastasis. In details, ginsenoside Rd inhibited migration, invasion and lung metastasis of BC cells *in vitro* and *in vivo*, respectively (Wang et al., 2016). Moreover, ginsenoside Rd can inhibit HIF-1α (hypoxia inducible factor-1)/VEGF (vascular endothelial growth factors) through Akt/mTOR/p70S6K signaling pathway and then inhibit angiogenesis and cut off nutrient supply (Zhang et al., 2017). VEGF, also known as vascular permeability factor, promotes endothelial cell proliferation, migration and invasion into surrounding tissues, and is an important factor in tumor angiogenesis (Adams and Alitalo

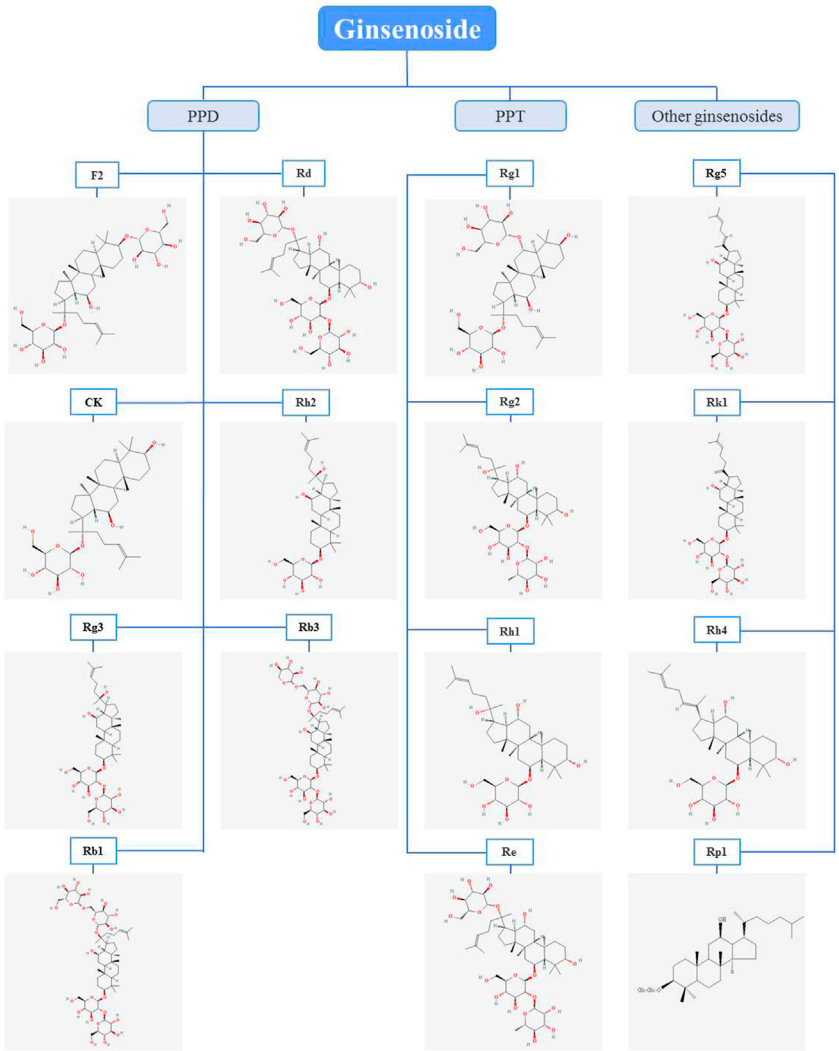


FIGURE 1
Chemical structures of ginsenosides with anti-breast cancer activity. Glc, glucose. The chemical structures of the ginsenosides included in this publication were obtained from pubchem, but ginsenoside Rp1 was drawn using the chemdraw program.

2007). Therefore, we speculate that ginsenoside Rd can not only inhibit angiogenesis but also inhibit the growth, metastasis and invasion of BC cells by targeting VEGF.

Ginsenoside CK Ginsenoside CK is the main metabolite and final absorption form of PPD type ginsenoside in the intestine (Jin et al., 2018). Ginsenoside CK can inhibit the proliferation, induce apoptosis of MCF-7 cells, as well as EMT, through down-regulating the PI3K/Akt pathway (Zhang and Li 2016). Moreover, ginsenoside CK can reduce glycogen synthase kinase-3β (GSK-3β) phosphorylation, which leads to decreased expression of oncoprotein β-catenin and cyclin D1, thereby induce programmed necrosis of cancer cells (Kwak et al., 2015). TNBC is particularly addicted to glutamine, which is an essential nutrient that replenishes energy to cancer cells (Edwards et al., 2021). Studies have shown that ginsenoside CK targets glutamine metabolism and exerts anti-cancer effects on high glutamine-dependent TNBC cells both *in vitro* and *in vivo*. In particularly, ginsenoside CK decreased the expression of glutaminase 1 (GLS1), resulting in reduced ATP production, attenuated amino acid availability, causing oxidative stress and TNBC cells growth inhibition and apoptosis (Zhang B. et al., 2022).

Ginsenoside Rh2 Ginsenoside Rh2 has several pharmacological activities, including improving cardiac function, anti-inflammatory and anti-cancer effects (Liu T. et al., 2022). It is reported that ginsenoside Rh2 mediates cell cycle arrest and inhibits proliferation of BC cells (Oh et al., 1999; Hu et al., 2021). Ginsenoside Rh2 can mediate G_1/S cell cycle

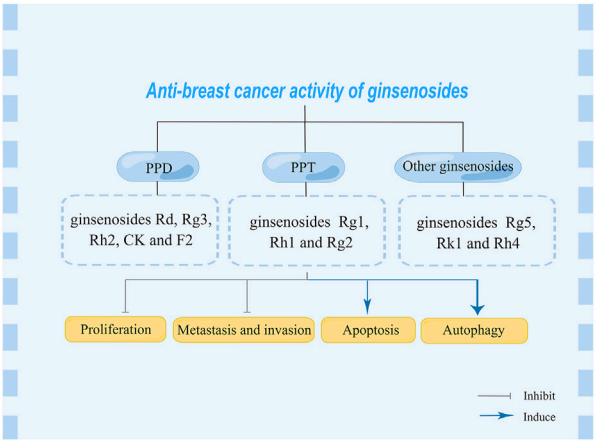


FIGURE 2
Anti-breast cancer effect of various ginsenosides.

arrest by activating p38 and decreasing cyclin D1 expression (Peng et al., 2022). Moreover, ginsenoside Rh2 mediates G1 phase arrest of BC cells, which is caused by p15 INK4B and p27 kip1 dependent inhibition of CDKs/cyclin complex (Choi et al., 2009). In addition, ginsenoside Rh2 can up-regulate the phosphorylation levels of p53, p38 and ASK1 (apoptosis signal-regulating kinase 1), whereas down-regulate the expression of TRAF2 (TNF receptor-associated factor 2), thereby inhibiting the proliferation of BC cells (Ren et al., 2018). It is reported that ginsenoside Rh2 can induce BC cells apoptosis (Park et al., 2022). Studies have shown that ginsenoside Rh2 can increase the expression of TNFα (tumor necrosis factor α) by up-regulating ERβ (estrogen receptor β), which in turn induces apoptosis of BC cells (Peng et al., 2022). Furthermore, ginsenoside Rh2 induced apoptosis of BC cells was associated with increased levels of pro-apoptotic proteins including Bak, Bax and Bim, and decreased levels of anti-apoptotic proteins including Bcl-2, Bcl-xL and Mcl-1 (Choi et al., 2011). On the other hand, ginsenoside Rh2 shows the activity of inhibiting the migration and invasion of cancer cells. In details, studies have shown that ginsenoside Rh2 can inhibit migration and invasion of BC cells promoted by the senescence-associated secretory phenotype of BC cells and normal epithelial cells (Hou et al., 2019).

Ginsenoside Rg3 Ginsenoside Rg3 has anti-cancer activity and has been demonstrated in many types of cancer, including BC (Liu M. Y. et al., 2021). It has been reported that two epimers of ginsenoside Rg3, 20(S)-ginsenoside Rg3 (SRg3) and 20(R)-ginsenoside Rg3 (RRg3), can inhibit the proliferation, migration and invasion of BC cells, respectively (Nakhjavani et al., 2019). In details, ginsenoside Rg3 can affect cell proliferation through a variety of pathways, including inducing protein synthesis, cell division, and inhibiting nuclear factor kappa-B (NF-κB) signaling (Zou et al., 2018), among which, SRg3 inhibits BC cells proliferation by arresting the cell cycle of G0/G1 (Nakhjavani et al., 2019). Furthermore, studies demonstrated that SRg3 induces apoptosis of BC cells through classical mitochondria-dependent caspase activation (Kim et al., 2013). In details, SRg3 inhibits the phosphorylation of ERK and Akt, as well as induces the instability of mutant p53, thereby blocking NF-κB signal transduction, which in turn decreases the expression of Bcl-2 and ultimately induces apoptosis of BC cells (Kim et al., 2014). Moreover, ginsenoside Rg3 shows the activity of inhibiting angiogenesis, driving autophagy, as well as mitastasis of BC cells (Zhang et al., 2016). SRg3 inhibits the expression of VEGFs and matrix metalloproteinases (MMPs), which in turn suppresses angiogenesis, and further enhances the autophagic process by inhibiting mTOR/PI3K/Akt and JNK/Beclin-1 signaling pathways (Zhang et al., 2016). While SRg3 can also target CXCR4 expression and function in inhibiting the migration of BC cells (Chen et al., 2011). On the other hand, ginsenoside Rg3 are able to reduce stem cell properties and EMT to exert anti-cancer effects. Cancer stem cell properties play critical roles in tumorigenesis, progression, and therapy, and SRg3 inhibits self-renewal activity in breast stem-like cancer cells by blocking Akt-induced HIF-1α activation and inhibiting HIF-1α-regulated expression of Bmi-1 and Sox-2 (Oh et al., 2019). Moreover, SRg3 inhibited PI3K/AKT pathway to decrease the expression of cellular "stemness", which in turn reduced mammosphere formation efficiency (Nakhjavani et al., 2021). And studies have shown that (S, R) Rg3 down-regulates cancer "stemness" and EMT suppresses myeloid-derived suppressor cell (MDSC) of BC cells, thereby exerting anti-cancer effects (Song et al., 2020).

Anti-breast cancer activity of PPT types

PPT has anti-cancer biological activity, including BC (Yu et al., 2018; Wang B. et al., 2020). For example, ginsenoside Re has the activity of inhibiting the proliferation of BC cells, while increasing the activity of cisplatin in a concentration-related manner and improving its anti-proliferative effect on BC (Aung et al., 2007).

Ginsenoside Rg1 Ginsenoside Rg1 is an effective anti-cancer component in total ginsenosides (Li J. et al., 2014). It is reported that ginsenoside Rg1 decreases cell viability, inhibits cell proliferation, induces reactive oxygen species (ROS) thereby drives apoptosis of BC cells (Chu et al., 2020). In details, ginsenoside Rg1 induces apoptosis by generating ROS of BC cells (Chu et al., 2020). On the other hand, ginsenoside Rg1 shows the activity of suppressing invasion and migration of BC cells. Studies have shown that ginsenoside Rg1 inhibits PMA-induced MMP-9 expression by inhibiting NF-κB activity, which in turn inhibits invasion and migration of BC cells (Li L. et al., 2014). Moreover, ginsenoside Rg1 can down-regulate the

expression of angiogenesis and EMT markers, thereby functions in inhibiting angiogenesis and EMT (Chu et al., 2020). Therefore, we speculate that ginsenoside Rg1 has the effect of blocking BC cells metastasis mediated by EMT.

Ginsenoside Rg2 Ginsenoside Rg2 has the functions of enhancing memory, improving metabolism, protecting heart, as well as anti-cancer (Liu X et al., 2022). It is reported that ginsenoside Rg2 induces cell cycle arrest, inhibits proliferation and drives apoptosis of BC cells (Jeon et al., 2021a). In details, ginsenoside Rg2 can induce ROS production by inhibiting the activation of ERK1/2 and Akt, which in turn inhibits proliferation of BC cells, further arrest the cell cycle of G1 and induce apoptosis of BC cells by promoting ROS-mediated AMPK activation (Jeon et al., 2021b). In addition, studies have shown that ginsenoside Rg2 can mediate the activation of p53, which in turn induces the up-regulation of AMPK, further functions in regulating apoptosis and autophagy of BC cells (Chung et al., 2018).

Ginsenoside Rh1 Ginsenoside Rh1 is a metabolite of ginsenoside Re and Rg1, and has been reported to possess anti-allergic, anti-inflammatory, anti-aging, anti-oxidant and anti-cancer activity (Lyu et al., 2019). It is reported that ginsenoside Rh1 induces cell cycle arrest and drives apoptosis of BC cells (Huynh et al., 2021; Jin et al., 2022). In details, ginsenoside Rh1 induces cell cycle arrest, apoptosis, as well as autophagy by inhibiting ROS mediated PI3K/Akt pathway (Huynh et al., 2021). Moreover, ginsenoside Rh1 increases cell cycle arrest and apoptosis by inducing mtROS (mitochondrial ROS)-activated intracellular calcium accumulation and ER stress signaling pathways (Jin et al., 2022). On the other hand, studies have shown that ginsenoside Rh1 increases the production of mtROS to induce mitochondrial dysfunction, thereby inhibiting STAT/NF-κB signaling pathway, which in turn inhibits migration, invasion of BC cells, as well as angiogenesis (Jin et al., 2021).

Anti-breast cancer activity of other ginsenosides

At present, rare ginsenosides such as Rg5, Rk1, Rh4 have also begun to be extensively studied, which are the deglycosylated forms of the main ginsenosides, and harvest greater drug potential (Qi et al., 2011). Ginsenoside Rg5 is a minor ginsenoside synthesized by deglycylation of ginsenoside Rb1 and dehydration of the carbon at the 20-position of ginsenoside Rg3 during ginsenoside cooking treatment, and exhibits excellent anti-BC effect (Liu M. Y. et al., 2021). Numerous studies have shown that ginsenoside Rg5 can induce apoptotic death in BC cells, one by inhibiting the PI3K/Akt/mTOR pathway and subsequently reducing Bad phosphorylation (Liu and Fan 2018; Liu and Fan 2020), and the other by regulating mitochondria-mediated pathway to induce caspase-dependent apoptosis including caspase-3, caspase-8, caspase-9 and Poly (ADP-ribose) polymerase (PARP) (Kim and Kim 2015; Liu and Fan 2018; Liu and Fan 2020). Moreover, ginsenoside Rg5 can up-regulate the expression of LC3-II, Beclin-1, Atg5 and Atg12 and down-regulate the expression of p62, thereby inducing autophagy and promoting BC cells death (Liu and Fan 2018; Liu and Fan 2020). Ginsenoside Rg5 can also induce G0/G1 cell cycle arrest by reducing the protein expression of cyclin D1, cyclin E2 and CDK4, and increasing the expression of p15 ink 4B, p53 and p21WAF1/CIP1, and subsequently inhibit the proliferation of BC cells (Kim and Kim 2015). Ginsenoside Rk1 is derived from ginsenoside Rg3 through a dehydration or heating process (Ryoo et al., 2020). The study found that ginsenoside Rk1 can also inhibit the growth of TNBC cells by inhibiting the expression of cycle-related proteins, causing cells to arrest in G0/G1 phase (Hong and Fan 2019). In addition, ginsenoside Rk1 can also regulate the production of intracellular ROS and reduce mitochondrial membrane potential, and then increase the expression of Bax and cytochrome C, thereby inducing apoptosis and death of TNBC cells (Hong and Fan 2019). Ginsenoside Rh4 is produced by Rg1 and Re by restoring antioxidant defense enzyme activity or inhibiting ROS generation (Baek et al., 2017), and has been shown to have inhibitory effects on the occurrence of colorectal cancer, lung cancer and esophageal cancer (Baek et al., 1996). Daidi Fan's research shows that ginsenoside Rh4 can not only effectively inhibit the proliferation of S-phase cells, but also induce apoptosis by reducing Bcl-2, increasing Bax and activating caspase-8, caspase-3 and PARP, thereby inhibiting BC cells growth (Duan et al., 2018). On the other hand, ginsenoside Rp1 is prepared from ginsenosides Rg5, Rk1, etc. through reduction and hydrogenation reactions (Cho et al., 2001). It has been reported that ginsenoside Rp1 can induce cell cycle arrest and apoptosis of BC cells through inhibit the insulin-like growth factor 1 receptor (IGF-1R)/Akt pathway (Kang et al., 2011). In details, MCF-7 cells were arrested in G1 phase and MDA-MB-231 and T-47D cells were arrested in G2/M phase. In this study, Ju-Hee Kang et al. proposed that ginsenoside Rp1 could be further investigated to inhibit metastasis of BC cells.

Anti-breast cancer activity of ginsenosides mediated by microRNAs

MiRNAs are a group of endogenous noncoding RNAs that regulate gene expression, ranging in size from 19 to 25 nucleotides (Lu and Rothenberg 2018). MiRNAs bind to the 3′-UTR (untranslated region) of a specific target gene to degrade mRNA or inhibit its protein translation (Lee and Dutta 2009). In general, miRNAs are aberrantly expressed in a variety of cancers, oncogenic miRNAs are frequently overexpressed, and tumor suppressor miRNAs are frequently downregulated

TABLE 1 Inhibition of ginsenosides on breast cancer cells through inducing cell cycle arrest and apoptosis, inhibiting proliferation, migration, invasion, autophagy, angiogenesis and EMT, etc.

Type of ginsenoside	Physiological effects	Cell lines	Related signaling pathways	References
Rh2	Arrest cell cycle	MCF-7, MDA-MB-231	N/A	(Choi et al., 2009; Peng et al., 2022)
Rg2		MCF-7	N/A	(Jeon et al., 2021a; Jeon et al., 2021b)
Rh1		MCF-7, HCC1428; MDA-MB-231	Inhibit ROS/PI3K/Akt pathway; Induce intracellular calcium accumulation and ER stress signaling pathways	(Huynh et al., 2021; Jin et al., 2022)
Rg5		MCF-7	N/A	Kim and Kim (2015)
Rk1		MDA-MB-231	N/A	Hong and Fan (2019)
Rp1		MCF-7, T-47D, MDA-MB-231	Inhibit the IGF-1R/Akt pathway	Kang et al. (2011)
F2	Inhibit proliferation	CSCs	N/A	Mai et al. (2012)
Rd		MCF-7; MDA-MB-231	Inhibit TRPM7	(Kim 2013; Liu H. et al., 2020)
CK		MCF-7	Inhibit PI3K/Akt pathway	Zhang and Li (2016)
Type of ginsenoside	**Physiological effects**	**Cell lines**	**Related signaling pathways**	**References**
Rh2		MCF-7, MDA-MB-231	N/A	Ren et al. (2018)
Rg3	Induce apoptosis	MDA-MB-231	N/A	Nakhjavani et al. (2019)
Rg1		MDA-MB-231	N/A	Chu et al. (2020)
Rg2		MCF-7	N/A	(Jeon et al., 2021a; Jeon et al., 2021b)
Rh4		MCF-7	N/A	Duan et al. (2018)
Rd		MCF-7	N/A	Kim (2013)
Rp1		MCF-7, T-47D, MDA-MB-231	Inhibit the IGF-1R/Akt pathway	Kang et al. (2011)
CK	Inhibit proliferation	MCF-7; TNBC cells (MDA-MB-231, etc.)	Inhibit PI3K/Akt pathway	(Zhang and Li 2016; Zhang B. et al., 2022)
Rh2		MCF-7, MDA-MB-231	N/A	(Choi et al., 2011; Peng et al., 2022)
Rg3		MDA-MB-231	Block NF-κB signal transduction	(Kim et al., 2013; Kim et al., 2014)
Type of ginsenoside	**Physiological effects**	**Cell lines**	**Related signaling pathways**	**References**
Rg1		MDA-MB-231	N/A	Chu et al. (2020)
Rg2		MCF-7	N/A	(Jeon et al., 2021a; Jeon et al., 2021b)
Rh1		MCF-7, HCC1428; MDA-MB-231	Inhibit ROS/PI3K/Akt pathway; Induce intracellular calcium accumulation and ER stress signaling pathways	(Huynh et al., 2021; Jin et al., 2022)
Rg5		MCF-7	Inhibit the PI3K/Akt/mTOR pathway; Regulat mitochondria-mediated pathway	(Kim and Kim 2015; Liu and Fan 2018; Liu and Fan 2020)
Rk1		MDA-MB-231	Inhibit ROS/PI3K/Akt pathway	Hong and Fan (2019)
Rh4		MCF-7	N/A	Duan et al. (2018)
Rd	Inhibit migration and invasion	4T1 (*in vitro* and *in vivo*)	N/A	Wang et al. (2016)
Rh2		MDA-MB-231	N/A	Hou et al. (2019)
Rg3		MDA-MB-231	N/A	Chen et al. (2011)
Type of ginsenoside	**Physiological effects**	**Cell lines**	**Related signaling pathways**	**References**
Rg1		MCF-7	Inhibit NF-κB activity	Li J. et al. (2014)

(Continued on following page)

TABLE 1 (*Continued*) Inhibition of ginsenosides on breast cancer cells through inducing cell cycle arrest and apoptosis, inhibiting proliferation, migration, invasion, autophagy, angiogenesis and EMT, etc.

Type of ginsenoside	Physiological effects	Cell lines	Related signaling pathways	References
Rh1	Induce autophagy	MDA-MB-231	Inhibit STAT/NF-κB signaling pathway	Jin et al. (2021)
Rg3		MCF-7	Inhibit mTOR/PI3K/Akt and JNK/Beclin-1 signaling pathways	Zhang et al. (2016)
Rg2		MCF-7	N/A	Chung et al. (2018)
Rh1		MCF-7, HCC1428	Inhibit ROS/PI3K/Akt pathway	Huynh et al. (2021)
Rg5		MCF-7	N/A	(Liu and Fan 2018; Liu and Fan 2020)
Rd	Inhibit angiogenesis Inhibit EMT	MDA-MB-231	Inhibit Akt/Mtor/p70S6K pathway	Zhang et al. (2017)
Rg3		MCF-7	N/A	Zhang et al. (2016)
CK		MCF-7	Inhibit PI3K/Akt pathway	Zhang and Li (2016)
Rg3		MDSCs	N/A	Song et al. (2020)

(Fridrichova and Zmetakova 2019). A growing number of studies have shown that some miRNAs are emerging as BC diagnostic, prognostic and therapeutic biomarkers and control BC hallmark functions, such as invasion, metastasis, proliferation and apoptosis, etc (Bertoli et al., 2015; Xu et al., 2020). For example, the miR-200 family is often present in BC cells as tumor suppressors, regulating EMT by targeting the transcriptional repressors ZEB1 and ZEB2, thereby inhibit the metastasis and invasion of BC cells (Liu 2012).

It has also been reported that ginsenosides can inhibit the occurrence and growth of cancer cells by targeting miRNAs (Table 2). Studies have shown that ginsenoside Rh2 can inhibit the proliferation of lung cancer cells and glioma cells by targeting miR-491 and miR-128 (Wu et al., 2011; Chen et al., 2019). Ginsenoside Rg3 regulates the expression of target genes by targeting miRNAs including miR-4425, miR-603 and miR-324-5p, which in turn inhibits ovarian cancer progression (Zheng et al., 2018; Lu et al., 2019; Lu et al., 2020). Ginsenosides Rh7 and Rg1 have been reported to inhibit the progression of non-small cell lung cancer (NSCLC) by targeting miR-212 and miR-126, respectively (Chen et al., 2021a; Chen et al., 2022). In particularly, several studies clarify that ginsenosides mediate the miRNAs expression profile of BC (Li et al., 2020). For example, ginsenoside Rh2 can up-regulate LncRNA STXBP5-AS1, whereas inhibit the level of miR-4425, further reduce the expression of target gene RNF217, thereby inhibit the growth of BC cells (Park et al., 2021). Ginsenoside Rh2 can also down-regulate the expression of miR-3614-3p mediated by CFAP20DC-AS1, and then reduce the expression of BBX and TNFAIP3, thereby inducing apoptosis of BC cells (Park et al., 2022). Moreover, ginsenoside Rh2 can reduce the drug resistance of BC by targeting miR-222, miR-34a and miR-29a to down-regulate the expression of target gene Bax (Wen et al., 2015). According to reports, ginsenoside Rd can down-regulate the expression of Smad2 by targeting miR-18a, thereby inhibit the metastasis and invasion of BC cells (Wang et al., 2016). Ginsenoside Rg3 can activate oncogenic CHRM3 and DACH1 by downregulating ATXN8OS-mediated miR-424-5p, thereby inhibiting BC cells proliferation (Kim et al., 2021). It has been reported that ginsenosides Rg3 can block the occurrence of EMT in ovarian cancer cells by targeting miR-145, which has been shown to play an important role in inhibiting the migration and invasion of BC cells by directly targeting the angiopoietin 2 gene (ANGPT2) of BC cells (Li et al., 2017; Liu et al., 2017; Jiang et al., 2019; Tang et al., 2019). Therefore, we speculate that ginsenoside Rg3 inhibits the growth of BC cells by regulating miR-145, which needs further demonstrated.

It has been reported that ginsenosides Rg3 can block the occurrence of EMT in ovarian cancer cells by targeting miR-145, which has been shown to play an important role in inhibiting the migration and invasion of BC cells by directly targeting the angiopoietin 2 gene (ANGPT2) of BC cells (Li et al., 2017; Liu et al., 2017; Jiang et al., 2019; Tang et al., 2019). Therefore, we speculate that ginsenoside Rg3 inhibits the growth of BC cells by regulating miR-145, which needs further demonstrated. Moreover, ginsenoside Rg3 can improve gastric precancerous lesions by targeting miR-21 (Liu W. et al., 2020), which has been shown to regulate the proliferation and invasion of BC (Ali et al., 2020) and TNBC cells (Fang et al., 2017). Afterwards, we speculate that ginsenoside Rg3 targeting miR-21 to regulate the growth of BC and TNBC requires more clinical studies. Notably, targeting miR-126 expression has been shown to inhibit PI3K/AKT signaling activity and thereby inhibit BC cells growth (McGuire et al., 2015). Ginsenoside Rg1 can also inhibit NSCLC by targeting miR-126 to inhibit the PI3K/AKT pathway (Chen et al., 2022). Therefore, we speculate that ginsenoside Rg1 may regulate the PI3K/AKT pathway by targeting miR-126, thereby inhibiting the progression of BC, which needs more experiments to confirm. Numerous studies have shown that ginsenoside Rh2 can effectively inhibit IL-6-induced

TABLE 2 Ginsenoside regulation of miRNAs in breast cancer.

Type of ginsenoside	Type of cancer	miRNAs	miRNA targets	References
Rh2	BC	miR-4425↓	RNF217↓	Park et al. (2021)
	BC	miR-222↓	Bax↓	Wen et al. (2015)
	BC	miR-34a↓	Bax↓	Wen et al. (2015)
	BC	miR-29a↓	Bax↓	Wen et al. (2015)
	BC	miR-3614-3p↓	BBX and TNFAIP3↓	Park et al. (2022)
Rd	BC	miR-18a↓	Smad2↓	Wang et al. (2016)

STAT3 phosphorylation and the expression of miR-214 in cultured normal colonic epithelial cells to relieve ulcerative colitis (Chen et al., 2021b). The regulation of miRNA-214 can also inhibit the biological activity of breast cancer cells (Ouyang et al., 2020). It can be speculated that ginsenoside Rh2 may inhibit the growth of BC by targeting miRNA-214.

In conclusion, ginsenosides have great potential as broad-spectrum anti-cancer drugs and effective chemosensitizers.

Future prospects

Currently, surgery, radiotherapy and chemotherapy are the main methods used for clinical cancer treatment (Colli et al., 2017). However, serious toxic and side effects of chemoradiotherapy drugs and multidrug resistance (MDR) interfered with therapeutic effect. (Colli et al., 2017). Conversely, combination therapy can reduce the incidence of drug resistance in cancer cells by targeting different pathways, and can reduce toxicity by reducing the required dose of a single drug (Aumeeruddy and Mahomoodally 2019). It is reported that ginsenosides can inhibit the growth of cancer cells through different pathways and can be used as adjuvant drugs to suppress MDR and increase chemosensitivity (Hashemi et al., 2021).

Recently, studies have confirmed that ginsenosides can be used in combination with a variety of drugs to enhance the inhibitory effect on cancer cells or increase the sensitivity to traditional chemotherapy drugs (Choi et al., 2013). For example, ginsenoside Rb1 and apatinib work synergistically to enhance the inhibition of growth of pharyngeal cancer cells (Li Y. et al., 2022). Combination therapy of ginsenoside Rg3 and chemotherapeutics drugs against BC has been demonstrated. Such as, ginsenoside Rg3 in combination with sorafenib has been shown to enhance the inhibitory effect on hepatocellular carcinoma growth by modulating HK2-mediated glycolysis and PI3K/Akt signaling pathways (Wei et al., 2022), whereas in combination with paclitaxel (PTX) and cisplatin (DDP) can enhance the anti-esophageal squamous cell carcinoma effect (Chang et al., 2014). Moreover, ginsenoside Rg3 also enhance the chemotherapy sensitivity of DDP-resistant human lung cancer cell and PTX-resistant TNBC by downregulating MDR-mediated proteins (including P-glycoprotein (P-gp), multidrug resistance-related protein (MPR1) and lung resistance protein 1 (LPR1)) (Liu et al., 2018) and inhibiting NF-κB signaling pathway, respectively (Yuan et al., 2017). Another ginsenoside Rh2, has been shown that in combination with regorafenib and more effectively inhibit the proliferation of liver cancer cells by regulating the expression of caspase-3 gene (Wang P. et al., 2020). In consistently, ginsenoside Rh2 can also inhibit the growth of human prostate cancer cells in combination with 1α, 25-dihydroxyvitamin D3 (Ben-Eltriki et al., 2021). In addition, lower doses of ginsenoside Rh2 combined with biotea protein A inhibited the proliferation, metastasis and invasion of BC cells by upregulating the expression of p53, p38 and ASK1, which was consistent with the effect of single drug administration (Ren et al., 2018). While *in vitro* and *in vivo* studies have shown that ginsenoside Rh2 can significantly enhance the anti BC effect of doxorubicin and reduce cardiotoxicity during the treatment phase (Hou et al., 2022).

Emerging studies demonstrate that multiple ginsenosides exhibit significant anti-cancer activity in several cancers *in vitro* and *in vivo*. In this review, we summarize the molecular mechanisms of various ginsenosides in inhibiting BC, including targeting miRNAs and their roles in inhibiting BC cells proliferation, inducing cell cycle arrest and apoptosis, inhibiting metastasis and invasion, and triggering autophagy aspects of the role. In addition, ginsenosides can also be used in combination with chemotherapeutic drugs to increase the chemosensitivity of drug-resistant cancer cells and enhance the anti-BC effect of chemotherapeutic drugs. In particular, a variety of ginsenosides can exert anti-BC effects by targeting miRNAs, among which ginsenoside Rh2 has been widely identified. From this, we speculate that ginsenosides have great potential as broad-spectrum anti-cancer drugs and effective chemosensitizers. However, with the continuous accumulation of evidence, more clinical studies are urgently needed for ginsenosides in the anti-BC mechanism and in improving oral bioavailability.

In summary, this review summarizes the molecular mechanism of ginsenosides including PPD, PPT and other three saponins inhibiting BC, among which the PPD type is

the most reported. Thus, ginsenosides have great potential as broad-spectrum anti-BC drugs and effective chemosensitizers.

Author contributions

HL and DW Designed the review; MF, MS, XL, XF, and DS contributed to the writing of the manuscirpt.

Funding

This work was supported by National Natural Science Foundation of China (Grant No. 81903876), Jilin Provincial Department of Education (JJKH20210982KJ), Jilin Province Youth Science and Technology Talent Support Project (2020025), Jilin Provincial Development and Reform Commission Innovation Capacity Building Project (2021C035-5), Jilin Province Science and Technology Development Plan Project (20200708081YY).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Adams, R. H., and Alitalo, K. (2007). Molecular regulation of angiogenesis and lymphangiogenesis. *Nat. Rev. Mol. Cell. Biol.* 8 (6), 464–478. doi:10.1038/nrm2183

Ali, S. A., Abdulrahman, Z. F. A., and Faraidun, H. N. (2020). Circulatory miRNA-155, miRNA-21 target PTEN expression and activity as a factor in breast cancer development. *Cell. Mol. Biol.* 66 (7), 44–50. doi:10.14715/cmb/2020.66.7.8

Aumeeruddy, M. Z., and Mahomoodally, M. F. (2019). Combating breast cancer using combination therapy with 3 phytochemicals: Piperine, sulforaphane, and thymoquinone. *Cancer* 125 (10), 1600–1611. doi:10.1002/cncr.32022

Aung, H. H., Mehendale, S. R., Wang, C. Z., Xie, J. T., McEntee, E., and Yuan, C. S. (2007). Cisplatin's tumoricidal effect on human breast carcinoma MCF-7 cells was not attenuated by American ginseng. *Cancer Chemother. Pharmacol.* 59 (3), 369–374. doi:10.1007/s00280-006-0278-6

Baek, N. I., Kim, D. S., Lee, Y. H., Park, J. D., Lee, C. B., and Kim, S. I. (1996). Ginsenoside Rh4, a genuine dammarane glycoside from Korean red ginseng. *Planta Med.* 62 (1), 86–87. doi:10.1055/s-2006-957816

Baek, S. H., Shin, B. K., Kim, N. J., Chang, S. Y., and Park, J. H. (2017). Protective effect of ginsenosides Rk3 and Rh4 on cisplatin-induced acute kidney injury *in vitro* and *in vivo*. *J. Ginseng Res.* 41 (3), 233–239. doi:10.1016/j.jgr.2016.03.008

Ben-Eltriki, M., Deb, S., and Guns, E. S. T. (2021). 1α, 25-Dihydroxyvitamin D(3) synergistically enhances anticancer effects of ginsenoside Rh2 in human prostate cancer cells. *J. Steroid Biochem. Mol. Biol.* 209, 105828. doi:10.1016/j.jsbmb.2021.105828

Bertoli, G., Cava, C., and Castiglioni, I. (2015). MicroRNAs: New biomarkers for diagnosis, prognosis, therapy prediction and therapeutic tools for breast cancer. *Theranostics* 5 (10), 1122–1143. doi:10.7150/thno.11543

Chang, L., Huo, B., Lv, Y., Wang, Y., and Liu, W. (2014). Ginsenoside Rg3 enhances the inhibitory effects of chemotherapy on esophageal squamous cell carcinoma in mice. *Mol. Clin. Oncol.* 2 (6), 1043–1046. doi:10.3892/mco.2014.355

Changizi, V., Gharekhani, V., and Motavaseli, E. (2021). Co-Treatment with ginsenoside 20(S)-Rg3 and curcumin increases radiosensitivity of MDA-MB-231 cancer cell line. *Iran. J. Med. Sci.* 46 (4), 291–297. doi:10.30476/ijms.2020.83977.1334

Chen, P., Li, X., Yu, X., and Yang, M. (2022). Ginsenoside Rg1 suppresses non-small-cell lung cancer via MicroRNA-126-PI3K-AKT-mTOR pathway. *Evid. Based. Complement. Altern. Med.* 2022, 1244836. doi:10.1155/2022/1244836

Chen, X., Liu, W., and Liu, B. (2021a). Ginsenoside Rh7 suppresses proliferation, migration and invasion of NSCLC cells through targeting ILF3-AS1 mediated miR-212/SMAD1 Axis. *Front. Oncol.* 11, 656132. doi:10.3389/fonc.2021.656132

Chen, X. P., Qian, L. L., Jiang, H., and Chen, J. H. (2011). Ginsenoside Rg3 inhibits CXCR4 expression and related migrations in a breast cancer cell line. *Int. J. Clin. Oncol.* 16 (5), 519–523. doi:10.1007/s10147-011-0222-6

Chen, X., Xu, T., Lv, X., Zhang, J., and Liu, S. (2021b). Ginsenoside Rh2 alleviates ulcerative colitis by regulating the STAT3/miR-214 signaling pathway. *J. Ethnopharmacol.* 274, 113997. doi:10.1016/j.jep.2021.113997

Chen, Y., Zhang, Y., Song, W., Zhang, Y., Dong, X., and Tan, M. (2019). Ginsenoside Rh2 inhibits migration of lung cancer cells under hypoxia via mir-491. *Anticancer. Agents Med. Chem.* 19 (13), 1633–1641. doi:10.2174/1871520619666190704165205

Cho, J. Y., Yoo, E. S., Baik, K. U., Park, M. H., and Han, B. H. (2001). *In vitro* inhibitory effect of protopanaxadiol ginsenosides on tumor necrosis factor (TNF)-alpha production and its modulation by known TNF-alpha antagonists. *Planta Med.* 67 (3), 213–218. doi:10.1055/s-2001-12005

Choi, J. S., Chun, K. S., Kundu, J., and Kundu, J. K. (2013). Biochemical basis of cancer chemoprevention and/or chemotherapy with ginsenosides (Review). *Int. J. Mol. Med.* 32 (6), 1227–1238. doi:10.3892/ijmm.2013.1519

Choi, S., Kim, T. W., and Singh, S. V. (2009). Ginsenoside Rh2-mediated G1 phase cell cycle arrest in human breast cancer cells is caused by p15 Ink4B and p27 Kip1-dependent inhibition of cyclin-dependent kinases. *Pharm. Res.* 26 (10), 2280–2288. doi:10.1007/s11095-009-9944-9

Choi, S., Oh, J. Y., and Kim, S. J. (2011). Ginsenoside Rh2 induces Bcl-2 family proteins-mediated apoptosis *in vitro* and in xenografts *in vivo* models. *J. Cell. Biochem.* 112 (1), 330–340. doi:10.1002/jcb.22932

Chu, Y., Zhang, W., Kanimozhi, G., Brindha, G. R., and Tian, D. (2020). Ginsenoside Rg1 induces apoptotic cell death in triple-negative breast cancer cell lines and prevents carcinogen-induced breast tumorigenesis in sprague dawley rats. *Evid. Based. Complement. Altern. Med.* 2020, 8886955. doi:10.1155/2020/8886955

Chung, Y., Jeong, S., Choi, H. S., Ro, S., Lee, J. S., and Park, J. K. (2018). Upregulation of autophagy by ginsenoside Rg2 in MCF-7 cells. *Anim. Cells Syst.* 22 (6), 382–389. doi:10.1080/19768354.2018.1545696

Colli, L. M., Machiela, M. J., Zhang, H., Myers, T. A., Jessop, L., Delattre, O., et al. (2017). Landscape of combination immunotherapy and targeted therapy to improve cancer management. *Cancer Res.* 77 (13), 3666–3671. doi:10.1158/0008-5472.CAN-16-3338

Duan, Z., Wei, B., Deng, J., Mi, Y., Dong, Y., Zhu, C., et al. (2018). The anti-tumor effect of ginsenoside Rh4 in MCF-7 breast cancer cells *in vitro* and *in vivo*. *Biochem. Biophys. Res. Commun.* 499 (3), 482–487. doi:10.1016/j.bbrc.2018.03.174

Edwards, D. N., Ngwa, V. M., Raybuck, A. L., Wang, S., Hwang, Y., Kim, L. C., et al. (2021). Selective glutamine metabolism inhibition in tumor cells improves antitumor T lymphocyte activity in triple-negative breast cancer. *J. Clin. Invest.* 131 (4), 140100. doi:10.1172/JCI140100

Fang, H., Xie, J., Zhang, M., Zhao, Z., Wan, Y., and Yao, Y. (2017). miRNA-21 promotes proliferation and invasion of triple-negative breast cancer cells through targeting PTEN. *Am. J. Transl. Res.* 9 (3), 953–961.

Fridrichova, I., and Zmetakova, I. (2019). MicroRNAs contribute to breast cancer invasiveness. *Cells* 8 (11), E1361. doi:10.3390/cells8111361

Hashemi, F., Zarrabi, A., Zabolian, A., Saleki, H., Farahani, M. V., Sharifzadeh, S. O., et al. (2021). Novel strategy in breast cancer therapy: Revealing the bright side of ginsenosides. *Curr. Mol. Pharmacol.* 14 (6), 1093–1111. doi:10.2174/1874467214666210120153348

He, B., Chen, D., Zhang, X., Yang, R., Yang, Y., Chen, P., et al. (2022). Oxidative stress and ginsenosides: An update on the molecular mechanisms. *Oxid. Med. Cell. Longev.* 2022, 9299574. doi:10.1155/2022/9299574

Hobbs, E. A., Chen, N., Kuriakose, A., Bonefas, E., and Lim, B. (2022). Prognostic/predictive markers in systemic therapy resistance and metastasis in breast cancer. *Ther. Adv. Med. Oncol.* 14, 17588359221112698. doi:10.1177/17588359221112698

Hong, H., Baatar, D., and Hwang, S. G. (2021). Anticancer activities of ginsenosides, the main active components of ginseng. *Evid. Based. Complement. Altern. Med.* 2021, 8858006. doi:10.1155/2021/8858006

Hong, Y., and Fan, D. (2019). Ginsenoside Rk1 induces cell cycle arrest and apoptosis in MDA-MB-231 triple negative breast cancer cells. *Toxicology* 418, 22–31. doi:10.1016/j.tox.2019.02.010

Hou, J. G., Jeon, B. M., Yun, Y. J., Cui, C. H., and Kim, S. C. (2019). Ginsenoside Rh2 ameliorates doxorubicin-induced senescence bystander effect in breast carcinoma cell MDA-MB-231 and normal epithelial cell MCF-10a. *Int. J. Mol. Sci.* 20 (5), E1244. doi:10.3390/ijms20051244

Hou, J., Yun, Y., Cui, C., and Kim, S. (2022). Ginsenoside Rh2 mitigates doxorubicin-induced cardiotoxicity by inhibiting apoptotic and inflammatory damage and weakening pathological remodelling in breast cancer-bearing mice. *Cell. Prolif.* 55 (6), e13246. doi:10.1111/cpr.13246

Hou, M., Wang, R., Zhao, S., and Wang, Z. (2021). Ginsenosides in Panax genus and their biosynthesis. *Acta Pharm. Sin. B* 11 (7), 1813–1834. doi:10.1016/j.apsb.2020.12.017

Hu, C., Yang, L., Wang, Y., Zhou, S., Luo, J., and Gu, Y. (2021). Ginsenoside Rh2 reduces m6A RNA methylation in cancer via the KIF26B-SRF positive feedback loop. *J. Ginseng Res.* 45 (6), 734–743. doi:10.1016/j.jgr.2021.05.004

Hu, X., Zhang, Q., Xing, W., and Wang, W. (2022). Role of microRNA/lncRNA intertwined with the wnt/β-catenin Axis in regulating the pathogenesis of triple-negative breast cancer. *Front. Pharmacol.* 13, 814971. doi:10.3389/fphar.2022.814971

Huynh, D. T. N., Jin, Y., Myung, C. S., and Heo, K. S. (2021). Ginsenoside Rh1 induces MCF-7 cell apoptosis and autophagic cell death through ROS-mediated Akt signaling. *Cancers (Basel)* 13 (8), 1892. doi:10.3390/cancers13081892

Iorio, M. V., Ferracin, M., Liu, C. G., Veronese, A., Spizzo, R., Sabbioni, S., et al. (2005). MicroRNA gene expression deregulation in human breast cancer. *Cancer Res.* 65 (16), 7065–7070. doi:10.1158/0008-5472.CAN-05-1783

Jeon, H., Huynh, D. T. N., Baek, N., Nguyen, T. L. L., and Heo, K. S. (2021b). Ginsenoside-Rg2 affects cell growth via regulating ROS-mediated AMPK activation and cell cycle in MCF-7 cells. *Phytomedicine.* 85, 153549. doi:10.1016/j.phymed.2021.153549

Jeon, H., Jin, Y., Myung, C. S., and Heo, K. S. (2021a). Ginsenoside-Rg2 exerts anti-cancer effects through ROS-mediated AMPK activation associated mitochondrial damage and oxidation in MCF-7 cells. *Arch. Pharm. Res.* 44 (7), 702–712. doi:10.1007/s12272-021-01345-3

Jiang, Y., Wang, D., Ren, H., Shi, Y., and Gao, Y. (2019). MiR-145-targeted HBXIP modulates human breast cancer cell proliferation. *Thorac. Cancer* 10 (1), 71–77. doi:10.1111/1759-7714.12903

Jin, X., Yang, Q., and Cai, N. (2018). Preparation of ginsenoside compound-K mixed micelles with improved retention and antitumor efficacy. *Int. J. Nanomedicine* 13, 3827–3838. doi:10.2147/IJN.S167529

Jin, Y., Huynh, D. T. N., and Heo, K. S. (2022). Ginsenoside Rh1 inhibits tumor growth in MDA-MB-231 breast cancer cells via mitochondrial ROS and ER stress-mediated signaling pathway. *Arch. Pharm. Res.* 45 (3), 174–184. doi:10.1007/s12272-022-01377-3

Jin, Y., Huynh, D. T. N., Myung, C. S., and Heo, K. S. (2021). Ginsenoside Rh1 prevents migration and invasion through mitochondrial ROS-mediated inhibition of STAT3/NF-κB signaling in MDA-MB-231 cells. *Int. J. Mol. Sci.* 22 (19), 10458. doi:10.3390/ijms221910458

Jin, Y., Huynh, D. T. N., Nguyen, T. L. L., Jeon, H., and Heo, K. S. (2020). Therapeutic effects of ginsenosides on breast cancer growth and metastasis. *Arch. Pharm. Res.* 43 (8), 773–787. doi:10.1007/s12272-020-01265-8

Kang, J. H., Song, K. H., Woo, J. K., Park, M. H., Rhee, M. H., Choi, C., et al. (2011). Ginsenoside Rp1 from Panax ginseng exhibits anti-cancer activity by down-regulation of the IGF-1R/Akt pathway in breast cancer cells. *Plant Foods Hum. Nutr.* 66 (3), 298–305. doi:10.1007/s11130-011-0242-4

Karami Fath, M., Azargoonjahromi, A., Kiani, A., Jalalifar, F., Osati, P., Akbari Oryani, M., et al. (2022). The role of epigenetic modifications in drug resistance and treatment of breast cancer. *Cell. Mol. Biol. Lett.* 27 (1), 52. doi:10.1186/s11658-022-00344-6

Kim, B. J. (2013). Involvement of melastatin type transient receptor potential 7 channels in ginsenoside Rd-induced apoptosis in gastric and breast cancer cells. *J. Ginseng Res.* 37 (2), 201–209. doi:10.5142/jgr.2013.37.201

Kim, B. M., Kim, D. H., Park, J. H., Na, H. K., and Surh, Y. J. (2013). Ginsenoside Rg3 induces apoptosis of human breast cancer (MDA-MB-231) cells. *J. Cancer Prev.* 18 (2), 177–185. doi:10.15430/jcp.2013.18.2.177

Kim, B. M., Kim, D. H., Park, J. H., Surh, Y. J., and Na, H. K. (2014). Ginsenoside Rg3 inhibits constitutive activation of NF-κB signaling in human breast cancer (MDA-MB-231) cells: ERK and Akt as potential upstream targets. *J. Cancer Prev.* 19 (1), 23–30. doi:10.15430/jcp.2014.19.1.23

Kim, H., Ji, H. W., Kim, H. W., Yun, S. H., Park, J. E., and Kim, S. J. (2021). Ginsenoside Rg3 prevents oncogenic long noncoding RNA ATXN8OS from inhibiting tumor-suppressive microRNA-424-5p in breast cancer cells. *Biomolecules* 11 (1), 118. doi:10.3390/biom11010118

Kim, S. J., and Kim, A. K. (2015). Anti-breast cancer activity of fine black ginseng (Panax ginseng meyer) and ginsenoside Rg5. *J. Ginseng Res.* 39 (2), 125–134. doi:10.1016/j.jgr.2014.09.003

Kwak, C. W., Son, Y. M., Gu, M. J., Kim, G., Lee, I. K., Kye, Y. C., et al. (2015). A bacterial metabolite, compound K, induces programmed necrosis in MCF-7 cells via GSK3β. *J. Microbiol. Biotechnol.* 25 (7), 1170–1176. doi:10.4014/jmb.1505.05057

Lahiani, M. H., Eassa, S., Parnell, C., Nima, Z., Ghosh, A., Biris, A. S., et al. (2017). Carbon nanotubes as carriers of Panax ginseng metabolites and enhancers of ginsenosides Rb1 and Rg1 anti-cancer activity. *Nanotechnology* 28 (1), 015101. doi:10.1088/0957-4484/28/1/015101

Lee, Y. S., and Dutta, A. (2009). MicroRNAs in cancer. *Annu. Rev. Pathol.* 4, 199–227. doi:10.1146/annurev.pathol.4.110807.092222

Li, J., Huang, Q., Yao, Y., Ji, P., Mingyao, E., Chen, J., et al. (2022). Biotransformation, pharmacokinetics, and pharmacological activities of ginsenoside Rd against multiple diseases. *Front. Pharmacol.* 13, 909363. doi:10.3389/fphar.2022.909363

Li, J., Lu, J., Ye, Z., Han, X., Zheng, X., Hou, H., et al. (2017). 20(S)-Rg3 blocked epithelial-mesenchymal transition through DNMT3A/miR-145/FSCN1 in ovarian cancer. *Oncotarget* 8 (32), 53375–53386. doi:10.18632/oncotarget.18482

Li, J., Wei, Q., Zuo, G. W., Xia, J., You, Z. M., Li, C. L., et al. (2014). Ginsenoside Rg1 induces apoptosis through inhibition of the EpoR-mediated JAK2/STAT5 signalling pathway in the TF-1/Epo human leukemia cell line. *Asian pac. J. Cancer Prev.* 15 (6), 2453–2459. doi:10.7314/apjcp.2014.15.6.2453

Li, L., Wang, Y., Qi, B., Yuan, D., Dong, S., Guo, D., et al. (2014). Suppression of PMA-induced tumor cell invasion and migration by ginsenoside Rg1 via the inhibition of NF-κB-dependent MMP-9 expression. *Oncol. Rep.* 32 (5), 1779–1786. doi:10.3892/or.2014.3422

Li, X., Chu, S., Lin, M., Gao, Y., Liu, Y., Yang, S., et al. (2020). Anticancer property of ginsenoside Rh2 from ginseng. *Eur. J. Med. Chem.* 203, 112627. doi:10.1016/j.ejmech.2020.112627

Li, Y., He, F., Zhang, Y., and Pan, Z. (2022). Apatinib and ginsenoside-Rb1 synergetically control the growth of hypopharyngeal carcinoma cells. *Dis. Markers* 2022, 3833489. doi:10.1155/2022/3833489

Liu, C., Gong, Q., Chen, T., Lv, J., Feng, Z., Liu, P., et al. (2018). Treatment with 20(S)-ginsenoside Rg3 reverses multidrug resistance in A549/DDP xenograft tumors. *Oncol. Lett.* 15 (4), 4376–4382. doi:10.3892/ol.2018.7849

Liu, G., Zhang, J., Sun, F., Ma, J., and Qi, X. (2022). Ginsenoside Rg2 attenuated trastuzumab-induced cardiotoxicity in rats. *Biomed. Res. Int.* 2022, 8866660. doi:10.1155/2022/8866660

Liu, H., Dilger, J. P., and Lin, J. (2020). The role of transient receptor potential melastatin 7 (TRPM7) in cell viability: A potential target to suppress breast cancer cell cycle. *Cancers (Basel)* 12 (1), E131. doi:10.3390/cancers12010131

Liu, H. (2012). MicroRNAs in breast cancer initiation and progression. *Cell. Mol. Life Sci.* 69 (21), 3587–3599. doi:10.1007/s00018-012-1128-9

Liu, J., Wang, Y., Yu, Z., Lv, G., Huang, X., Lin, H., et al. (2022). Functional mechanism of ginsenoside compound K on tumor growth and metastasis. *Integr. Cancer Ther.* 21, 15347354221101203. doi:10.1177/15347354221101203

Liu, L., Wang, H., Chai, X., Meng, Q., Jiang, S., and Zhao, F. (2022). Advances in biocatalytic synthesis, pharmacological activities, pharmaceutical preparation and metabolism of ginsenoside Rh2. *Mini Rev. Med. Chem.* 22 (3), 437–448. doi:10.2174/1389557521666210913114631

Liu, M. Y., Liu, F., Gao, Y. L., Yin, J. N., Yan, W. Q., Liu, J. G., et al. (2021). Pharmacological activities of ginsenoside Rg5 (review). *Exp. Ther. Med.* 22 (2), 840. doi:10.3892/etm.2021.10272

Liu, S. Y., Li, X. Y., Chen, W. Q., Hu, H., Luo, B., Shi, Y. X., et al. (2017). Demethylation of the MIR145 promoter suppresses migration and invasion in breast cancer. *Oncotarget* 8 (37), 61731–61741. doi:10.18632/oncotarget.18686

Liu, T., Zhu, L., and Wang, L. (2022). A narrative review of the pharmacology of ginsenoside compound K. *Ann. Transl. Med.* 10 (4), 234. doi:10.21037/atm-22-501

Liu, W., Pan, H. F., Yang, L. J., Zhao, Z. M., Yuan, D. S., Liu, Y. L., et al. (2020). Panax ginseng C.A. Meyer (Rg3) ameliorates gastric precancerous lesions in Atp4a(-/-) mice via inhibition of glycolysis through PI3K/AKT/miRNA-21 pathway. *Evid. Based. Complement. Altern. Med.* 2020, 2672648. doi:10.1155/2020/2672648

Liu, X., Papukashvili, D., Wang, Z., Liu, Y., Chen, X., Li, J., et al. (2022). Potential utility of miRNAs for liquid biopsy in breast cancer. *Front. Oncol.* 12, 940314. doi:10.3389/fonc.2022.940314

Liu, Y., and Fan, D. (2018). Ginsenoside Rg5 induces apoptosis and autophagy via the inhibition of the PI3K/Akt pathway against breast cancer in a mouse model. *Food Funct.* 9 (11), 5513–5527. doi:10.1039/c8fo01122b

Liu, Y., and Fan, D. (2020). The preparation of ginsenoside Rg5, its antitumor activity against breast cancer cells and its targeting of PI3K. *Nutrients* 12 (1), E246. doi:10.3390/nu12010246

Liu, Z., Liu, T., Li, W., Li, J., Wang, C., and Zhang, K. (2021). Insights into the antitumor mechanism of ginsenosides Rg3. *Mol. Biol. Rep.* 48 (3), 2639–2652. doi:10.1007/s11033-021-06187-2

Lu, J., Wang, L., Chen, W., Wang, Y., Zhen, S., Chen, H., et al. (2019). miR-603 targeted hexokinase-2 to inhibit the malignancy of ovarian cancer cells. *Arch. Biochem. Biophys.* 661, 1–9. doi:10.1016/j.abb.2018.10.014

Lu, J., Zhou, Y., Zheng, X., Chen, L., Tuo, X., Chen, H., et al. (2020). 20(S)-Rg3 upregulates FDFT1 via reducing miR-4425 to inhibit ovarian cancer progression. *Arch. Biochem. Biophys.* 693, 108569. doi:10.1016/j.abb.2020.108569

Lu, T. X., and Rothenberg, M. E. (2018). MicroRNA. *J. Allergy Clin. Immunol.* 141 (4), 1202–1207. doi:10.1016/j.jaci.2017.08.034

Lyu, X., Xu, X., Song, A., Guo, J., Zhang, Y., and Zhang, Y. (2019). Ginsenoside Rh1 inhibits colorectal cancer cell migration and invasion *in vitro* and tumor growth *in vivo*. *Oncol. Lett.* 18 (4), 4160–4166. doi:10.3892/ol.2019.10742

Mai, T. T., Moon, J., Song, Y., Viet, P. Q., Phuc, P. V., Lee, J. M., et al. (2012). Ginsenoside F2 induces apoptosis accompanied by protective autophagy in breast cancer stem cells. *Cancer Lett.* 321 (2), 144–153. doi:10.1016/j.canlet.2012.01.045

McGuire, A., Brown, J. A., and Kerin, M. J. (2015). Metastatic breast cancer: The potential of miRNA for diagnosis and treatment monitoring. *Cancer Metastasis Rev.* 34 (1), 145–155. doi:10.1007/s10555-015-9551-7

Meng, H., Liu, X. K., Li, J. R., Bao, T. Y., and Yi, F. (2022). Bibliometric analysis of the effects of ginseng on skin. *J. Cosmet. Dermatol.* 21 (1), 99–107. doi:10.1111/jocd.14450

Nakhjavani, M., Palethorpe, H. M., Tomita, Y., Smith, E., Price, T. J., Yool, A. J., et al. (2019). Stereoselective anti-cancer activities of ginsenoside Rg3 on triple negative breast cancer cell models. *Pharm. (Basel)* 12 (3), E117. doi:10.3390/ph12030117

Nakhjavani, M., Smith, E., Palethorpe, H. M., Tomita, Y., Yeo, K., Price, T. J., et al. (2021). Anti-cancer effects of an optimised combination of ginsenoside Rg3 epimers on triple negative breast cancer models. *Pharm. (Basel)* 14 (7), 633. doi:10.3390/ph14070633

Oh, J., Yoon, H. J., Jang, J. H., Kim, D. H., and Surh, Y. J. (2019). The standardized Korean Red Ginseng extract and its ingredient ginsenoside Rg3 inhibit manifestation of breast cancer stem cell-like properties through modulation of self-renewal signaling. *J. Ginseng Res.* 43 (3), 421–430. doi:10.1016/j.jgr.2018.05.004

Oh, M., Choi, Y. H., Choi, S., Chung, H., Kim, K., Kim, S. I., et al. (1999). Anti-proliferating effects of ginsenoside Rh2 on MCF-7 human breast cancer cells. *Int. J. Oncol.* 14 (5), 869–875. doi:10.3892/ijo.14.5.869

Ouyang, Q., Cui, Y., Yang, S., Wei, W., Zhang, M., Zeng, J., et al. (2020). lncRNA MT1JP suppresses biological activities of breast cancer cells *in vitro* and *in vivo* by regulating the miRNA-214/RUNX3 Axis. *Onco. Targets. Ther.* 13, 5033–5046. doi:10.2147/OTT.S241503

Pan, W., Xue, B., Yang, C., Miao, L., Zhou, L., Chen, Q., et al. (2018). Biopharmaceutical characters and bioavailability improving strategies of ginsenosides. *Fitoterapia* 129, 272–282. doi:10.1016/j.fitote.2018.06.001

Park, J. E., Ji, H. W., Kim, H. W., Baek, M., Jung, S., and Kim, S. J. (2022). Ginsenoside Rh2 regulates the cfap20dc-AS1/MicroRNA-3614-3p/BBX and TNFAIP3 Axis to induce apoptosis in breast cancer cells. *Am. J. Chin. Med.* 50 (6), 1703–1717. doi:10.1142/S0192415X22500720

Park, J. E., Kim, H. W., Yun, S. H., and Kim, S. J. (2021). Ginsenoside Rh2 upregulates long noncoding RNA STXBP5-AS1 to sponge microRNA-4425 in suppressing breast cancer cell proliferation. *J. Ginseng Res.* 45 (6), 754–762. doi:10.1016/j.jgr.2021.08.006

Peng, K., Luo, T., Li, J., Huang, J., Dong, Z., Liu, J., et al. (2022). *Ginsenoside Rh2 inhibits breast cancer cell growth viaERβ-TNFα pathway*. Shanghai): Acta Biochim Biophys Sin.

Qi, L. W., Wang, C. Z., and Yuan, C. S. (2011). Ginsenosides from American ginseng: Chemical and pharmacological diversity. *Phytochemistry* 72 (8), 689–699. doi:10.1016/j.phytochem.2011.02.012

Qiu, D., Zhang, G., Yan, X., Xiao, X., Ma, X., Lin, S., et al. (2021). Prospects of immunotherapy for triple-negative breast cancer. *Front. Oncol.* 11, 797092. doi:10.3389/fonc.2021.797092

Ren, G., Shi, Z., Teng, C., and Yao, Y. (2018). Antiproliferative activity of combined biochanin A and ginsenoside Rh_2 on MDA-MB-231 and MCF-7 human breast cancer cells. *Molecules* 23 (11), E2908. doi:10.3390/molecules23112908

Ryoo, N., Rahman, M. A., Hwang, H., Ko, S. K., Nah, S. Y., Kim, H. C., et al. (2020). Ginsenoside Rk1 is a novel inhibitor of NMDA receptors in cultured rat hippocampal neurons. *J. Ginseng Res.* 44 (3), 490–495. doi:10.1016/j.jgr.2019.04.002

Shekari, N., Asadi, M., Akbari, M., Baradaran, B., Zarredar, H., Mohaddes-Gharamaleki, F., et al. (2022). Autophagy-regulating microRNAs: Two-sided coin in the therapies of breast cancer. *Eur. Rev. Med. Pharmacol. Sci.* 26 (4), 1268–1282. doi:10.26355/eurrev_202202_28120

Song, J. H., Eum, D. Y., Park, S. Y., Jin, Y. H., Shim, J. W., Park, S. J., et al. (2020). Inhibitory effect of ginsenoside Rg3 on cancer stemness and mesenchymal transition in breast cancer via regulation of myeloid-derived suppressor cells. *PLoS One* 15 (10), e0240533. doi:10.1371/journal.pone.0240533

Song, X., Wang, L., and Fan, D. (2022). Insights into recent studies on biotransformation and pharmacological activities of ginsenoside Rd. *Biomolecules* 12 (4), 512. doi:10.3390/biom12040512

Tang, W., Zhang, X., Tan, W., Gao, J., Pan, L., Ye, X., et al. (2019). miR-145-5p suppresses breast cancer progression by inhibiting SOX2. *J. Surg. Res.* 236, 278–287. doi:10.1016/j.jss.2018.11.030

Wang, B., Wang, F., Ding, A., Zhao, H., and Bu, X. (2020). Regorafenib and ginsenoside combination therapy: Inhibition of HepG2 cell growth through modulating survivin and caspase-3 gene expression. *Clin. Transl. Oncol.* 22 (9), 1491–1498. doi:10.1007/s12094-019-02283-9

Wang, P., Du, X., Xiong, M., Cui, J., Yang, Q., Wang, W., et al. (2016). Ginsenoside Rd attenuates breast cancer metastasis implicating derepressing microRNA-18a-regulated Smad2 expression. *Sci. Rep.* 6, 33709. doi:10.1038/srep33709

Wang, P., Song, D., Wan, D., Li, L., Mei, W., Li, X., et al. (2020). Ginsenoside panaxatriol reverses TNBC paclitaxel resistance by inhibiting the IRAK1/NF-κB and ERK pathways. *PeerJ* 8, e9281. doi:10.7717/peerj.9281

Wei, Q., Ren, Y., Zheng, X., Yang, S., Lu, T., Ji, H., et al. (2022). Ginsenoside Rg3 and sorafenib combination therapy relieves the hepatocellular carcinomaprogression through regulating the HK2-mediated glycolysis and PI3K/Akt signaling pathway. *Bioengineered* 13 (5), 13919–13928. doi:10.1080/21655979.2022.2074616

Wen, X., Zhang, H. D., Zhao, L., Yao, Y. F., Zhao, J. H., and Tang, J. H. (2015). Ginsenoside Rh2 differentially mediates microRNA expression to prevent chemoresistance of breast cancer. *Asian pac. J. Cancer Prev.* 16 (3), 1105–1109. doi:10.7314/apjcp.2015.16.3.1105

Wu, N., Wu, G. C., Hu, R., Li, M., and Feng, H. (2011). Ginsenoside Rh2 inhibits glioma cell proliferation by targeting microRNA-128. *Acta Pharmacol. Sin.* 32 (3), 345–353. doi:10.1038/aps.2010.220

Xu, J., Wu, K. J., Jia, Q. J., and Ding, X. F. (2020). Roles of miRNA and lncRNA in triple-negative breast cancer. *J. Zhejiang Univ. Sci. B* 21 (9), 673–689. doi:10.1631/jzus.B1900709

Xu, L., Xiao, S., Lee, J. J., Li, X., and Zhao, Y. (2022). Gender-related differences in tissue distribution, excretion, and metabolism studies of panaxadiol in rats and anti-inflammatory study. *J. Agric. Food Chem.* 70 (28), 8672–8679. doi:10.1021/acs.jafc.2c02618

Yang, L., Zhang, X. Y., Li, K., Li, A. P., Yang, W. D., Yang, R., et al. (2019). Protopanaxadiol inhibits epithelial-mesenchymal transition of hepatocellular carcinoma by targeting STAT3 pathway. *Cell. Death Dis.* 10 (9), 630. doi:10.1038/s41419-019-1733-8

Yu, R., Zhang, Y., Xu, Z., Wang, J., Chen, B., and Jin, H. (2018). Potential antitumor effects of panaxatriol against DU-15 human prostate cancer cells is mediated via mitochondrial mediated apoptosis, inhibition of cell migration and sub-G1 cell cycle arrest. *J. buon* 23 (1), 200–204.

Yuan, Z., Jiang, H., Zhu, X., Liu, X., and Li, J. (2017). Ginsenoside Rg3 promotes cytotoxicity of Paclitaxel through inhibiting NF-κB signaling and regulating Bax/Bcl-2 expression on triple-negative breast cancer. *Biomed. Pharmacother.* 89, 227–232. doi:10.1016/j.biopha.2017.02.038

Zarneshan, S. N., Fakhri, S., and Khan, H. (2022). Targeting akt/CREB/BDNF signaling pathway by ginsenosides in neurodegenerative diseases: A mechanistic approach. *Pharmacol. Res.* 177, 106099. doi:10.1016/j.phrs.2022.106099

Zeng, L., Li, W., and Chen, C. S. (2020). Breast cancer animal models and applications. *Zool. Res.* 41 (5), 477–494. doi:10.24272/j.issn.2095-8137.2020.095

Zhang, B., Fu, R., Duan, Z., Shen, S., Zhu, C., and Fan, D. (2022). Ginsenoside CK induces apoptosis in triple-negative breast cancer cells by targeting glutamine metabolism. *Biochem. Pharmacol.* 202, 115101. doi:10.1016/j.bcp.2022.115101

Zhang, E., Shi, H., Yang, L., Wu, X., and Wang, Z. (2017). Ginsenoside Rd regulates the Akt/mTOR/p70S6K signaling cascade and suppresses angiogenesis and breast tumor growth. *Oncol. Rep.* 38 (1), 359–367. doi:10.3892/or.2017.5652

Zhang, K., and Li, Y. (2016). Effects of ginsenoside compound K combined with cisplatin on the proliferation, apoptosis and epithelial mesenchymal transition in MCF-7 cells of human breast cancer. *Pharm. Biol.* 54 (4), 561–568. doi:10.3109/13880209.2015.1101142

Zhang, M., Wang, Y., Wu, Y., Li, F., Han, M., Dai, Y., et al. (2021). *In vitro* transformation of protopanaxadiol saponins in human intestinal flora and its effect on intestinal flora. *Evid. Based. Complement. Altern. Med.* 2021, 1735803. doi:10.1155/2021/1735803

Zhang, Y., Liu, Q. Z., Xing, S. P., and Zhang, J. L. (2016). Inhibiting effect of Endostar combined with ginsenoside Rg3 on breast cancer tumor growth in tumor-bearing mice. *Asian pac. J. Trop. Med.* 9 (2), 180–183. doi:10.1016/j.apjtm.2016.01.010

Zhao, A., Liu, N., Yao, M., Zhang, Y., Yao, Z., Feng, Y., et al. (2022). A review of neuroprotective effects and mechanisms of ginsenosides from Panax ginseng in treating ischemic stroke. *Front. Pharmacol.* 13, 946752. doi:10.3389/fphar.2022.946752

Zhao, L., Zhang, Y., Li, Y., Li, C., Shi, K., Zhang, K., et al. (2022). Therapeutic effects of ginseng and ginsenosides on colorectal cancer. *Food Funct.* 13 (12), 6450–6466. doi:10.1039/d2fo00899h

Zheng, X., Zhou, Y., Chen, W., Chen, L., Lu, J., He, F., et al. (2018). Ginsenoside 20(S)-Rg3 prevents PKM2-targeting miR-324-5p from H19 sponging to antagonize the warburg effect in ovarian cancer cells. *Cell. Physiol. biochem.* 51 (3), 1340–1353. doi:10.1159/000495552

Zou, M., Wang, J., Gao, J., Han, H., and Fang, Y. (2018). Phosphoproteomic analysis of the antitumor effects of ginsenoside Rg3 in human breast cancer cells. *Oncol. Lett.* 15 (3), 2889–2898. doi:10.3892/ol.2017.7654

Zuo, S., Wang, J., An, X., Wang, Z., Zheng, X., and Zhang, Y. (2022). Fabrication of ginsenoside-based nanodrugs for enhanced antitumor efficacy on triple-negative breast cancer. *Front. Bioeng. Biotechnol.* 10, 945472. doi:10.3389/fbioe.2022.945472

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 20 October 2022
DOI 10.3389/fphar.2022.1031438

Check for updates

OPEN ACCESS

EDITED BY
Yongye Huang,
Northeastern University, China

REVIEWED BY
Yupeng Wang,
Southern Medical University, China
Alessandra Ferraresi,
Università degli Studi del Piemonte Orientale, Italy
Junhong Lü,
Shanghai Advanced Research Institute (CAS), China

*CORRESPONDENCE
Zhiguang Chang,
changzhg@mail.sysu.edu.cn
Xiaobo Wang,
wangxiaobo@sysush.com

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Pharmacology of Anti-Cancer Drugs, a section of the journal Frontiers in Pharmacology

RECEIVED 30 August 2022
ACCEPTED 23 September 2022
PUBLISHED 20 October 2022

CITATION
Zhang W, Zhang R, Chang Z and Wang X (2022), Resveratrol activates CD8+ T cells through IL-18 bystander activation in lung adenocarcinoma. *Front. Pharmacol.* 13:1031438. doi: 10.3389/fphar.2022.1031438

COPYRIGHT
© 2022 Zhang, Zhang, Chang and Wang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Resveratrol activates CD8$^+$ T cells through IL-18 bystander activation in lung adenocarcinoma

Wei Zhang[1†], Ruohao Zhang[2†], Zhiguang Chang[3*] and Xiaobo Wang[4*]

[1]Emergency and Disaster Medical Center, The Seventh Affiliated Hospital, Sun Yat-Sen University, Shenzhen, China, [2]School of Medicine, Sun Yat-sen University, Shenzhen, China, [3]Edmond H. Fischer Translational Medical Research Laboratory, Scientific Research Center, The Seventh Affiliated Hospital, Sun Yat-sen University, Shenzhen, China, [4]Department of Hematology, The Seventh Affiliated Hospital, Sun Yat-sen University, Shenzhen, China

Resveratrol, a natural product, has demonstrated anti-tumor effects in various kinds of tumor types, including colon, breast, and pancreatic cancers. Most research has focused on the inhibitory effects of resveratrol on tumor cells themselves rather than resveratrol's effects on tumor immunology. In this study, we found that resveratrol inhibited the growth of lung adenocarcinoma in a subcutaneous tumor model by using the β-cyclodextrin-resveratrol inclusion complex. After resveratrol treatment, the proportion of M2-like tumor-associated macrophages (TAMs) was reduced and tumor-infiltrating CD8T cells showed significantly increased activation. The results of co-culture and antibody neutralization experiments suggested that macrophage-derived IL-18 may be a key cytokine in the resveratrol anti-tumor effect of CD8T cell activation. The results of this study demonstrate a novel view of the mechanisms of resveratrol tumor suppression. This natural product could reprogram TAMs and CD8T effector cells for tumor treatment.

KEYWORDS

resveratrol (CID: 445154), CD8+T cell, IL-18, bystander activation of T cells, tumor-associated macrophage, lung adenocarcinoma

Introduction

Natural products with free radical scavenging activity have shown potential for tumor treatment and prevention. Polyphenols include a large class of antioxidants, including flavonoids, anthocyanins, phenolic acids, lignans, and stilbenes. These compounds are all derived from phenylalanine and contain aromatic rings with active hydroxyl groups (Dixon, 2001). Resveratrol, a member of the stilbene subclass, is a type of defensive molecule called phytoalexin, which can prevent infection and damage caused by exposure to ultraviolet (UV) irradiation. Resveratrol and its analogs have been identified in several edible natural products such as grapevine, peanut (*Arachis* spp.) (Sanders et al., 2000), berries (Rimando et al., 2004), and rhubarb (*Rheum* spp.) (Matsuda et al., 2001).

As a health care product, resveratrol has been widely confirmed to be safe. Moreover, substantial evidence has shown that resveratrol and its analogs exert cardiovascular protective effects by anti-inflammatory activities and scavenging free radicals generated by oxidation (Ashraf et al., 2015). Recently, resveratrol has also been widely assessed in tumor growth inhibition studies. The anti-tumor effects of resveratrol were first reported in 1997 (Jang et al., 1997). Since then, the antioxidative, anti-inflammatory, anti-proliferative, and anti-angiogenic effects of resveratrol have been extensively studied. Recent reports have shown that resveratrol inhibits the proliferation of several cancers, including colon cancer, breast cancer, pancreatic cancer, prostate cancer, ovarian cancer, and lymphoma through different pathways (Ulrich et al., 2006; Bjorklund et al., 2011; Frazzi et al., 2013).

Resveratrol is considered to directly inhibit cell proliferation by inducing tumor cell senescence (Yang et al., 2013), modulating sub-pools of sphingolipids (Signorelli et al., 2009; Shin et al., 2012), or inducing apoptosis (Aggarwal et al., 2004; Savio et al., 2022). Mechanistically, resveratrol can inhibit PKC (Atten et al., 2001) or MEK1/2-ERK1/2-c-Jun (Aquilano et al., 2009) activity. Reports have also demonstrated that resveratrol can also exert anti-tumor effects by affecting other cells, including immune cells (Chhabra et al., 2021; Mu and Najafi, 2021), stromal cells (Guo et al., 2021) and cancer stem-like cells (Pradhan et al., 2021) in tumors.

Lung cancer is the leading cause of cancer deaths (18.0% of total cancer deaths) (Sung et al., 2021). Lung adenocarcinoma accounts for about 40% of the total number of lung patients. At present, chemotherapy for lung adenocarcinoma has a high effective rate and a significantly improved survival rate. The incidence of EGFR, ALK, and other sensitive mutations in lung adenocarcinoma is also significantly higher than that in other lung cancers. However, the 5-year survival rate for stage II lung adenocarcinoma is only 40–50%. The 5-year survival rates for stage 3A and 3B disease are 25%–30%, and 5%–17%, respectively. The prolongation of the survival time of patients with lung adenocarcinoma by using new tumor treatment methods is a key question for lung adenocarcinoma research. Effective drugs with fewer side effects are needed. In this study, we evaluated the potential of resveratrol for the treatment of lung adenocarcinoma by investigating the changes in the activation of innate and adaptive immune cells during tumor treatment.

Materials and methods

Cell lines

Lewis lung carcinoma (LLC) cells were obtained from American Type Culture Collection (ATCC). The LLC cell line was established from the lung of a C57BL mouse bearing a tumor resulting from the implantation of primary LLC. The cell line is highly tumorigenic and is primarily used to model metastasis and evaluate the efficacy of chemotherapeutic agents *in vivo* (Sakai et al., 2006). THP-1 cells were also obtained from ATCC. THP1 is a human leukemia monocytic cell line that has been extensively used to study monocyte/macrophage function (Chanput et al., 2014). Finally, the RAW 264.7 murine macrophage cell line was obtained from Procell Life Science & Technology Co., Ltd., LLC. The Raw264.7 cells were maintained in DMEM+10% FBS+1% P/S/ The THP1 cells were maintained in RPMI-1640 + 10% FBS+1% P/S. All cell lines were cultured in 5% CO_2 at 37°C.

Resveratrol-βcd preparation

β-Cyclodextrin-resveratrol inclusion complex was prepared as previously described, with some modifications (Venuti et al., 2014). Sulfobutylether-β-cyclodextrin (Captisol, βcd, Shanghai Chineway Pharmaceutical Tech. Co., Ltd.) was solubilized in water at room temperature and added to an amount of resveratrol (MCE, HY-16561) exceeding its intrinsic solubility. The mix was sonicated in a water bath and then stirred. The suspension was filtered through 0.22 μm filters and freeze-dried. The stoichiometry of the complex was confirmed using Job's plotting method. A maximum value at R = 0.5 and a symmetrical shape indicated a 1:1 complex. The water solubilities of the resveratrol and the resveratrol-βcd complex were determined by suspending excess drug amounts in 10 ml water and stirring at 25 ± 0.1°C for 24 h. The suspensions were then filtered through 0.22 μm filters and analyzed by UV–vis spectroscopy at 305 nm.

CCK8 assay

CCK8 (Solarbio, CA1210) was used for the analysis of cell proliferation according to the manufacturer's instructions. Briefly, cells were seeded in 96-well plates and treated with different drugs, then placed in a 37°C, 5% CO_2 incubator for pre-culture. For the analysis of CD8T cell proliferation, the upper layer of co-culture was collected and centrifuged at 500 *g* for 5 min to collect the cell pellets, which were then resuspended in 100 μl of culture medium and added to a 96-well plate. Next, 10 μl of CCK-8 solution was added to each well. The culture plates were incubated at 37°C for 60 min. The absorbance at 450 nm was measured using a microplate reader.

Development and treatment of the lung adenocarcinoma subcutaneous tumor model

Six to eight-week-old C57BL/6J mice were purchased from GemPharmatech Co., Ltd. The study protocols were approved by

the Institutional Animal Care and Use Committee of Sun Yat-Sen University. To construct the tumor subcutaneous model, each mouse was shaved and subsequently subcutaneously inoculated with 10^6 LLC cancer cells. After the tumor volume reached 300 mm^3, 5 mg/kg resveratrol-βcd was injected peritumorally every 2 days. The resveratrol-βcd injection site was sterilized with 70% alcohol, the injection needle was inserted obliquely, and the drug was injected 3–5 mm under the skin. Syringe draw-back was avoided and the volume for each injection did not exceed 50 μl to avoid necrosis. The mice were sacrificed when the tumor volumes reached approximately 2000 mm^3 (tumor volume = $1/2 \times a \times b^2$; a = length and b = width). The tumor tissues were collected for subsequent experiments.

Flow cytometry

The following antibodies were used for the analysis of tumor infiltration by immune cells: CD45 -eFluor 450 (eBioscience 48-0451-82), CD11b-APC cy7 (eBioscience 47-0112-82), F4/80-PE (eBioscience 12-4801-82), CD8-PE cy7 (eBioscience 25-0081-82), CD4-FITC (eBioscience 11-0042-85), IFNgama-APC (eBioscience 17-7311-82), CD206-APC (eBioscience 17-2061-82). Single-cell suspensions were prepared from the tumor tissues after homogenization or from culture suspension cells. For staining, 100 μl of single-cell suspensions were incubated with antibodies for 30 min. Individual single-color controls were prepared for compensation adjustment. After staining, the samples were washed twice with PBS and resuspended in 600 μl PBS. Flow cytometry data were acquired using a CytoFLEX LX system (Beckman Coulter). FlowJo version 10.0 was used for data analysis. The absolute numbers of $CD8^+$ T cell or macrophage numbers per gram of tumor were the corresponding cell counts from 60 μl of cell suspension (10% of total volume) divided by the tumor weight. The gating strategy is described in each figure's caption. An example of the gating strategy is given in Supplementary Figure S1.

Cell apoptosis assay

The Annexin V-FITC/PI Kit (Yeasen Biotechnology, Shanghai) was used for the cell apoptosis assay according to the manufacturer's instructions. Briefly, treated LLC cells were digested with EDTA-free trypsin and harvested by centrifugation at 200 *g* at 4°C for 5 min. Trypsin digestion time was controlled to prevent false positives. The cells were washed with pre-cooled PBS twice at 200 *g* at 4°C for 5 min. The cells were then resuspended in 100 μl of 1× Binding Buffer. Next, 5 μl Annexin V-FITC and 10 μl PI Staining Solution were added. The cells were stained in the dark and at room temperature for 15 min. Next, 400 μl of 1×Binding Buffer was added to the samples and cell apoptosis was detected by flow cytometry within 1 h.

A commercial TUNEL Apoptosis Detection Kit (Alexa Fluor 488) (Yeasen Biotechnology, Shanghai) was used for the analysis of subcutaneous tumor apoptosis. The tumor tissues were embedded in paraffin and sectioned. The sections were immersed in xylene for 5 min twice at room temperature to completely remove the paraffin. The sections were then soaked in 100% ethanol for 5 min at room temperature and then in a 90, 80, and 70% ethanol gradient for 3 min each. The sections were then gently rinsed with PBS. Next, 100 μl of Proteinase K solution at 20 μg/ml was added to each sample and incubated at room temperature for 20 min. The samples were then rinsed three times with PBS solution and the excess liquid was gently removed. Next, 100 μl of 1× Equilibration Buffer was added to each sample to completely equilibrate the sample area and then incubated for 30 min. After removal of the Equilibration Buffer, 50 μl of TdT Incubation Buffer was added to the sample and incubated at 37°C for 60 min in the dark. The samples were then washed three times with PBS and the slides were immersed in a staining tank containing DAPI solution (2 μg/ml, freshly prepared and diluted with PBS) in the dark for 5 min. The samples were then washed three times with deionized water, and 100 μl PBS was added to the sample area to keep the samples moist. The samples were immediately analyzed under a fluorescence microscope.

Cell senescence analysis

At 1–7 days after resveratrol-βcd treatment, the cells were digested with EDTA-free trypsin and harvested by centrifugation at 200 *g* at 4°C for 5 min. Next, they were washed with PBS, and flow cytometry was performed using p16 as a marker of cell senescence. After 30 min of incubation with p16INK4a antibody (ThermoFisher, PA5-119712), the cells were stained with Alexa Fluor 488-conjugated goat anti-rabbit IgG secondary antibody at a 1:2000 dilution for 30 min. Flow cytometry data were acquired on a CytoFLEX LX system (Beckman Coulter). FlowJo version 10.0 was used for data analysis. For β-gal analysis, a commercial Senescence β-Galactosidase Staining Kit (Beyotime Biotechnology) was used according to the manufacturer's instructions. Cells in 24-well culture dishes were washed once with PBS. Next, 250 μl β-galactosidase staining fixation solution was added and the samples were incubated for 15 min at room temperature. After fixation, the cells were washed three times with PBS for 3 min each. Next, 250 μl of staining working solution was added to each well and the plates were incubated overnight at 37°C. The plates were sealed with Parafilm or plastic wrap to prevent evaporation. The results were observed and counted by light microscopy.

Macrophage polarization and CD8T cell separation

Macrophage polarization was performed as previously described (Smith et al., 2015). Briefly, 10 ng/ml PMA was first used to activate THP1 cells for 24 h, making the cells adhere to the plate wall as M0 macrophages. The M0 macrophages were further activated with LPS or IL-4+IL-13 to polarize to M1 or M2 for subsequent experiments. The polarized macrophages were washed three times with serum-free culture medium and then co-cultured with separated CD8T cells. In some co-culture experiments, an anti-hIL18 antibody (InvivoGen) was added to neutralize IL-18 in the culture. A CD8a+ T Cell Isolation Kit (Miltenyi, 130-104-075) was used for CD8T cell separation according to the manufacturer's instructions. For spleen or tumor CD8T cell isolation, tissue homogenization was performed first. Red Blood Cell Lysis Buffer (Beyotime Biotechnology, C3702) was then used to lyse the red blood cells, followed by CD8T cell purification. Purified CD8T cells were identified by flow cytometry as >90% isolated cells with $CD8^+$ phenotypes.

Enzyme-linked immunosorbent assay (ELISA)

The ELISA assays used 50 μl of culture supernatant after centrifugation at 16,000 *g* at 4°C for 10 min. The mouse cytokines IL-12 (Biolegend 431704), Il-15 (Biolegend 435104), and IL-18 (Elabscience) were used according to the manufacturers' instructions. Mouse IL-10 ELISA (Biolegend, 431414), Mouse TNFα ELISA (Biolegend, 430901), and Mouse IL-12p70 ELISA (Biolegend, 433604) were used for intratumor cytokine analysis. For the THP-1 supernatant experiment, Human IL-10 ELISA (Biolegend, 430604) and human IL-6 ELISA (Biolegend, 430504) were used for macrophage polarization analysis. The chemiluminescent signals were measured at 450 nm.

Quantitative PCR (qPCR)

Total RNA was extracted using a Total RNA Extraction Kit (Solarbio, R1200) according to the manufacturer's instructions. cDNA was synthesized using the TransScript® Uni All-in-One First-Strand cDNA Synthesis mix (TransGen Biotech, AU341-02). Quantitative PCR (qPCR) was performed using a qPCR Mix (Takara). The relative expression levels of genes were determined using the $2^{-\Delta\Delta Ct}$ method and normalized to h β-actin expression levels. The gene-specific PCR primers are listed below:

IL6: F: ACTCACCTCTTCAGAACGAATTG; R: CCATCTTTGGAAGGTTCAGGTTG;
TNF: F: CCTCTCTCTAATCAGCCCTCTG; R: GAGGACCTGGGAGTAGATGAG;
IL10: F: GACTTTAAGGGTTACCTGGGTTG; R: TCACATGCGCCTTGATGTCTG;
MRC1: F: TCCGGGTGCTGTTCTCCTA; R: CCAGTCTGTTTTTGATGGCACT;
h β-actin, F: ACTCTTCCAGCCTTCCTTCC; R: CGTACAGGTCTTTGCGGATG;

Statistical analysis

Data were analyzed using GraphPad 9.0 software. Student's *t*-tests or one-way ANOVA were used to analyze the significance of differences between groups. $P < 0.05$ was considered statistically significant. $^{****}p < 0.0001$, $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$.

Results

Resveratrol-βcd induces lung adenocarcinoma cell apoptosis and senescence *in vitro*

Due to the low solubility of resveratrol in water, its bioavailability is quite low. Previous studies have reported that inclusion complexes formed by resveratrol-loaded glutathione responsive cyclodextrin nanosponges can increase the water solubility of resveratrol (Palminteri et al., 2021), Therefore, this study used a β-cyclodextrin-resveratrol inclusion complex to improve the resveratrol solubility. The water solubility of resveratrol-β-cyclodextrin (resveratrol-βcd) increased from 0.026 mg/ml to 0.967 mg/ml, with a rapid dissolution rate (Supplementary Figure S2).

As reported previously, resveratrol can induce tumor cell apoptosis or senescence. Therefore, we first analyzed the therapeutic mechanism of resveratrol-βcd on tumor cells *in vitro*. First, we treated LLC (a lung adenocarcinoma cell line) with 0-50 μM resveratrol-βcd *in vitro* and analyzed cell proliferation at 24, 48, and 72 h post-treatment. The results showed that resveratrol-βcd inhibited tumor growth at 10 μM (Figure 1A). Analysis of apoptosis and senescence after resveratrol-βcd treatment showed that only about 6% of the cells showed apoptosis (both early and late stages) 48 h after treatment (Figures 1B,C). At 72 h after treatment, the level of apoptosis reached approximately 15%. We further analyzed cell senescence by flow cytometry using p16 as a maker of senescence. At 7 days post-treatment, the LLC cells had developed a senescence phenotype (Figures 1D,E). The β-gal analysis showed a similar trend in cell senescence as that observed in flow cytometry analysis (Figures 1F,G). However, the β-gal-positive cell ratio was quite low compared to the $p16^+$ ratio.

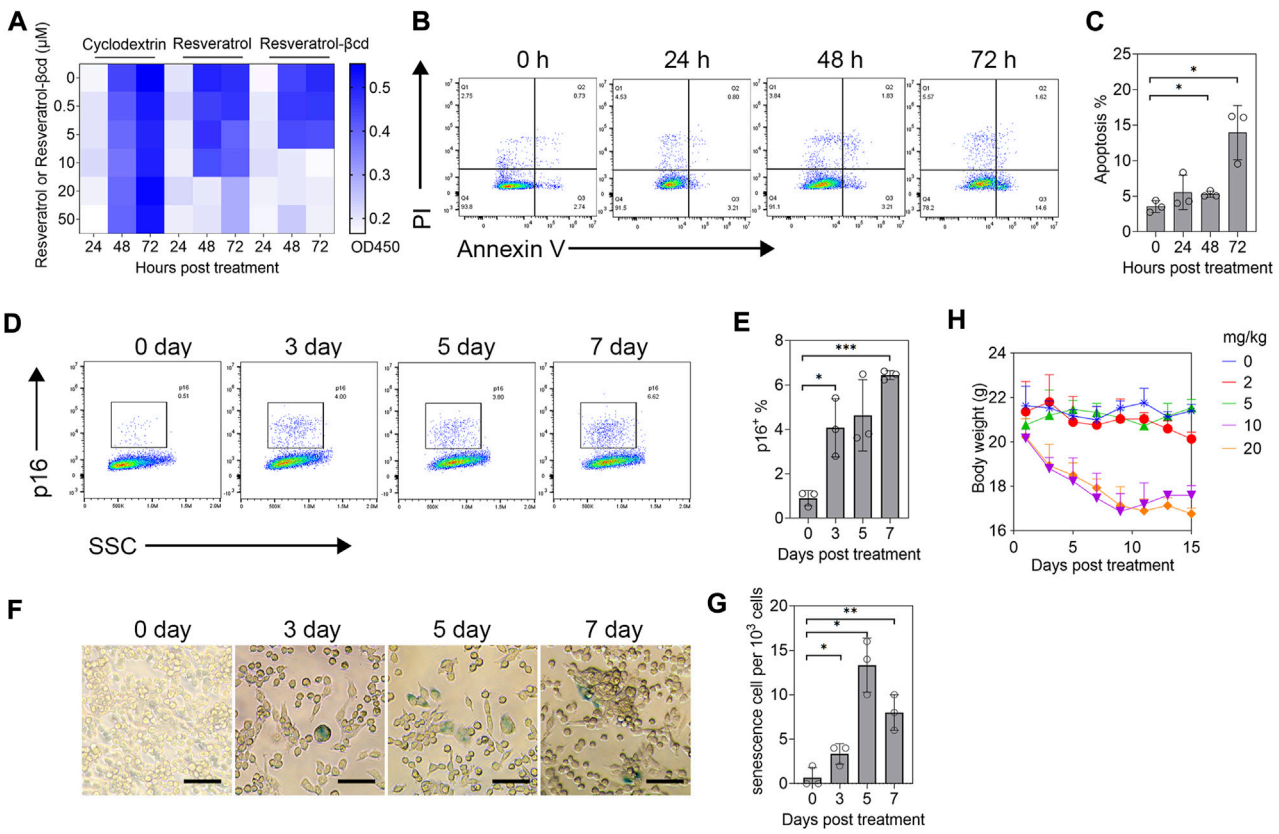


FIGURE 1
Evaluation of resveratrol-βcd anti-tumor effects *in vitro*. **(A)** CCK-8 assessment of the inhibitory effects of resveratrol-βcd or resveratrol or an equal volume of cyclodextrin on cell proliferation at the indicated times and concentrations. The heatmap values are the average values of three replicates. **(B)** Typical diagram of apoptosis flow cytometry after treatment of LLC cells with 10 μM resveratrol-βcd at different time points. **(C)** Statistical results of apoptosis at the indicated times. Error bar = mean ± S.D. **(D)** Typical flow cytometry diagram of LLC cell senescence analysis after treatment with 10 μM resveratrol-βcd for the indicated times. p16 was used as the marker of senescence. **(E)** Statistical results of the senescence analysis. Error bar = mean ± S.D. **(F)** Typical β-gal graph of LLC cells after treatment with 10 μM resveratrol-βcd at the indicated times. Cyan-stained cells were considered positive for senescence. Scale bar = 50 μm; positive cells per 10^3 cells were counted. **(G)** Statistical results. Error bar = mean ± S.D. **(H)** Time course of mouse body weight after treatment with the indicated concentrations of resveratrol-βcd. Error bar = mean ± S.D., n = 5.

Thus, resveratrol-βcd showed a modest effect on cell senescence. To minimize the effects of the drug on the mice, we evaluated the side effects of different doses of resveratrol-βcd *in vivo*. Subcutaneous injections of resveratrol-βcd were administered every 2 days. At a concentration of 10 mg/kg, the body weight of mice decreased by about 10%, and reduced liver and renal function were observed (Figure 1H, Supplementary Figure S3).

Resveratrol-βcd activated tumor-infiltrating CD8T cells significantly inhibited subcutaneous tumor growth

We used a murine subcutaneous lung adenocarcinoma model to evaluate the therapeutic effects of resveratrol-βcd (Figure 2A). As shown in Figure 1H, we chose 5 mg/kg resveratrol-βcd for tumor treatment and performed peritumoral injections every 2 days. Compared to the control group, the mice in the treatment group showed obvious tumor inhibition (Figure 2B). On the 17th day after treatment, we sacrificed the mice (Figure 2C). A TUNEL analysis showed increased apoptosis in the treatment group (Figure 2D). In the group administered resveratrol-βcd, the tumor mass was 82% lower (Figure 2E). However, this inhibitory effect only lasted for approximately 10–14 days. In the survival experiment, the resveratrol-βcd-treated mice reached the humane endpoint at about 40 days, about 10 days longer than that in the control group (Figure 2F). Our analysis of the immune cells in the tumor environment on the 17th day after treatment showed that almost all CD8T cells in the tumor were activated in the treatment group (Figures 2G,H); however, the ratio of CD8T cells was significantly decreased (Figures 2I,J). These results suggested that resveratrol may have inhibited tumor growth by activating tumor-infiltrating CD8T cells.

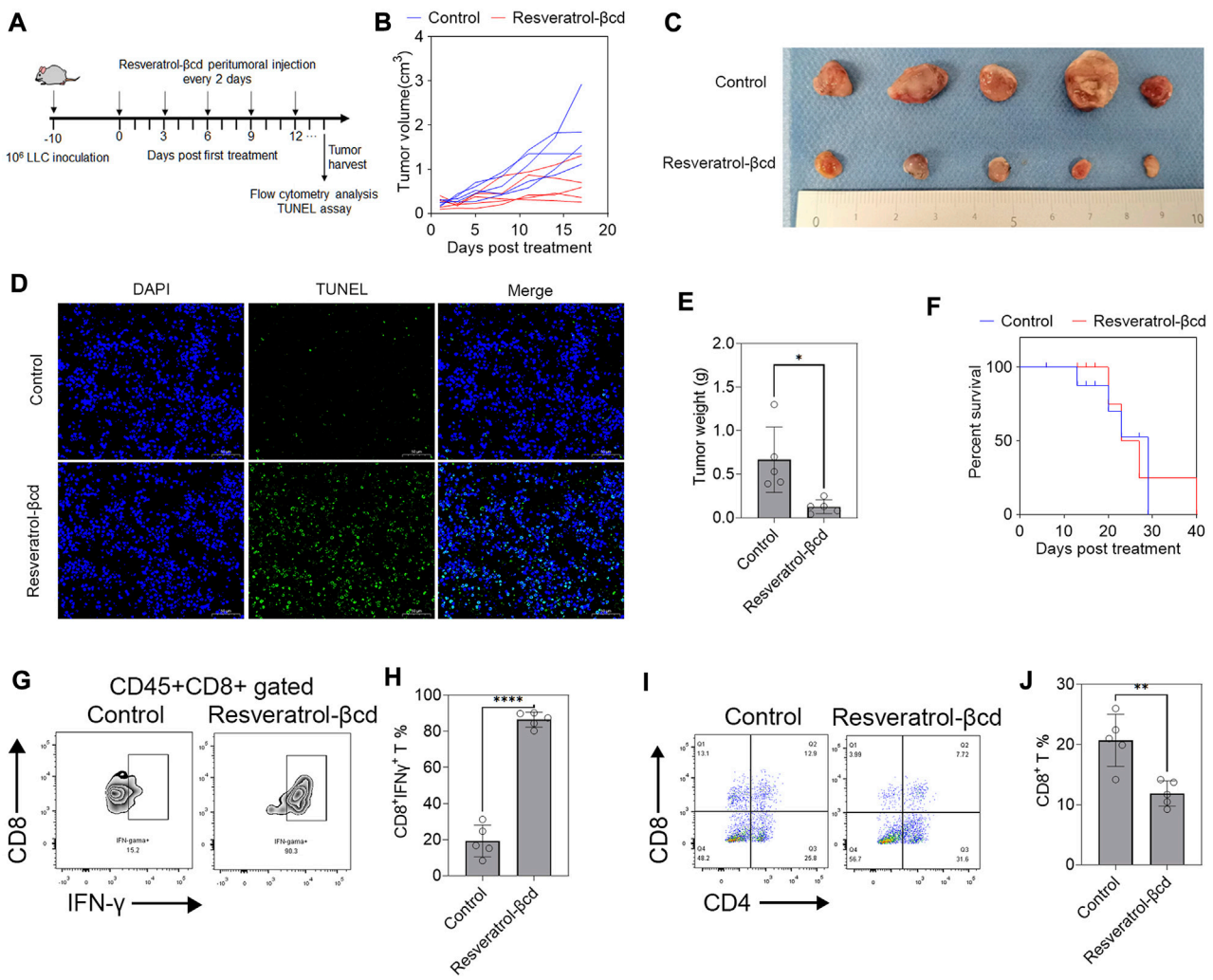


FIGURE 2
Resveratrol-βcd activates tumor-infiltrating CD8T cells against lung adenocarcinoma. **(A)** A graphical representation showing the timeline of *in vivo* experiments. A total of 10^6 LLC cells were inoculated into the right flank of mice. Approximately 10 days post-inoculation, the tumor volume reached around 300 mm^3 and drug administration began. The first day of drug administration was recorded as Day 0 post-treatment. Paratumoral injections were administered every 2 days until reaching the humane endpoint. **(B)** Resveratrol-βcd, equivalent to 5 mg/kg resveratrol, was injected adjacent to the tumor every 2 days, while the control group received the same amount of βcd. The results showed significant inhibition of tumor growth in the treatment group (n = 5). **(C)** Typical images of tumors after the mice were sacrificed 17 days after treatment. **(D)** A typical graph of TUNEL analysis of tumor apoptosis 17 days after treatment. Scale bar = 50 μm. **(E)** The mean tumor masses after dissection were 0.126 g and 0.666 g in the treatment and control groups, respectively. Error bar = mean ± S.D., n = 5; *p = 0.014. **(F)** Survival curves of the resveratrol-βcd and control groups. All mice in the control group reached the humane endpoint at 29 days post-treatment, compared to 40 days in the resveratrol-βcd group. n = 5. **(G)** Typical flow cytometry diagram of CD8T activation in tumors. The cells were $CD45^+CD8^+$ gated. **(H)** CD8T cell activation in the treatment and control groups. Error bar = mean ± S.D., ****p < 0.0001. **(I)** Typical flow cytometry diagram of CD4T and CD8T cells in tumors. The cells were $CD45^+$ gated. **(J)** The ratio of CD8T in the treatment and control groups. Error bar = mean ± S.D., **p < 0.01.

Tumor-associated macrophages showed a polarization switch during resveratrol-βcd treatment

The levels of tumor-associated macrophages (TAMs) decreased significantly in resveratrol-βcd treated mice (Figures 3A,B). Using CD206 as a marker of M2-like macrophages, a decreased proportion of M2 macrophages was also observed in the treated group (Figures 3C,D). The number of M2-like macrophages decreased by 42.4% in the treatment group compared to that in the control group (Figure 3E), while the numbers of M1-like macrophages were similar in the treatment and control groups (Figure 3F). Previous studies showed that resveratrol can activate M2 macrophages and show anti-inflammatory effects (Liu et al., 2019; Yu et al., 2019; Ma et al., 2020).

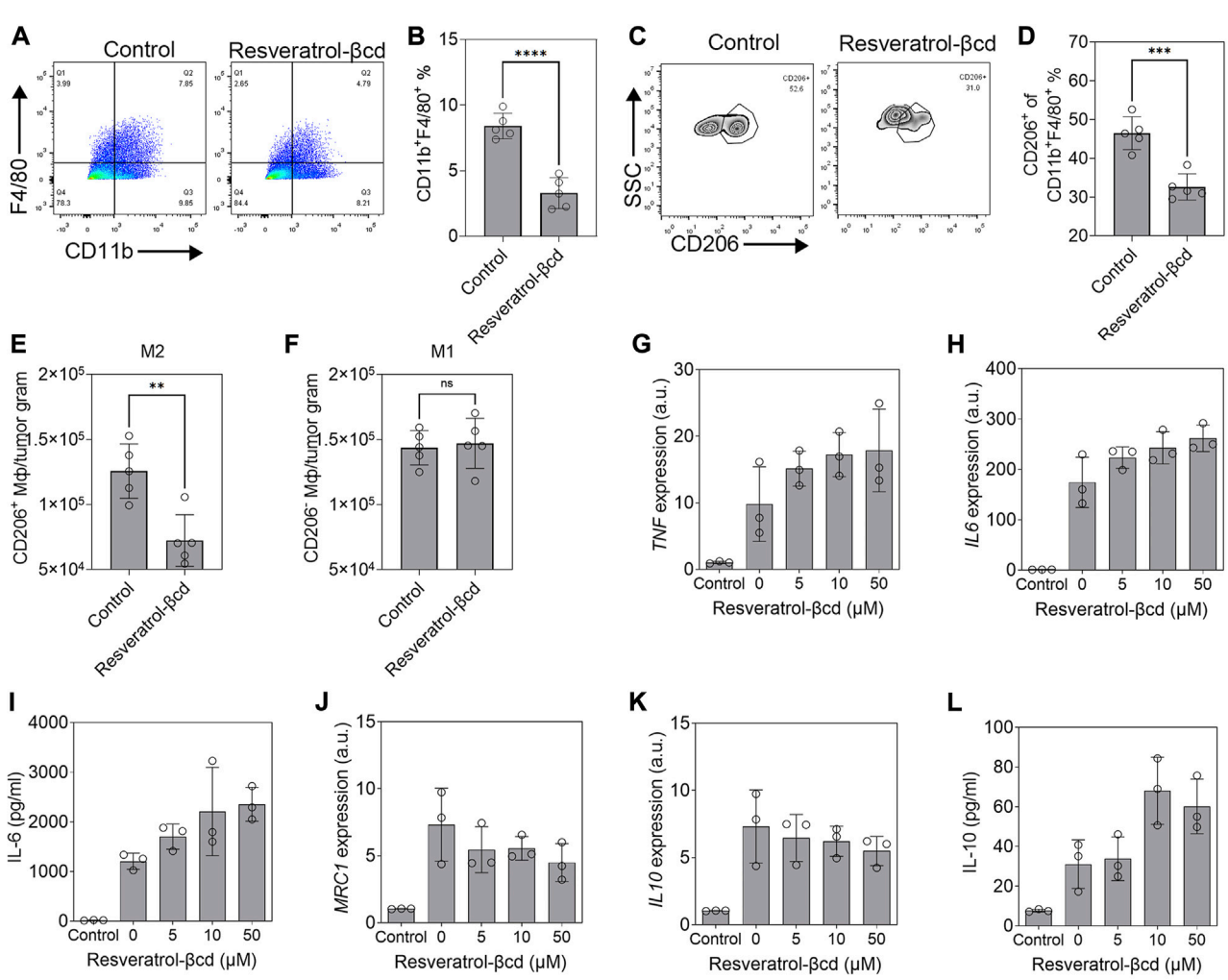


FIGURE 3
Resveratrol-βcd switch tumor-associated macrophage M2-type polarization. **(A,C)** Typical flow cytometry diagram of TAM ($CD11b^+F4/80^+$) or M2 macrophages ($CD11b^+F4/80^+$ $CD206^+$). The cells were $CD45^+$ gated. **(B,D)** The proportions of TAM or M2-like macrophages in the treatment and control groups. Error bar = mean ± S.D. ***$p < 0.001$. ****$p < 0.0001$. **(E)** The numbers of M2-like and **(F)** M1-like ($CD206^-F4/80^+CD11b^+$) macrophages per tumor gram. **$p < 0.01$. **(G)** THP1 as the control group and THP1-derived M1 macrophages treated with resveratrol-βcd at the indicated concentrations. *TNF* and **(H)** *IL6* transcript levels and **(I)** IL-6 levels in the medium 48 h after treatment were measured to identify M1 polarization. Error bar = mean ± S.D. **(J)** *MRC1*, **(K)** *IL10*, and **(L)** IL-10 levels 48 h after treatment with the indicated concentration of resveratrol-βCd. Error bar = mean ± S.D.

We used resveratrol-βcd to treat M1 or M2 macrophages derived from THP1 to determine the effects of resveratrol-βcd on macrophage polarization. The results showed that resveratrol-βcd indistinctly but directly activated THP-1 derived M1 macrophages in a dose-dependent manner (Figures 3G,H) and that IL-6 protein and transcription levels show consistent trends after treatment (Figure 3I). After resveratrol-βcd treatment, the activation levels of different typical markers of M2-like macrophages were not consistent (Figures 3J–L). Resveratrol-βcd had almost no effect on *IL10* transcription levels; however, IL-10 levels increased significantly in the supernatant (Figure 3L). *MRC1(CD206)* transcription levels decreased by 34% after treatment with 50 μM resveratrol-βcd (Figure 3K).

Resveratrol-βcd activates $CD8^+$ T cells through macrophage-derived IL-18

We co-cultured Raw 264.7-derived M1 (LPS) or M2(IL-4+IL-13) with spleen-derived CD8T cells in different concentrations of resveratrol-βcd and analyzed CD8T cell activation. CD8T cells were partially activated under 5 μM resveratrol-βcd (Figure 4A). Moreover, M1 macrophages

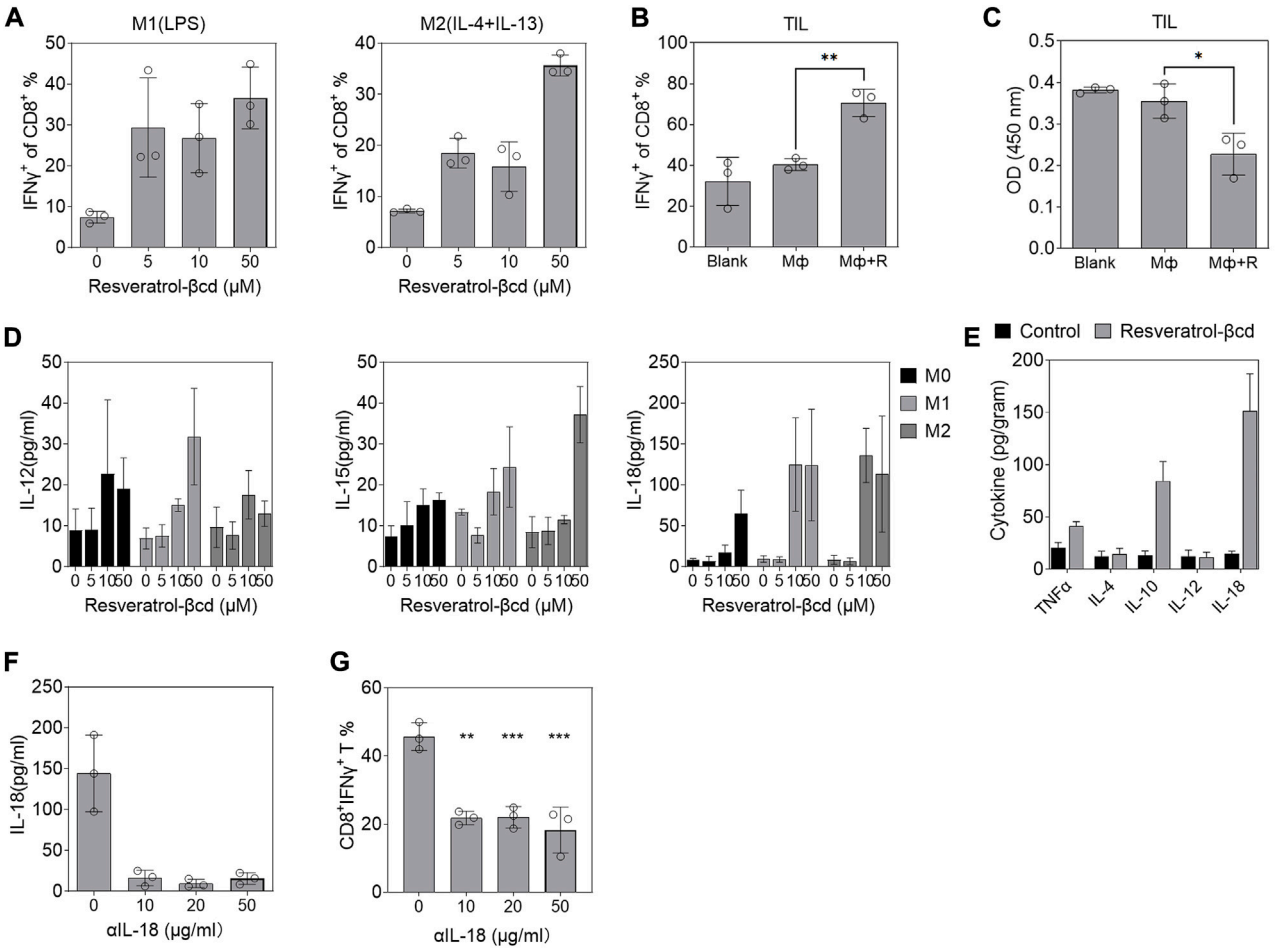


FIGURE 4
Resveratrol-βcd induced macrophages to secrete IL-18 and promote CD8T bystander activation. **(A)** CD8T activation in co-cultured Raw264.7-derived M1 or M2 macrophages with the indicated resveratrol-βcd and CD8T cells derived from the spleen. Error bar = mean ± S.D. **(B)** Activation of tumor-derived CD8T cells and cell proliferation **(C)** in co-culture with M1 macrophages or M1 macrophages + resveratrol-βcd. Error bar = mean ± S.D. **(D)** M1 or M2 macrophages were activated and incubated with the indicated resveratrol-βcd concentrations for 48 h. The levels of IL-12, IL-15, and IL-18 in the supernatant were analyzed by ELISA. **(E)** Subcutaneous tumors were harvested 4 days after the first treatment. The intratumoral cytokine levels were measured by ELISA. $n = 5$. Error bar = mean ± S.D. **(F)** IL-18 in the supernatant of M1 macrophages was neutralized by the indicated anti-IL-18 antibody (αIL-18) and the effect of neutralization was analyzed. Error bar = mean ± S.D. **(G)** After neutralization, M1 macrophages treated with resveratrol-βcd were co-cultured with spleen-derived CD8Ts. CD8T activation was analyzed 24 h after co-culture. Error bar = mean ± S.D., $^{**}p < 0.01$, $^{***}p < 0.001$.

elicited significantly higher levels of CD8T activation compared to M2 macrophages (Figure 4A). However, these activation levels (40%) were not consistent with the results observed in the tumor (Figure 2H), which showed >90% activation. This difference might be due to the specificity of tumor-infiltrating CD8T cells. Therefore, we sorted tumor-infiltrating CD8T cells from mice LLC tumors for co-culture. The results showed that tumor-derived CD8T was significantly activated with macrophages and 10 μM resveratrol-βcd (Figure 4B), indicating that tumor-derived CD8T cells had a phenotype that could be activated by macrophages. Interestingly, CD8T cells barely proliferated after co-culture, and the cell population decreased even in culture with resveratrol-βcd (Figure 4C). Our analysis of IL-12, IL-15, and IL-18 levels, which are associated with CD8T activation, in the macrophage culture medium 48 h after resveratrol-βcd treatment showed that IL-12 and IL-15 levels were not significantly increased in M0, M1, or M2 macrophages (Figure 4D), while IL-18 levels were greatly increased in both M1 and M2 macrophages. In the tumors, IL-10 and IL-18 levels also increased 4 days after the first treatment (Figure 4E). An antibody neutralization experiment was performed to clarify the role of macrophage-derived IL-18 in CD8T activation. The addition of 10 μg/ml αIL-18 (anti-IL-18 antibody) to the medium reduced the IL-18 level to approximately 10 pg/ml (Figure 4F) and

significantly inhibited CD8T cell activation (Figure 4G). These results suggested that IL-18 is a factor involved in resveratrol-βcd-induced CD8T cell activation through macrophages.

Discussion

Immune cells are highly abundant in the tumor microenvironment of lung adenocarcinoma. CD8T cells are the most important type of immune cells involved in tumor immunity and have direct cytotoxic effects. Regarding CD8T cell activation, in addition to the classical TCR pathway mediated activation, there also exists a bystander mode of activation. Various types of human cancers show many $CD8^+$ T cells without tumor antigen specificity (Simoni et al., 2018). This type of CD8T cell can be activated in a T-cell receptor-independent, cytokine-dependent manner. The cytokines that can participate in the bystander activation of CD8T cells include type I interferon (Tough et al., 1996), interleukin-18 (Freeman et al., 2012), and interleukin-15 (Kim et al., 2018). Bystander-activated CD8T cells are cytotoxic and secrete cytokines such as interferon-γ to perform their effects; however, bystander activation is nonspecific and tends to promote T cell activation rather than proliferation. The results of the present study demonstrated a significant increase in the level of T cell activation after treatment but a significant decrease in the absolute number of T cells. This indicated that resveratrol-βcd may activate T cells through bystander activation. Cytotoxic activity and the production of TNFα and IFN-γ are the main mechanisms of the $CD8^+$ T cell-mediated tumor cytotoxic effect. Both IFN-γ and TNF-α can be induced when $CD8^+$ T cells are stimulated with peptide antigens. However, during bystander activation, $CD8^+$ T cells only produce IFN-γ, rather than TNFα (Beadling and Slifka, 2005). Resveratrol reportedly attenuates the proliferative effect of TNF-α on CD8T cells *in vitro* (Silva et al., 2014), likely because resveratrol can activate and cause the apoptosis of CD8T *via* the bystander pathway.

In addition, macrophages are among the most abundant immune cells in tumors. TAMs usually exhibit M2-like polarization, which is associated with tumor immune escape, angiogenesis, and invasion during tumor development. TAMs mainly play a role in tumor immunosuppression during the development of lung adenocarcinoma. This immunosuppressive effect is partly caused by crosstalk with CD8T cells.TAM can express PD-L1, PD-L2, and CTLA-4 ligands CD80 and CD86. These immune checkpoints mainly target CD8 T cells and lead to the inhibition of CD8T cytotoxic effects. TAM also plays an important role in $CD8^+$ T cell infiltration in malignant tumors (Wynn and Barron, 2010; Jiang et al., 2017). *In vivo* studies showed that TAM-derived TGF-β inhibited T cell-mediated tumor clearance (Thomas and Massague, 2005). The neutralization of TGF-β by an antagonist upregulated cytotoxic gene expression in $CD8^+$ T cells in a mouse model (Thomas and Massague, 2005). TAM also modulated T cell metabolism (Noy and Pollard, 2014; Proto et al., 2018).

Infectious disease studies reported that resveratrol activates M2 macrophages (Liu et al., 2019; Yu et al., 2019; Ma et al., 2020) and reduces the proportion of M1 macrophages (Shabani et al., 2020; Cheuk et al., 2022). Resveratrol inhibits macrophage infiltration and IL-6 secretion during infection (Li et al., 2020). These findings suggest that resveratrol may alleviate the suppressive state of T cells in the tumor environment by altering macrophage polarization or attenuating macrophage M2 polarization. Resveratrol and its analogs can inhibit hematological tumors *in vitro* (Anisimova et al., 2011). Resveratrol has a concentration-dependent bidirectional function: while it significantly activates NK cells at low concentrations, it shows inhibitory effects at high concentrations (Falchetti et al., 2001). Resveratrol also slightly inhibits the production of interleukin-10 by approximately 10% and monocyte chemoattractant protein-1 production by approximately 50% in M2 macrophages, while promoting the production of transforming growth factor-β1 (Kimura and Sumiyoshi, 2016). The results of the present and previous studies suggest that resveratrol likely inhibits macrophage polarization, reduces cytokine secretion, and indirectly promotes T cell activation.

In conclusion, we used a subcutaneous tumor model of lung adenocarcinoma to evaluate the antitumor effects of resveratrol. The results demonstrated the anti-tumor effects of resveratrol at concentrations that did not affect the normal physiological activity of mice. In addition to its direct inhibitory effect on tumor cell growth, resveratrol also indirectly activated T cells by switching macrophage polarization. Macrophage-derived IL-18 may be a key bystander activation cytokine during resveratrol treatment. During treatment, the proportions and immune state of macrophages and T cells within the tumor environment showed obvious changes, suggesting that resveratrol could switch the tumor immune microenvironment, reverse the immunosuppression state of these immune cells, and activate CD8 T cells, thus providing an anti-tumor effect. The reduced proportion of T cells during treatment suggested further research to assess the combination of resveratrol with immune stimulators such as IL-2 to potentially improve the therapeutic effect.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by the Ethics Committee of The Seventh Affiliated Hospital, Sun Yat-sen University.

Author contributions

WZ and RZ performed the experiments, prepared the figures, and wrote the manuscript. ZC and XW collected and analyzed the data. ZC and XW initiated and directed the research. All authors contributed to the manuscript revision and read and approved the submitted version.

Funding

This work was supported by a Project of Traditional Chinese Medicine Bureau of Guangdong Province, China (Grant No. 20221095), a Sanming Project of Medicine in Shenzhen (SZSM201911004), a Shenzhen Science and Technology Plan Basic Research Project (JCYJ20180307150408596 and JCYJ20190809172403604), and the Natural Science Foundation of Guangdong Province (2019A1515110703).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors, and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.1031438/full#supplementary-material

References

Aggarwal, B. B., Bhardwaj, A., Aggarwal, R. S., Seeram, N. P., Shishodia, S., and Takada, Y. (2004). Role of resveratrol in prevention and therapy of cancer: Preclinical and clinical studies. *Anticancer Res.* 24 (5A), 2783–2840.

Anisimova, N. Y., Kiselevsky, M. V., Sosnov, A. V., Sadovnikov, S. V., Stankov, I. N., and Gakh, A. A. (2011). Trans-cis-and dihydro-resveratrol: A comparative study. *Chem. Cent. J.* 5, 88. doi:10.1186/1752-153X-5-88

Aquilano, K., Baldelli, S., Rotilio, G., and Ciriolo, M. R. (2009). trans-Resveratrol inhibits H2O2-induced adenocarcinoma gastric cells proliferation via inactivation of MEK1/2-ERK1/2-c-Jun signalling axis. *Biochem. Pharmacol.* 77 (3), 337–347. doi:10.1016/j.bcp.2008.10.034

Ashraf, M. I., Shahzad, M., and Shabbir, A. (2015). Oxyresveratrol ameliorates allergic airway inflammation via attenuation of IL-4, IL-5, and IL-13 expression levels. *Cytokine* 76 (2), 375–381. doi:10.1016/j.cyto.2015.09.013

Atten, M. J., Attar, B. M., Milson, T., and Holian, O. (2001). Resveratrol-induced inactivation of human gastric adenocarcinoma cells through a protein kinase C-mediated mechanism. *Biochem. Pharmacol.* 62 (10), 1423–1432. doi:10.1016/s0006-2952(01)00788-2

Beadling, C., and Slifka, M. K. (2005). Differential regulation of virus-specific T-cell effector functions following activation by peptide or innate cytokines. *Blood* 105 (3), 1179–1186. doi:10.1182/blood-2004-07-2833

Bjorklund, M., Roos, J., Gogvadze, V., and Shoshan, M. (2011). Resveratrol induces SIRT1- and energy-stress-independent inhibition of tumor cell regrowth after low-dose platinum treatment. *Cancer Chemother. Pharmacol.* 68 (6), 1459–1467. doi:10.1007/s00280-011-1640-x

Chanput, W., Mes, J. J., and Wichers, H. J. (2014). THP-1 cell line: An *in vitro* cell model for immune modulation approach. *Int. Immunopharmacol.* 23 (1), 37–45. doi:10.1016/j.intimp.2014.08.002

Cheuk, I. W., Chen, J., Siu, M., Ho, J. C., Lam, S. S., Shin, V. Y., et al. (2022). Resveratrol enhanced chemosensitivity by reversing macrophage polarization in breast cancer. *Clin. Transl. Oncol. official Publ. Fed. Span. Oncol. Soc. Natl. Cancer Inst. Mexico* 24 (5), 854–863. doi:10.1007/s12094-021-02731-5

Chhabra, G., Singh, C. K., Amiri, D., Akula, N., and Ahmad, N. (2021). Recent advancements on immunomodulatory mechanisms of resveratrol in tumor microenvironment. *Molecules* 26 (5), 1343. doi:10.3390/molecules26051343

Dixon, R. A. (2001). Natural products and plant disease resistance. *Nature* 411 (6839), 843–847. doi:10.1038/35081178

Falchetti, R., Fuggetta, M. P., Lanzilli, G., Tricarico, M., and Ravagnan, G. (2001). Effects of resveratrol on human immune cell function. *Life Sci.* 70 (1), 81–96. doi:10.1016/s0024-3205(01)01367-4

Frazzi, R., Valli, R., Tamagnini, I., Casali, B., Latruffe, N., and Merli, F. (2013). Resveratrol-mediated apoptosis of hodgkin lymphoma cells involves SIRT1 inhibition and FOXO3a hyperacetylation. *Int. J. Cancer* 132 (5), 1013–1021. doi:10.1002/ijc.27748

Freeman, B. E., Hammarlund, E., Raue, H. P., and Slifka, M. K. (2012). Regulation of innate CD8+ T-cell activation mediated by cytokines. *Proc. Natl. Acad. Sci. U. S. A.* 109 (25), 9971–9976. doi:10.1073/pnas.1203543109

Guo, K., Feng, Y., Zheng, X., Sun, L., Wasan, H. S., Ruan, S., et al. (2021). Resveratrol and its analogs: Potent agents to reverse epithelial-to-mesenchymal transition in tumors. *Front. Oncol.* 11, 644134. doi:10.3389/fonc.2021.644134

Jang, M., Cai, L., Udeani, G. O., Slowing, K. V., Thomas, C. F., Beecher, C. W., et al. (1997). Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* 275 (5297), 218–220. doi:10.1126/science.275.5297.218

Jiang, H., Hegde, S., and DeNardo, D. G. (2017). Tumor-associated fibrosis as a regulator of tumor immunity and response to immunotherapy. *Cancer Immunol. Immunother.* 66 (8), 1037–1048. doi:10.1007/s00262-017-2003-1

Kim, J., Chang, D. Y., Lee, H. W., Lee, H., Kim, J. H., Sung, P. S., et al. (2018). Innate-like cytotoxic function of bystander-activated CD8(+) T cells is associated with liver injury in acute hepatitis A. *Immunity* 48 (1), 161–173. doi:10.1016/j.immuni.2017.11.025

Kimura, Y., and Sumiyoshi, M. (2016). Resveratrol prevents tumor growth and metastasis by inhibiting lymphangiogenesis and M2 macrophage activation and differentiation in tumor-associated macrophages. *Nutr. Cancer* 68 (4), 667–678. doi:10.1080/01635581.2016.1158295

Li, Y., Feng, L., Li, G., An, J., Zhang, S., Li, J., et al. (2020). Resveratrol prevents ISO-induced myocardial remodeling associated with regulating polarization of macrophages through VEGF-B/AMPK/NF-kB pathway. *Int. Immunopharmacol.106508* 84. doi:10.1016/j.intimp.2020.106508

Liu, S., Du, Y., Shi, K., Yang, Y., and Yang, Z. (2019). Resveratrol improves cardiac function by promoting M2-like polarization of macrophages in mice with myocardial infarction. *Am. J. Transl. Res.* 11 (8), 5212–5226.

Ma, S., Fan, L., Li, J., Zhang, B., and Yan, Z. (2020). Resveratrol promoted the M2 polarization of microglia and reduced neuroinflammation after cerebral ischemia by inhibiting miR-155. *Int. J. Neurosci.* 130 (8), 817–825. doi:10.1080/00207454.2019.1707817

Matsuda, H., Tomohiro, N., Hiraba, K., Harima, S., Ko, S., Matsuo, K., et al. (2001). Study on anti-Oketsu activity of rhubarb II. Anti-allergic effects of stilbene components from Rhei undulati Rhizoma (dried rhizome of Rheum undulatum cultivated in Korea). *Biol. Pharm. Bull.* 24 (3), 264–267. doi:10.1248/bpb.24.264

Mu, Q., and Najafi, M. (2021). Resveratrol for targeting the tumor microenvironment and its interactions with cancer cells. *Int. Immunopharmacol.* 98, 107895. doi:10.1016/j.intimp.2021.107895

Noy, R., and Pollard, J. W. (2014). Tumor-associated macrophages: From mechanisms to therapy. *Immunity* 41 (1), 49–61. doi:10.1016/j.immuni.2014.06.010

Palminteri, M., Dhakar, N. K., Ferraresi, A., Caldera, F., Vidoni, C., Trotta, F., et al. (2021). Cyclodextrin nanosponge for the GSH-mediated delivery of Resveratrol in human cancer cells. *Nanotheranostics* 5 (2), 197–212. doi:10.7150/ntno.53888

Pradhan, R., Chatterjee, S., Hembram, K. C., Sethy, C., Mandal, M., and Kundu, C. N. (2021). Nano formulated Resveratrol inhibits metastasis and angiogenesis by reducing inflammatory cytokines in oral cancer cells by targeting tumor associated macrophages. *J. Nutr. Biochem.* 92, 108624. doi:10.1016/j.jnutbio.2021.108624

Proto, J. D., Doran, A. C., Gusarova, G., Yurdagul, A., Jr., Sozen, E., Subramanian, M., et al. (2018). Regulatory T cells promote macrophage efferocytosis during inflammation resolution. *Immunity* 49 (4), 666–677. doi:10.1016/j.immuni.2018.07.015

Rimando, A. M., Kalt, W., Magee, J. B., Dewey, J., and Ballington, J. R. (2004). Resveratrol, pterostilbene, and piceatannol in vaccinium berries. *J. Agric. Food Chem.* 52 (15), 4713–4719. doi:10.1021/jf040095e

Sakai, Y., Sasahira, T., Ohmori, H., Yoshida, K., and Kuniyasu, H. (2006). Conjugated linoleic acid reduced metastasized LL2 tumors in mouse peritoneum. *Virchows Arch.* 449 (3), 341–347. doi:10.1007/s00428-006-0249-7

Sanders, T. H., McMichael, R. W., Jr., and Hendrix, K. W. (2000). Occurrence of resveratrol in edible peanuts. *J. Agric. Food Chem.* 48 (4), 1243–1246. doi:10.1021/jf990737b

Savio, M., Ferraresi, A., Corpina, C., Vandenberghe, S., Scarlata, C., Sottile, V., et al. (2022). Resveratrol and its analogue 4, 4'-Dihydroxy-trans-stilbene inhibit Lewis lung carcinoma growth *in vivo* through apoptosis, autophagy and modulation of the tumour microenvironment in a murine model. *Biomedicines* 10 (8), 1784. doi:10.3390/biomedicines10081784

Shabani, M., Sadeghi, A., Hosseini, H., Teimouri, M., Babaei Khorzoughi, R., Pasalar, P., et al. (2020). Resveratrol alleviates obesity-induced skeletal muscle inflammation via decreasing M1 macrophage polarization and increasing the regulatory T cell population. *Sci. Rep.* 10 (1), 3791. doi:10.1038/s41598-020-60185-1

Shin, K. O., Park, N. Y., Seo, C. H., Hong, S. P., Oh, K. W., Hong, J. T., et al. (2012). Inhibition of sphingolipid metabolism enhances resveratrol chemotherapy in human gastric cancer cells. *Biomol. Ther.* 20 (5), 470–476. doi:10.4062/biomolther.2012.20.5.470

Signorelli, P., Munoz-Olaya, J. M., Gagliostro, V., Casas, J., Ghidoni, R., and Fabrias, G. (2009). Dihydroceramide intracellular increase in response to resveratrol treatment mediates autophagy in gastric cancer cells. *Cancer Lett.* 282 (2), 238–243. doi:10.1016/j.canlet.2009.03.020

Silva, A. M., Oliveira, M. I., Sette, L., Almeida, C. R., Oliveira, M. J., Barbosa, M. A., et al. (2014). Resveratrol as a natural anti-tumor necrosis factor-alpha molecule: Implications to dendritic cells and their crosstalk with mesenchymal stromal cells. *PloS one* 9 (3), e91406. doi:10.1371/journal.pone.0091406

Simoni, Y., Becht, E., Fehlings, M., Loh, C. Y., Koo, S. L., Teng, K. W. W., et al. (2018). Bystander CD8(+) T cells are abundant and phenotypically distinct in human tumour infiltrates. *Nature* 557 (7706), 575–579. doi:10.1038/s41586-018-0130-2

Smith, M. P., Young, H., Hurlstone, A., and Wellbrock, C. (2015). Differentiation of THP1 cells into macrophages for transwell Co-culture assay with melanoma cells. *Bio. Protoc.* 5 (21), e1638. doi:10.21769/bioprotoc.1638

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., et al. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Ca. Cancer J. Clin.* 71 (3), 209–249. doi:10.3322/caac.21660

Thomas, D. A., and Massague, J. (2005). TGF-beta directly targets cytotoxic T cell functions during tumor evasion of immune surveillance. *Cancer Cell* 8 (5), 369–380. doi:10.1016/j.ccr.2005.10.012

Tough, D. F., Borrow, P., and Sprent, J. (1996). Induction of bystander T cell proliferation by viruses and type I interferon *in vivo*. *Science* 272 (5270), 1947–1950. doi:10.1126/science.272.5270.1947

Ulrich, S., Loitsch, S. M., Rau, O., von Knethen, A., Brune, B., Schubert-Zsilavecz, M., et al. (2006). Peroxisome proliferator-activated receptor gamma as a molecular target of resveratrol-induced modulation of polyamine metabolism. *Cancer Res.* 66 (14), 7348–7354. doi:10.1158/0008-5472.CAN-05-2777

Venuti, V., Cannava, C., Cristiano, M. C., Fresta, M., Majolino, D., Paolino, D., et al. (2014). A characterization study of resveratrol/sulfobutyl ether-beta-cyclodextrin inclusion complex and *in vitro* anticancer activity. *Colloids Surf. B Biointerfaces* 115, 22–28. doi:10.1016/j.colsurfb.2013.11.025

Wynn, T. A., and Barron, L. (2010). Macrophages: Master regulators of inflammation and fibrosis. *Semin. Liver Dis.* 30 (3), 245–257. doi:10.1055/s-0030-1255354

Yang, Q., Wang, B., Zang, W., Wang, X., Liu, Z., Li, W., et al. (2013). Resveratrol inhibits the growth of gastric cancer by inducing G1 phase arrest and senescence in a Sirt1-dependent manner. *PloS one* 8 (11), e70627. doi:10.1371/journal.pone.0070627

Yu, B., Qin, S. Y., Hu, B. L., Qin, Q. Y., Jiang, H. X., and Luo, W. (2019). Resveratrol improves CCL4-induced liver fibrosis in mouse by upregulating endogenous IL-10 to reprogramme macrophages phenotype from M(LPS) to M(IL-4). *Biomed. Pharmacother. = Biomedecine Pharmacother.* 117, 109110. doi:10.1016/j.biopha.2019.109110

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 28 November 2022
DOI 10.3389/fphar.2022.1045179

Check for updates

OPEN ACCESS

EDITED BY
Peng Qu,
National Institutes of Health (NIH),
United States

REVIEWED BY
Wenjie Liu,
Virginia Commonwealth University,
United States
Tao Yi,
Hong Kong Baptist University, Hong
Kong SAR, China

*CORRESPONDENCE
Huanfa Yi,
yihuanfa@jlu.edu.cn

SPECIALTY SECTION
This article was submitted to
Pharmacology of Anti-Cancer Drugs,
a section of the journal
Frontiers in Pharmacology

RECEIVED 15 September 2022
ACCEPTED 14 November 2022
PUBLISHED 28 November 2022

CITATION
Zhang Y, Qin H, Bian J, Ma Z and Yi H
(2022), *SLC2As* as diagnostic markers
and therapeutic targets in LUAD patients
through bioinformatic analysis.
Front. Pharmacol. 13:1045179.
doi: 10.3389/fphar.2022.1045179

COPYRIGHT
© 2022 Zhang, Qin, Bian, Ma and Yi. This
is an open-access article distributed
under the terms of the Creative
Commons Attribution License (CC BY).
The use, distribution or reproduction in
other forums is permitted, provided the
original author(s) and the copyright
owner(s) are credited and that the
original publication in this journal is
cited, in accordance with accepted
academic practice. No use, distribution
or reproduction is permitted which does
not comply with these terms.

SLC2As as diagnostic markers and therapeutic targets in LUAD patients through bioinformatic analysis

Yanli Zhang[1,2,3], Han Qin[1,2], Jing Bian[4], Zhanchuan Ma[1,2] and Huanfa Yi[1,2]*

[1]Central Laboratory, The First Hospital of Jilin University, Changchun, China, [2]Key Laboratory of Organ Regeneration and Transplantation, Ministry of Education, Changchun, Jilin, China, [3]Echocardiography Department, The First Hospital of Jilin University, Changchun, China, [4]Department of Respiratory Medicine, The First Hospital of Jilin University, Changchun, China

Facilitative glucose transporters (GLUTs), which are encoded by solute carrier 2A (*SLC2A*) genes, are responsible for mediating glucose absorption. In order to meet their higher energy demands, cancer cells are more likely than normal tissue cells to have elevated glucose transporters. Multiple pathogenic processes, such as cancer and immunological disorders, have been linked to GLUTs. Few studies, meanwhile, have been conducted on individuals with lung adenocarcinoma (LUAD) to evaluate all 14 *SLC2A* genes. We first identified increased protein levels of *SLC2A1*, *SLC2A5*, *SLC2A6*, and *SLC2A9 via* HPA database and downregulated mRNA levels of *SLC2A3*, *SLC2A6*, *SLC2A9*, and *SLC2A14* by ONCOMINE and UALCAN databases in patients with LUAD. Additionally, lower levels of *SLC2A3*, *SLC2A6*, *SLC2A9*, *SLC2A12*, and *SLC2A14* and higher levels of *SLC2A1*, *SLC2A5*, *SLC2A10*, and *SLC2A11* had an association with advanced tumor stage. *SLC2A1*, *SLC2A7*, and *SLC2A11* were identified as prognostic signatures for LUAD. Kaplan-Meier analysis, Univariate Cox regression, multivariate Cox regression and ROC analyses further revealed that these three genes signature was a novel and important prognostic factor. Mechanistically, the aberrant expression of these molecules was caused, in part, by the hypomethylation of *SLC2A3*, *SLC2A10*, and *SLC2A14* and by the hypermethylation of *SLC2A1*, *SLC2A2*, *SLC2A5*, *SLC2A6*, *SLC2A7*, and *SLC2A11*. Additionally, *SLC2A3*, *SLC2A5*, *SLC2A6*, *SLC2A9*, and *SLC2A14* contributed to LUAD by positively modulating M2 macrophage and T cell exhaustion. Finally, pathways involving *SLC2A1*/BUB1B/mitotic cell cycle, *SLC2A5*/CD86/negative regulation of immune system process, *SLC2A6*/PLEK/lymphocyte activation, *SLC2A9*/CD4/regulation of cytokine production might participate in the pathogenesis of LUAD. In summary, our results will provide the theoretical basis on *SLC2As* as diagnostic markers and therapeutic targets in LUAD.

KEYWORDS

***SLC2As*, lung adenocarcinoma, diagnostic maker, therapeutic targets, glucose transporters**

Introduction

Lung adenocarcinoma (LUAD), the most prevalent NSCLC subtype, has a high morbidity and mortality rate that makes it a global public health concern (Liu et al., 2015). Even though traditional treatment approaches involving curative resection, targeted therapy, or immunotherapy have seen enormous advancements in recent years, it is crucial to comprehend the molecular pathogenesis and etiology of LUAD to develop new prognostic and therapeutic targets for LUAD (Tong et al., 2018).

Tumor cells exhibit a distinct metabolism from normal cells, converting to higher glycolysis demands (the Warburg effect) and glucose intake for ATP synthesis to meet their energy needs. Facilitative glucose transporters (GLUTs), which are encoded by 14 *SLC2A* genes, are responsible for mediating this process. Based on function and sequence similarity, these transporters can be divided into three groups. Additionally, they differ in terms of tissue location and their affinity for the substrate (glucose and other hexoses like fructose). GLUT-1-4 and GLUT-14 are classified as Class 1 GLUTs, whereas GLUT-5, GLUT-7, GLUT-9, and GLUT-11 are Class 2 GLUTs. GLUT-6, GLUT-8, GLUT-10, GLUT-12, and GLUT-13 (H(+)-myo-inositol transporter HMIT) are classified as Class 3 GLUTs (Cura and Carruthers, 2012). Several clinical diseases, including cancer and autoimmune illness, are associated with *SLC2As* (Hu et al., 1999; Macintyre et al., 2014; Yin et al., 2015; Jodeleit et al., 2018; Tilekar et al., 2020a). Previous research has determined the upregulation of *SLC2As* and their roles in certain malignancies. Additionally, numerous types of researches have focused on GLUT-inhibitors to slow the growth of tumors (including breast cancer, osteosarcoma, and NSCLC) and other proliferative illnesses (Sanli et al., 2010; Storozhuk et al., 2012; Storozhuk et al., 2013; Hardie and Ashford, 2014). GLUT inhibitors have shown encouraging results in the treatment of tumors, particularly when combined with other chemotherapy, radiation, immunotherapy, pathway-specific oncogenic-targeted medicines, other anti-metabolic, or epigenetic medications (Granchi et al., 2016). However, off-targets and low potency, which is a result of cancer cell potency to adopt alternative strategies for glucose supply, still exist (Tilekar et al., 2020b). Additionally, in specific tumor forms, glucose uptake does not coincide with GLUT-1 expression, suggesting the participation of other transporters. To put it another way, GLUT-1 may have an impact *via* working with other GLUTs. All these reports serve as a reminder that to evaluate the transport members' roles in a particular cancer type, they must be considered as a whole. The same is true for LUAD; we could only reliably anticipate outcomes and identify patients who would respond better to particular GLUT inhibitors if we thoroughly evaluated the metabolic profiles in LUAD.

To explore the molecular landscape of LUAD patients, we first evaluated the DNA methylation, mRNA, and protein levels of 14 *SLC2As* in this study. Then, to better grasp the potential of *SLC2As* as diagnostic biomarkers and prognosis indicators of LUAD, we further investigated the relationship between the expressions of 14 *SLC2As* and clinical data. A theoretical foundation for *SLC2As* as therapeutic targets in LUAD is provided by our exploration of the molecular mechanisms that *SLC2As* contribute to the pathophysiology of LUAD.

Materials and methods

Ethic statement

Samples of tumor tissues and adjacent tissues were taken from 6 LUAD patients from The First Hospital of Jilin University. Each participant gave their written informed consent in accordance with the principles of the Declaration of Helsinki. Additionally, the hospital's institutional ethics committee gave its approval for this investigation.

Transcriptional level of *SLC2As* analysis *via* ONCOMINE and UALCAN database

ONCOMINE (Rhodes et al., 2004) is the largest integrated database mining platform and cancer gene chip database. The difference in *SLC2A* mRNA expression between LUAD patients and healthy lung tissues was examined. The p threshold was set at 1E-4 and the fold change threshold was set at 2. UALCAN (Chandrashekar et al., 2017) is a comprehensive online analytic tool that uses RNA-seq and clinical data from 31 different cancer types from the TCGA database. The *SLC2As* mRNA expression profiles and DNA methylation levels in individuals with LUAD were compared to those in healthy tissues using the UALCAN database. Additionally, the UALCAN database was used to evaluate the relationship between the expression of *SLC2As* and clinicopathologic characteristics. The p-value was calculated using Student's t-test, and the threshold was set at 0.05.

Analysis of *SLC2As* protein levels using the human protein altas database

The Human Protein Altas (HPA) (Uhlén et al., 2015) is a website that allows users to investigate each protein's expression level and location in 20 cancer types that are extremely prevalent. We employed HPA to examine how the expression of GLUTs varied between LUAD patients and healthy lung tissue.

Construction and evaluation of the prognostic nomogram

RNA-sequencing expression (level 3) profiles and corresponding clinical information for LUAD were

downloaded from the TCGA dataset (https://portal.gdc.com). To choose the appropriate terms for the nomogram, univariate and multivariate cox regression analysis were used. With the use of the R program "forestplot," the forest was utilized to display the *p*-value, HR, and 95% confidence interval for each variable (Xiong et al., 2020). Based on the outcomes of the multivariate cox proportional hazards analyses a nomogram was created to forecast the overall recurrence over the next 1, 2, 3, 5 year. The nomogram offered a graphical depiction of the elements that may be used to determine a patient's unique risk of recurrence based on the points assigned to each risk factor using the "rms" R program (Jeong et al., 2020; Xiong et al., 2020).

Construction and evaluation of the prognostic risk model of *SLC2As*

Kaplan-Meier plotter (Győrffy et al., 2013) was employed to examine the relationship between the transcriptional levels of *SLC2As* and the survival of LUAD patients. $p \leq 0.05$ was used to define the statistical significance level.

From the TCGA dataset, RNA-sequencing expression (level 3) profiles and associated clinical data for LUAD were obtained (https://portal.gdc.com). Univariate Cox regression was used to assess the 14 genes in question, and candidate genes were chosen if they satisfied the screening requirement of $p < 0.05$. Following that, we used the R software's "glment" package to perform LASSO regression on high-dimensional data to identify the most effective prognostic factors (Xu et al., 2021). Three genes were chosen, and a risk score was also computed for each of them. Based on the median expression of *SLC2A* genes, patients were split into high-risk and low-risk groups. Using the KM survival approach, the association between *SLC2A* genes and survival rates was examined. The *p*-value of KM survival curves was determined using log-rank testing. The receiver operating characteristic (ROC) curve was created to evaluate the model's accuracy. To find LUAD prognostic variables, univariate and multivariate Cox regression analyses were performed. R (foundation for statistical computing 2020) version 4.0.3 was used to implement all the analysis techniques and R packages (Supplementary Material S1). A *p*-value of 0.05 was regarded as statistically significant.

Co-expression genes of *SLC2As* mRNA in lung adenocarcinoma

We wanted to further explore the underlying mechanism *SLC2As* regulating LUAD. Thus, we analyzed the co-expression profiles *in* LUAD using the cBioPortal database (Cerami et al., 2012). The top 50 positively co-expressed genes and 50 negatively co-expressed genes were selected based on Spearman's correlation.

Gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis

Metascape (Zhou et al., 2019) is a web-based database that incorporates over 40 different gene functions. The co-expressed genes were enriched using GO and KEGG analyses. We defined minimum overlap as 3, minimum enrichment as 1.5, and $p \leq 0.05$ as significant.

Protein-protein interaction (PPI) networks of the member of *SLC2As via* Cytoscape

Cytoscape (Smoot et al., 2011), a software focusing on open source network visualization and analysis, provides the basic function layout and query network. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database was used to build a PPI network to evaluate the potential PPI of co-expressed genes (Szklarczyk et al., 2021). PPI pairs with a minimum interaction value of 0.4 were chosen, and Cytoscape 3.8.2 was used to display the network. According to the degree score of each gene node, the CytoHubba plugin for Cytoscape approved the top 10 core genes.

The association between *SLC2As* and immune cells infiltration in patients with LUAD

TIMER (Li et al., 2017) investigates in detail the immune infiltration status of different cancer types. In our study, the correlation between the immune cell marker genes and *SLC2As* in the correlation module was used to evaluate the relationship between *SLC2As* expressions and the infiltration of immune subtypes in LUAD patients. All the markers' genes with coefficients greater than 0.35 were chosen.

Quantitative polymerase chain reaction

We reanalyzed our prior projected *SLC2A3*, *SLC2A6*, *SLC2A9*, and *SLC2A14* expression level results, including paired tumor and surrounding tissues from 6 primary LUAD patients diagnosed in The First Hospital of Jilin University from June 2021 to June 2022, to see if the mRNA expressions of *SLA2As* are parallel to those in clinical samples. A TRIzol-based (Invitrogen) technique was used to extract the RNA, and RT EasyTM (with gDNase) was used to create complementary deoxyribonucleic acid (FORE GENE). On an ABI StepOnePlus

TABLE 1 The primer sequence of *SLC2A3*, *SLC2A6*, *SLC2A9*, *SLC2A14*.

	Forward primer (5′-3′)	Reverse primer (5′-3′)
Human-*SLC2A3*	GGTCGCTTGGTTATTGGC	ACCGCTGGAGGATCTGCT
Human-*SLC2A6*	GGTGTACGTGTCTGAGATTGC	CCTGGATCTGCTCGAACTCC
Human-*SLC2A9*	CAATAGACCCAGACACTCTGACT	TCTTCACAATTAACGTCCCCAC
Human-*SLC2A14*	GAGATGGACAACAGACAGAATGT	AAACAGGCCACAAACGACC

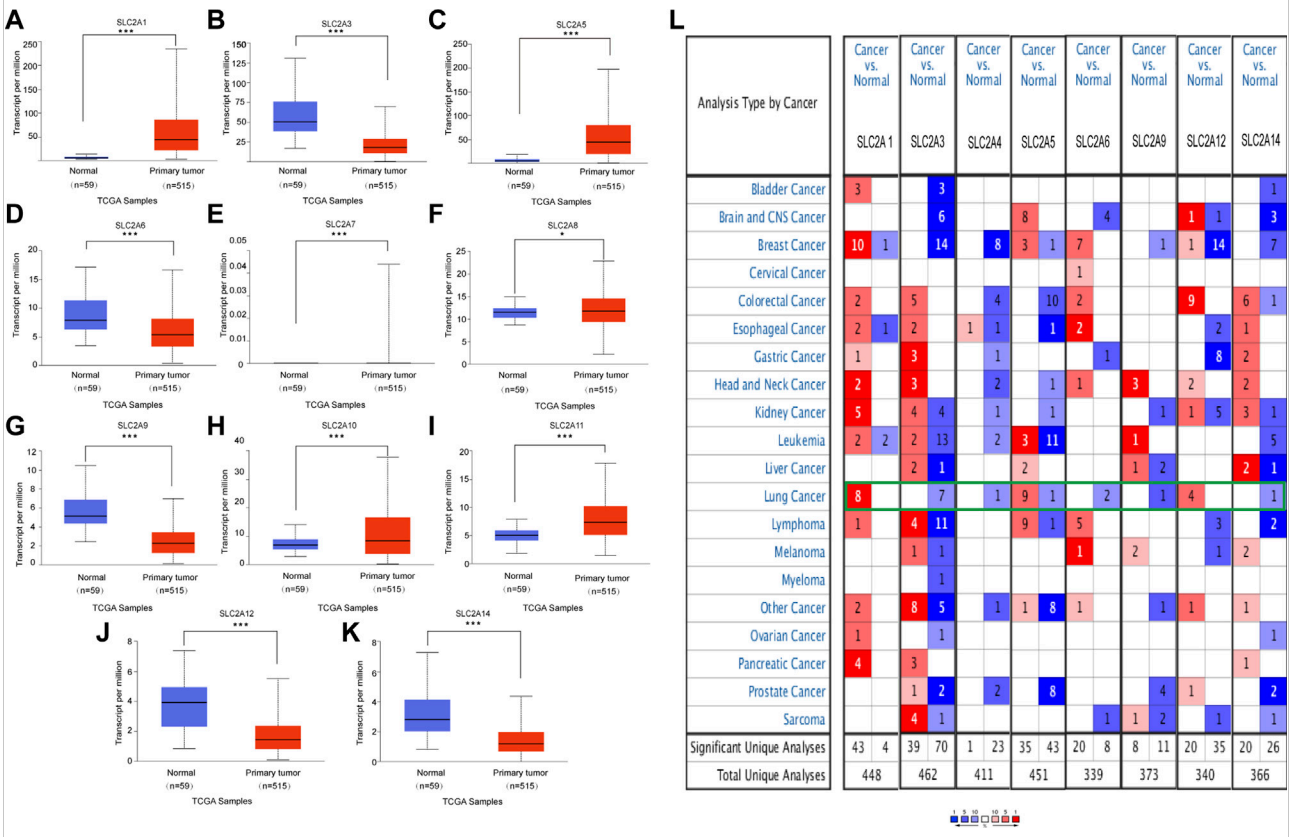


FIGURE 1
mRNA expression of distinct *SLC2As* in LUAD tissues and adjacent normal lung tissues from UALCAN and ONCOMINE database. **(A–K)** (UALCAN) mRNA expressions of *SLC2A1*, *SLC2A5*, *SLC2A7*, *SLC2A8*, *SLC2A10*, *SLC2A11* were over-expressed while *SLC2A3*, *SLC2A6*, *SLC2A9*, *SLC2A12*, *SLC2A14* were downregulated in primary LUAD tissues compared to normal lung tissues. ***$p < 0.001$, **<0.01, *<0.05. **(L)** Transcriptional expression of *SLC2As* in 20 different types of cancer diseases (ONCOMINE database). Difference of transcriptional expression was compared by Students' *t*-test. Cut-off of *p* value and fold change were as following: *p* value: 0.05, fold change: 2.0, gene rank: 10%, data type: mRNA (ONCOMINE).

machine from Applied Biosystems, using the Power q-PCR SYBR Green Mix, all PCRs were run in triplicate (Yeasen). Using the Cttechnique, the relative mRNA expression of several genes was measured. One of the housekeeping genes was GAPDH. The additional information included a list of the primer sequences (Comate Bioscience) (Table 1). We calculated relative gene expression levels with $2^{-\Delta\Delta CT}$, visualizing data with Graphpad 9.0.

Stastical analysis

Univariate survival analysis with Kaplan-Meier and log-rank tests. Multifactor survival analysis was conducted using COX regression models. By calculating the area under the curve using the pROC software package, we were able to assess the specificity and sensitivity of *SLC2As* in prognosis of LUAD. The continuous variables

were compared using independent *t*-tests. Categorical data were tested with chi-square tests. Statistical significance was defined as a two-sided p-value < 0.05. All data processing was done in R4.0.3 software.

Results

Functional annotation of *SLC2As*

The overview of the research design is present in Supplementary Material S2; Figure 1. First and foremost, we are interested in the functional annotation of *SLC2As*. As shown in Supplementary Material S2; Figure 2, Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis in Metascape database were employed to analyze the function and pathways *SLC2A* family members participated in. Biological processes (BP) such as GO: 1904659 (glucose transmembrane transport), GO:0070837 (dehydroascorbic acid transport), GO: 0098704: (carbohydrate import across plasma membrane) and GO: 0032868 (response to insulin) were closely correlated with *SLC2As* (Supplementary Material S2; Figure 2A). Cellular components (CC), involving GO: 0016324 (apical plasma membrane), GO: 0098793 (presynapse), GO: 0048471 (perinuclear region of cytoplasm) (Supplementary Material S2; Figure 2B) and molecular functions (MF) such as GO: 0005355 (glucose transmembrane transporter activity), GO: 0033300 (dehydroascorbic acid transmembrane transporter activity), GO: 0005353 (fructose transmembrane transporter activity), GO: 0015295 (solute: proton symporter activity) were enriched for *SLC2As* (Supplementary Material S2; Figure 2C). KEGG pathway analysis indicates pathway 04931: *SLC2As* and insulin resistance were linked (Supplementary Material S2; Figure 2D). Additionally, *SLC2As* were linked to many diseases, such as intestinal atresia, pregnancy-related diabetes, hypouricemia, Lesch-Nyhan syndrome, gestational diabetes, arterial tortuosity syndrome, meningomyelocele, and addictive behavior (Supplementary Material S2; Figure 2E).

Abnormal expressions of *SLC2As* in patients with LUAD

We initially determined the mRNA and protein expression of *SLC2As* using the ONCOMINE, UALCAN, and HPA databases to investigate the diagnostic and therapeutic relevance of *SLC2As* in LUAD patients. In the UALCAN database, we first verified the mRNA expression of SLC2A family members, The findings in Figures 1A–K demonstrated that LUAD tissues had lower levels of *SLC2A3*, *SLC2A6*, *SLC2A9*, *SLC2A12*, and *SLC2A14* than normal lung tissues and higher levels of *SLC2A1*, *SLC2A5*, *SLC2A7*, *SLC2A8*, *SLC2A10*, and *SLC2A11*. The ONCOMINE database, which was different from UALCAN database, was used to quantify the mRNA expressions of 14 *SLC2A* genes in 20 different types of malignancies and compare them to normal tissues. LUAD tumor tissues showed higher mRNA expressions of *SLC2A1*, *SLC2A5*, and *SLC2A12* and lower expressions of *SLC2A3*, *SLC2A4*, *SLC2A6*, *SLC2A9*, and *SLC2A14* (Figure 1L). Together, these findings demonstrated that individuals with LUAD had transcriptionally upregulated levels of *SLC2A1*, *SLC2A5*, and downregulated levels of *SLC2A3*, *SLC2A6*, *SLC2A9*, and *SLC2A14*. Using the HPA database, we attempted to identify the protein levels of *SLC2As* in LUAD. According to the findings in Figure 2, GLUT-1 protein expression was shown to be low in lung tissues and high in LUAD tissues (Figure 2A). Additionally, while low or medium protein levels were expressed in LUAD tissues, GLUT-5, GLUT-6, and GLUT-9 were not detected in normal lung tissues (Figures 2B–D). In summary, GLUT-1, GLUT-5 GLUT-6, and GLUT-9 protein levels were overexpressed in patients with LUAD.

Correlation between *SLC2As* family mRNA expressions and clinical tumor stage

To further explore the relation of *SLC2As* with clinical characters, the association between *SLC2As* expressions and patients' cancer stages in LUAD patients by UALCAN database was then investigated. As shown in Figure 3, *SLC2A1*, *SLC2A5*, *SLC2A10*, and *SLC2A11* levels tended to be higher in patients with advanced tumor stages, which shows that these molecules may be involved in the development of malignancies in LUAD patients. Furthermore, the highest expression of *SLC2A1*, *SLC2A5*, *SLC2A10*, and *SLC2A11* was observed in stage 4, whereas *SLC2A5* and *SLC2A11* expression in stage 3 was lower than in stage 2. This suggests that the difference in sample sizes between stage 2 ($n = 125$) and stage 3 ($n = 85$) may be a reasonable explanation for the contradictory results. Furthermore, *SLC2A3*, *SLC2A6*, *SLC2A9*, *SLC2A12*, and *SLC2A14* levels were lower in patients with advanced tumor stages, suggesting that these genes might act as a barrier to the progression of LUAD. Interestingly, the expression levels of *SLC2A1*, *SLC2A5*, *SLC2A10*, *SLC2A11*, *SLC2A3*, *SLC2A6*, *SLC2A9*, *SLC2A12*, and *SLC2A14* differed in stage I LUAD tissues compared to normal tissues, suggesting that these molecules could act as non-invasive biomarkers for LUAD early detection. All of these findings open the door for these *SLC2A* genes to be used as possible biomarkers for the early identification and precise stratification of LUAD patients.

Construction and evaluation of *SLC2As*-related prognostic model

Kaplan-Meier plotter online tool was used to predict the value of SLC2As in gauging the prognosis of LUAD. Lower

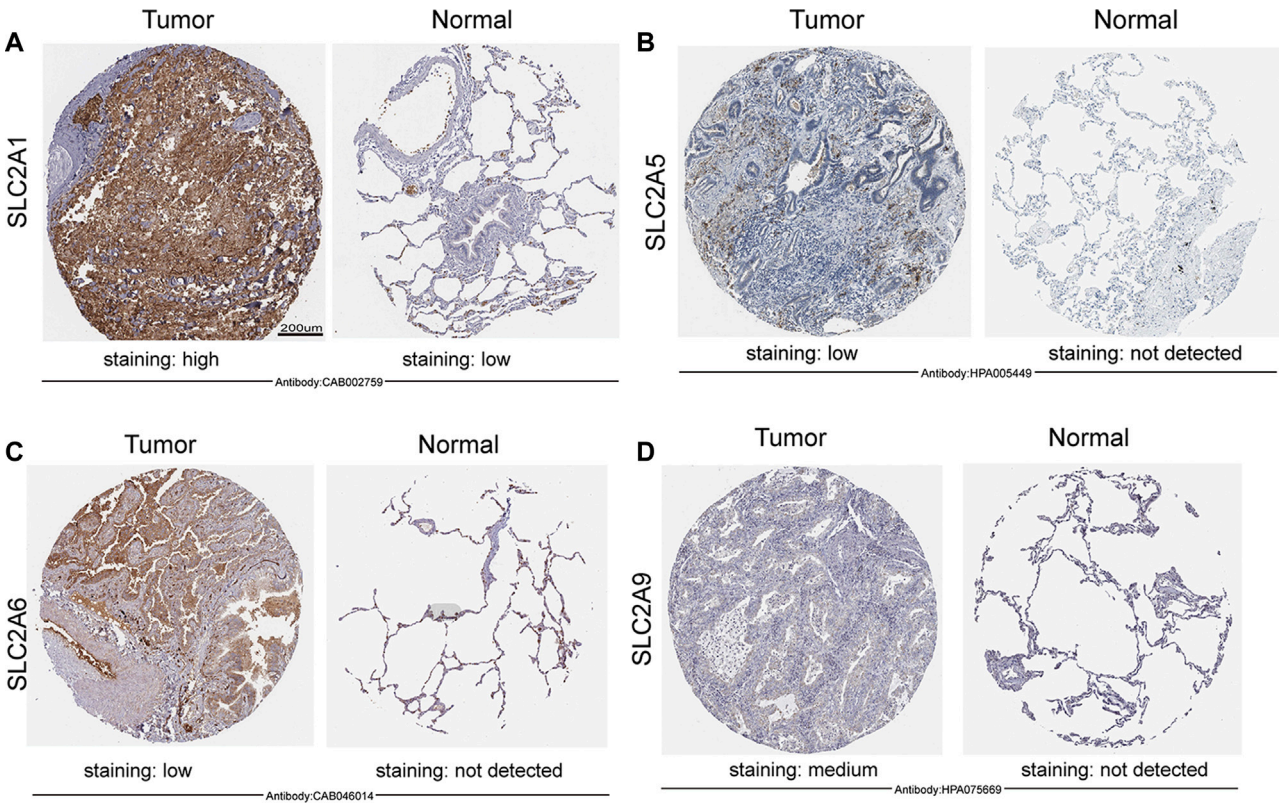


FIGURE 2
Expression of *SLC2As* protein in LUAD tissues and adjacent lung tissues (HPA). Scale bar, 200 μm. **(A)** Lung tissues displayed low GLUT 1 expression while LUAD tumors showed high protein expressions of it. **(B–D)** GLUT 5, GLUT 6, GLUT 9 were not expressed in normal lung tissues, while low or medium protein levels were expressed in LUAD tissues.

mRNA expressions of *SLC2A1*, *SLC2A2*, *SLC2A3*, *SLC2A4*, *SLC2A5*, *SLC2A9*, *SLC2A14* and higher mRNA level of *SLC2A10*, *SLC2A12*, *SLC2A13* were significantly associated with favorable Overall Survial (OS) of LUAD (Supplementary Material S2; Figure 3). Furthermore, we ran univariate and multivariate Cox regression tests to see if the risk model of 14 SLC2As had distinct prognostic features for LUAD. *SLC2A1*, *SLC2A7*, and *SLC2A11* were chosen as independent predictors for survival in LUAD patients in univariate Cox regression analysis, with *SLC2A1*, *SLC2A7* serving as risk factors and *SLC2A11* as a favorable factor in LUAD (Figure 4A). *SLC2A1*, *SLC2A7*, and *SLC2A11* were also statistically significant in multivariate Cox regression analysis (Figure 4B), suggesting that these three genes may be the reliable predictors in LUAD. A nomogram based on *SLC2A1*, *SLC2A7*, and *SLC2A11* to forecast the 1, 2, 3, and 5 year overall recurrence was created (Figure 4C). The observed vs. predicted rates of the 1-, 2-, and 3-year OS showed excellent consistency in the correlation plots (Figure 4D). While attempting to uncover predictive gene sets for LUAD using the LASSO approach (Zhang et al., 2020), λ was chosen when the minimum sum of squared residuals was found. We ultimately discovered three genes, including *SLC2A1*, *SLC2A7*, and *SLC2A11* for subsequent multivariate analysis (Figures 5A,B). The risk score of 3 genes was also calculated for further univariate and multivariate Cox regression analyses. The hazard ratio between the low-expression sample and the high-expression sample is represented as HR (High risk). Based on the integrated model with cutoff values at the median expression of the three candidate genes, patients were categorized into high-risk and low-risk groups. The selected dataset's risk score, survival period, and survival status. Risk score is shown in the top scatterplot, ranging from low to high (Figure 5C). The risk score of various samples, which corresponds to survival time and survival status, is represented by the scatter plot distribution (Figure 5D). The gene expression from the signature is displayed in the bottom heatmap. *SLC2A1* and *SLC2A7* levels that were higher and *SLC2A11* levels that were lower indicated significant risk in LUAD patients (Figure 5E). The risk model from the dataset underwent a Kaplan-Meier survival analysis, and a log-rank test was used to compare

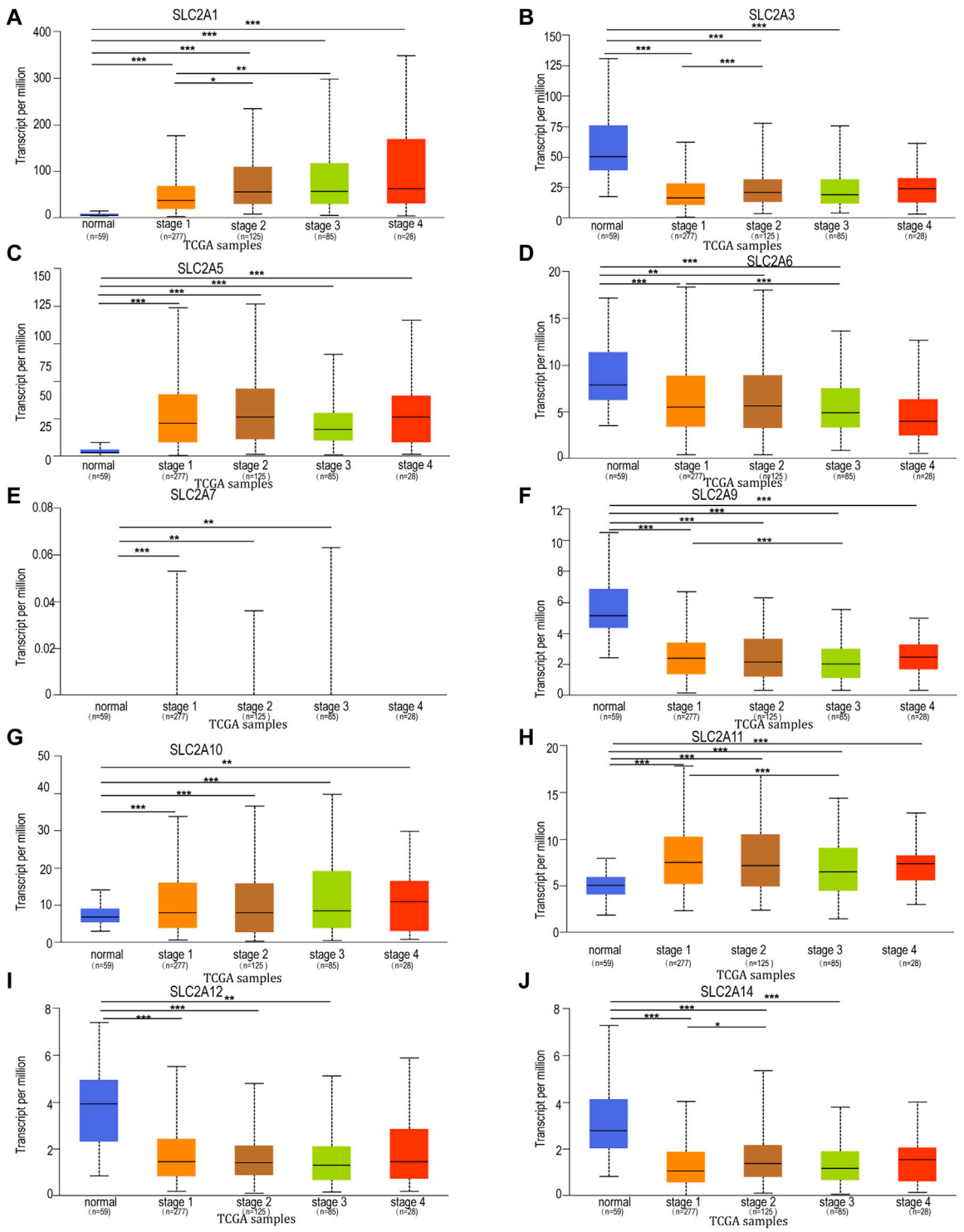


FIGURE 3
Relationship between mRNA expression of distinct *SLC2As* and individual cancer progression of LUAD patients. mRNA expressions of *SLC2As* were remarkably correlated with patients' individual cancer stages. **(A,C,G,H)** Patients who were in more advanced stages tended to express higher mRNA expression of *SLC2A1*, *SLC2A5*, *SLC2A10*, *SLC2A11*. The highest mRNA expressions of, *SLC2A5*, *SLC2A10*, *SLC2A11* were found in stage 4. **(B,D,F,I,J)** Patients who were in more advanced stages tended to express lower mRNA expression of *SLC2A3*, *SLC2A6*, *SLC2A9*, *SLC2A12* and *SLC2A14*. **(A–I)** In addition, *SLC2A1*, *SLC2A3*, *SLC2A5*, *SLC2A6*, *SLC2A7*, *SLC2A9*, *SLC2A10*, *SLC2A11*, *SLC2A12*, *SLC2A14* expressions were different in stage 1 from that in normal group. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

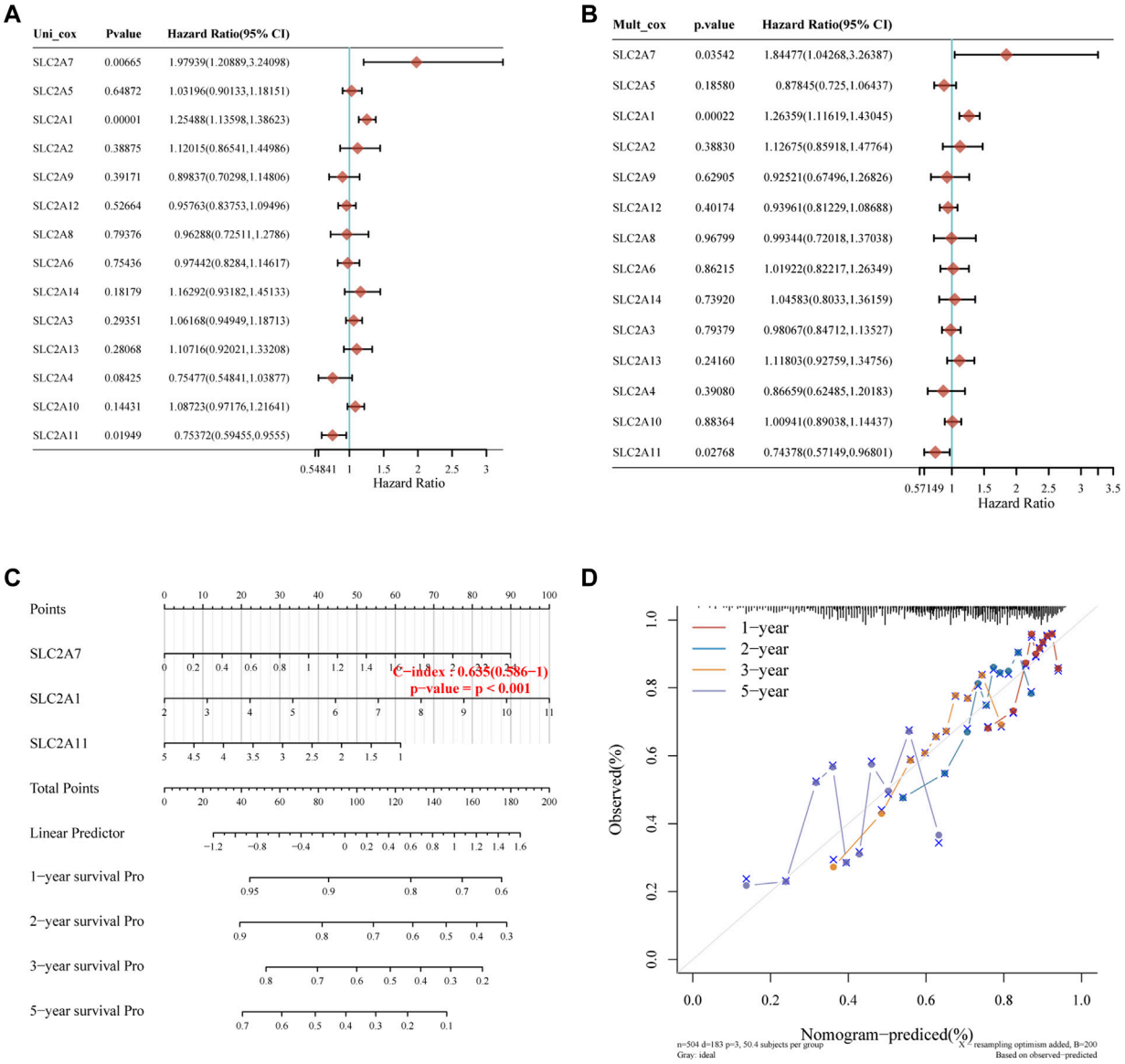


FIGURE 4
Construction of the nomogram model. **(A,B)** Forest plots for hazard ratios of survival-associated SLC2As in LUAD through univariate and multivariate Cox regression tests. *SLC2A1*, *SLC2A7*, and *SLC2A11* were independent predictors of prognosis according to univariate and multivariate Cox regression analysis of the *p* value, risk coefficient (HR), and confidence interval of *SLC2As*. **(C)** Nomogram can forecast the overall survival of LUAD patients at 1, 2, 3, and 5 years. **(D)** Calibration curve for the overall survival nomogram model in the discovery group. The blue line, red line, and orange line indicate the 1-year, 2-year, 3-year, and 5-year of the observed nomogram, respectively. This suggests that the prognostic model could successfully predict the prognosis of LUAD. The dashed diagonal line represents the ideal nomogram.

the outcomes of the various groups. Compared to the low-risk group, the high-risk group consistently had a poor prognosis (Figure 5F). By using ROC curves, we also compared the prognostic effect of risk factors. Areas under the curve (AUC) for 1-year OS, 3-year OS and 5-year OS were 64.8%, 67.1%, and 61.3%, respectively (Figure 5G), demonstrating that the combination of *SLC2A1*, *SLC2A7*, and *SLC2A11* might serve as a marker of prognosis of LUAD.

Methylation levels of *SLC2As* in LUAD patients

The upstream factor that dysregulates the expression of SLC2A was then identified. DNA methylation, one type of epigenetic change, involves adding a methyl group to the cytosine's C5 position to create 5-methylcytosine. This occurs primarily at CG and CH (CH = CA, CT, and CC)

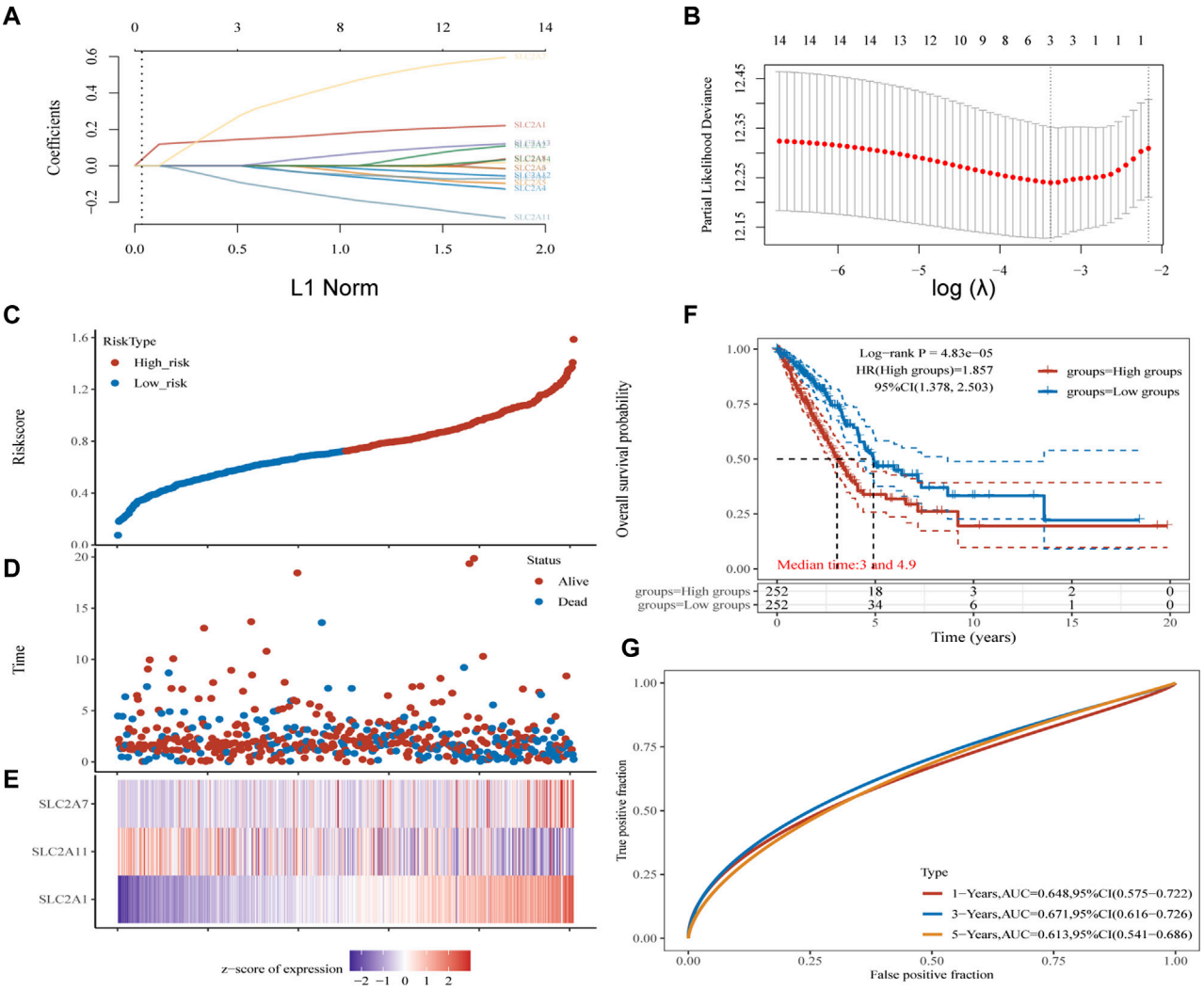


FIGURE 5
Construction and validation of *SLC2As*-related signature. **(A)** The coefficients of *SLC2As* are shown by lambda parameter. **(B)** Partial likehood versus log(λ) were manifested using LASSO model. **(C–E)** The Riskscore, survival time and survival status of LUAD. **(C)** The top scatterplot represents the Riskscore from low to high. Blue represents low risk group and red represents high risk group. **(D)** The middle scatter plot distribution represents the Riskscore of different samples correspond to the survival time and survival status. **(E)** The bottom heatmap is the gene *SLC2A1*, *SLC2A7*, *SLC2A11* expression from the signature. **(F)** Kaplan-Meier survival analysis of the risk model from dataset, comparison among high and low group was made by log-rank test. HR = 1.857 indicates the gene set is a risk factor. The median survival time (LT50) for the high group and low group is 3 and 4.9 years, respectively, and the HR (95% CI) ranges from 1.378 to 2.503.**(G)** The ROC curve and AUC of the gene set for 1,3, and 5 year (64.8%, 67.1%, 61.3%).

locations (Wang et al., 2021). It has been suggested that DNA methylation has a role in a variety of biological processes, particularly the genesis of cancer. Next, we attempted to investigate how DNA methylation by *SLC2As* affects LUAD. According to data from the UALCAN database, LUAD patients' tumor tissues had hypomethylation of *SLC2A1*, *SLC2A2*, *SLC2A5*, *SLC2A6*, *SLC2A7*, *SLC2A11* and hypermethylation of *SLC2A3*, *SLC2A10*, and *SLC2A14* compared to normal tissues (Figure 6).

Construction of a network of *SLC2As* and co-expressed genes and identification of potential "hub" genes

The functions and pathways in which *SLC2As* are involved in LUAD patients will next be further clarified. We performed GO and KEGG enrichment analyses to investigate the biological functions of the co-expression profiles of *SLC2A1*, *SLC2A5*, *SLC2A6*, and *SLC2A9* in LUAD patients (whose protein

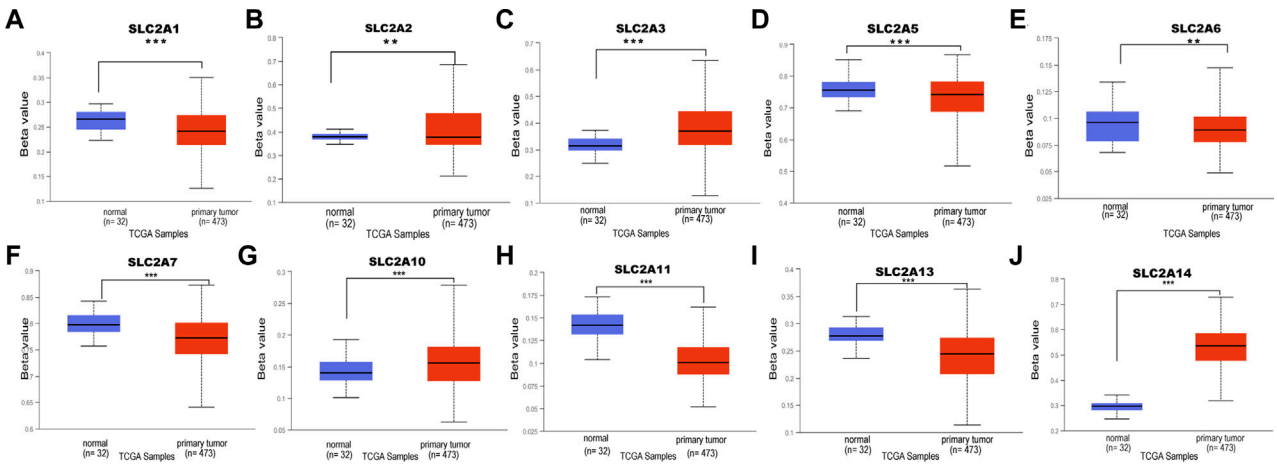


FIGURE 6
DNA methylation level of distinct SLC2As in LUAD tissues and adjacent normal lung tissues (UALCAN). Hypomethylation of *SLC2A1*, *SLC2A2*, *SLC2A5*, *SLC2A9*, *SLC2A11* **(A,B,D–F,H,I)** and hypermethylation of *SLC2A3*, *SLC2A10*, *SLC2A14* **(C,G,J)** were found in primary LUAD tissues compared with those in normal lung tissues. ***$p < 0.001$, **<0.01, *<0.05.

expression level was different in LUAD from that in normal tissues). The Metascape database was enriched for the top 100 genes (Supplementary Material S3) substantially linked with *SLC2A1*, *SLC2A5*, *SLC2A6*, and *SLC2A9* (according to *p* value) (Figures 7A–D). The genes that co-express *SLC2A1* were primarily enriched in the centromeric region of the chromosome and were grouped during the mitotic cell cycle process. *SLC2A5*-coexpressed genes were particularly rich in those that negatively regulated immune system function, immunological receptor activation, and osteoclast differentiation. Lymphocyte activation, cytokine-mediated signaling pathways, and the positive modulation of immune response were among the gene functions enriched in *SLC2A6* co-expression. The regulation of cytokine production, the positive regulation of immune response, and the regulation of cell activation were all enriched in the genes co-expressed with *SLC2A9*. According to the degree score of each gene node, the CytoHubba plugin for Cytoscape selected the top 10 core genes (Figures 7E–H, e–h). The core gene BUB1B (e), which was engaged in the mitotic cell cycle process and might be the most significant gene among those connected with *SLC2A1*, had the highest degree score. CD86 (f), a gene involved in the negative regulation of immune system activity, was the most significant gene for *SLC2A5*. The gene PLEK (g), which was involved in lymphocyte activation, was the most significant gene for *SLC2A6*. The CD4 (h) gene, which controlled cytokine production, was the most significant gene for *SLC2A9*. These results suggest that *SLC2As* are implicated in LUAD through orchestrating immune cells.

SLC2As expressions are correlated with infiltration of immune cells

Different illness states can stimulate immune cells (Fridlender et al., 2009; Murray, 2017). In LUAD patients, we attempted to determine whether *SLC2As* are correlated with the marker genes of different immune cell types, such as $CD8^+$ T cells, monocytes, T cells (general), M1 and M2 macrophages, B cells, neutrophils, tumor-associated macrophages (TAMs), natural killer cells, and DCs. We also examined T cells with various subtypes, including fatigued T cells and T-helper type 1 (Th1), Th17, Th2, and Treg (Supplementary Material S4). *SLC2As* substantially linked with the majority of marker genes of various types of immune cells and diverse functional T cells in LUAD after tumor purity-correlated modifications. *SLC2A3*, *SLC2A5*, and *SLC2A14* expressions were all shown to be substantially linked with M2 macrophages and monocytes. Additionally, *SLC2A6* expression was highly connected with M2 macrophages, M-DC, monocytes, and Treg cells, while *SLC2A9* expression was significantly correlated with T cells, Th1 cells, and monocyte infiltration (Supplementary Material S2; Figure 4). Expressions of *SLC2A6* and *SLC2A9* were positively correlated with TAMs (Supplementary Material S4). These findings show how these molecules are involved in enlisting and activating various immune cell subtypes to take part in the pathogenesis of LUAD. Additionally, there was a positive correlation between the levels of *SLC2A3*, *SLC2A5*, *SLC2A6*, *SLC2A9*, and *SLC2A14* and markers of T cell exhaustion (TCE) (PDCD1, CTLA-4, LAG3, HAVCR2, GZMB), identifying that the elevated level of these molecules implied the presence of TCE in LUAD (Figure 8).

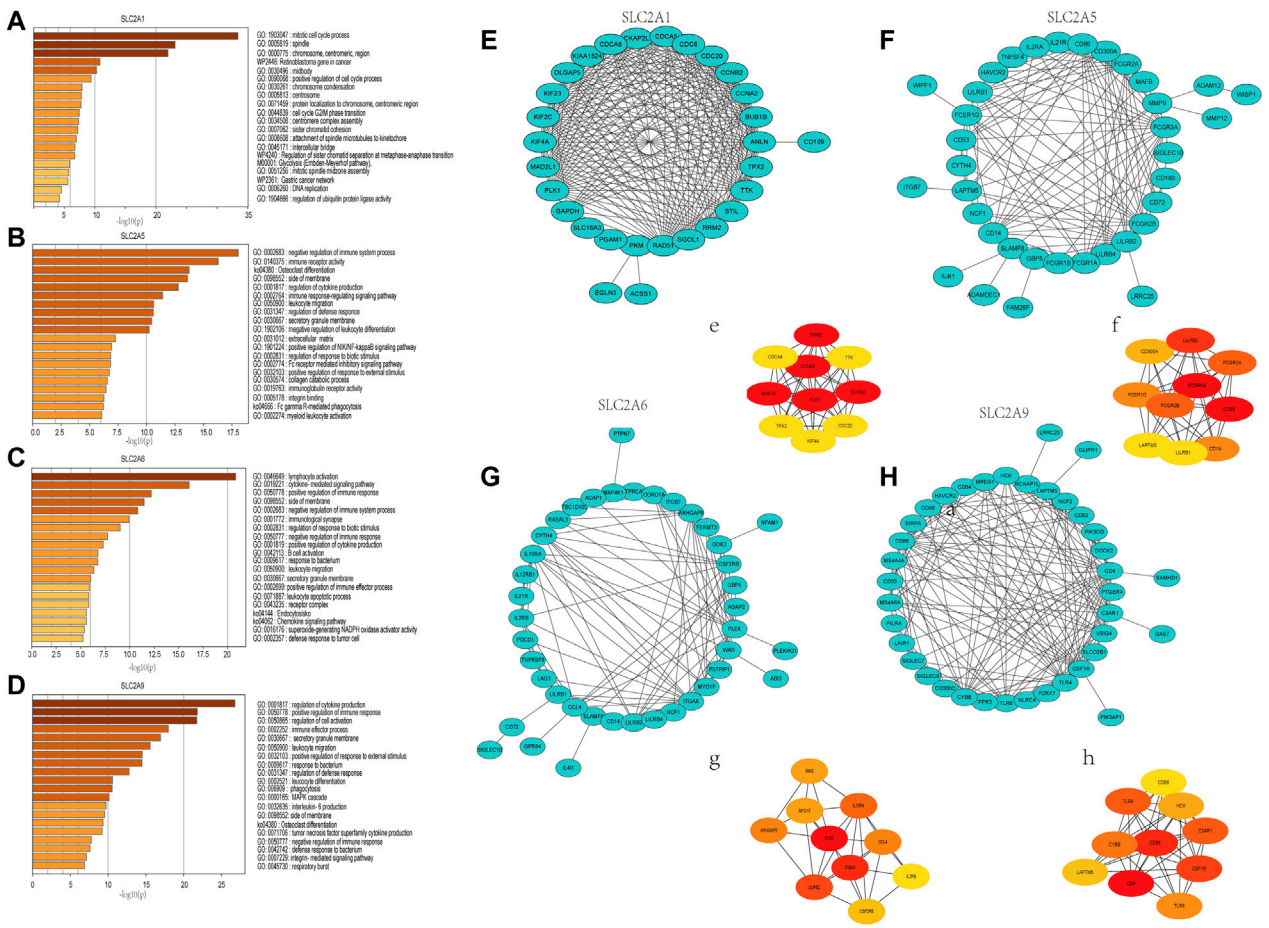


FIGURE 7
Gene Ontology (GO) annotations and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses of genes that ranked top 100 co-expressed genes with *SLC2A1*, *SLC2A5*, *SLC2A6*, *SLC2A9*. **(A)** *SLC2A1*. **(B)** *SLC2A5*. **(C)** *SLC2A6*. **(D)** *SLC2A9*. Construction of an interaction network with *SLC2As* top 100 correlated genes and identification of potential hub genes. **(E–H)** PPI network of *SLC2A1*, *SLC2A5*, *SLC2A6*, and *SLC2A9* co-expressed genes. (e–h) The top 10 hub genes identified in the PPI network.

The mRNA expression in clinical samples of LUAD

We selected *SLC2A3*, *SLC2A6*, *SLC2A9*, and *SLC2A14* as verified targets in our clinical samples since prior research has demonstrated higher expression of *SLC2A1* and *SLC2A5* in LUAD patients. Patients numbers 2 and 6 had lower levels of *SLC2A3*, *SLC2A6*, *SLC2A9*, and *SLC2A14* than the surrounding normal tissue. However, patient number 1 had elevated levels of *SLC2A3*, *SLC2A6*, and *SLC2A14*, whereas patient number 3 had elevated levels of *SLC2A3*, *SLC2A6*, and *SLC2A9*. In patient number 4 and 5, elevated levels of *SLC2A3*, *SLC2A6*, *SLC2A9*, and *SLC2A14* were discovered (As shown in Figure 9; Supplementary Material S5).

Discussion

Three classifications have been established for GLUTs (Hardie and Ashford, 2014). Class 1, which includes GLUTs 1-4 and GLUT-14 and contains glucose as its substrate, has a substantially lower affinity for fructose (Mueckler and Thorens, 2013). GLUT-5, GLUT-7, GLUT-9, and GLUT-11 are all members of class 2. Fructose serves as GLUT-5's primary substrate, and it has a higher affinity for it than for glucose (Tatibouët et al., 2000). While GLUT- 7 and GLUT-11 have the same affinity for glucose as fructose (Manolescu et al., 2007; Cheeseman, 2008) GLUT-9 transports urate along with fructose in the physiological range (Cheeseman, 2008; So and Thorens, 2010). Class 3, which consists of GLUT-6, GLUT-8, GLUT-10,

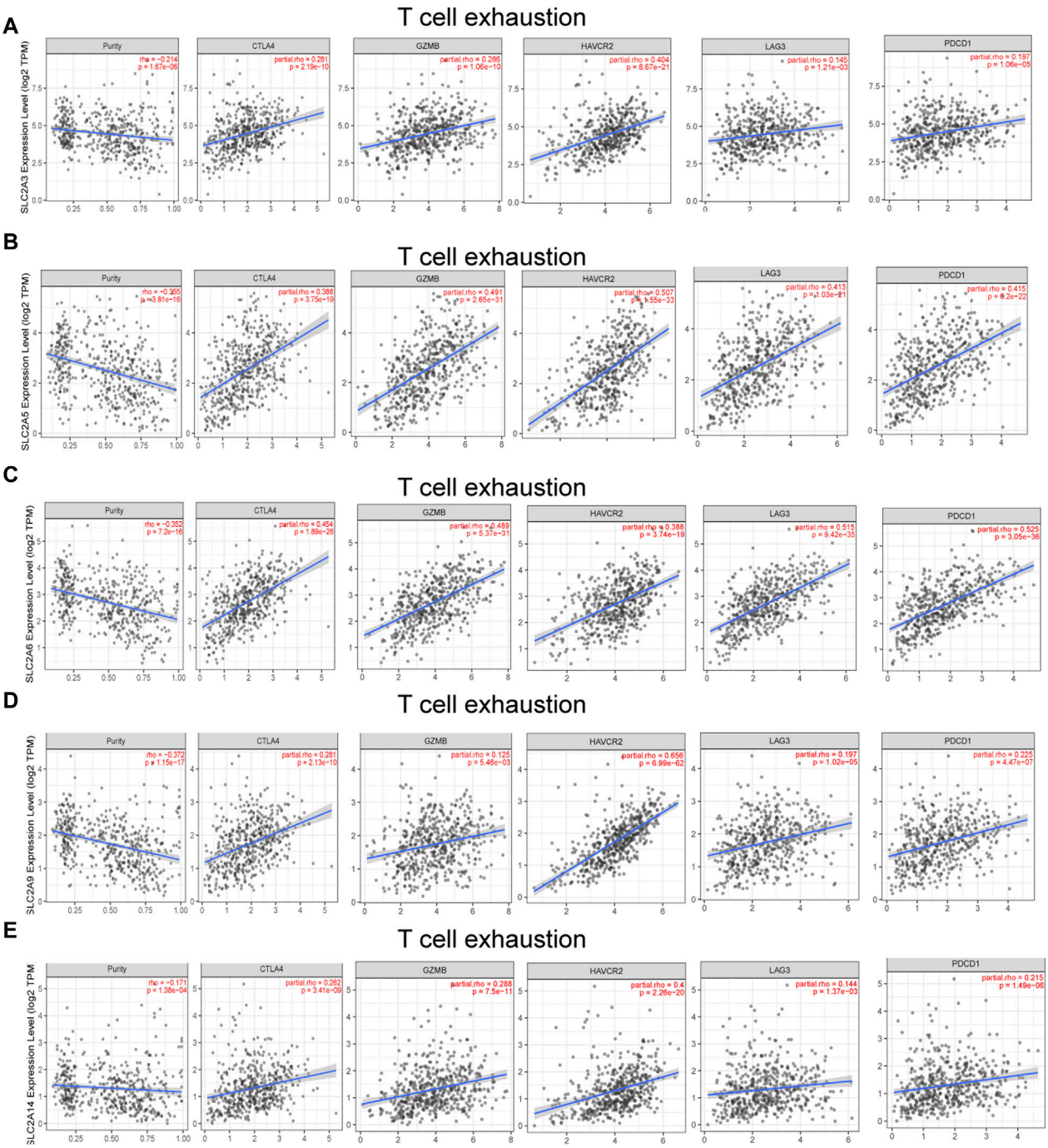


FIGURE 8
SLC2As expression has significant relations with infiltration of T cell exhaustion in LUAD. **(A)** *SLC2A3*. **(B)** *SLC2A5*. **(C)** *SLC2A6*. **(D)** *SLC2A9*. **(E)** *SLC2A14*. TCE marker genes include PDCD1, CTLA4, LAG3, HAVCR2, and GZMB.

GLUT-12, and GLUT-13, is challenging to characterize. The existing evidence indicated that GLUT-12 transports glucose (Macheda et al., 2005) and GLUT-13 transports myo-inositol (Holman, 2020). However, it is still unknown what the other members substrates are (Holman, 2020). *SLC2As* have reportedly been implicated in multiple diseases, including cancer. This study is the first comprehensive biological analysis of the 14 *SLC2A* genes in LUAD patients.

The results of the researchers' numerous studies on *SLC2As* expressions in LUAD at the mRNA and protein levels are

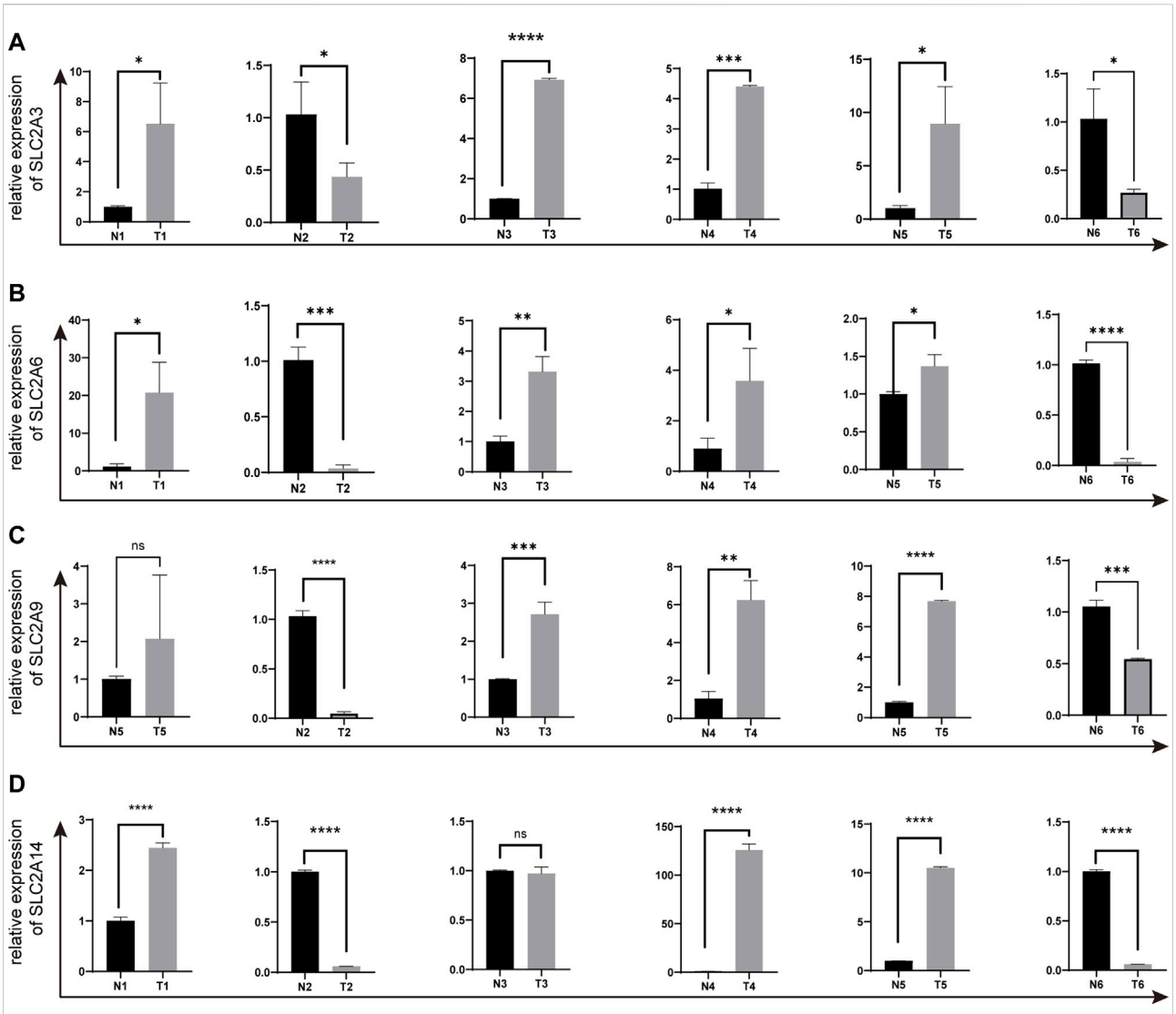


FIGURE 9
The expressions of *SLC2A3*, *SLC2A6*, *SLC2A9* and *SLC2A14* in clinical LUAD samples were validated by q-PCR **(A)** *SLC2A3*. **(B)** *SLC2A6*. **(C)** *SLC2A9*. **(D)** *SLC2A14*. T, Tumor. N, Normal adjacent tissues.

inconsistent or even conflicting. One study identifies *SLC2A1* may be a crucial glucose transporter in LUAD patients as *SLC2A1* mRNA and protein levels are elevated in LUAD patients, and its overexpression is a potential indicator for lower differentiated tumor grade and poor prognosis (Younes et al., 1997a; Minami et al., 2002; Maki et al., 2013; Chai et al., 2017; Weng et al., 2018a; Guo et al., 2020). However, another study demonstrates that GLUT-1 protein expression is absent in healthy lung tissues but positive in 14 of 24 adenocarcinomas and that the levels of expression are correlated with greater tumor sizes, less differentiation of the tumor, and positive lymph node metastases (Ito et al., 1998). LUAD patients did not exhibit altered *SLC2A3* and *SLC2A5* expressions at the mRNA level when compared to healthy donors. However, compared to the primary tumor, lung cancer metastases have higher levels of *SLC2A3* and *SLC2A5*, highlighting their significance in tumor metastasis (Kurata et al., 1999). Another study uses immunohistochemical analysis to show that GLUT-3 and GLUT-5 are expressed at the same levels as those of healthy lung tissues (Holman, 2020), while GLUT-4 expression is positive and GLUT-2, GLUT-3, and GLUT-5 are negative in LUAD patients (Brown et al., 1999). Regarding GLUT-3 protein levels, numerous investigations show that they are absent in healthy lung tissues (Younes et al., 1997b) and increased in non-small cell lung cancer (de Geus-Oei et al., 2007; Suzawa et al., 2011). Compared to healthy tissues, the level of *SLC2A5* mRNA is higher in LUAD tissues. Additionally, a worse prognosis is connected to *SLC2A5* overexpression (Weng et al., 2018a;

Weng et al., 2018b). GLUT-3, GLUT-4, and GLUT-5 have been discovered to be overexpressed in LUAD patients at the protein level (Younes et al., 1997b; Flavahan et al., 2013; Chai et al., 2017). In a different study, GLUT-2, GLUT-3, GLUT-4, GLUT-5, GLUT-6, GLUT-9 expression levels were tested in lung adenocarcinoma and only GLUT-3 is positive in 1 of 8 lung adenocarcinoma patients (Godoy et al., 2006). Neither GLUT-3 nor GLUT-4 is positively stained in normal lung epithelia while lung cancers have elevated levels of *SLC2A3* and *SLC2A4* (Ito et al., 1998). H1299 (NSCLC cell line) has high *SLC2A12* mRNA expression (Zawacka-Pankau et al., 2011), while lung cancer cell line A549 has high levels of GLUT-12 expression (Pujol-Gimenez et al., 2015). The predictive value of the *SLC2As* in LUAD patients has also been examined. Increased GLUT-3 levels are a potential indicator of a poor prognosis and a biomarker for lower tumor differentiation in Stage I non-small cell lung cancer patients, highlighting the significance of GLUT-3 for early diagnosis and prognostic accuracy (Younes et al., 1997b; Maki et al., 2013). According to a different study, patients with NSCLC who have high levels of the mRNAs *SLC2A1*, *SLC2A2*, *SLC2A3*, *SLC2A4*, *SLC2A5*, *SLC2A6*, *SLC2A7*, *SLC2A9*, *SLC2A11*, and *SLC2A14* have a considerably worse overall survival (Du et al., 2020). Downregulated levels of *SLC2A10* in lung cancer indicate a poor prognosis and increased aggressiveness of tumors, suggesting that *SLC2A10* may play a crucial role in the progression of cancer (Jian et al., 2021).

In our study, we show that transcriptional levels of *SLC2A1*, *SLC2A5* are upregulated and those of *SLC2A3*, *SLC2A6*, *SLC2A9*, *SLC2A14* are downregulated in LUAD patients, supporting the role of *SLC2A1*, *SLC2A5* as oncogenes and *SLC2A3*, *SLC2A6*, *SLC2A9*, *SLC2A14* as tumor suppressor genes. Inhibiting GLUT-1, GLUT-5, GLUT-6, and GLUT-9 may be used as a therapy for LUAD patients as a result of the increased protein levels of these enzymes that were shown in our study. *SLC2As*' mismatched mRNA and protein levels in LUAD patients could be explained by changes in turnover (transcription and degradation), as well as post-transcriptional and post-translational modifications like phosphorylation, acetylation, ubiquitination, and methylation, as well as sumo of the *SLC2As* or GLUTs. Furthermore, the upregulation of GLUT-1, GLUT-5, GLUT-6, and GLUT-9 suggests that fructose and glucose may both play important roles in LUAD metabolism, supporting the development of blockades for these molecules as potential new medications or biomarkers for the treatment or detection of LUAD. Re-examining the mRNA expression of *SLC2A3*, *SLC2A6*, *SLC2A9*, and *SLC2A14* revealed that 2 of 6 patients had lower levels of these genes than those predicted by RNA-seq methods in LUAD samples. The results from the other four LUAD patients were different, showing higher levels of *SLC2A3*, *SLC2A6*, *SLC2A9*, and *SLC2A14* in LUAD patients. The differing experimental platforms could be the cause of the varied expression levels between RNA-seq and q-PCR. Nearly the whole exon region of a gene is covered by RNA-seq, which also considers the expression levels of each exon region. However, q-PCR does not account for the entire length of the gene when designing primers or amplifying.

Because patients in the same phase might have quite different clinical outcomes, even at an early stage, it is important to identify novel tumor biomarkers in order to accurately estimate patient prognosis and predict responsiveness to a specific therapy. The next step is to assess if tumor stages can be identified based on *SLC2As* expression levels. A lower differentiation grade tumor is implied by overexpression of *SLC2A1*, *SLC2A5*, *SLC2A10*, *SLC2A11*and lower levels of *SLC2A3*, *SLC2A6*, *SLC2A9*, *SLC2A12*, and *SLC2A14*, emphasizing the possibility of these molecular roles for predicting the course of the tumor. Interestingly, the expression levels of *SLC2A1*, *SLC2A3*, *SLC2A5*, *SLC2A6*, *SLC2A7*, *SLC2A9*, *SLC2A10*, *SLC2A11*, *SLC2A12*, and *SLC2A14* all differed in stage 1 compared to normal tissues, strongly proposing these molecules as possible markers for the early diagnosis of LUAD. Secondly, we establish the importance of low levels of *SLC2A1*, *SLC2A2*, *SLC2A3*, *SLC2A4*, *SLC2A5*, *SLC2A9*, *SLC2A14* and high levels of *SLC2A10*, *SLC2A12*, and *SLC2A13* as biomarkers for favourable prognosis. Results for *SLC2A1*, *SLC2A3*, *SLC2A5*, and *SLC2A10* are in line with earlier investigations. It is intriguing that individuals with LUAD who had high levels of *SLC2A9* and *SLC2A14*, which were previously identified as suppressor genes in our investigations, have lower survival rates. We think that these molecules may be the source of the resistance to chemotherapy and radiation. Analyses using the LASSO technique are used to investigate the most important factors. *SLC2A1*, *SLC2A7*, and *SLC2A11* are three *SLC2As* that have been discovered as possible predictive biomarkers. *SLC2A11* is an indicator of a favorable outcome in LUAD, but high expression of *SLC2A1* and *SLC2A7* is a characteristic of an adverse prognosis. The combination of *SLC2A1*, *SLC2A7*, and *SLC211* is also supported by the ROC curve as a possible biomarker for 1-year, 3- year, and 5-year survival. In conclusion, *SLC2As* may someday serve as critical clinical markers for LUAD patients, allowing for earlier identification, more precise risk classification, and individualized survival prediction.

The role of epigenetic regulators, such as DNA methylation, on *SLC2As* deregulation, was identified using the UALCAN database to investigate the upstream mechanisms causing the abnormal *SLC2As* expression. Focusing on how epigenetic alteration affects cancer etiology, both suppressor gene suppression caused by excessive methylation in gene promoters and oncogene activation or chromosomal instability caused by widespread hypomethylation can occur in malignancies (Merlo et al., 1995; Rosty et al., 2002; Gaudet et al., 2003). Particularly, the development of DNA methylation liquid biopsy has made early cancer diagnosis and treatment recommendations

possible (Brikun et al., 2018; Shen et al., 2018; Zhao et al., 2018). In our research, hypomethylation occurs on oncogenes including *SLC2A1*, *SLC2A5*, *SLC2A7*, and *SLC2A11*. Additionally, hypermethylation of tumor suppressor genes, such as *SLC2A3* and *SLC2A14*, is observed in LUAD patients, suggesting that DNA methylation may be the cause of the aberrant expression of *SLC2As* in LUAD. There is a lot of interest in treatments that target epigenome alterations, such as DNA methyltransferases (DNMTs). DNMTs have been used as anti-cancer drugs, specifically as potential chemotherapy or radiotherapy sensitizers, to increase treatment efficacy (Gravina et al., 2010). Given the significance of DNA methylation affects *SLC2As*, DNMTs have a potential for correcting these aberrant *SLC2As* expressions in LUAD. To determine the level of DNA methylation in LUAD, future studies will need to carefully implement animal and cell line research. These results will lay the groundwork for DNA methylation as sensitive prognostic, predictive biomarkers, and therapeutic targets in LUAD.

What role did the aberrant *SLC2As* play in LUAD pathogenesis? Due to the co-expressed genes' enrichment in GO and KEGG pathways associated with immune cells, we concentrate on the relationship between *SLC2As* and immunological signatures. Recently, numerous tumors have been successfully treated using a variety of immunotherapy techniques, including immune checkpoint blockades (PD-1, CTLA4), cellular therapy, and therapeutic vaccinations, including LUAD (Del Paggio, 2018; Christofi et al., 2019). However, not every immunotherapy is successful in treating patients due to patients' limited tumor immunity and the heterogeneity of checkpoint inhibitors (Sasidharan Nair and Elkord, 2018; Liu et al., 2020). In addition, tumor cells are prone to switch from oxidative metabolism to glycolysis and lactic acid fermentation even in normal conditions. The metabolic alteration leads to hypoxia, production of acid, poor in nutrients and abundance of immune-modulatory metabolites, consequently contributing to the resistance to immunotherapy (Ramapriyan et al., 2019). Therefore, we aim to better understand the connection between *SLC2As* and immune cell infiltration in LUAD, which will aid in identifying patients who may benefit from immune therapy. The alteration of the tumor microenvironment caused by circulating monocytes and macrophages, which are drawn to the tumor, promotes the growth of malignancies (Chanmee et al., 2014). Macrophages are generally classified into two subpopulations, classically activated pro-inflammatory macrophages (M1) and alternatively activated macrophages (M2). M2 macrophages are involved in promoting the growth and invasion of lung cancer cell, while M1 mac rophages inhibit lung cancer cell proliferation and activity *via* increasing cancer cell chemical sensitivity and decreasing angiogenesis (Yuan et al., 2015). It is interesting to note that in LUAD patients, M2 and monocyte expression levels are favorably linked with *SLC2A3* and *SLC2A5* expression levels. Th1, monocytes, and T cells had a positive correlation with *SLC2A6* levels. M2, TAM, M-DC, monocytes, and Treg cells all exhibit favorable correlations with *SLC2A9* expression levels. In LUAD patients, *SLC2A14* expression is favorably linked with M2, monocytes, and TAM. These findings reveal that members of the *SLC2A* family may contribute to carcinogenesis by controlling M2 polarization and drawing monocytes and Tregs to tumor locations. However, more research into the underlying mechanism is still required. Additionally, it has been suggested that TCE plays a role in various malignancies and chronic infections, a condition marked by a decline in T cell activity, persistent expression of inhibitory receptors, and a transcriptional state distinct from that of functioning effector or memory T cells and inhibiting pathways elevated in exhaustion can reverse the dysregulated state and reinvigorate immune responses (Wherry and Kurachi, 2015). According to our findings, expression of *SLC2A3*, *SLC2A5*, *SLC2A6*, *SLC2A9*, and *SLC2A14* is positively correlated with the TCE marker gene, suggesting that these molecules may reflect the presence of TCE in LUAD. All these findings supported the use of *SLC2A3*, *SLC2A5*, *SLC2A6*, *SLC2A9*, and *SLC2A14* as therapeutic targets and biomarkers for evaluating the effectiveness of immunotherapy.

Finally, we suggest multiple pathways in which *SLC2As* were involved in LUAD, including *SLC2A1*/BUB1B/mitotic cell cycle, *SLC2A5*/CD86/negative immune system process regulation, *SLC2A6*/PLEK/lymphocyte activation, and *SLC2A9*/CD4/cytokine production regulation.

The results that an increased level of GLUT-1 is positively correlated with F-18 fluorodeoxyglucose (FDG) uptake indicate the involvement of GLUT-1 in the accumulation of FDG in LUAD patients (Brown et al., 1999; Gu et al., 2006; Wink et al., 2017; Vanhove et al., 2019). However, as confounders in PET positron emission tomography-computer tomography (PET-CT) analysis, inflammatory pseudotumor, tuberculoma, and organizing pneumonia may present a similar picture with lung malignancies. We hypothesize that the combination of GLUT-1, GLUT-5, GLUT-6, and GLUT-9 may distinguish the inflammation (necrosis) lesions from malignancies in light of the increased protein expression of GLUT-1, GLUT-5, GLUT-6, and GLUT-9 in LUAD patients in our study. However, the hypothesis needs further investigation.

GLUT inhibitors have been used in numerous studies to treat NSCLC. A GLUT-1 inhibitor called WZB117 prevents the proliferation of the NSCLC cancer cells (H1299 and A549) (Liu et al., 2012). Oxime-based GLUT-1 inhibitors reduce the proliferation of the cancerous H1299 cell line (Tuccinardi et al., 2013). The GLUT-2 inhibitor quercetin lowers the risk of developing lung cancer (Rastogi et al., 2007). Small interfering RNA (siRNA) for GLUT-1 increases NSCLC cell susceptibility to gefitinib treatment (Suzuki et al., 2018). Anti-GLUT-1 antibodies reduce non-small cell lung cancer cell growth by 50% (Rastogi

et al., 2007). However, the findings that both glucose and fructose are energy sources in LUAD remind us that cancer cells can switch to a different option for their energy requirements, so we recommend that the best course of action may be to completely inhibit GLUT-transporters. Blocking GLUT-1, GLUT-5, GLUT-6, and GLUT-9 may display beneficial efficiency in LUAD therapy. A better understanding of the universal expression profiles of GLUTs and the tissue distribution of GLUTs will pave the way for transforming the GLUT inhibitors into clinical application in LUAD.

Conclusion and outlooks

The prognosis of patients with LUAD can be correctly stratified with the use of a thorough examination of their metabolic profiles. Our findings might suggest *SLC2As* as biomarkers that could help distinguish between low-risk LUAD patients and advanced LUAD patients. Moreover, *SLC2As* could serve as prognostic signatures to predict 1, 3, and 5 year survival in LUAD patients. *SLC2As* are abnormally expressed in LUAD due to DNA methylation, and *SLC2As* that are dysregulated promote LUAD by changing the immunological microenvironment. We also suggest several pathways that could be involved in LUAD, including *SLC2A1*/BUB1B/mitotic cell cycle, *SLC2A5*/CD86/negative immune system process regulation, *SLC2A6*/PLEK/lymphocyte activation, and *SLC2A9*/CD4/cytokine production regulation. These findings open the door to the use of these SLC2A molecules as LUAD biomarkers and treatment targets.

Due to their propensity to mimic other inflammatory diseases, cancers diagnosed by PET-CT were likewise less sensitive and specific. GLUTs should work together as radiotracers to efficiently image LUAD tumors and provide early identification, accurate tumor grading, and prognosis prediction.

The future trend will be to completely inhibit GLUT-1, GLUT-5, GLUT-6, and GLUT-9 as therapeutic targets. Our findings revealed that LUAD uses fructose (a GLUT-5 and GLUT-9 substrate) as a different type of energy source. Since GLUT-5 and GLUT-9 are not universally expressed as GLUT-1, blocking the transport of fructose may be an appealing method to inhibit the metabolism of glucose. In other words, it is important to consider the high specificity. We advise exosomes or nanoparticles loaded with GLUT inhibitors as a viable option that might be safely and conveniently delivered to combat LUAD.

The GLUT function has previously been studied using cell lines, but this method has the disadvantage of not compressing other tissue-matrix components. To shed light on the significance of glucose carriers in LUAD patients, it is necessary to conduct these experimental and clinical trial tests in *SLC2A* conditional knockout mice, which enable GLUT knocking out in specific cells. Another related problem is that we still do not fully comprehend how *SLC2As* regulate various immune cell subtypes, such as TCE, M2, T cells, and monocyte in LUAD.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by The Academic Committee of The First Hospital of Jilin University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

YZ wrote the manuscript. HQ, JB, and ZM consulted relevant literature. HY revised the manuscript.

Funding

This research is supported by a grant from Jilin Province direct health special project (3D5205184428).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.1045179/full#supplementary-material

References

Brikun, I., Nusskern, D., Decatus, A., Harvey, E., Li, L., and Freije, D. (2018). A panel of DNA methylation markers for the detection of prostate cancer from FV and DRE urine DNA. *Clin. Epigenetics* 10, 91. doi:10.1186/s13148-018-0524-x

Brown, R. S., Leung, J. Y., Kison, P. V., Zasadny, K. R., Flint, A., and Wahl, R. L. (1999). Glucose transporters and FDG uptake in untreated primary human non-small cell lung cancer. *J. Nucl. Med.* 40, 556–565.

Cerami, E., Gao, J., Dogrusoz, U., Gross, B. E., Sumer, S. O., Aksoy, B. A., et al. (2012). The cBio cancer genomics portal: An open platform for exploring multidimensional cancer genomics data. *Cancer Discov.* 2, 401–404. doi:10.1158/2159-8290.Cd-12-0095

Chai, Y. J., Yi, J. W., Oh, S. W., Kim, Y. A., Yi, K. H., Kim, J. H., et al. (2017). Upregulation of SLC2 (GLUT) family genes is related to poor survival outcomes in papillary thyroid carcinoma: Analysis of data from the Cancer Genome Atlas. *Surgery* 161, 188–194. doi:10.1016/j.surg.2016.04.050

Chandrashekar, D. S., Bashel, B., Balasubramanya, S. A. H., Creighton, C. J., Ponce-Rodriguez, I., Chakravarthi, B., et al. (2017). Ualcan: A portal for facilitating tumor subgroup gene expression and survival analyses. *Neoplasia (New York, N.Y.)* 19, 649–658. doi:10.1016/j.neo.2017.05.002

Chanmee, T., Ontong, P., Konno, K., and Itano, N. (2014). Tumor-associated macrophages as major players in the tumor microenvironment. *Cancers* 6, 1670–1690. doi:10.3390/cancers6031670

Cheeseman, C. (2008). GLUT7: A new intestinal facilitated hexose transporter. *Am. J. Physiol. Endocrinol. Metab.* 295, E238–E241. doi:10.1152/ajpendo.90394.2008

Christofi, T., Baritaki, S., Falzone, L., Libra, M., and Zaravinos, A. (2019). Current perspectives in cancer immunotherapy. *Cancers* 11, E1472. doi:10.3390/cancers11101472

Cura, A. J., and Carruthers, A. (2012). Role of monosaccharide transport proteins in carbohydrate assimilation, distribution, metabolism, and homeostasis. *Compr. Physiol.* 2, 863–914. doi:10.1002/cphy.c110024

de Geus-Oei, L. F., van Krieken, J. H., Aliredjo, R. P., Krabbe, P. F., Frielink, C., Verhagen, A. F., et al. (2007). Biological correlates of FDG uptake in non-small cell lung cancer. *Lung cancer (Amsterdam, Neth.* 55, 79–87. doi:10.1016/j.lungcan.2006.08.018

Del Paggio, J. C. (2018). Immunotherapy: Cancer immunotherapy and the value of cure. *Nat. Rev. Clin. Oncol.* 15, 268–270. doi:10.1038/nrclinonc.2018.27

Du, H., Liu, Y., Yuan, Y., Zhang, Y., and Geng, H. (2020). Distinct prognostic values of the mRNA expression of glucose transporters in non-small cell lung cancer. *Ann. Clin. Lab. Sci.* 50, 481–489.

Flavahan, W. A., Wu, Q., Hitomi, M., Rahim, N., Kim, Y., Sloan, A. E., et al. (2013). Brain tumor initiating cells adapt to restricted nutrition through preferential glucose uptake. *Nat. Neurosci.* 16, 1373–1382. doi:10.1038/nn.3510

Fridlender, Z. G., Sun, J., Kim, S., Kapoor, V., Cheng, G., Ling, L., et al. (2009). Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. *Cancer cell* 16, 183–194. doi:10.1016/j.ccr.2009.06.017

Gaudet, F., Hodgson, J. G., Eden, A., Jackson-Grusby, L., Dausman, J., Gray, J. W., et al. (2003). Induction of tumors in mice by genomic hypomethylation. *Sci. (New York, N.Y.)* 300, 489–492. doi:10.1126/science.1083558

Godoy, A., Ulloa, V., Rodríguez, F., Reinicke, K., Yañez, A. J., García Mde, L., et al. (2006). Differential subcellular distribution of glucose transporters GLUT1-6 and GLUT9 in human cancer: Ultrastructural localization of GLUT1 and GLUT5 in breast tumor tissues. *J. Cell. Physiol.* 207, 614–627. doi:10.1002/jcp.20606

Granchi, C., Fortunato, S., and Minutolo, F. (2016). Anticancer agents interacting with membrane glucose transporters. *MedChemComm* 7, 1716–1729. doi:10.1039/c6md00287k

Gravina, G. L., Festuccia, C., Marampon, F., Popov, V. M., Pestell, R. G., Zani, B. M., et al. (2010). Biological rationale for the use of DNA methyltransferase inhibitors as new strategy for modulation of tumor response to chemotherapy and radiation. *Mol. Cancer* 9, 305. doi:10.1186/1476-4598-9-305

Gu, J., Yamamoto, H., Fukunaga, H., Danno, K., Takemasa, I., Ikeda, M., et al. (2006). Correlation of GLUT-1 overexpression, tumor size, and depth of invasion with 18F-2-fluoro-2-deoxy-D-glucose uptake by positron emission tomography in colorectal cancer. *Dig. Dis. Sci.* 51, 2198–2205. doi:10.1007/s10620-006-9428-2

Guo, W., Sun, S., Guo, L., Song, P., Xue, X., Zhang, H., et al. (2020). Elevated SLC2A1 expression correlates with poor prognosis in patients with surgically resected lung adenocarcinoma: A study based on immunohistochemical analysis and bioinformatics. *DNA Cell Biol.* 39, 631–644. doi:10.1089/dna.2019.5291

Győrffy, B., Surowiak, P., Budczies, J., and Lánczky, A. (2013). Online survival analysis software to assess the prognostic value of biomarkers using transcriptomic data in non-small-cell lung cancer. *PloS one* 8, e82241. doi:10.1371/journal.pone.0082241

Hardie, D. G., and Ashford, M. L. (2014). Ampk: Regulating energy balance at the cellular and whole body levels. *Physiology* 29, 99–107. doi:10.1152/physiol.00050.2013

Holman, G. D. (2020). Structure, function and regulation of mammalian glucose transporters of the SLC2 family. *Pflugers Arch.* 472, 1155–1175. doi:10.1007/s00424-020-02411-3

Hu, H. Z., Li, G. L., Lim, Y. K., Chan, S. H., and Yap, E. H. (1999). Kinetics of interferon-gamma secretion and its regulatory factors in the early phase of acute graft-versus-host disease. *Immunology* 98, 379–385. doi:10.1046/j.1365-2567.1999.00881.x

Ito, T., Noguchi, Y., Satoh, S., Hayashi, H., Inayama, Y., and Kitamura, H. (1998). Expression of facilitative glucose transporter isoforms in lung carcinomas: Its relation to histologic type, differentiation grade, and tumor stage. *Mod. Pathol.* 11, 437–443.

Jeong, S. H., Kim, R. B., Park, S. Y., Park, J., Jung, E. J., Ju, Y. T., et al. (2020). Nomogram for predicting gastric cancer recurrence using biomarker gene expression. *Eur. J. Surg. Oncol.* 46, 195–201. doi:10.1016/j.ejso.2019.09.143

Jian, L., Zhang, M., Wu, Q., Min, X., Li, B., Wu, Z., et al. (2021). Glut10 is a novel immune regulator involved in lung cancer immune cell infiltration and predicts worse survival when transcriptionally down-regulated. *Res. square*. doi:10.21203/rs.3.rs-386785/v1

Jodeleit, H., Al-Amodi, O., Caesar, J., Villarroel Aguilera, C., Holdt, L., Gropp, R., et al. (2018). Targeting ulcerative colitis by suppressing glucose uptake with ritonavir. *Dis. Model. Mech.* 11, dmm036210. doi:10.1242/dmm.036210

Kurata, T., Oguri, T., Isobe, T., Ishioka, S., and Yamakido, M. (1999). Differential expression of facilitative glucose transporter (GLUT) genes in primary lung cancers and their liver metastases. *Jpn. J. Cancer Res.* 90, 1238–1243. doi:10.1111/j.1349-7006.1999.tb00702.x

Li, T., Fan, J., Wang, B., Traugh, N., Chen, Q., Liu, J. S., et al. (2017). Timer: A web server for comprehensive analysis of tumor-infiltrating immune cells. *Cancer Res.* 77, e108–e110. doi:10.1158/0008-5472.Can-17-0307

Liu, J., Cho, S. N., Akkanti, B., Jin, N., Mao, J., Long, W., et al. (2015). ErbB2 pathway activation upon Smad4 loss promotes lung tumor growth and metastasis. *Cell Rep.* 10, 1599–1613. doi:10.1016/j.celrep.2015.02.014

Liu, J., Nie, S., Wu, Z., Jiang, Y., Wan, Y., Li, S., et al. (2020). Exploration of a novel prognostic risk signatures and immune checkpoint molecules in endometrial carcinoma microenvironment. *Genomics* 112, 3117–3134. doi:10.1016/j.ygeno.2020.05.022

Liu, Y., Cao, Y., Zhang, W., Bergmeier, S., Qian, Y., Akbar, H., et al. (2012). A small-molecule inhibitor of glucose transporter 1 downregulates glycolysis, induces cell-cycle arrest, and inhibits cancer cell growth *in vitro* and *in vivo*. *Mol. Cancer Ther.* 11, 1672–1682. doi:10.1158/1535-7163.Mct-12-0131

Macheda, M. L., Rogers, S., and Best, J. D. (2005). Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer. *J. Cell. Physiol.* 202, 654–662. doi:10.1002/jcp.20166

Macintyre, A. N., Gerriets, V. A., Nichols, A. G., Michalek, R. D., Rudolph, M. C., Deoliveira, D., et al. (2014). The glucose transporter Glut1 is selectively essential for CD4 T cell activation and effector function. *Cell Metab.* 20, 61–72. doi:10.1016/j.cmet.2014.05.004

Maki, Y., Soh, J., Ichimura, K., Shien, K., Furukawa, M., Muraoka, T., et al. (2013). Impact of GLUT1 and Ki-67 expression on early-stage lung adenocarcinoma diagnosed according to a new international multidisciplinary classification. *Oncol. Rep.* 29, 133–140. doi:10.3892/or.2012.2087

Manolescu, A. R., Augustin, R., Moley, K., and Cheeseman, C. (2007). A highly conserved hydrophobic motif in the exofacial vestibule of fructose transporting SLC2A proteins acts as a critical determinant of their substrate selectivity. *Mol. Membr. Biol.* 24, 455–463. doi:10.1080/09687680701298143

Merlo, A., Herman, J. G., Mao, L., Lee, D. J., Gabrielson, E., Burger, P. C., et al. (1995). 5' CpG island methylation is associated with transcriptional silencing of the tumour suppressor p16/CDKN2/MTS1 in human cancers. *Nat. Med.* 1, 686–692. doi:10.1038/nm0795-686

Minami, K., Saito, Y., Imamura, H., and Okamura, A. (2002). Prognostic significance of p53, Ki-67, VEGF and Glut-1 in resected stage I adenocarcinoma of the lung. *Lung cancer (Amsterdam, Neth.* 38, 51–57. doi:10.1016/s0169-5002(02)00108-3

Mueckler, M., and Thorens, B. (2013). The SLC2 (GLUT) family of membrane transporters. *Mol. Asp. Med.* 34, 121–138. doi:10.1016/j.mam.2012.07.001

Murray, P. J. (2017). Macrophage polarization. *Annu. Rev. Physiol.* 79, 541–566. doi:10.1146/annurev-physiol-022516-034339

Pujol-Gimenez, J., de Heredia, F. P., Idoate, M. A., Airley, R., Lostao, M. P., and Evans, A. R. (2015). Could GLUT12 be a potential therapeutic target in cancer treatment? A preliminary report. *J. Cancer* 6, 139–143. doi:10.7150/jca.10429

Ramapriyan, R., Caetano, M. S., Barsoumian, H. B., Mafra, A. C. P., Zambalde, E. P., Menon, H., et al. (2019). Altered cancer metabolism in mechanisms of immunotherapy resistance. *Pharmacol. Ther.* 195, 162–171. doi:10.1016/j.pharmthera.2018.11.004

Rastogi, S., Banerjee, S., Chellappan, S., and Simon, G. R. (2007). Glut-1 antibodies induce growth arrest and apoptosis in human cancer cell lines. *Cancer Lett.* 257, 244–251. doi:10.1016/j.canlet.2007.07.021

Rhodes, D. R., Yu, J., Shanker, K., Deshpande, N., Varambally, R., Ghosh, D., et al. (2004). Oncomine: A cancer microarray database and integrated data-mining platform. *Neoplasia (New York, N.Y.)* 6, 1–6. doi:10.1016/s1476-5586(04)80047-2

Rosty, C., Ueki, T., Argani, P., Jansen, M., Yeo, C. J., Cameron, J. L., et al. (2002). Overexpression of S100A4 in pancreatic ductal adenocarcinomas is associated with poor differentiation and DNA hypomethylation. *Am. J. Pathol.* 160, 45–50. doi:10.1016/s0002-9440(10)64347-7

Sanli, T., Rashid, A., Liu, C., Harding, S., Bristow, R. G., Cutz, J. C., et al. (2010). Ionizing radiation activates AMP-activated kinase (AMPK): A target for radiosensitization of human cancer cells. *Int. J. Radiat. Oncol. Biol. Phys.* 78, 221–229. doi:10.1016/j.ijrobp.2010.03.005

Sasidharan Nair, V., and Elkord, E. (2018). Immune checkpoint inhibitors in cancer therapy: A focus on T-regulatory cells. *Immunol. Cell Biol.* 96, 21–33. doi:10.1111/imcb.1003

Shen, S. Y., Singhania, R., Fehringer, G., Chakravarthy, A., Roehrl, M. H. A., Chadwick, D., et al. (2018). Sensitive tumour detection and classification using plasma cell-free DNA methylomes. *Nature* 563, 579–583. doi:10.1038/s41586-018-0703-0

Smoot, M. E., Ono, K., Ruscheinski, J., Wang, P. L., and Ideker, T. (2011). Cytoscape 2.8: New features for data integration and network visualization. *Bioinforma. Oxf. Engl.* 27, 431–432. doi:10.1093/bioinformatics/btq675

So, A., and Thorens, B. (2010). Uric acid transport and disease. *J. Clin. Invest.* 120, 1791–1799. doi:10.1172/jci42344

Storozhuk, Y., Hopmans, S. N., Sanli, T., Barron, C., Tsiani, E., Cutz, J. C., et al. (2013). Metformin inhibits growth and enhances radiation response of non-small cell lung cancer (NSCLC) through ATM and AMPK. *Br. J. Cancer* 108, 2021–2032. doi:10.1038/bjc.2013.187

Storozhuk, Y., Sanli, T., Hopmans, S. N., Schultz, C., Farrell, T., Cutz, J. C., et al. (2012). Chronic modulation of AMP-Kinase, Akt and mTOR pathways by ionizing radiation in human lung cancer xenografts. *Radiat. Oncol.* 7, 71. doi:10.1186/1748-717x-7-71

Suzawa, N., Ito, M., Qiao, S., Uchida, K., Takao, M., Yamada, T., et al. (2011). Assessment of factors influencing FDG uptake in non-small cell lung cancer on PET/CT by investigating histological differences in expression of glucose transporters 1 and 3 and tumour size. *Lung cancer (Amsterdam, Neth.* 72, 191–198. doi:10.1016/j.lungcan.2010.08.017

Suzuki, S., Okada, M., Takeda, H., Kuramoto, K., Sanomachi, T., Togashi, K., et al. (2018). Involvement of GLUT1-mediated glucose transport and metabolism in gefitinib resistance of non-small-cell lung cancer cells. *Oncotarget* 9, 32667–32679. doi:10.18632/oncotarget.25994

Szklarczyk, D., Gable, A. L., Nastou, K. C., Lyon, D., Kirsch, R., Pyysalo, S., et al. (2021). The STRING database in 2021: Customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res.* 49, D605–d612. doi:10.1093/nar/gkaa1074

Tatibouët, A., Yang, J., Morin, C., Holman, G. D., and Tatibouet, A. (2000). Synthesis and evaluation of fructose analogues as inhibitors of the D-fructose transporter GLUT5. *Bioorg. Med. Chem.* 8, 1825–1833. doi:10.1016/s0968-0896(00)00108-5

Tilekar, K., Upadhyay, N., Iancu, C. V., Pokrovsky, V., Choe, J. Y., and Ramaa, C. S. (2020). Power of two: Combination of therapeutic approaches involving glucose transporter (GLUT) inhibitors to combat cancer. *Biochim. Biophys. Acta. Rev. Cancer* 1874, 188457. doi:10.1016/j.bbcan.2020.188457

Tilekar, K., Upadhyay, N., Schweipert, M., Hess, J. D., Macias, L. H., Mrowka, P., et al. (2020). Permuted 2, 4-thiazolidinedione (TZD) analogs as GLUT inhibitors and their *in-vitro* evaluation in leukemic cells. *Eur. J. Pharm. Sci.* 154, 105512. doi:10.1016/j.ejps.2020.105512

Tong, L., Liu, J., Yan, W., Cao, W., Shen, S., Li, K., et al. (2018). RDM1 plays an oncogenic role in human lung adenocarcinoma cells. *Sci. Rep.* 8, 11525. doi:10.1038/s41598-018-30071-y

Tuccinardi, T., Granchi, C., Iegre, J., Paterni, I., Bertini, S., Macchia, M., et al. (2013). Oxime-based inhibitors of glucose transporter 1 displaying antiproliferative effects in cancer cells. *Bioorg. Med. Chem. Lett.* 23, 6923–6927. doi:10.1016/j.bmcl.2013.09.037

Uhlén, M., Fagerberg, L., Hallström, B. M., Lindskog, C., Oksvold, P., Mardinoglu, A., et al. (2015). Proteomics. Tissue-based map of the human proteome. *Sci. (New York, N.Y.)* 347, 1260419. doi:10.1126/science.1260419

Vanhove, K., Thomeer, M., Derveaux, E., Shkedy, Z., Owokotomo, O. E., Adriaensens, P., et al. (2019). Correlations between the metabolic profile and (18)F-FDG-Positron Emission Tomography-Computed Tomography parameters reveal the complexity of the metabolic reprogramming within lung cancer patients. *Sci. Rep.* 9, 16212. doi:10.1038/s41598-019-52667-8

Wang, M., Ngo, V., and Wang, W. (2021). Deciphering the genetic code of DNA methylation. *Brief. Bioinform.* 22, bbaa424. doi:10.1093/bib/bbaa424

Weng, Y., Fan, X., Bai, Y., Wang, S., Huang, H., Yang, H., et al. (2018). SLC2A5 promotes lung adenocarcinoma cell growth and metastasis by enhancing fructose utilization. *Cell Death Discov.* 4, 38. doi:10.1038/s41420-018-0038-5

Weng, Y., Zhu, J., Chen, Z., Fu, J., and Zhang, F. (2018). Fructose fuels lung adenocarcinoma through GLUT5. *Cell Death Dis.* 9, 557. doi:10.1038/s41419-018-0630-x

Wherry, E. J., and Kurachi, M. (2015). Molecular and cellular insights into T cell exhaustion. *Nat. Rev. Immunol.* 15, 486–499. doi:10.1038/nri3862

Wink, K. C. J., van Baardwijk, A., Troost, E. G. C., and De Ruysscher, D. (2017). Nodal recurrence after stereotactic body radiotherapy for early stage non-small cell lung cancer: Incidence and proposed risk factors. *Cancer Treat. Rev.* 56, 8–15. doi:10.1016/j.ctrv.2017.04.001

Xiong, Y., Yuan, L., Xiong, J., Xu, H., Luo, Y., Wang, G., et al. (2020). An outcome model for human bladder cancer: A comprehensive study based on weighted gene co-expression network analysis. *J. Cell. Mol. Med.* 24, 2342–2355. doi:10.1111/jcmm.14918

Xu, F., Huang, X., Li, Y., Chen, Y., and Lin, L. (2021). m^6A-related lncRNAs are potential biomarkers for predicting prognoses and immune responses in patients with LUAD. *Mol. Ther. Nucleic Acids* 24, 780–791. doi:10.1016/j.omtn.2021.04.003

Yin, Y., Choi, S. C., Xu, Z., Perry, D. J., Seay, H., Croker, B. P., et al. (2015). Normalization of CD4+ T cell metabolism reverses lupus. *Sci. Transl. Med.* 7, 274ra18. doi:10.1126/scitranslmed.aaa0835

Younes, M., Brown, R. W., Stephenson, M., Gondo, M., and Cagle, P. T. (1997). Overexpression of Glut1 and Glut3 in stage I nonsmall cell lung carcinoma is associated with poor survival. *Cancer* 80, 1046–1051. doi:10.1002/(sici)1097-0142(19970915)80:6<1046::aid-cncr6>3.0.co;2-7

Younes, M., Lechago, L. V., Somoano, J. R., Mosharaf, M., and Lechago, J. (1997). Immunohistochemical detection of Glut3 in human tumors and normal tissues. *Anticancer Res.* 17, 2747–2750.

Yuan, A., Hsiao, Y. J., Chen, H. Y., Chen, H. W., Ho, C. C., Chen, Y. Y., et al. (2015). Opposite effects of M1 and M2 macrophage subtypes on lung cancer progression. *Sci. Rep.* 5, 14273. doi:10.1038/srep14273

Zawacka-Pankau, J., Grinkevich, V. V., Hünten, S., Nikulenkov, F., Gluch, A., Li, H., et al. (2011). Inhibition of glycolytic enzymes mediated by pharmacologically activated p53: Targeting Warburg effect to fight cancer. *J. Biol. Chem.* 286, 41600–41615. doi:10.1074/jbc.M111.240812

Zhang, Z., Lin, E., Zhuang, H., Xie, L., Feng, X., Liu, J., et al. (2020). Construction of a novel gene-based model for prognosis prediction of clear cell renal cell carcinoma. *Cancer Cell Int.* 20, 27. doi:10.1186/s12935-020-1113-6

Zhao, F., Olkhov-Mitsel, E., Kamdar, S., Jeyapala, R., Garcia, J., Hurst, R., et al. (2018). A urine-based DNA methylation assay, ProCUrE, to identify clinically significant prostate cancer. *Clin. Epigenetics* 10, 147. doi:10.1186/s13148-018-0575-z

Zhou, Y., Zhou, B., Pache, L., Chang, M., Khodabakhshi, A. H., Tanaseichuk, O., et al. (2019). Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat. Commun.* 10, 1523. doi:10.1038/s41467-019-09234-6

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 01 December 2022
DOI 10.3389/fphar.2022.1053556

Check for updates

OPEN ACCESS

EDITED BY
Peng Qu,
National Institutes of Health (NIH),
United States

REVIEWED BY
Shuai Liu,
National Heart, Lung, and Blood
Institute (NIH), United States
Kun Wang,
National Institutes of Health (NIH),
United States

*CORRESPONDENCE
Zhixia Zhou,
zhou_zhixia@126.com
Shoushi Wang,
wangshoushi1226@126.com
Peifeng Li,
peifli@qdu.edu.cn

SPECIALTY SECTION
This article was submitted to
Pharmacology of Anti-Cancer Drugs,
a section of the journal
Frontiers in Pharmacology

RECEIVED 25 September 2022
ACCEPTED 21 November 2022
PUBLISHED 01 December 2022

CITATION
Zhou Z, Cao Q, Diao Y, Wang Y, Long L, Wang S and Li P (2022), Non-coding RNA-related antitumor mechanisms of marine-derived agents.
Front. Pharmacol. 13:1053556.
doi: 10.3389/fphar.2022.1053556

COPYRIGHT
© 2022 Zhou, Cao, Diao, Wang, Long, Wang and Li. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Non-coding RNA-related antitumor mechanisms of marine-derived agents

Zhixia Zhou[1]*, Qianqian Cao[2], Yujing Diao[2], Yin Wang[1], Linhai Long[1], Shoushi Wang[2]* and Peifeng Li[1]*

[1]Institute for Translational Medicine, The Affiliated Hospital of Qingdao University, College of Medicine, Qingdao University, Qingdao, China, [2]Qingdao Central Hospital, Central Hospital Affiliated to Qingdao University, Qingdao, China

In the last two decades, natural active substances have attracted great attention in developing new antitumor drugs, especially in the marine environment. A series of marine-derived compounds or derivatives with potential antitumor effects have been discovered and developed, but their mechanisms of action are not well understood. Emerging studies have found that several tumor-related signaling pathways and molecules are involved in the antitumor mechanisms of marine-derived agents, including noncoding RNAs (ncRNAs). In this review, we provide an update on the regulation of marine-derived agents associated with ncRNAs on tumor cell proliferation, apoptosis, cell cycle, invasion, migration, drug sensitivity and resistance. Herein, we also describe recent advances in marine food-derived ncRNAs as antitumor agents that modulate cross-species gene expression. A better understanding of the antitumor mechanisms of marine-derived agents mediated, regulated, or sourced by ncRNAs will provide new biomarkers or targets for potential antitumor drugs from preclinical discovery and development to clinical application.

KEYWORDS

marine, antitumor, drug, ncRNA, biomarker

1 Introduction

High-throughput sequencing technologies have accelerated the study of biological systems related to the biological activity and function of the human genome, improving our understanding of gene expression and regulation (Dutta et al., 2021). The International Human Genome Consortium revealed that the estimated number of human protein-coding genes has shrunk from 35,000 to 20,500, or less than 3% of the total genome sequence, a surprisingly low number for our species (Liang et al., 2000; Clamp et al., 2007). As a result, there has been an explosion in research into the possible functional roles of the other nearly 98% of the human genome that does not encode proteins (Santosh et al., 2015). In fact, almost 90% of the human genome is transcribed, which means that the RNA pool must contain tens of thousands of noncoding RNA (ncRNA) transcripts with little or no protein-forming capacity (Djebali et al., 2012). Many

classes of ncRNAs with very different lengths, structures, and functions have been identified and studied by developing next-generation sequencing techniques over the past two decades (Esteller, 2011; Mehta et al., 2021). Accumulating evidence suggests that they have emerged as important regulators of gene expression by affecting chromatin architecture, DNA/RNA/histone modifications, RNA/protein scaffolds, RNA splicing, stability, and translation (Mehta et al., 2021). Hence, they are not only closely related to various physiological processes, such as cell development, differentiation, and DNA repair but also participate in the pathophysiological processes of various diseases, especially tumors (Dutta et al., 2021).

A large body of evidence indicates that ncRNAs are abnormally expressed in all types of human cancers and are associated with patient treatment, prognosis and recurrence (Slack and Chinnaiyan, 2019; Anastasiadou et al., 2018; an and Bu, 2021). Many ncRNAs have been shown to be released from tumor cells into human body fluids, such as blood saliva, urine, and breast milk, which provides strong support for ncRNAs as cancer diagnostic markers or prognostic indicators (Lei et al., 2019; Bautista-Sanchez et al., 2020; Sil et al., 2020). Meanwhile, ncRNAs have also been shown to act as oncogenes or tumor suppressor genes to regulate the occurrence and development of tumors (Figure 1), suggesting their potential as therapeutic targets for antitumor drugs (Arun et al., 2018; Toden et al., 2021; Yan et al., 2022). Moreover, recent studies have shown that some ncRNAs are also implicated in the mechanism of antitumor drugs of natural origin, including marine-derived compounds or derivatives (agents) (Schnekenburger et al., 2014; Liu et al., 2020).

In this review, we first briefly describe the main information known about ncRNAs in tumors and marine-derived antitumor agents. We then focus on the scientific progress made regarding the antitumor mechanisms of various marine-derived agents associated with ncRNAs. In addition, we recapitulated the potential value of marine food-derived ncRNAs as antitumor agents by mediating gene expression across species. This helps to elucidate the antitumor mechanism of marine-derived antitumor agents and reveal the potential value of ncRNAs as biomarkers for marine drug-related tumor treatment, prognosis and recurrence and even as sources of marine antitumor drugs.

2 ncRNA in tumors

According to length, ncRNAs can be divided into two categories: short/small ncRNAs and long ncRNAs (lncRNAs). Short/small ncRNAs are transcribed RNA molecules less than 200 nt in length and usually <30 nt. This type of ncRNA includes at least four types: microRNAs (miRNAs), small nucleolar RNAs (snoRNAs), transfer RNA-derived small RNAs (tsRNAs), and piwi-interacting RNAs (piRNAs) (Cech and Steitz, 2014; Slack and Chinnaiyan, 2019). Among them, the most abundant and most investigated short/small ncRNAs are miRNAs, which generally act as negative gene regulators of their target RNAs, especially messenger RNAs (mRNAs), through RNA cleavage, degradation, or translational repression (Vishnoi and Rani, 2017; Slack and Chinnaiyan, 2019). Long ncRNAs are generally longer than 200 nt, and their transcripts consist of multiple exons, 5′ caps and 3′ poly(A) tails but lack open reading frames (ORFs) (Clark and Mattick, 2011). Compared with miRNAs, most lncRNAs have lower sequence conservation among species, but they are implicated in almost all levels of gene expression: epigenetic, transcriptional, and posttranscriptional. They usually interact with other nucleic acid molecules (such as DNA, mRNA, and miRNA) or proteins to act as molecular signals, decoys, guides, or scaffolds (Wang and Chang, 2011; Robinson et al., 2020). In addition, two special cases of lncRNAs have received extensive attention, namely, pseudogenes and circular RNAs (circRNAs) (Groen et al., 2014; Grander and Johnsson, 2016; Wilusz, 2018; Kristensen et al., 2019; Zhou et al., 2020), whose roles and mechanisms remain to be further explored. Dysregulation of both short/small and long ncRNAs can contribute to tumorigenesis and progression (Lekka and Hall, 2018).

2.1 miRNAs

miRNAs are by far the most well-characterized ncRNA group in cancer, with more than 70,000 publications in PubMed concerning cancer-related miRNAs. This miRNA dysregulation or genetic changes have been observed in nearly all cancer types studied, and more than 50% of miRNA genes are located in cancer-associated genomic regions or in fragile sites, indicating the important role for miRNAs in the pathogenesis of human cancers (Zhang et al., 2007; Di Leva et al., 2014). According to their effects on tumors, these cancer-associated miRNAs are mainly divided into two subclasses: oncogenic miRNAs (or "tumor-miRs") and tumor suppressor miRNAs (Yan et al., 2022). Examples of well-characterized oncogenic miRNAs include miR-21, miR-141, miR-155, and miR-215, which are widely overexpressed in a variety of systemic tumors, including gastrointestinal, respiratory, hematological, and reproductive system tumors (Bayraktar and Van Roosbroeck, 2018; Vychytilova-Faltejskova and Slaby, 2019; Luo et al., 2022; Yan et al., 2022). Conversely, the let-7 family, miR-34a, miR-145, and miR-29 targets are regarded as tumor suppressors and can inhibit tumor formation, proliferation, migration, spread, and angiogenesis by targeting many oncogenic transcription factors, including E2F1, K-RAS and c-MYC (Yang et al., 2015; Alizadeh et al., 2019; Zhang et al., 2019; Xu et al., 2020). Interestingly, several miRNAs appear to possess dual functionality, acting as both tumor suppressors and oncogenes. Interestingly, some miRNAs seem to have a double-edged sword-like effect on tumor progression, playing

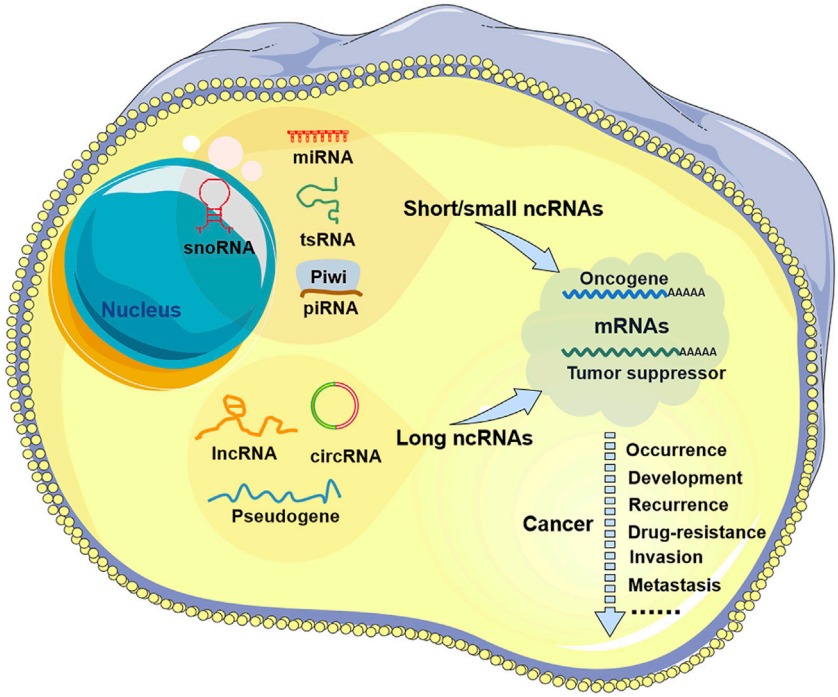


FIGURE 1
Noncoding RNA in tumors. There are two types of non-coding RNAs (ncRNAs) in tumor cells: short/small ncRNAs and long ncRNAs. Short/small ncRNAs include at least four types: microRNAs (miRNAs), small nucleolar RNAs (snoRNAs), transfer RNA-derived small RNAs (tsRNAs), and piwi-interacting RNAs (piRNAs). Long ncRNAs include common lncRNAs, circular RNAs (circRNAs), and pseudogenes. These ncRNAs are aberrantly expressed in all types of human cancers and are involved in tumor progression, including tumor occurrence, development, recurrence, drug resistance, invasion, and metastasis.

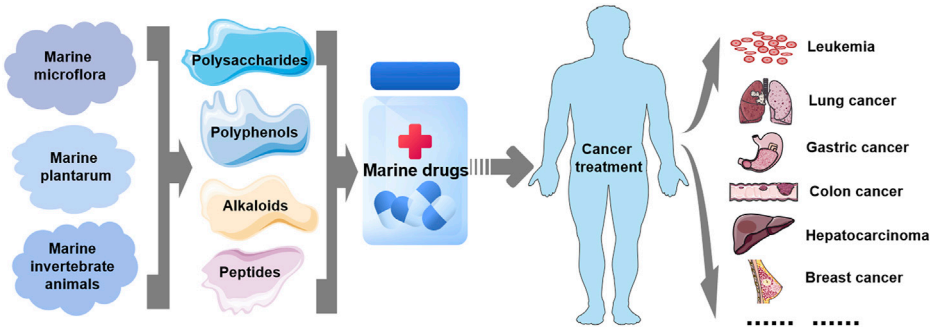


FIGURE 2
Marine-derived antitumor drugs. Antitumor substances of marine natural origin include polysaccharides, polyphenols, alkaloids, and peptides extracted from marine organisms, including microflora, plants and invertebrates. At present, several marine-derived antitumor substances have been used as drugs for the treatment of clinical human tumors, including leukemia and lung, gastric, colon, liver, breast, and liver cancers.

diametrically opposite promotion or inhibition effects on tumors at different stages, such as miR-200c (Korpal et al., 2011; Pecot et al., 2013; Toiyama et al., 2014; Yan et al., 2022). In agreement with their oncogenic or tumor-suppressive roles, miRNA-targeting cancer therapeutic strategies include miRNA mimics, small-molecule antagonists/drugs, or miRNA inhibitors based on oligonucleotide, sponge, or CRISPR/Cas9 genome editing (Regazzi, 2018; He et al., 2020; Yan et al., 2022). The therapeutic strategies for miRNA delivery mainly rely on liposomes, cationic polymers, peptide-based transmembrane structures, adeno-associated viral vectors, and exosomes (Arun et al., 2018; Liu et al., 2020; Sil et al., 2020).

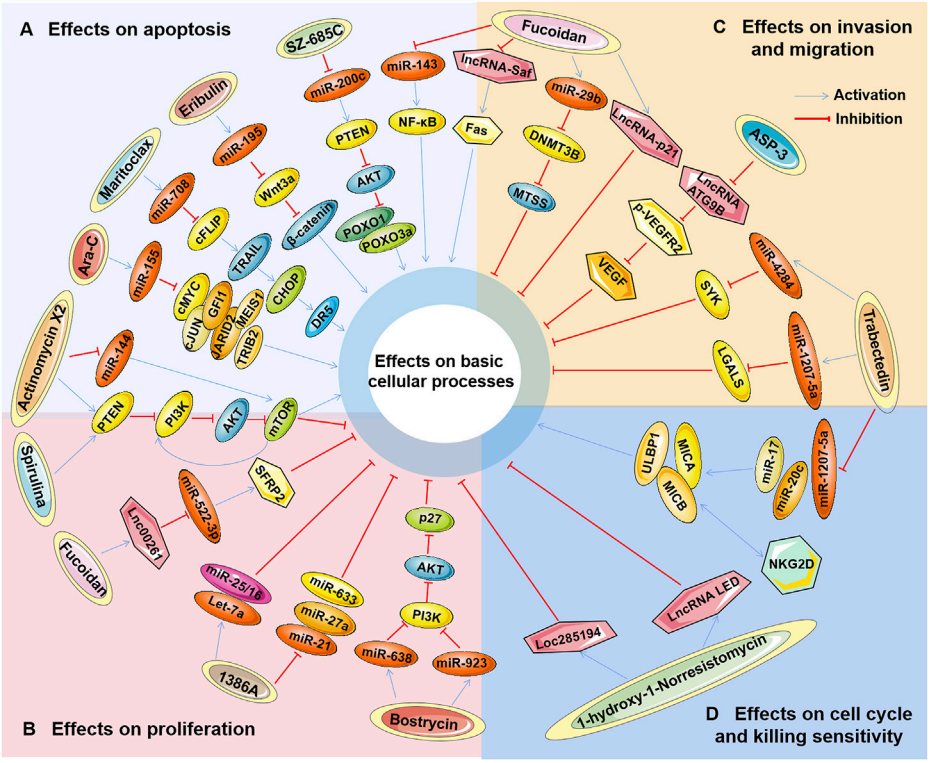


FIGURE 3
The antitumor effects of marine-derived antitumor agents mediated by non-coding RNAs in basic cellular processes. Marine-derived antitumor agents (SZ-685C, eribulin, maritoclax, trabectedin, spirulina, bostrycin, cytarabine, fucoidan, 1386A, actinomycin X2,1-hydroxy-1-norresistomycin, and ASP-3) can induce tumor cell apoptosis **(A)**, inhibit cell proliferation **(B)**, invasion, and metastasis **(C)**, inhibit cell cycle arrest, and enhance tumor cell-killing sensitivity to immune cells **(D)** by upregulating oncogenic noncoding RNA (ncRNA), or downregulate tumor suppressor ncRNAs.

2.2 lncRNAs

To date, approximately 60,000 aberrantly expressed lncRNAs have been identified from human tumor tissues and cancer cell lines, and although the functions of most RNAs in tumors are not well defined, there are approximately 30,000 publications about cancer-related lncRNAs on PubMed (Iyer et al., 2015). Similar to miRNAs, cancer-related lncRNAs also act as oncogenes or suppressors to regulate cancer initiation, angiogenesis, metastasis, and drug resistance. Among them, the more well-studied oncogenic lncRNAs are mainly GHET1, ANRIL, MALAT1, PCAT-1, HOTAIR, HOTTIP, and PCA3, which are often overexpressed in cancer and are associated with poor patient outcomes (Clark and Mattick, 2011; Regazzi, 2018). In contrast, GAS5, MEG3, LincRNA-p21, NCRNACCND1, and CASC15-S, which are cancer suppressors, are generally underexpressed in cancer and are related to good patient prognosis (an and Bu, 2021; Yan et al., 2022; Tang et al., 2017). The functional mechanism of these lncRNAs in tumor cells involves a variety of cell signaling pathways related to cellular processes, such as p53, nuclear transcription factor-κB (NF-κB), phosphatidyl inositol 3-kinase (PI3K)/protein kinase B (AKT), extracellular regulated protein kinases (ERK)/mitogen-activated protein kinase (MAPK), wingless-type MMTV integration site family (Wnt)/β-chain protein (β-catenin), and hypoxia-inducible factor-1α (HIF1α) (Peng et al., 2017). In addition, the competing endogenous RNA (ceRNA) regulatory network of lncRNA–miRNA-mRNA is an important molecular mechanism for ncRNA functions in tumor pathogenesis (Lei et al., 2019). Emerging therapeutic strategies targeting lncRNAs mainly focus on the clearance of oncogenic lncRNAs mediated by RNA interference (RNAi), morpholino-based antisense oligonucleotides (MO-ASOs), natural antisense RNAs (NATs), CRISPR–Cas9 genome editing, *etc.* (Schnekenburger et al., 2014; Slack and Chinnaiyan, 2019).

2.3 Other ncRNAs

The success of miRNA-based cancer research has promoted not only the research progress of lncRNAs but also research on other ncRNA families and identified them as potential therapeutic targets for cancer, such as circRNAs, tsRNAs,

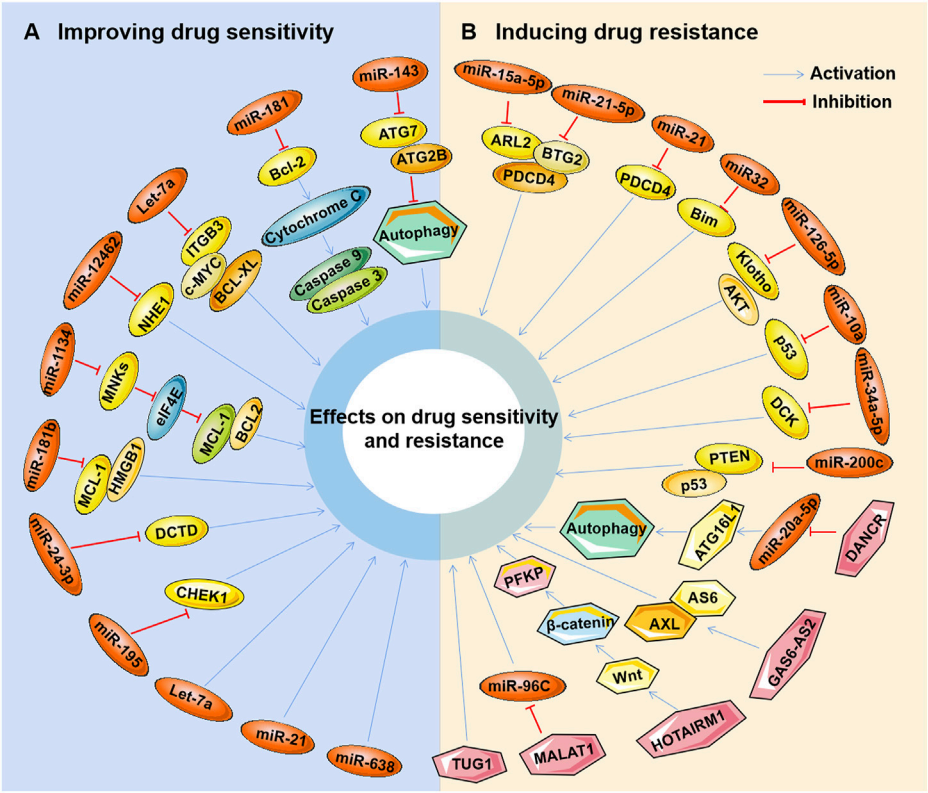


FIGURE 4
Marine-derived antitumor agent-related non-coding RNAs in tumor cell drug sensitivity and resistance. Non-coding RNAs (ncRNAs) regulated by marine-derived antitumor agents (cytarabine or trabectedin) can be used as tumor suppressors to induce the drug sensitivity of tumor cells **(A)** or as oncogenes to induce cellular drug resistance **(B)**.

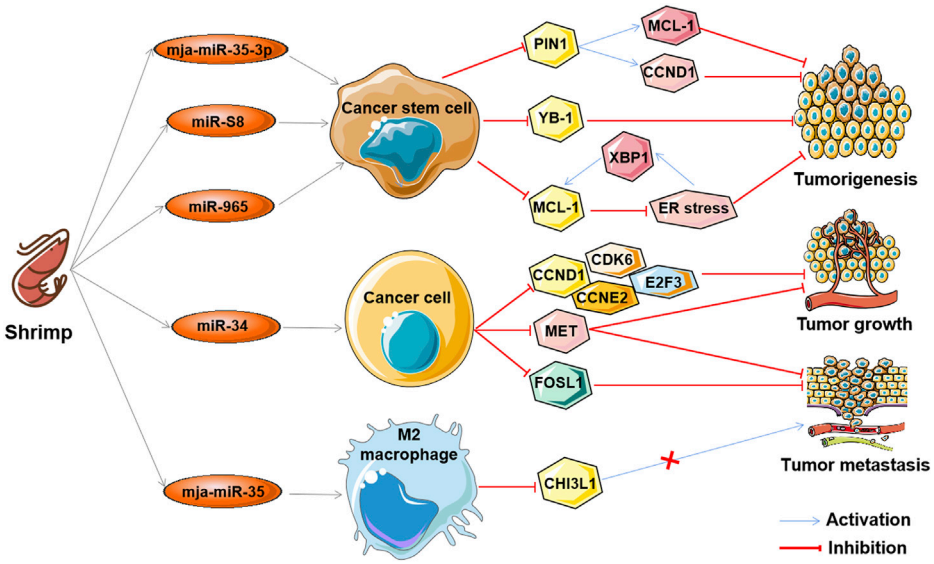


FIGURE 5
The antitumor effects of marine shrimp-derived microRNAs. microRNAs (miRNAs) derived from marine shrimp (*Marsupenaeus japonicus*) exhibit antitumor ability by regulating cross-species gene expression to inhibit human tumorigenesis, tumor growth, and tumor metastasis.

10.3389/fphar.2022.1053556

TABLE 1 Marine-derived agents associated with ncRNAs in tumor fundamental cellular processes.

Marine-derived agents (source)	Related ncRNAs	ncRNA expression regulated by agents	ncRNA roles in tumor	Related cellular processes	Related genes	Tumor types	References
miRNA-related marine-derived agents							
SZ-685C (Angrove endophytic fungus)	miR-200c	Down-regulated	Oncogene	Inhibition of cell proliferation, induction of apoptosis	PTEN, p-AKT, FOXO1, FOXO3a	Pituitary tumor	Xie et al., (2010); Chen et al., (2013a); Liao et al., (2013)
Eribulin (*Halichondria okadai*)	miR-195	Up-regulated	Suppressor	Induction of apoptosis	Wnt3a, β-catenin	Breast cancer	Green et al., (2013); Furuya et al., (2016); Yu et al., (2018a)
Maritoclax (*Streptomyces*)	miR-708	Up-regulated	Suppressor	Induction of apoptosis	cFLIP, TRAIL, CHOP, DR5	Renal carcinoma	Jeon et al., (2018); Sun et al., (2019)
Trabectedin (*Ecteinascidia turbinata*)	miR-4284, miR-1207-5, miR-1225-5p	Up-regulated	Suppressor	Induction of apoptosis, inhibition of cell migration and motility	SYK, LGALS1	Biliary tract cancer	Peraldo Neia et al. (2016)
	pri-miR-17-92, miR-17, miR-20a	Up-regulated	Suppressor	Induction of apoptosis, cell cycle arrest, cellular stress, and DNA damage	IRF4, IKZF1, MICA, MICB, ULBP1, NKG2D	Multiple myeloma	Cuce et al. (2019)
Spirulina (*Cyanobacteria*)	miR-34a, miR-125b	Up-regulated	Suppressor	Inhibition of cell proliferation	PI3K, AKT, mTOR	Glioma	Arab et al., (2021); Braune et al., (2021)
Bostrycin (*Alternaria eichhorniae*)	miR-638, miR-923	Up-regulated	Suppressor	Inhibition of cell proliferation	PT3K, AKT, p27	Lung cancer	Chen et al., (2011); Chen et al., (2012a)
Cytarabine (*Cryptotethia crypta*)	miR-155	Up-regulated	Suppressor	Inhibition of cell proliferation, induction of cell differentiation and apoptosis	cMYC, cJUN, GFI1, JARID2, MEIS1, TRIB2	Acute myeloid leukemia	Palma et al. (2014)
Fucoidan (Brown seaweed)	miR-29, miR-143	Up-regulated	Suppressor	Inhibition of cell survival, proliferation, invasive, and EMT process	DNMT3B, MTSS1, NF-κB pathway	Hepatocellular carcinoma	Yan et al., (2015); Zheng et al., (2018); El-Far et al., (2021)
1386A (Mangrove fungus)	let-7a, miR-15/16	Up-regulated	Suppressor	ND	ND	Breast cancer	Tang et al. (2012)
	miR-21, miR-27a, miR-633	Down-regulated	Oncogene	ND	ND	Breast cancer	Tang et al. (2012)
Actinomycin X2 (*Streptomyces*)	miR-144	Up-regulated	Oncogene	Inhibition of cell proliferation, induction of apoptosis	PTEN, PI3K, AKT, mTOR	Prostate cancer	Liu et al. (2016)
lncRNA-related marine-derived agentsH							
Fucoidan (Brown seaweed)	LINC00261	Up-regualted	Suppressor	Inhibition of cell proliferation and invasion	miR-522-3p, SFRP2	Hepatocellular carcinoma	Ma et al. (2021)
	lncRNA-p21	Up-regualted	Suppressor	Inhibition of cell invasion and metastasis	ND	Hepatocellular carcinoma	Yan et al. (2019)
	lncRNA-Saf	Down-regualted	Oncogene	Induction of apoptosis	Fas	Hepatocellular carcinoma	Yan et al., (2005); Yan et al., (2019)
1-hydroxy-1-Norresistomycin (*Streptomyces*)	LED, LOC285194	Up-regualted	Suppressor	Inhibition of cell proliferation, invasion, and metastasis, induction of apoptosis and cell cycle arrest	p53, p21	Lung cancer	Ramalingam et al. (2018)
ASP-3 (*Arca subcrenata*)	ATG9B	Down-regualted	Oncogene	Inhibition of invasion and metastasis	VEGFR2	Hepatocellular carcinoma	Guo et al. (2019)

ND, not determined.

piRNAs, and snoRNAs. Compared with the first two ncRNAs, there are relatively few studies on these ncRNAs. For example, there are fewer than 5,000 literature reports on tumor-related circRNAs on PubMed. The expression profiles of circRNAs in human cancers are diverse, depending on the type of cancer (Li et al., 2021). That is, each type of tumor has only its own unique abnormally expressed

RNA, and the expression of a given circRNA is significantly different in different cancer types. Therefore, the tissue and cancer specificity of circRNA expression profiles makes them potentially reliable prognostic markers for cancer diagnosis and therapy. At present, well-characterized tumor-related circRNAs are mainly from breast, lung, liver, and gastric cancers. These circRNAs have been reported to be involved in cancer development as oncogenes or tumor suppressor genes, ranging from tumor growth and metastasis to drug resistance, such as circAGFG1, CircDnmt1, circHER2 circTADA2A-E6, and Circ0025202 (Kristensen et al., 2018; He et al., 2021; Zhou et al., 2021). tsRNAs are the second most abundant member of the sncRNA family after miRNAs (Lee et al., 2009). They are often highly expressed in cancers under stress and correlate with the clinical stage of cancer (Fu et al., 2009; Gebetsberger and Polacek, 2013). Moreover, tsRNA expression levels are also elevated in the serum and urine of cancer patients, suggesting their potential as diagnostic markers for cancer (Lakings et al., 1977; Gehrke et al., 1979). piRNAs were originally thought to be mainly expressed in the reproductive system but not in cancer (Siddiqi and Matushansky, 2012). Recently, increasing evidence has shown that piRNAs are not only abnormally expressed in cancer but also related to the mechanism of tumor occurrence and development. For example, piRNA-823, piRNA-54265A, and piR-651 are highly expressed in tumors and play a role in promoting cancer development (Feng et al., 2020; Mai et al., 2020). Similarly, the functions of snoRNAs have been considered for many years to be those of housekeeping genes; however, there is increasing evidence that high expression of certain snoRNAs also has oncogenic effects (Thorenoor and Slaby, 2015), such as snoRNA U50 and SNORA42 (Tanaka et al., 2000; Mei et al., 2012). However, despite the important roles of these ncRNAs in cancer, their functional and mechanistic studies in tumorigenesis are still at an early stage and require further investigation.

3 Marine-derived antitumor agents

As described above, the emergence of tumor-related ncRNAs provides a new alternative for cancer diagnosis and therapy. However, compared with many other potential targets (oncogenes, mutations, gene fusion, *etc.*), the advantages of targeted ncRNA therapy in cancer are still unclear, and their effective application in clinical cancer intervention remains challenging and is limited by many obstacles, such as off-target effects, toxicity of the delivery system, and synthetic ncRNA immunogenicity (Peng et al., 2017). At present, chemotherapy is one of the most effective methods for the treatment of cancer, especially for patients with advanced tumors or with a tendency to spread throughout the body. However, it is a systemic treatment that allows them to destroy or eliminate tumor cells while also causing damage to normal cells, bringing adverse reactions to patients. In addition, tumor cells have a certain resistance to the effects of chemotherapeutic drugs through self-regulation, which significantly reduces the efficacy of chemotherapeutic drugs (Perez-Herrero and Fernandez-Medarde, 2015). Therefore, these limitations limit the clinical application of chemotherapeutic drugs and are further incentives to find other effective treatments with fewer side effects.

Over the past 20 years, emerging evidence has shown that natural products, including marine-derived compounds or derivatives, represent an available source of antitumor drugs, new drugs and chemical entities, whose biologically active ingredients exhibit potent cytotoxic effects on tumor cells with few side effects (Newman et al., 2003; Wu et al., 2021). The antitumor effects of these marine-derived substances have been or are being demonstrated *in vitro*, *in vivo* (animal models), and in preclinical and clinical trials (Newman and Cragg, 2012; Newman and Cragg, 2014). Many have been approved as antitumor drugs for clinical cancer treatment, such as cytarabine, eribulin mesylate, and trabectidine (Khalifa et al., 2019). As shown in Figure 2 these marine-derived agents derived from marine plants and microbes, the Caribbean sponges (*Cryptotethya crypta*), were the first to be studied with tumor cytotoxicity, followed by those derived from many other organisms, ranging from marine microflora (bacteria, actinomycetes, cyanobacteria, and fungi) and plantarum (microalgae and macroalgae) to invertebrate animals (e.g., sponges, soft corals, nudibranchs, and tunicates) (Khalifa et al., 2019). However, the antitumor mechanisms of these marine-derived agents remain to be further studied. With the advancement of science and technology and extensive research on the ocean, such as deep-sea mining, extraction and separation, molecular modification, genetic engineering, and organic synthesis (Wu et al., 2021), an increasing number of organisms and their metabolites have been used in the study of marine natural antitumor agents. However, the antitumor mechanism, clinical safety and treatment strategy of the active ingredients of marine-derived agents still need to be further studied. Evidence suggests that their toxic effects on tumor cells are achieved by targeting cancer-related molecules expressed by cancer cells, such as macromolecules in signal transduction pathways (Newman et al., 2003; Wu et al., 2021). Moreover, recent studies have shown that several small molecules are also involved in the antitumor mechanism of marine-derived substances, including ncRNAs.

4 ncRNAs as oncogenes or tumor suppressors involved in the antitumor mechanism of marine-derived agents

Studies have found that marine-derived agents exert antitumor activity by interfering with basic cellular processes of tumor cells, such as proliferation, apoptosis, autophagy and

TABLE 2 Marine-derived agents associated with ncRNAs in tumor drug sensitivity and resistance.

Aghents (source)	Related ncRNAs	ncRNA expression regulated by agents	ncRNA roles in tumor	Related cellular processes	Related genes	Tumor types	References
miRNA-related marine-derived agents							
Cytarabine (*Cryptotethia crypta*)	miR-143	Down-regulated	Suppressor	Promotion or maintenance of cellular drug sensitivity	ATG7, ATG2B	Acute myeloid leukemia	Zhang et al. (2020c)
	miR-181a	Down-regulated	Suppressor	Promotion or maintenance of cellular drug sensitivity	Bcl-2, Caspase 9/3, Cytochrome C	Acute myeloid leukemia	Bai et al. (2012)
	miR-12462	ND	Suppressor	Promotion or maintenance of cellular drug sensitivity	NHE1	Acute myeloid leukemia	Jia et al. (2020)
	let-7a	ND	Suppressor	Promotion or maintenance of cellular drug sensitivity	SDF-1α, CXCR4, YY1, ITGB3, c-MYC, BCL-XL	Acute myeloid leukemia	Chen et al. (2013b)
	miR-29a, miR-30c, miR-625-3p	ND	Suppressor	Promotion or maintenance of cellular drug sensitivity	ND	Acute myeloid leukemia	Russ et al., (2011); Jiang et al., (2018)
	miR-134	Down-regulated	Suppressor	Promotion or maintenance of cellular drug sensitivity	eIF4E, MNKs, MCL-1, BCL2	Acute myeloid leukemia	Chen et al. (2018a)
	miR-181b	Down-regulated	Suppressor	Promotion or maintenance of cellular drug sensitivity	Mcl-1, HMGB1	Acute myeloid leukemia	Lu et al. (2014)
	miR-24-3p	Down-regulated	Suppressor	Promotion or maintenance of cellular drug sensitivity, inhibition of autophagy	DCTD	Acute myeloid leukemia	Bhise et al. (2019)
	miR-15a-5p, miR-21-5p	UP-regulated	Oncogene	Induction of cellular drug resistance	PDCD4, ARL2, BTG2	Acute myeloid leukemia	Vandewalle et al. (2021)
	miR-21	UP-regulated	Oncogene	Induction of cellular drug resistance	PDCD4	Acute myeloid leukemia	Vandewalle et al. (2021)
	miR-32	UP-regulated	Oncogene	Induction of cellular drug resistance	Bim	Acute myeloid leukemia	Vandewalle et al. (2021)
	miR-126-5p	UP-regulated	Oncogene	Induction of cellular drug resistance	*Klotho*, Akt	Acute myeloid leukemia	Shibayama et al. (2015)
	miR-10a	UP-regulated	Oncogene	Induction of cellular drug resistance	p53	Acute myeloid leukemia	Vu et al. (2021)
	miR-34a-5p	UP-regulated	Oncogene	Induction of cellular drug resistance	DCK	Acute myeloid leukemia	Bhise et al. (2019)
	miR-200	UP-regulated	Oncogene	Induction of cellular drug resistance	PTEN, p53	Colon cancer	Chen et al. (2014)
Trabectedin (*Ecteinascidia turbinata*)	miR-130a, miR-19a, miR-125a-5p...	Up-regulated	Oncogene	Induction of cellular drug resistance	FUS-CHOP fusion gene	Myxoid liposarcoma	Grosso et al., (2007); Forni et al., (2009); Uboldi et al., (2012)
	miR-195	ND	Suppressor	Promotion or maintenance of cellular drug sensitivity	CHEK1	Lung cancer	Yu et al. (2018b)
lncRNA-related marine-derived agents							
Cytarabine (*Cryptotethia crypta*)	DANCR	Up-regualted	Oncogene	Induction of cellular drug resistance and autophagy	miR-20a-5p, ATG16L1	Acute myeloid leukemia	Zhang et al. (2021b)

(Continued on following page)

TABLE 2 (*Continued*) Marine-derived agents associated with ncRNAs in tumor drug sensitivity and resistance.

Aghents (source)	Related ncRNAs	ncRNA expression regulated by agents	ncRNA roles in tumor	Related cellular processes	Related genes	Tumor types	References
	GAS6-AS2	Up-regualted	Oncogene	Induction of cellular drug resistance	AS6, AXL	Acute myeloid leukemia	Bester et al. (2018)
	HOTAIRM1	ND	Oncogene	Induction of cellular drug resistance, glucose consumption, and lactate production	Wnt, β-catenin, PFKP	Acute myeloid leukemia	Chen et al. (2020)
	MALAT1	ND	Oncogene	Induction of cellular drug resistance	miR-96C	Acute myeloid leukemia	Goyal et al. (2021)
	TUG1	Up-regualted	Oncogene	Induction of cellular drug resistance	ND	Acute myeloid leukemia	Luo et al. (2018)

ND, not determined.

the cell cycle. Mechanistically, it was found that several marine substances could affect the expression levels of ncRNAs (mainly miRNAs and lncRNAs) in tumor cells and then affect their functions so that the antitumor functions of these marine-derived substances depend at least to some extent on the ncRNAs they affect, including SZ-685C, eribulin, maritoclax, trabectedin, spirulina, bostrycin, cytarabine, fucoidan, 1386A, actinomycin X2, 1-hydroxy-1-norresistomycin, and ASP-3 Figure 3 and (Table 1). Furthermore, the sensitivity or tolerance of tumor cells to these marine-derived agents (cytarabine and trabectedin) can also be affected by these marine-agent-related ncRNAs, as shown in Figure 4 and Table 2. The data suggest that marine-derived agent-related ncRNAs can act as oncogenes or tumor suppressor genes to mediate or regulate the antitumor activity of marine-derived agents.

4.1 Regulation of basic cellular processes in tumor cells (apoptosis, proliferation, cell cycle, invasion, and migration) by ncRNA-related marine-derived drugs

4.1.1 miRNA-related marine-derived agents

SZ-685C is a marine anthraquinone isolated from a mangrove endophytic fungus, and its anticancer effects have been demonstrated in various tumors, including breast, prostate, liver, and pituitary cancers and glioma (Xie et al., 2010; Chen et al., 2013a). SZ-685C inhibited the proliferation ability of tumor cells *in vitro* and xenograft tumor growth *in vivo* (Xie et al., 2010; Chen et al., 2013a). In addition, SZ-685C has a direct proapoptotic effect by downregulating the phosphorylation of AKT (p-AKT) and its downstream factors forkhead box protein O1 (FOXO1) and FOXO3a (Xie et al., 2010). Regarding pituitary tumor cells, the apoptotic capacity induced by Z-685C exhibited a microRNA-200c (miR-200c) dependency (Chen et al., 2013a). Treatment with SZ-685C significantly downregulated the expression level of miR-200c in tumor cells, while overexpression of miR-200c significantly attenuated SZ-685C-induced apoptosis (Chen et al., 2013a). MicroRNA-200c has been reported to act as an oncogene to inhibit the apoptosis of pituitary adenoma cells by decreasing phosphatase tensin homolog (PTEN) expression but upregulating the expression of p-AKT (Liao et al., 2013). These results suggest that SZ-685C exerts antitumor effects by targeting the miR-200c/PTEN/Akt/FOXO pathway to induce apoptosis.

Eribulin (eribulin mesylate), a synthetic analog of a natural polyether macrolide derived from marine sponges, is a tubulin-interacting cytotoxic agent that was approved for the treatment of metastatic breast cancer and soft tissue sarcoma (STS) (Seetharam et al., 2018). The antitumor activity of eribulin is mainly manifested by downregulating Rho to inhibit cell migration and by promoting cell apoptosis through upregulating p53 and BAX (Wiemer et al., 2017). Moreover, studies have shown that miRNAs are also involved in the antitumor mechanism of eribulin. Furuya reported that the expression profiles of miRNAs in different types of triple-negative breast cancer (TNBC) cells treated with eribulin were significantly different, including miR-195, which has been reported to significantly impact oncogenicity in various cancers (Furuya et al., 2016; Yu et al., 2018a). Compared with the basal-like TNBC cells of HCC1143, the expression level of miR-195 was significantly decreased compared to the nonbasal-like TNBC cells of MDA-MB 231. Correspondingly, the expression of Wnt3a, a target of miR-195, was significantly lower in MDA-MB-231 cells treated with eribulin (Furuya

et al., 2016). Since the inhibition of Wnt3a can lead to the inactivation of the Wnt/β-catenin signaling pathway and induce cancer cell apoptosis, the apoptosis level of MDA-MB-231 cells after eribulin treatment is significantly higher than that of HCC1143 cells (Green et al., 2013; Furuya et al., 2016). These results confirm the role of miRNAs in the antitumor effect of eribulin and may explain the different susceptibilities to eribulin in patients with different breast cancer types.

Maritoclax, a natural compound isolated from marine *Streptomyces*, is a B-cell lymphoma-2 (BCL-2) family inhibitor that induces proteasomal degradation of myeloid cell leukemia-1 (MCL-1) (Haste et al., 2011; Doi et al., 2012). The antitumor effect of maritoclax by inducing apoptosis has been demonstrated in tumors (Pandey et al., 2013; Doi et al., 2014; Varadarajan et al., 2015; Jeon et al., 2018). In addition, maritoclax was recently shown to enhance TRAIL-induced apoptosis in tumor cells. Jeon found that combined treatment with maritoclax and TRAIL markedly induced apoptosis in different types of tumor cells, including kidney, lung, and liver cancer cells (Jeon et al., 2018). C/EBP homologous protein (CHOP)-mediated upregulation of death receptor 5 (DR5) was then proven to be involved in the enhancement of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis by maritoclax (Jeon et al., 2018). Meanwhile, the expression of miR-708, which is considered an oncogene or a tumor suppressor gene in different tumors, was significantly increased by maritoclax (Jeon et al., 2018; Sun et al., 2019). Furthermore, it was found that miR-708 could mediate the downregulation of cellular FLICE-inhibitory protein (cFLIP), which played a negative role in the enhancement of TRAIL-induced apoptosis by maritoclax (Jeon et al., 2018). This suggests that miR-708 acts as a tumor suppressor gene involved in the apoptosis induced by maritoclax combined with TRAIL.

Trabectedin (Ecteinascidin-743 or NSC 684766) is a tetrahydroisoquinoline alkaloid isolated from the marine tunicate *Ecteinascidia Turbinata* that has a potent antitumor effect by regulating various transcription factors involved in cell proliferation and the cell cycle (Trabectedin, 2006). Trabectedin has been approved for the clinical treatment of cancer, primarily ovarian cancer and soft tissue sarcoma (Krasner et al., 2012; Di Giandomenico et al., 2014). Trabectedin was also found to have antitumor activity against other types of tumors, including biliary tract cancer (BTC) and multiple myeloma (MM) (Peraldo Neia et al., 2016; Cuce et al., 2019). In trabectedin-treated BTC cells, the expression of genes involved in tissue development and apoptotic processes was activated, whereas genes involved in cell migration and motility were inhibited, including spleen tyrosine kinase (SYK) and lectin galactoside-binding soluble 1 (LGALS1). Further research showed that silencing SYK or LGALS1 significantly inhibited the migration of BTC cells (Peraldo Neia et al., 2016). miRNA expression profiling of trabectedin-treated BTC cells revealed that 24 microRNAs were deregulated by trabectedin, while 5 microRNAs were perturbed. Interestingly, SYK is a putative target of all upregulated microRNAs, including miR-4284, while LGALS1 is a predicted target of miR-1207-5p and miR-1225-5p (Peraldo Neia et al., 2016). All three miRNAs have been reported to function as tumor suppressor genes in cancer (Aydogdu et al., 2012; Yang et al., 2014), which suggests further investigation into the interaction of these miRNAs with their targets SYK and LGALS1. Regarding multiple myeloma (MM) cells, trabectedin also exerts potent antitumor activity by inducing apoptosis, cell cycle arrest, cellular stress, and DNA damage *in vitro* and in 3D models (Baruchel et al., 2012). Moreover, trabectedin inhibited the expression of interferon regulatory factor 4 (IRF4), IKAROS family zinc finger 1 (IKZF1) and the miR-17 family (pri-miR-17-92, miR-17, and miR-20a) in MM cells, resulting in the upregulation of MICA, MICB, and ULBP1, ligands of natural killer (NK) cell activating receptor NKG2D, which ultimately lead to the activation and degranulation of NK cells (Cuce et al., 2019). These results suggest that trabectedin treatment not only induces tumor cell suppression but also enhances their lysis susceptibility to immune cells.

Spirulina (Spi), a nutritionally valuable dietary supplement isolated from blue–green algae (*Cyanobacteria*), has long been reported to have antitumor properties (ElFar et al., 2022). Further study found that Spi exhibits significant anti-proliferative effects on glioma cells in both *in vitro* and *in vivo* models by decreasing the expression of PI3K/AKT/mammalian target of rapamycin (mTOR) and increasing the expression of miR-34a and miR-125b (Arab et al., 2021; Braune et al., 2021). Both miR-34a and miR-125a-5p were reported to have suppressive effects in cancer *via* the PI3K/AKT/mTOR pathway (Li et al., 2018; Zhang et al., 2020a). Reports suggest that Spi exerts antitumor effects by targeting the miR-34a/PI3K/AKT/mTOR or miR-125b/PI3K/AKT/mTOR pathway to inhibit cell proliferation.

Bostrycin is a classical compound isolated from marine fungi, and its antitumor activity has been demonstrated in prostate, gastric, and lung cancers (Chen et al., 2011; Chen et al., 2012a; Jie et al., 2020). It can significantly inhibit the proliferation of tumor cells, promote apoptosis, and cause cell cycle arrest in the G0/G1 phase (Chen et al., 2011; Chen et al., 2012a). The expression levels of microRNA-638 and microRNA-923 were significantly upregulated in bostrycin-treated lung adenocarcinoma cells. Furthermore, the downregulation of AKT and the upregulation of the cell cycle protein p27 were shown in bostrycin-treated cells (Chen et al., 2011). microRNA-638 has been reported to function as a tumor suppressor gene in tumor cells to regulate cell proliferation, apoptosis, metastasis and autophagy (Wang et al., 2017a; Zhang et al., 2021a; Xu et al., 2021). However, the role of microRNA-923 in tumors and its mechanism still need further study.

Cytarabine (Ara-C), a nucleoside analog derived from sponge, is the first marine-derived drug approved for the treatment of human leukemia and lymphomatous meningitis by disrupting DNA synthesis to induce cell apoptosis (Burnett

et al., 2011). Palma found that Ara-C significantly increased the expression level of miR-155 in acute myeloid leukemia (AML) cells, and inhibition of miR-155 made cells resistant to cytarabine-induced apoptosis and inhibited the ability of cells to differentiate (Palma et al., 2014). Ectopic expression of miR-155 in AML cells resulted in enhanced cell differentiation capacity, increased apoptosis, and decreased cell growth and clonogenic capacity, suggesting that miR-155 has tumor-suppressive effects. It was further confirmed that multiple apoptosis- and differentiation-related transcriptional regulators were target genes of miR-155, including c-MYC, c-JUN, Jumonji (JARID2), myeloid ecotropic viral integration site 1 (MEIS1), growth factor independent 1 transcriptional repressor (GFI1) and tribbles pseudokinase 2 (TRIB2) (Palma et al., 2014). These results suggest that Ara-C could induce apoptosis and differentiation of AML cells by upregulating the tumor suppressor miR-155.

Fucoidan is a sulfated polysaccharide isolated from brown seaweed and has various pharmacological effects, including antitumor effects (Wu et al., 2016; Deepika et al., 2019). It is able to induce apoptosis of various tumor cells *in vitro* by regulating many signaling pathways, such as caspase family proteins, transforming growth factor (TGF) receptor, Smad 4 protein, and secreted frizzled-related protein 2 (SFRP2) (Senthilkumar and Kim, 2014; Ma et al., 2021). A total of 53 miRNAs were affected by fucoidan in a 24-hour test study of healthy volunteers ingesting a single dose of 1 g of fucoidan for 24 h (Gueven et al., 2020). These miRNAs affected 29 different pathways and processes, including cancer-related pathways, consistent with most previous results obtained in the laboratory. This study shows that even a single dose of a fucoidan drug has the potential to affect the expression of genes involved in basic cellular processes (Gueven et al., 2020). Analysis of the microRNA expression profiles of fucoidan-treated HCC cells revealed that the expression level of miR-29b was markedly upregulated by fucoidan. miR-29b was subsequently shown to act as a tumor suppressor gene to inhibit the EMT process by targeting the DNA methyltransferase (DNMT) 3B-metastasis suppressor (MTSS) 1 axis, ultimately leading to a reduction in cell-invasive activity (Yan et al., 2015). Furthermore, in an *in vivo* model of hepatocellular carcinoma (HCC), fucoidan significantly improved survival, inhibited cellular proliferation, and decreased serum AFP expression levels in HCC rats. Additionally, fucoidan reduced the upregulation of miR-143 and NF-κB expression levels induced by HCC (El-Far et al., 2021). The miR-143/NF-κB pathway was proven to be an important component involved in tumor progression and invasion (Zheng et al., 2018). This suggests that fucoidan may inhibit the metastasis of liver cancer by inhibiting the miR-143-mediated signaling pathway but promoting the miR-29-mediated signaling pathway.

The marine fungal metabolite 1386A, a small-molecule compound derived from a mangrove fungus, was reported to significantly inhibit MCF-7 cell proliferation in a time- and dose-dependent manner (Tang et al., 2012). Analysis of miRNA expression profiles induced by 1386A revealed that several deregulated miRNAs may play complex roles in 1386A-induced breast cancer cytotoxicity, including the unregulated tumor suppressor miRNAs (let-7a and miR-15/16) and the downregulated oncogenic miRNAs (miR-21, miR-27a and miR-633) (Tang et al., 2012). This suggests that 1386A may be an effective antitumor drug, and its molecular mechanism may be related to miRNAs, which needs further study.

Marine-derived *Streptomyces* MS449 produces high yields of the polypeptide antibiotics actinomycin X2 (AX2), actinomycin D, and actinomycin X0β. Among them, AX2 and actinomycin D showed strong anti-tuberculosis activity (Wang et al., 2017b). However, all three of them showed strong antitumor activity, especially AX2 (Chen et al., 2012b). Liu Jun found that AX2 could significantly reduce the proliferation rate and induce the apoptosis of prostate cancer cells by regulating the PTEN/PI3K/AKT/mTOR signaling pathway (Liu et al., 2016). Meanwhile, it was also found that AX2 decreased the expression level of miRNA144, and the direct target gene of miR-144 was subsequently identified as mTOR (Liu et al., 2016). However, miR-144 acts as a tumor suppressor gene to regulate cell proliferation, apoptosis and autophagy (Xiao et al., 2015; Wang et al., 2017c; Zhang et al., 2020b). In addition, it is well known that mTOR is a positive feedback regulator of the PI3K/Akt/mTOR pathway. Therefore, these data suggest that the antitumor effect of AX2 *via* modulation of the PTEN/PI3K/AKT/mTOR pathway may be somewhat attenuated by downregulation of miR-144.

4.1.2 lncRNA-related marine-derived agents

In addition to miRNAs, several lncRNAs have also been implicated in the antitumor activity of marine-derived drugs, including the tumor suppressors LINC00261 (Ma et al., 2021), lncRNA-p21 (Yan et al., 2019), lncRNA LED and LOC285194 (Ramalingam et al., 2018) and the oncogenic lncRNA-Saf (Yan et al., 2019). As mentioned above, miR-29b- and miR-143-mediated signaling pathways play important regulatory roles in the activity of fucoidan against HCC (Yan et al., 2015; El-Far et al., 2021). It was further found that the lncRNA-mediated signaling pathway was also involved in the antitumor mechanism of fucoidan (Yan et al., 2019; Ma et al., 2021). LINC00261 was significantly upregulated by fucoidan in HCC cells and then acted as a tumor suppressor gene by regulating the miR-522-3p/secreted frizzled-related protein 2 (SFRP2) signaling pathway to inhibit cell proliferation and invasion (Ma et al., 2021). Similarly, lncRNA-p21 was also upregulated by fucoidan, which has been shown to inhibit the invasion and metastasis of HCC cells (Yan et al., 2019). In contrast, the expression of lncRNA-Saf was significantly downregulated by fucoidan in HCC cells, which has been shown to increase the anti-apoptotic ability of cells by affecting the alternative splicing of

tumor necrosis factor (TNF) receptor superfamily member 6 (Fas) (Yan et al., 2005; Yan et al., 2019). These data suggest that fucoidan-mediated antitumor effects are closely related to the regulation of oncogene lncRNAs and tumor suppressor lncRNAs in tumors.

1-hydroxy-1-norresistomycin (HNM) is a secondary metabolite isolated from the marine actinomycete *Streptomyces* variabilis and has potent antimicrobial and antioxidant activity (Ramalingam and Rajaram, 2016). Recently, the antitumor effect of HNM was reported in human adenocarcinoma lung cancer cells. HNM can significantly inhibit cell proliferation, metastasis and invasion and promote cell apoptosis and cell cycle arrest. Mechanistically, p53-mediated transcriptional regulation of long noncoding RNAs (lncRNAs LED and LOC285194) was shown to be attributed to the repressive effect of HNM (Ramalingam et al., 2018). Among them, HNM-induced upregulation of the lncRNA LED can induce cell cycle arrest by targeting p21, while upregulation of LOC285194 can inhibit cell growth (Ramalingam et al., 2018). These findings also suggest the potential value of HNM as an anticancer drug by regulating tumor suppressor lncRNAs.

ASP-3 is a protein purified from marine *Arca subcrenata* and exhibits a strong inhibitory effect on the proliferation of HCC cells (Guo et al., 2019). Mechanistically, ASP-3 was found to effectively reduce vascular endothelial growth factor receptor (VEGFR) 2 phosphorylation (p-VEGFR2) in tumor cells and affect the vascular endothelial growth factor (VEGF) signaling pathway. Moreover, some tumor-related lncRNAs are regulated by ASP-3, including lncRNA ATG9B, which targets the VEGF signaling pathway (Guo et al., 2019). This suggests that ASP-3 can be used as an antitumor drug, and its molecular mechanism may be achieved by activating the ATG9B/VEGF signaling pathway to inhibit tumor angiogenesis-mediated cell invasion, which needs further study.

4.2 Regulation of ncRNA-related marine-derived agents in tumor drug sensitivity and resistance

4.2.1 miRNA-related marine-derived agents

Cytarabine (AraC) serves as an essential cytotoxic agent in AML treatment, and AraC resistance is the main cause of treatment failure in patients (Dombret and Gardin, 2016). In the past two decades, many cytarabine resistance-related oncogenic or tumor-suppressive miRNAs have been identified in AML and other tumors. Generally, the expression of drug resistance-related tumor-suppressive miRNAs is depressed by AraC in tumor cells. For example, the expression levels of miR-143 and miR-181a were significantly reduced in Ara-C-treated AML cell lines and primary AML cells (Bai et al., 2012; Zhang et al., 2020c). Ectopic expression of miR-143 or miR-181a significantly reduced cell viability in Ara-C-treated AML cells, suggesting that these two miRNAs could enhance the sensitivity of AML cells to Ara-C (Bai et al., 2012; Zhang et al., 2020c). Mechanistically, miR-143 inhibited the induction of autophagy by targeting the autophagy-related genes (ATG)7 and ATG2B, thereby eliminating the inhibitory effect of autophagy in cytarabine-induced AML cell-dependent apoptosis and cytotoxicity (Zhang et al., 2020c). miR-181a was found to target Bcl-2 to enhance cytochrome C release, which in turn activates caspases 9/3 and ultimately initiates the apoptosis pathway (Bai et al., 2012). Moreover, overexpression of miR-12462, let-7a, miR-29a, or miR-30c was also found to sensitize AML cells to Ara-C *in vitro* and *in vivo* (Russ et al., 2011; Chen et al., 2013b; Jia et al., 2020). Furthermore, let-7a was proven to be involved in stromal-derived factor 1α (SDF-1α)/C-X-C chemokine receptor type (CXCR)4 axis-induced Ara-C tolerance through the SDF-1α/CXCR4/Yin Yang 1 (YY1)/let-7a/c-MYC/B-cell lymphoma extra-large (BCL-XL) pathway (Chen et al., 2013b). Downregulation of sodium/hydrogen exchanger 1 (NHE1), an isoform in the Na+/H+ exchanger family, was then found in miR-12462-overexpressing AML cells (Jia et al., 2020), while downregulation of ITGB3, c-MYC and BCL-XL was shown in let-7a-overexpressing AML cells (Chen et al., 2013b). In addition, low expression of miR-625-3p was found in ALL cell lines, and its overexpression significantly increased cell sensitivity to Ara-C-induced apoptosis (Jiang et al., 2018).

Similarly, miR-134 and miR-181b were significantly downregulated in multidrug-resistant leukemia cells and samples from patients with relapsed/refractory AML (Lu et al., 2014; Chen et al., 2018a). Overexpression of miR-134 or miR-181b can significantly increase the sensitivity of cells to Ara-C and enhance the ability of Ara-C to induce apoptosis (Lu et al., 2014; Chen et al., 2018a). miR-134 was then proven to inhibit the phosphorylation of eukaryotic initiation factor 4E (eIF4E) by targeting mitogen-activated protein kinase-interacting kinases (MNKs), leading to the downregulation of the antiapoptotic proteins MCL-1 and BCL2 (Chen et al., 2018a). Among them, Mcl-1, together with HMGB1, was also involved in the miR-181b-mediated enhancement of cellular sensitivity to Ara-C (Lu et al., 2014), which suggests the positive regulatory effect of miRNAs on Ara-C-induced apoptosis. In addition, miR-24-3p was found to be significantly more highly expressed in Ara-C-sensitive AML cell lines than in Ara-C-resistant cell lines (Bhise et al., 2019), indicating the role of miR-24-3p in the maintenance of cellular sensitivity to Ara-C. dCMP deaminase (DCTD), an enzyme involved in the metabolic inactivation of cytarabine expression, was suggested to possibly mediate the function of miR-24-3p (Bhise et al., 2019). These results suggest that Ara-C resistance-associated tumor suppressor miRNAs can inhibit the development of resistance by enhancing cellular chemosensitivity, inhibiting drug-induced autophagy, or maintaining drug cytotoxicity in tumor cells.

Unlike the tumor suppressor miRNAs associated with Ara-C resistance, the expression of oncogenic miRNAs associated with Ara-C resistance is induced by this drug in chemoresistant AML cell lines or patients, such as miR-15a-5p, miR-21-5p

(Vandewalle et al., 2021), miR-126-5p (Shibayama et al., 2015), miR-10a (Vu et al., 2021), miR-331 (Butrym et al., 2015), miR-21 (Riccioni et al., 2015), and miR-335 (Yingchun et al., 2015). Moreover, it was found that overexpression of miR-15a-5p, miR-21-5p (Vandewalle et al., 2021), or miR-126-5 (Shibayama et al., 2015) significantly decreased cytarabine-induced apoptosis in AML cells, while inhibition of miR-10 (Vu et al., 2021), miR-21 (Li et al., 2010), or miR-32 (Gocek et al., 2011) could effectively inhibit cell viability and enhance the sensitivity of cells to Ara-C-induced apoptosis. Mechanistically, miR-15a-5p and miR-21-5p contribute to cytarabine resistance by targeting programmed cell death 4 (PDCD4), ADP-ribosylation factor 2 (ARL2), and B-cell translocation gene 2 (BTG2) (Vandewalle et al., 2021). miR-21 and miR-32 negatively regulate the expression levels of PDCD4- and Bcl-2-interacting mediator of cell death (Bim), respectively (Li et al., 2010; Gocek et al., 2011). The expression of the antiaging gene *Klotho* and the phosphorylation level of AKT were significantly decreased by miR-126-5p (Shibayama et al., 2015). miR-10a was proven to be a regulator of the p53 signaling network (Vu et al., 2021). Its inhibition could induce a synergistic antitumor effect between the MDM2 inhibitor Nutlin-3a and cytarabine through the activation of apoptosis in AML cells mediated by the p53 signaling network and the elimination of the inhibitory effect of autophagy on apoptosis (Vu et al., 2021). In addition, miR-34a-5p may also be an oncogenic miRNA that reduces the cytotoxic activity of cytarabine in AML cells by targeting and regulating deoxycytidine kinase (DCK), an enzyme involved in the activation of cytarabine (Bhise et al., 2019), a possible disease pathway that needs further study. Moreover, the regulatory role of miRNAs, such as miR-200, on cellular cytarabine tolerance is also seen in colon cancer. miR-200c expression was significantly upregulated in colon cancer patients, and its silencing in tumor cells induced apoptosis and inhibited cell survival, migration, and invasion. Moreover, silencing miR-200c sensitized cellular chemosensitivity to Ara-C *in vitro* and *in vivo* by modulating the expression of PTEN and the phosphorylation of p53 (Chen et al., 2014). These results suggest that the response of tumor cells to therapeutic cytotoxic agents can be increased by decreasing the expression of these cellular drug resistance oncogenic miRNAs.

Regarding trabectedin, myxoid liposarcoma (MLS) is the most sensitive type of soft tissue sarcoma to this drug, which may be correlated to the expression of the FUS-CHOP fusion gene generated by the chromosomal translocation t (12; 16) (q13; p11) (Grosso et al., 2007; Forni et al., 2009). Nonetheless, it has been observed in the clinic that some patients bearing MLS remain resistant to long-term treatment with trabectedin. Analysis of miRNA expression profiles in trabectedin-sensitive myxoid liposarcoma (MLS) cells and trabectedin-resistant cells revealed that 47 miRNAs were differentially expressed, including 24 overexpressed miRNAs and 23 underexpressed miRNAs (Uboldi et al., 2012). The target genes predicted by these differentially expressed miRNAs mainly existed in the immune response, the apoptosis regulatory system and the cell cycle. Several miRNAs have been shown to be associated with drug resistance, such as miR-130a, miR-19a, and miR-125a-5p (Huang et al., 2018; Huang et al., 2019; Zhang et al., 2020d), which suggests their value as indicators in tumor treatment, prognosis, or recurrence. Compared with patient-bearing MLS, resistance to trabectedin is more common in patients with non-small cell lung cancer (NSCLC) with a low response rate (20%–30%) for many chemotherapy drugs (Fossella, 2002; Rosell et al., 2002). miR-195 has been shown to be downregulated in NSCLC and acts as a tumor suppressor gene to inhibit the progression of tumor cells by regulating proliferation, apoptosis, and senescence (Arima et al., 2014; Yongchun et al., 2014; Liu et al., 2015). Furthermore, miR-195 was found to sensitize NSCLC to microtubule-targeting agents, especially trabectedin, *in vitro* and *in vivo*. Moreover, it was found that the facilitative effect of miR-195 on trabectedin was achieved by targeting checkpoint kinase 1 (CHEK1) (Yu et al., 2018b). Inhibition of miR-195 or overexpression of CHEK1 contributes to the resistance of NSCLC cells to trabectedin (Yu et al., 2018b). This suggests the potential value of trabectedin tolerance-related miRNAs as indicators of efficacy, prognosis or relapse in this drug therapy.

4.2.2 lncRNA-related marine-derived agents

Similar to marine-derived agent-related miRNAs, lncRNAs associated with marine-derived agent resistance are also common in AML to Ara-C. However, these lncRNAs associated with Ara-C resistance mainly act as oncogenes to suppress cellular chemosensitivity and induce drug resistance, including lncRNA DANCR, GAS6-AS2, HOTAIRM1 and MALAT1. The lncRNA DANCR is highly expressed as an oncogene in many tumors, including AML (Thin et al., 2018; Zhang et al., 2021b). Interestingly, Ara-C further increased the expression level of DANCR in AML cells, and DANCR knockdown significantly reduced the resistance of AML cells to Ara-C. Further studies revealed that DANCR was able to promote autophagy in Ara-C-treated AML cells *via* the miR-20a-5p/ATG16L1 signaling pathway (Zhang et al., 2021b). This suggests that DANCR enhances the antagonism of autophagy against cytarabine-induced apoptosis by promoting autophagy, thereby inducing resistance to cytarabine in AML cells. LncRNA GAS6-AS2, a tumor-associated lncRNA first discovered in AML cells, is abnormally highly expressed and exhibits oncogenic effects in various tumors, including AML (Bester et al., 2018). The first oncogene function identified for GAS6-AS2 was involved in mediating Ara-C resistance in AML cells. GAS6-AS2 overexpression significantly promoted AML cell resistance to Ara-C-induced apoptosis *in vitro* and *in vivo*. Mechanistically, it was proven that GAS6-AS2 could increase the upregulation of growth arrest-specific gene 6 (GAS6) ligand and its receptor tyrosine kinase (AXL) to promote cellular survival and resistance (Bester et al., 2018). Similarly, HOTAIRM1 and MALAT1 have usually been shown to act as oncogenes that are highly expressed

10.3389/fphar.2022.1053556

TABLE 3 ncRNA-associated marine-derived anti-tumor agents.

Agent name	Source	Late-stage of clinical development	Related ncRNA types	ncRNA roles in tumor	ncRNA-related tumor types
Eribulin	*Halichondria okadai*	Approved	miRNA	Suppressor	Breast cancer
Maritoclax	*Streptomyces*	Approved	miRNA	Suppressor	Renal carcinoma
Trabectedin	*Ecteinascidia turbinata*	Approved	miRNA	Oncogene or suppressor	Biliary tract cancer; Myxoid liposarcoma; Lung cancer
Cytarabine	*Cryptotethia crypta*	Approved	miRNA; lncRNA	Oncogene or suppressor	Acute myeloid leukemia; Colon cancer
Spirulina	*Cyanobacteria*	*In vivo*	miRNA	Suppressor	Glioma
ASP-3	*Arca subcrenata*	*In vivo*	lncRNA	Oncogene	Hepatocellular carcinoma
Fucoidan	Brown seaweed	*In vivo*	miRNA; lncRNA	Suppressor	Hepatocellular carcinoma
SZ-685C	Angrove endophytic fungus	*In vitro*	miRNA	Oncogene	Pituitary tumor
Bostrycin	*Alternaria eichhorniae*	*In vitro*	miRNA	Suppressor	Lung cancer
1386A	Mangrove fungus	*In vitro*	miRNA	Suppressor	Breast cancer
Actinomycin X2	*Streptomyces*	*In vitro*	miRNA	Oncogene	Prostate cancer
1-hydroxy-1 -Norresistomycin	*Streptomyces*	*In vitro*	lncRNA	Suppressor	Lung cancer

in AML and various other human tumors. Knockdown of both enhanced the sensitivity of AML to Ara-C, decreased cell viability and increased apoptosis (Chen et al., 2020; Goyal et al., 2021). HOTAIRM1 knockdown also suppressed glucose consumption and lactate production in AML cells (Chen et al., 2020). Subsequently, the role of HOTAIRM1 was confirmed to be related to the Wnt/β-catenin/platelet-type phosphofructokinase (PFKP) signaling pathway, while MALAT1 could target and regulate the tumor suppressor gene miR-96C (Chen et al., 2020; Goyal et al., 2021). In addition, TUG1 expression was elevated in refractory or relapsed acute myeloid leukemia (R/R AML) patients who received Ara-C combined with other drugs. It was further found that patients with high TUG1 expression had a lower overall response rate (ORR), lower complete response (CR) value, and shorter overall survival (OS) (Luo et al., 2018). This suggests that TUG1 is a potential biomarker for poor prognosis in R/R AML patients treated with Ara-C, but the relationship between TUG1 and tumor cell resistance to Ara-C requires further study.

5 The antitumor effects of marine food-derived ncRNAs

The aforementioned ncRNAs in tumors play important regulatory roles as oncogenes or tumor suppressors in the antitumor effects of marine-derived drugs in different clinical research stages, as shown in Table 3. Interestingly, recent studies have found that ncRNA in marine food can play an anti-tumor role by mediating gene expression across species, which further confirms the anti-tumor strategy of marine ncRNA targeting (shown in Figure 5). These marine food-related ncRNAs were mainly from shrimp fed by miRNA-expressing bacteria, including mja-miR-35-3p, miR-S8, miR-965, miR-34, and mja-miR-35 (Table 4). This suggests that the active ingredients of marine-derived antitumor drugs are not limited to polyphenols, polysaccharides, alkaloids, or peptides but may also be ncRNAs.

mja-miR-35-3p is a microRNA derived from marine shrimp (*Marsupenaeus japonicus*), and its expression can be significantly upregulated by white spot syndrome virus (WSSV), which in turn plays an important role in the antiviral infection of shrimp (Huang et al., 2012). Overexpression of mja-miR-35-3p in human melanoma and breast cancer stem cells inhibited cell proliferation, arrested the cell cycle, and induced cellular apoptosis (Zhang and Zhang, 2019). Furthermore, it was found that mja-miR-35-3p could target and decrease the upregulated peptidylprolyl cis/trans isomerase NIMA-interacting 1 (PIN1) in human melanoma and breast cancer stem cells, thereby leading to the elimination of the stemness of tumor stem cells (Zhang and Zhang, 2019). In addition, the anti-human tumor effect of mja-miR-35 on the tumorigenic ability of melanoma stem cells *in vivo* was confirmed. Similarly, the shrimp microRNAs miR-S8 and miR-965 were also found to inhibit the stemness of human melanoma stem cells *in vitro* and *in vivo* (Yang et al., 2017; Wu et al., 2020). Mechanistic studies showed that miR-S8 targeted human Y-box binding protein 1 (YB-1) and inhibited its expression, thereby mediating the reduction in stem cell tumorigenicity (Yang et al., 2017). miR-965 suppresses the expression of human myeloid cell leukemia sequence 1 (MCL-1) and disrupts the balance of the MCL-1-endoplasmic reticulum (ER) stress-human X-box-binding protein 1 (XBP1) feedback

TABLE 4 Marine food-derived miRNAs as anti-tumor agents.

miRNAs	Source	Related human cellular processes	Related human genes	Tumor cell types	References
mja-miR-35-3p	*Marsupenaeus japonicus*	Inhibition of cell proliferation, Induction of cell cycle arrest and apoptosis	PIN1, CCND1, MCL-1	Melanoma stem cells, breast cancer stem cells	Zhang and Zhang, (2019)
miR-S8	*Marsupenaeus japonicus*	Inhibition of cell stemness and tumorigenic ability	YB-1	Melanoma stem cells	Yang et al. (2017)
miR-965	*Marsupenaeus japonicus*	Inhibition of cell stemness and tumorigenic ability	MCL-1, XBP1, ER stress	Melanoma stem cells	Wu et al. (2020)
miR-34	*Marsupenaeus japonicus*	Inhibition of cell tumorigenesis, tumor growth, and metastasis	CCND1, CDK6, MET, CCNE2, E2F3, FOSL1	Breast cancer cells	Cui et al., (2017); Cui et al., (2021)
mja-miR-35	*Marsupenaeus japonicus*	Inhibition of cell tumorigenesis, tumor growth, and metastasis	M2 macrophage, CHI3L1	Breast cancer cells	Chen et al. (2018b)

loop by interacting with the human Ago2 protein (Wu et al., 2020). These results suggest that shrimp microRNAs can inhibit the stemness of human tumor stem cells by regulating human tumor-related genes in a cross-species manner.

Similar to mja-miR-35, shrimp miR-34 and mja-miR-35 are two other WSSV-associated miRNAs whose expression can be upregulated by the virus, which then suppress the virus by targeting the viral genes wsv330 and wsv359 or wsv140, wsv279, wsv309, and wsv361, respectively (Cui et al., 2017; Chen et al., 2018b; Cui et al., 2021). Overexpression of shrimp miR-34 or mja-miR-35 in human breast cancer cells significantly suppressed cell metastasis *in vitro* and *in vivo* (Cui et al., 2017; Chen et al., 2018b). The molecular mechanism of shrimp miR-34 against human tumors was shown to be related to shrimp miR-34-mediated expression of multiple human tumor-related genes, including cyclin D1 (CCND1), cyclin dependent kinase 6 (CDK6), cyclin E2 (CCNE2), E2F transcription factor 3 (E2F3) FOS-like antigen-1 (FOSL1), and hepatocyte growth factor receptor (MET) (Cui et al., 2017), while the tumor suppressor function of mja-miR-35 is achieved by targeting M2 macrophages and human chitinase-3-like protein 1 (CHI3L1) (Chen et al., 2018b). Interestingly, elevated levels of mature miR-34 or mja-miR-35 expression were also found in shrimp fed bacteria overexpressing miRNAs (bacteria-miR-34 or bacteria-mja-miR-35), even in cooked shrimp (Chen et al., 2018b; Cui et al., 2021). Moreover, food (bacteria-miR-34 or bacteria-mja-miR-35)-derived shrimp miR-34 or mja-miR-35 still contribute to the antiviral immune response of shrimp (Chen et al., 2018b). Further studies found that transfection of human breast cancer cells with shrimp miR-34 or mja-miR-35 extracted from the muscle of bacteria-miRNA-fed shrimp could significantly inhibit cell growth and metastasis (Chen et al., 2018b; Cui et al., 2021). In addition, the antitumor effects of these two shrimp miRNAs were also demonstrated in human breast cancer xenograft model mice that were fed the cooked muscle of shrimp fed with bacteria-miRNAs (Chen et al., 2018b; Cui et al., 2021). These results demonstrate that xenogeneic miRNAs can inhibit not only human tumorigenesis but also tumor growth and progression, suggesting their potential as antitumor drugs. Furthermore, these results also suggest that miRNAs in food may be an effective strategy for the simultaneous control of viral diseases in food animals and human cancers.

6 Conclusion and discussion

ncRNAs are critical regulators of gene expression at multiple biological levels and are involved in the pathophysiology of various diseases, especially tumors. Substantial evidence indicates the potential of ncRNAs as novel biomarkers for tumor diagnosis and prognosis and even drug targets. In addition, as mentioned above, ncRNAs have recently been shown to play important roles in the antitumor mechanisms of marine-derived agents as oncogenes or tumor suppressors. This provides a new understanding of the antitumor mechanism of clinical (including cytarabine, fucoidan, and trabectedin) or potential marine-derived agents (other compounds or derivative derivatives mentioned in the text), which can promote their more rational clinical application or clinical translation. This also suggests that ncRNAs from marine life might be an important source for discovering human antitumor agents by mediating cross-species gene expression. Moreover, this further supports the potential of marine-derived agent-associated ncRNAs as biomarkers for tumor therapy, prognosis and recurrence, especially miRNAs. For example, Natsuko showed that serum miRNAs could serve as biomarkers of response to eribulin therapy in patients with metastatic breast cancer (Satomi-Tsushita et al., 2019). Of the 147 patients, 52 developed at least one new distant metastasis after treatment, and the remaining 95 did not develop new distant metastases. A combination of 8 serum miRNAs was predicted to be related to new distant metastases in patients after eribulin treatment, including miR-4483, miR-8089, miR-4755-3p, miR-296-3p, miR-575, miR-4710, miR-5698, and miR-3160-5p. Among them, miR-8089 and miR-5698 were also significantly associated with the overall survival of patients treated with

eribulin (Satomi-Tsushita et al., 2019). This study demonstrates that serum miRNA profiles can serve as biomarkers of responsiveness to eribulin and predict new distant metastases and prognostic development in metastatic breast cancer. In a phase II and phase III clinical trial investigating the antitumor activity of eribulin in patients with metastatic STS, 26 microRNA biomarkers in tumor samples were associated with sensitive or resistant patients (Wiemer et al., 2017). This suggests that the expression levels of these specific microRNAs in STS tissue samples can be used as predictors of patient response to eribulin. In addition, Li found in the serum and bone marrow of 204 Ara-C-treated adult AML patients that the expression level of miR-335 was significantly higher in patients who achieved complete remission than in those who did not (Yingchun et al., 2015). Interestingly, high bone marrow miR-335 levels, but not serum miR-335 levels, were significantly associated with poorer treatment response and predicted poorer prognosis (Yingchun et al., 2015). This suggests that bone marrow miR-335 levels can be used as a biomarker for predicting chemotherapy response and prognosis in Ara-C-treated adult AML patients.

However, the antitumor activity of some marine-derived substances that may have antitumor effects and their molecular mechanisms related to ncRNAs need to be further studied, such as sponge FAU and sponge FAU-derived SNORA62. Sponge FAU is a gene/protein isolated from the marine sponge *Suberites domuncula* (Perina et al., 2015). It has been shown to promote apoptosis of HEK293T cells, which suggests that sponge FAU may have the same potential for cancer treatment as human FAU (Pickard et al., 2011; Perina et al., 2015). Moreover, the snoRNA SNORA62 was identified in the sponge FAU gene, which is the sponge ortholog of human SNORA62 (E2) (Perina et al., 2015). Human SNORA62 is the most upregulated gene in diallyl sulfide (DAS)-induced apoptosis in HeLa cells (Moodley and Weiss, 2013). This finding indicates that FAU-derived SNORA62 may be a tumor-related snoRNA involved in the regulation of the apoptotic system. In addition, research on the role of other types of ncRNAs in marine-derived antitumor drugs has not been reported and is worthy of study. After all, they also play very important regulatory functions in the occurrence and development of tumors, such as circRNAs. Moreover.

Overall, the available data on ncRNAs as mediators, inhibitors, or drug sources support their indispensable roles in marine-derived antitumor agents by regulating tumor cell tumor cell proliferation, apoptosis, the cell cycle, autophagy, drug sensitivity, or resistance. Therefore, upregulation of drug-related tumor suppressor ncRNAs or elimination of drug-related oncogenic ncRNAs in tumor cells is one of the important options for promising biomarkers for diagnosis, prognosis, or targets for cancer treatment. However, it should be noted that, in most cases, ncRNAs are not the direct targets of these marine antitumor agents. The effect of these drugs on ncRNAs is most likely an indirect or incidental result, which may only contribute a small part of the anti-tumor activity. In addition, we also found that the correlation between ncRNA-related marine-derived agents and tumor drug sensitivity and drug resistance was mainly found in AML, which may be related to the complexity of the recurrence and treatment of hematological tumors, and may also indicate the cancer specificity of marine-derived agents, which all need to be further studied. Therefore, more mechanism studies are needed to clarify the role of ncRNA in the anti-tumor of these agents, including the identification of suitable ncRNA detection methods, the screening of ncRNA biomarkers, and the development of drug-specific ncRNA modulators or adjuvants. Moreover, the research mechanism of some compounds (or foods) mentioned in this review is still limited to the laboratory stage, even only at the level of cell experiment, which still needs to be fully tested *in vivo* and follow-up clinical trials. With the development of marine biology, marine biochemistry, and marine pharmacy based on high-throughput sequencing and screening techniques, an increasing number of early-stage antitumor drug candidates in preclinical research will be translated into clinical treatment, new marine-derived substances are being used in the development of antitumor drugs, and new marine-derived antitumor drug-related ncRNAs will be identified. Additionally, the efficacy and safety of ncRNA-related marine-derived antitumor drug or food in the cancer treatment will gradually become clear.

Author contributions

ZZ, PL, and SW designed the review and contributed to manuscript preparation. ZZ wrote the manuscript. QC, YD, and LL provided technical and administrative support. ZZ and YW prepared figures. ZZ and SW revised the manuscript. All authors read and approved the final manuscript.

Funding

This study was supported by the Natural Science Foundation of Shandong Province of China (ZR2020MH250), the National Natural Science Foundation of China (91849209 and 81502063), and the Medical Health Science and Technology Development Plan of Shandong Province (202004111322).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Alizadeh, M., Safarzadeh, A., Beyranvand, F., Ahmadpour, F., Hajiasgharzadeh, K., Baghbanzadeh, A., et al. (2019). The potential role of miR-29 in health and cancer diagnosis, prognosis, and therapy. *J. Cell. Physiol.* 234 (11), 19280–19297. doi:10.1002/jcp.28607

an, H., and Bu, P. (2021). Non-coding RNA in cancer. *Essays Biochem.* 65 (4), 625–639. doi:10.1042/EBC20200032

Anastasiadou, E., Jacob, L. S., and Slack, F. J. (2018). Non-coding RNA networks in cancer. *Nat. Rev. Cancer* 18 (1), 5–18. doi:10.1038/nrc.2017.99

Arab, S., Ghasemi, S., Ghanbari, A., Bahraminasab, M., Satari, A., Mousavi, M., et al. (2021). Chemopreventive effect of spirulina microalgae on an animal model of glioblastoma via down-regulation of PI3K/AKT/mTOR and up-regulation of miR-34a/miR-125B expression. *Phytother. Res.* 35 (11), 6452–6461. doi:10.1002/ptr.7298

Arima, C., Kajino, T., Tamada, Y., Imoto, S., Shimada, Y., Nakatochi, M., et al. (2014). Lung adenocarcinoma subtypes definable by lung development-related miRNA expression profiles in association with clinicopathologic features. *Carcinogenesis* 35 (10), 2224–2231. doi:10.1093/carcin/bgu127

Arun, G., Diermeier, S. D., and Spector, D. L. (2018). Therapeutic targeting of long non-coding RNAs in cancer. *Trends Mol. Med.* 24 (3), 257–277. doi:10.1016/j.molmed.2018.01.001

Aydogdu, E., Katchy, A., Tsouko, E., Lin, C. Y., Haldosen, L. A., Helguero, L., et al. (2012). MicroRNA-regulated gene networks during mammary cell differentiation are associated with breast cancer. *Carcinogenesis* 33 (8), 1502–1511. doi:10.1093/carcin/bgs161

Bai, H., Cao, Z., Deng, C., Zhou, L., and Wang, C. (2012). miR-181a sensitizes resistant leukaemia HL-60/Ara-C cells to Ara-C by inducing apoptosis. *J. Cancer Res. Clin. Oncol.* 138 (4), 595–602. doi:10.1007/s00432-011-1137-3

Baruchel, S., Pappo, A., Krailo, M., Baker, K. S., Wu, B., Villaluna, D., et al. (2012). A phase 2 trial of trabectedin in children with recurrent rhabdomyosarcoma, ewing sarcoma and non-rhabdomyosarcoma soft tissue sarcomas: A report from the children's oncology group. *Eur. J. Cancer* 48 (4), 579–585. doi:10.1016/j.ejca.2011.09.027

Bautista-Sanchez, D., Arriaga-Canon, C., Pedroza-Torres, A., De La Rosa-Velazquez, I. A., Gonzalez-Barrios, R., Contreras-Espinosa, L., et al. (2020). The promising role of miR-21 as a cancer biomarker and its importance in RNA-based therapeutics. *Mol. Ther. Nucleic Acids* 20, 409–420. doi:10.1016/j.omtn.2020.03.003

Bayraktar, R., and Van Roosbroeck, K. (2018). miR-155 in cancer drug resistance and as target for miRNA-based therapeutics. *Cancer Metastasis Rev.* 37 (1), 33–44. doi:10.1007/s10555-017-9724-7

Bester, A. C., Lee, J. D., Chavez, A., Lee, Y. R., Nachmani, D., Vora, S., et al. (2018). An integrated genome-wide CRISPRa approach to functionalize lncRNAs in drug resistance. *Cell.* 173 (3), 649–664. doi:10.1016/j.cell.2018.03.052

Bhise, N. S., Elsayed, A. H., Cao, X., Pounds, S., and Lamba, J. K. (2019). MicroRNAs mediated regulation of expression of nucleoside analog pathway genes in acute myeloid leukemia. *Genes (Basel)* 10 (4), E319. doi:10.3390/genes10040319

Braune, S., Kruger-Genge, A., Kammerer, S., Jung, F., and Kupper, J. H. (2021). Phycocyanin from arthrospira platensis as potential anti-cancer drug: Review of *in vitro* and *in vivo* studies. *Life (Basel)* 11 (2), 91. doi:10.3390/life11020091

Burnett, A., Wetzler, M., and Lowenberg, B. (2011). Therapeutic advances in acute myeloid leukemia. *J. Clin. Oncol.* 29 (5), 487–494. doi:10.1200/JCO.2010.30.1820

Butrym, A., Rybka, J., Baczynska, D., Tukiendorf, A., Kuliczkowski, K., and Mazur, G. (2015). Expression of microRNA-331 can be used as a predictor for response to therapy and survival in acute myeloid leukemia patients. *Biomark. Med.* 9 (5), 453–460. doi:10.2217/bmm.14.112

Cech, T. R., and Steitz, J. A. (2014). The noncoding RNA revolution-trashing old rules to forge new ones. *Cell.* 157 (1), 77–94. doi:10.1016/j.cell.2014.03.008

Chen, C. H., Xiao, W. W., Jiang, X. B., Wang, J. W., Mao, Z. G., Lei, N., et al. (2013). A novel marine drug, SZ-685C, induces apoptosis of MMQ pituitary tumor cells by downregulating miR-200c. *Curr. Med. Chem.* 20 (16), 2145–2154. doi:10.2174/0929867311320160007

Chen, C., Song, F., Wang, Q., Abdel-Mageed, W. M., Guo, H., Fu, C., et al. (2012). A marine-derived Streptomyces sp. MS449 produces high yield of actinomycin X2 and actinomycin D with potent anti-tuberculosis activity. *Appl. Microbiol. Biotechnol.* 95 (4), 919–927. doi:10.1007/s00253-012-4079-z

Chen, H., Zhong, L., Long, Y., Li, J., Wu, J., Liu, L., et al. (2012). Studies on the synthesis of derivatives of marine-derived bostrycin and their structure-activity relationship against tumor cells. *Mar. Drugs* 10 (4), 932–952. doi:10.3390/md10040932

Chen, J., Wang, W., Zhang, Y., Hu, T., and Chen, Y. (2014). The roles of miR-200c in colon cancer and associated molecular mechanisms. *Tumour Biol.* 35 (7), 6475–6483. doi:10.1007/s13277-014-1860-x

Chen, K., Chen, Y., Chen, Z., Shi, Y., He, Z., Ding, B., et al. (2018). miR-134 increases the antitumor effects of cytarabine by targeting Mnks in acute myeloid leukemia cells. *Onco. Targets. Ther.* 11, 3141–3147. doi:10.2147/OTT.S143465

Chen, L., Hu, N., Wang, C., and Zhao, H. M. (2020). HOTAIRM1 knockdown enhances cytarabine-induced cytotoxicity by suppression of glycolysis through the Wnt/β-catenin/PFKP pathway in acute myeloid leukemia cells. *Arch. Biochem. Biophys.* 680, 108244. doi:10.1016/j.abb.2019.108244

Chen, W. S., Hou, J. N., Guo, Y. B., Yang, H. L., Xie, C. M., Lin, Y. C., et al. (2011). Bostrycin inhibits proliferation of human lung carcinoma A549 cells via downregulation of the PI3K/Akt pathway. *J. Exp. Clin. Cancer Res.* 30, 17. doi:10.1186/1756-9966-30-17

Chen, Y., Jacamo, R., Konopleva, M., Garzon, R., Croce, C., and Andreeff, M. (2013). CXCR4 downregulation of let-7a drives chemoresistance in acute myeloid leukemia. *J. Clin. Invest.* 123 (6), 2395–2407. doi:10.1172/JCI66553

Chen, Y., Zhang, S., Cao, J., and Zhang, X. (2018). Shrimp antiviral mja-miR-35 targets CHI3L1 in human M2 macrophages and suppresses breast cancer metastasis. *Front. Immunol.* 9, 2071. doi:10.3389/fimmu.2018.02071

Clamp, M., Fry, B., Kamal, M., Xie, X., Cuff, J., Lin, M. F., et al. (2007). Distinguishing protein-coding and noncoding genes in the human genome. *Proc. Natl. Acad. Sci. U. S. A.* 104 (49), 19428–19433. doi:10.1073/pnas.0709013104

Clark, M. B., and Mattick, J. S. (2011). Long noncoding RNAs in cell biology. *Semin. Cell. Dev. Biol.* 22 (4), 366–376. doi:10.1016/j.semcdb.2011.01.001

Cuce, M., Gallo Cantafio, M. E., Siciliano, M. A., Riillo, C., Caracciolo, D., Scionti, F., et al. (2019). Trabectedin triggers direct and NK-mediated cytotoxicity in multiple myeloma. *J. Hematol. Oncol.* 12 (1), 32. doi:10.1186/s13045-019-0714-9

Cui, Y., Wan, H., and Zhang, X. (2021). miRNA in food simultaneously controls animal viral disease and human tumorigenesis. *Mol. Ther. Nucleic Acids* 23, 995–1006. doi:10.1016/j.omtn.2021.01.011

Cui, Y., Yang, X., and Zhang, X. (2017). Shrimp miR-34 from shrimp stress response to virus infection suppresses tumorigenesis of breast cancer. *Mol. Ther. Nucleic Acids* 9, 387–398. doi:10.1016/j.omtn.2017.10.016

Deepika, M. S., Thangam, R., Sheena, T. S., Sasirekha, R., Sivasubramanian, S., Babu, M. D., et al. (2019). A novel rutin-fucoidan complex based phytotherapy for cervical cancer through achieving enhanced bioavailability and cancer cell apoptosis. *Biomed. Pharmacother.* 109, 1181–1195. doi:10.1016/j.biopha.2018.10.178

Di Giandomenico, S., Frapolli, R., Bello, E., Uboldi, S., Licandro, S. A., Marchini, S., et al. (2014). Mode of action of trabectedin in myxoid liposarcomas. *Oncogene* 33 (44), 5201–5210. doi:10.1038/onc.2013.462

Di Leva, G., Garofalo, M., and Croce, C. M. (2014). MicroRNAs in cancer. *Annu. Rev. Pathol.* 9, 287–314. doi:10.1146/annurev-pathol-012513-104715

Djebali, S., Davis, C. A., Merkel, A., Dobin, A., Lassmann, T., Mortazavi, A., et al. (2012). Landscape of transcription in human cells. *Nature* 489 (7414), 101–108. doi:10.1038/nature11233

Doi, K., Li, R., Sung, S. S., Wu, H., Liu, Y., Manieri, W., et al. (2012). Discovery of marinopyrrole A (maritoclax) as a selective Mcl-1 antagonist that overcomes ABT-737 resistance by binding to and targeting Mcl-1 for proteasomal degradation. *J. Biol. Chem.* 287 (13), 10224–10235. doi:10.1074/jbc.M111.334532

Doi, K., Liu, Q., Gowda, K., Barth, B. M., Claxton, D., Amin, S., et al. (2014). Maritoclax induces apoptosis in acute myeloid leukemia cells with elevated Mcl-1 expression. *Cancer Biol. Ther.* 15 (8), 1077–1086. doi:10.4161/cbt.29186

Dombret, H., and Gardin, C. (2016). An update of current treatments for adult acute myeloid leukemia. *Blood* 127 (1), 53–61. doi:10.1182/blood-2015-08-604520

Dutta, A., Roy, A., and Chatterjee, S. (2021). Long noncoding RNAs in cancer immunity: A new avenue in drug discovery. *Drug Discov. Today* 26 (1), 264–272. doi:10.1016/j.drudis.2020.07.026

El-Far, Y. M., Khodir, A. E., Emarah, Z. A., Ebrahim, M. A., and Al-Gayyar, M. M. H. (2021). Fucoidan ameliorates hepatocellular carcinoma induced in rats: Effect on miR143 and inflammation. *Nutr. Cancer* 73 (8), 1498–1510. doi:10.1080/01635581.2020.1798478

ElFar, O. A., Billa, N., Lim, H. R., Chew, K. W., Cheah, W. Y., Munawaroh, H. S. H., et al. (2022). Advances in delivery methods of Arthrospira platensis (spirulina) for enhanced therapeutic outcomes. *Bioengineered* 13 (6), 14681–14718. doi:10.1080/21655979.2022.2100863

Esteller, M. (2011). Non-coding RNAs in human disease. *Nat. Rev. Genet.* 12 (12), 861–874. doi:10.1038/nrg3074

Feng, J., Yang, M., Wei, Q., Song, F., Zhang, Y., Wang, X., et al. (2020). Novel evidence for oncogenic piRNA-823 as a promising prognostic biomarker and a potential therapeutic target in colorectal cancer. *J. Cell. Mol. Med.* 24 (16), 9028–9040. doi:10.1111/jcmm.15537

Forni, C., Minuzzo, M., Virdis, E., Tamborini, E., Simone, M., Tavecchio, M., et al. (2009). Trabectedin (ET-743) promotes differentiation in myxoid liposarcoma tumors. *Mol. Cancer Ther.* 8 (2), 449–457. doi:10.1158/1535-7163.MCT-08-0848

Fossella, F. V. (2002). Docetaxel for previously treated non-small-cell lung cancer. *Oncol. Willist. Park)* 16, 45–51.

Fu, H., Feng, J., Liu, Q., Sun, F., Tie, Y., Zhu, J., et al. (2009). Stress induces tRNA cleavage by angiogenin in mammalian cells. *FEBS Lett.* 583 (2), 437–442. doi:10.1016/j.febslet.2008.12.043

Furuya, K., Sasaki, A., Tsunoda, Y., Tsuji, M., Udaka, Y., Oyamada, H., et al. (2016). Eribulin upregulates miR-195 expression and downregulates Wnt3a expression in non-basal-like type of triple-negative breast cancer cell MDA-MB-231. *Hum. Cell.* 29 (2), 76–82. doi:10.1007/s13577-015-0126-2

Gebetsberger, J., and Polacek, N. (2013). Slicing tRNAs to boost functional ncRNA diversity. *RNA Biol.* 10 (12), 1798–1806. doi:10.4161/rna.27177

Gehrke, C. W., Kuo, K. C., Waalkes, T. P., and Borek, E. (1979). Patterns of urinary excretion of modified nucleosides. *Cancer Res.* 39 (4), 1150–1153.

Gocek, E., Wang, X., Liu, X., Liu, C. G., and Studzinski, G. P. (2011). MicroRNA-32 upregulation by 1, 25-dihydroxyvitamin D3 in human myeloid leukemia cells leads to Bim targeting and inhibition of AraC-induced apoptosis. *Cancer Res.* 71 (19), 6230–6239. doi:10.1158/0008-5472.CAN-11-1717

Goyal, B., Yadav, S. R. M., Awasthee, N., Gupta, S., Kunnumakkara, A. B., and Gupta, S. C. (2021). Diagnostic, prognostic, and therapeutic significance of long non-coding RNA MALAT1 in cancer. *Biochim. Biophys. Acta. Rev. Cancer* 1875 (2), 188502. doi:10.1016/j.bbcan.2021.188502

Grander, D., and Johnsson, P. (2016). Pseudogene-expressed RNAs: Emerging roles in gene regulation and disease. *Curr. Top. Microbiol. Immunol.* 394, 111–126. doi:10.1007/82_2015_442

Green, J. L., La, J., Yum, K. W., Desai, P., Rodewald, L. W., Zhang, X., et al. (2013). Paracrine Wnt signaling both promotes and inhibits human breast tumor growth. *Proc. Natl. Acad. Sci. U. S. A.* 110 (17), 6991–6996. doi:10.1073/pnas.1303671110

Groen, J. N., Capraro, D., and Morris, K. V. (2014). The emerging role of pseudogene expressed non-coding RNAs in cellular functions. *Int. J. Biochem. Cell. Biol.* 54, 350–355. doi:10.1016/j.biocel.2014.05.008

Grosso, F., Jones, R. L., Demetri, G. D., Judson, I. R., Blay, J. Y., Le Cesne, A., et al. (2007). Efficacy of trabectedin (ecteinascidin-743) in advanced pretreated myxoid liposarcomas: A retrospective study. *Lancet. Oncol.* 8 (7), 595–602. doi:10.1016/S1470-2045(07)70175-4

Gueven, N., Spring, K. J., Holmes, S., Ahuja, K., Eri, R., Park, A. Y., et al. (2020). Micro RNA expression after ingestion of fucoidan; A clinical study. *Mar. Drugs* 18 (3), E143. doi:10.3390/md18030143

Guo, Z., Shi, H., Li, C., Luo, Y., Bi, S., Yu, R., et al. (2019). Identification and characterization of a novel protein ASP-3 purified from Arca subcrenata and its antitumor mechanism. *Mar. Drugs* 17 (9), E528. doi:10.3390/md17090528

Haste, N. M., Hughes, C. C., Tran, D. N., Fenical, W., Jensen, P. R., Nizet, V., et al. (2011). Pharmacological properties of the marine natural product marinopyrrole A against methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 55 (7), 3305–3312. doi:10.1128/AAC.01211-10

He, A. T., Liu, J., Li, F., and Yang, B. B. (2021). Targeting circular RNAs as a therapeutic approach: Current strategies and challenges. *Signal Transduct. Target. Ther.* 6 (1), 185. doi:10.1038/s41392-021-00569-5

He, B., Zhao, Z., Cai, Q., Zhang, Y., Zhang, P., Shi, S., et al. (2020). miRNA-based biomarkers, therapies, and resistance in Cancer. *Int. J. Biol. Sci.* 16 (14), 2628–2647. doi:10.7150/ijbs.47203

Huang, J., Zhao, M., Hu, H., Wang, J., Ang, L., and Zheng, L. (2019). MicroRNA-130a reduces drug resistance in breast cancer. *Int. J. Clin. Exp. Pathol.* 12 (7), 2699–2705.

Huang, T. Z., Xu, D. D., and Zhang, X. B. (2012). Characterization of host microRNAs that respond to DNA virus infection in a crustacean. *BMC Genomics* 13, 159. doi:10.1186/1471-2164-13-159

Huang, W. K., Akcakaya, P., Gangaev, A., Lee, L., Zeljic, K., Hajeri, P., et al. (2018). miR-125a-5p regulation increases phosphorylation of FAK that contributes to imatinib resistance in gastrointestinal stromal tumors. *Exp. Cell. Res.* 371 (1), 287–296. doi:10.1016/j.yexcr.2018.08.028

Iyer, M. K., Niknafs, Y. S., Malik, R., Singhal, U., Sahu, A., Hosono, Y., et al. (2015). The landscape of long noncoding RNAs in the human transcriptome. *Nat. Genet.* 47 (3), 199–208. doi:10.1038/ng.3192

Jeon, M. Y., Min, K. J., Woo, S. M., Seo, S. U., Choi, Y. H., Kim, S. H., et al. (2018). Maritoclax enhances TRAIL-induced apoptosis via CHOP-mediated upregulation of DR5 and miR-708-mediated downregulation of cFLIP. *Molecules* 23 (11), E3030. doi:10.3390/molecules23113030

Jia, Y., Liu, W., Zhan, H. E., Yi, X. P., Liang, H., Zheng, Q. L., et al. (2020). Roles of hsa-miR-12462 and SLC9A1 in acute myeloid leukemia. *J. Hematol. Oncol.* 13 (1), 101. doi:10.1186/s13045-020-00935-w

Jiang, Q., Lu, X., Huang, P., Gao, C., Zhao, X., Xing, T., et al. (2018). Expression of miR-652-3p and effect on apoptosis and drug sensitivity in pediatric acute lymphoblastic leukemia. *Biomed. Res. Int.* 2018, 5724686. doi:10.1155/2018/5724686

Jie, J., Shi, L., Yue, S., Wang, M., and Zhang, J. (2020). Bostrycin inhibits growth of tongue squamous cell carcinoma cells by inducing mitochondrial apoptosis. *Transl. Cancer Res.* 9 (6), 3926–3936. doi:10.21037/tcr-19-2076

Khalifa, S. A. M., Elias, N., Farag, M. A., Chen, L., Saeed, A., Hegazy, M. F., et al. (2019). Marine natural products: A source of novel anticancer drugs. *Mar. Drugs* 17 (9), E491. doi:10.3390/md17090491

Korpal, M., Ell, B. J., Buffa, F. M., Ibrahim, T., Blanco, M. A., Celia-Terrassa, T., et al. (2011). Direct targeting of Sec23a by miR-200s influences cancer cell secretome and promotes metastatic colonization. *Nat. Med.* 17 (9), 1101–1108. doi:10.1038/nm.2401

Krasner, C. N., Poveda, A., Herzog, T. J., Vermorken, J. B., Kaye, S. B., Nieto, A., et al. (2012). Patient-reported outcomes in relapsed ovarian cancer: Results from a randomized phase III study of trabectedin with pegylated liposomal doxorubicin (PLD) versus PLD alone. *Gynecol. Oncol.* 127 (1), 161–167. doi:10.1016/j.ygyno.2012.06.034

Kristensen, L. S., Andersen, M. S., Stagsted, L. V. W., Ebbesen, K. K., Hansen, T. B., and Kjems, J. (2019). The biogenesis, biology and characterization of circular RNAs. *Nat. Rev. Genet.* 20 (11), 675–691. doi:10.1038/s41576-019-0158-7

Kristensen, L. S., Hansen, T. B., Veno, M. T., and Kjems, J. (2018). Circular RNAs in cancer: Opportunities and challenges in the field. *Oncogene* 37 (5), 555–565. doi:10.1038/onc.2017.361

Lakings, D. B., Waalkes, T. P., Borek, E., Gehrke, C. W., Mrochek, J. E., Longmore, J., et al. (1977). Composition, associated tissue methyltransferase activity, and catabolic end products of transfer RNA from carcinogen-induced hepatoma and normal monkey livers. *Cancer Res.* 37 (1), 285–292.

Lee, Y. S., Shibata, Y., Malhotra, A., and Dutta, A. (2009). A novel class of small RNAs: tRNA-derived RNA fragments (tRFs). *Genes Dev.* 23 (22), 2639–2649. doi:10.1101/gad.1837609

Lei, B., Tian, Z., Fan, W., and Ni, B. (2019). Circular RNA: A novel biomarker and therapeutic target for human cancers. *Int. J. Med. Sci.* 16 (2), 292–301. doi:10.7150/ijms.28047

Lekka, E., and Hall, J. (2018). Noncoding RNAs in disease. *FEBS Lett.* 592 (17), 2884–2900. doi:10.1002/1873-3468.13182

Li, F., Yang, Q., He, A. T., and Yang, B. B. (2021). Circular RNAs in cancer: Limitations in functional studies and diagnostic potential. *Semin. Cancer Biol.* 75, 49–61. doi:10.1016/j.semcancer.2020.10.002

Li, Y., Zeng, C., Hu, J., Pan, Y., Shan, Y., Liu, B., et al. (2018). Long non-coding RNA-SNHG7 acts as a target of miR-34a to increase GALNT7 level and regulate PI3K/Akt/mTOR pathway in colorectal cancer progression. *J. Hematol. Oncol.* 11 (1), 89. doi:10.1186/s13045-018-0632-2

Li, Y., Zhu, X., Gu, J., Hu, H., Dong, D., Yao, J., et al. (2010). Anti-miR-21 oligonucleotide enhances chemosensitivity of leukemic HL60 cells to arabinosylcytosine by inducing apoptosis. *Hematology* 15 (4), 215–221. doi:10.1179/102453310X12647083620840

Liang, F., Holt, I., Pertea, G., Karamycheva, S., Salzberg, S. L., and Quackenbush, J. (2000). Gene index analysis of the human genome estimates approximately 120, 000 genes. *Nat. Genet.* 25 (2), 239–240. doi:10.1038/76126

Liao, C., Chen, W., Fan, X., Jiang, X., Qiu, L., Chen, C., et al. (2013). MicroRNA-200c inhibits apoptosis in pituitary adenoma cells by targeting the PTEN/Akt

signaling pathway. *Oncol. Res.* 21 (3), 129–136. doi:10.3727/096504013X13832473329999

Liu, B., Qu, J., Xu, F., Guo, Y., Wang, Y., Yu, H., et al. (2015). MiR-195 suppresses non-small cell lung cancer by targeting CHEK1. *Oncotarget* 6 (11), 9445–9456. doi:10.18632/oncotarget.3255

Liu, J., Xie, S., Wu, Y., Xu, M., Ao, C., Wang, W., et al. (2016). Apoptosis of human prostate cancer cells induced by marine actinomycin X2 through the mTOR pathway compounded by MiRNA144. *Anticancer. Drugs* 27 (3), 156–163. doi:10.1097/CAD.0000000000000309

Liu, T. J., Hu, S., Qiu, Z. D., Liu, D., Hu, Y., Wu, D., et al. (2020). Anti-tumor mechanisms associated with regulation of non-coding RNA by active ingredients of Chinese medicine: A review. *Front. Oncol.* 10, 602104. doi:10.3389/fonc.2020.602104

Lu, F., Zhang, J. R., Ji, M., Li, P., Du, Y. H., Wang, H. C., et al. (2014). miR-181b increases drug sensitivity in acute myeloid leukemia via targeting HMGB1 and Mcl-1. *Int. J. Oncol.* 45 (1), 383–392. doi:10.3892/ijo.2014.2390

Luo, Q. Q., Tian, Y., Qu, G. J., Kun, H., and Luo, S. S. (2022). Functional mechanism and clinical implications of miR-141 in human cancers. *Cell. Signal.* 95, 110354. doi:10.1016/j.cellsig.2022.110354

Luo, W. F., Yu, H. L., Zou, X. L., Ni, X., and Wei, J. (2018). Long non-coding RNA taurine-upregulated gene 1 correlates with unfavorable prognosis in patients with refractory or relapsed acute myeloid leukemia treated by purine analogue based chemotherapy regimens. *Cancer Biomark.* 23 (4), 485–494. doi:10.3233/Cbm-181405

Ma, D., Wei, J., Chen, S., Wang, H., Ning, L., Luo, S. H., et al. (2021). Fucoidan inhibits the progression of hepatocellular carcinoma via causing lncRNA LINC00261 overexpression. *Front. Oncol.* 11, 653902. doi:10.3389/fonc.2021.653902

Mai, D., Zheng, Y., Guo, H., Ding, P., Bai, R., Li, M., et al. (2020). Serum piRNA-54265 is a New Biomarker for early detection and clinical surveillance of Human Colorectal Cancer. *Theranostics* 10 (19), 8468–8478. doi:10.7150/thno.46241

Mehta, S. L., Chokkalla, A. K., and Vemuganti, R. (2021). Noncoding RNA crosstalk in brain health and diseases. *Neurochem. Int.* 149, 105139. doi:10.1016/j.neuint.2021.105139

Mei, Y. P., Liao, J. P., Shen, J., Yu, L., Liu, B. L., Liu, L., et al. (2012). Small nucleolar RNA 42 acts as an oncogene in lung tumorigenesis. *Oncogene* 31 (22), 2794–2804. doi:10.1038/onc.2011.449

Moodley, K., and Weiss, S. F. T. (2013). Downregulation of the non-integrin laminin receptor reduces cellular viability by inducing apoptosis in lung and cervical cancer cells. *PloS One* 8, e57409. doi:10.1371/journal.pone.0057409

Newman, D. J., and Cragg, G. M. (2014). Marine-sourced anti-cancer and cancer pain control agents in clinical and late preclinical development. *Mar. Drugs* 12 (1), 255–278. doi:10.3390/md12010255

Newman, D. J., and Cragg, G. M. (2012). Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J. Nat. Prod.* 75 (3), 311–335. doi:10.1021/np200906s

Newman, D. J., Cragg, G. M., and Snader, K. M. (2003). Natural products as sources of new drugs over the period 1981-2002. *J. Nat. Prod.* 66 (7), 1022–1037. doi:10.1021/np030096l

Palma, C. A., Al Sheikha, D., Lim, T. K., Bryant, A., Vu, T. T., Jayaswal, V., et al. (2014). MicroRNA-155 as an inducer of apoptosis and cell differentiation in Acute Myeloid Leukaemia. *Mol. Cancer* 13, 79. doi:10.1186/1476-4598-13-79

Pandey, M. K., Gowda, K., Doi, K., Sharma, A. K., Wang, H. G., and Amin, S. (2013). Proteasomal degradation of Mcl-1 by maritoclax induces apoptosis and enhances the efficacy of ABT-737 in melanoma cells. *PLoS One* 8 (11), e78570. doi:10.1371/journal.pone.0078570

Pecot, C. V., Rupaimoole, R., Yang, D., Akbani, R., Ivan, C., Lu, C., et al. (2013). Tumour angiogenesis regulation by the miR-200 family. *Nat. Commun.* 4, 2427. doi:10.1038/ncomms3427

Peng, W. X., Koirala, P., and Mo, Y. Y. (2017). LncRNA-mediated regulation of cell signaling in cancer. *Oncogene* 36 (41), 5661–5667. doi:10.1038/onc.2017.184

Peraldo Neia, C., Cavalloni, G., Chiorino, G., Ostano, P., Aglietta, M., and Leone, F. (2016). Gene and microRNA modulation upon trabectedin treatment in a human intrahepatic cholangiocarcinoma paired patient derived xenograft and cell line. *Oncotarget* 7 (52), 86766–86780. doi:10.18632/oncotarget.13575

Perez-Herrero, E., and Fernandez-Medarde, A. (2015). Advanced targeted therapies in cancer: Drug nanocarriers, the future of chemotherapy. *Eur. J. Pharm. Biopharm.* 93, 52–79. doi:10.1016/j.ejpb.2015.03.018

Perina, D., Korolija, M., Hadzija, M. P., Grbesa, I., Beluzic, R., Imesek, M., et al. (2015). Functional and structural characterization of FAU gene/protein from marine sponge *Suberites domuncula*. *Mar. Drugs* 13 (7), 4179–4196. doi:10.3390/md13074179

Pickard, M. R., Mourtada-Maarabouni, M., and Williams, G. T. (2011). Candidate tumour suppressor fau regulates apoptosis in human cells: An essential role for Bcl-G. *Biochim. Biophys. Acta* 1812 (9), 1146–1153. doi:10.1016/j.bbadis.2011.04.009

Ramalingam, V., and Rajaram, R. (2016). Antioxidant activity of 1-hydroxy-1-norresistomycin derived from Streptomyces variabilis KP149559 and evaluation of its toxicity against zebra fish *Danio rerio*. *RSC Adv.* 6 (20), 16615–16623. doi:10.1039/c5ra22558b

Ramalingam, V., Varunkumar, K., Ravikumar, V., and Rajaram, R. (2018). p53 mediated transcriptional regulation of long non-coding RNA by 1-hydroxy-1-norresistomycin triggers intrinsic apoptosis in adenocarcinoma lung cancer. *Chem. Biol. Interact.* 287, 1–12. doi:10.1016/j.cbi.2018.03.016

Regazzi, R. (2018). MicroRNAs as therapeutic targets for the treatment of diabetes mellitus and its complications. *Expert Opin. Ther. Targets* 22 (2), 153–160. doi:10.1080/14728222.2018.1420168

Riccioni, R., Lulli, V., Castelli, G., Biffoni, M., Tiberio, R., Pelosi, E., et al. (2015). miR-21 is overexpressed in NPM1-mutant acute myeloid leukemias. *Leuk. Res.* 39 (2), 221–228. doi:10.1016/j.leukres.2014.11.001

Robinson, E. K., Covarrubias, S., and Carpenter, S. (2020). The how and why of lncRNA function: An innate immune perspective. *Biochim. Biophys. Acta. Gene Regul. Mech.* 1863 (4), 194419. doi:10.1016/j.bbagrm.2019.194419

Rosell, R., Gatzemeier, U., Betticher, D. C., Keppler, U., Macha, H. N., Pirker, R., et al. (2002). Phase III randomised trial comparing paclitaxel/carboplatin with paclitaxel/cisplatin in patients with advanced non-small-cell lung cancer: A cooperative multinational trial. *Ann. Oncol.* 13 (10), 1539–1549. doi:10.1093/annonc/mdf332

Russ, A. C., Sander, S., Luck, S. C., Lang, K. M., Bauer, M., Rucker, F. G., et al. (2011). Integrative nucleophosmin mutation-associated microRNA and gene expression pattern analysis identifies novel microRNA - target gene interactions in acute myeloid leukemia. *Haematologica* 96 (12), 1783–1791. doi:10.3324/haematol.2011.046888

Santosh, B., Varshney, A., and Yadava, P. K. (2015). Non-coding RNAs: Biological functions and applications. *Cell. biochem. Funct.* 33 (1), 14–22. doi:10.1002/cbf.3079

Satomi-Tsushita, N., Shimomura, A., Matsuzaki, J., Yamamoto, Y., Kawauchi, J., Takizawa, S., et al. (2019). Serum microRNA-based prediction of responsiveness to eribulin in metastatic breast cancer. *PLoS One* 14 (9), e0222024. doi:10.1371/journal.pone.0222024

Schnekenburger, M., Dicato, M., and Diederich, M. (2014). Epigenetic modulators from "the Big blue": A treasure to fight against cancer. *Cancer Lett.* 351 (2), 182–197. doi:10.1016/j.canlet.2014.06.005

Seetharam, M., Kolla, K. R., and Chawla, S. P. (2018). Eribulin therapy for the treatment of patients with advanced soft tissue sarcoma. *Future Oncol.* 14 (16), 1531–1545. doi:10.2217/fon-2017-0461

Senthilkumar, K., and Kim, S. K. (2014). Anticancer effects of fucoidan. *Adv. Food Nutr. Res.* 72, 195–213. doi:10.1016/B978-0-12-800269-8.00011-7

Shibayama, Y., Kondo, T., Ohya, H., Fujisawa, S. I., Teshima, T., and Iseki, K. (2015). Upregulation of microRNA-126-5p is associated with drug resistance to cytarabine and poor prognosis in AML patients. *Oncol. Rep.* 33 (5), 2176–2182. doi:10.3892/or.2015.3839

Siddiqi, S., and Matushansky, I. (2012). Piwis and piwi-interacting RNAs in the epigenetics of cancer. *J. Cell. Biochem.* 113 (2), 373–380. doi:10.1002/jcb.23363

Sil, S., Dagur, R. S., Liao, K., Peeples, E. S., Hu, G., Periyasamy, P., et al. (2020). Strategies for the use of extracellular vesicles for the delivery of therapeutics. *J. Neuroimmune Pharmacol.* 15 (3), 422–442. doi:10.1007/s11481-019-09873-y

Slack, F. J., and Chinnaiyan, A. M. (2019). The role of non-coding RNAs in oncology. *Cell.* 179 (5), 1033–1055. doi:10.1016/j.cell.2019.10.017

Sun, S. N., Hu, S., Shang, Y. P., Li, L. Y., Zhou, H., Chen, J. S., et al. (2019). Relevance function of microRNA-708 in the pathogenesis of cancer. *Cell. Signal.* 63, 109390. doi:10.1016/j.cellsig.2019.109390

Tanaka, R., Satoh, H., Moriyama, M., Satoh, K., Morishita, Y., Yoshida, S., et al. (2000). Intronic U50 small-nucleolar-RNA (snoRNA) host gene of no protein-coding potential is mapped at the chromosome breakpoint t(3;6)(q27;q15) of human B-cell lymphoma. *Genes cells.* 5 (4), 277–287. doi:10.1046/j.1365-2443.2000.00325.x

Tang, B., He, W. L., Zheng, C., Cheang, T. Y., Zhang, X. F., Wu, H., et al. (2012). Marine fungal metabolite 1386A alters the microRNA profile in MCF-7 breast cancer cells. *Mol. Med. Rep.* 5 (3), 610–618. doi:10.3892/mmr.2011.697

Tang, Y., Cheung, B. B., Atmadibrata, B., Marshall, G. M., Dinger, M. E., Liu, P. Y., et al. (2017). The regulatory role of long noncoding RNAs in cancer. *Cancer Lett.* 391, 12–19. doi:10.1016/j.canlet.2017.01.010

Thin, K. Z., Liu, X., Feng, X., Raveendran, S., and TuLncRNA-Dancr, J. C. (2018). LncRNA-DANCR: A valuable cancer related long non-coding RNA for human cancers. *Pathol. Res. Pract.* 214 (6), 801–805. doi:10.1016/j.prp.2018.04.003

Thorenoor, N., and Slaby, O. (2015). Small nucleolar RNAs functioning and potential roles in cancer. *Tumour Biol.* 36 (1), 41–53. doi:10.1007/s13277-014-2818-8

Toden, S., Zumwalt, T. J., and Goel, A. (2021). Non-coding RNAs and potential therapeutic targeting in cancer. *Biochim. Biophys. Acta. Rev. Cancer* 1875 (1), 188491. doi:10.1016/j.bbcan.2020.188491

Toiyama, Y., Hur, K., Tanaka, K., Inoue, Y., Kusunoki, M., Boland, C. R., et al. (2014). Serum miR-200c is a novel prognostic and metastasis-predictive biomarker in patients with colorectal cancer. *Ann. Surg.* 259 (4), 735–743. doi:10.1097/SLA.0b013e3182a6909d

Trabectedin (2006). Trabectedin: Ecteinascidin 743, ecteinascidin-743, ET 743, ET-743, NSC 684766. *Drugs R. D.* 7 (5), 317–328. doi:10.2165/00126839-200607050-00005

Uboldi, S., Calura, E., Beltrame, L., Fuso Nerini, I., Marchini, S., Cavalieri, D., et al. (2012). A systems biology approach to characterize the regulatory networks leading to trabectedin resistance in an *in vitro* model of myxoid liposarcoma. *PLoS One* 7 (4), e35423. doi:10.1371/journal.pone.0035423

Vandewalle, V., Essaghir, A., Bollaert, E., Lenglez, S., Graux, C., Schoemans, H., et al. (2021). miR-15a-5p and miR-21-5p contribute to chemoresistance in cytogenetically normal acute myeloid leukaemia by targeting PDCD4, ARL2 and BTG2. *J. Cell. Mol. Med.* 25 (1), 575–585. doi:10.1111/jcmm.16110

Varadarajan, S., Poornima, P., Milani, M., Gowda, K., Amin, S., Wang, H. G., et al. (2015). Maritoclax and dinaciclib inhibit MCL-1 activity and induce apoptosis in both a MCL-1-dependent and -independent manner. *Oncotarget* 6 (14), 12668–12681. doi:10.18632/oncotarget.3706

Vishnoi, A., and Rani, S. (2017). MiRNA biogenesis and regulation of diseases: An overview. *Methods Mol. Biol.* 1509, 1–10. doi:10.1007/978-1-4939-6524-3_1

Vu, T. T., Stolzel, F., Wang, K. W., Rollig, C., Tursky, M. L., Molloy, T. J., et al. (2021). miR-10a as a therapeutic target and predictive biomarker for MDM2 inhibition in acute myeloid leukemia. *Leukemia* 35 (7), 1933–1948. doi:10.1038/s41375-020-01095-z

Vychytilova-Faltejskova, P., and Slaby, O. (2019). MicroRNA-215: From biology to theranostic applications. *Mol. Asp. Med.* 70, 72–89. doi:10.1016/j.mam.2019.03.002

Wang, D., Wang, C., Gui, P., Liu, H., Khalaf, S. M. H., Elsayed, E. A., et al. (2017). Identification, Bioactivity, and productivity of actinomycins from the marine-derived Streptomyces heliomycini. *Front. Microbiol.* 8, 1147. doi:10.3389/fmicb.2017.01147

Wang, K. C., and Chang, H. Y. (2011). Molecular mechanisms of long noncoding RNAs. *Mol. Cell.* 43 (6), 904–914. doi:10.1016/j.molcel.2011.08.018

Wang, X. X., Liu, J., Tang, Y. M., Hong, L., Zeng, Z., and Tan, G. H. (2017). MicroRNA-638 inhibits cell proliferation by targeting suppress PIM1 expression in human osteosarcoma. *Tumor Biol.* 37, 16367–16375. doi:10.1007/s13277-016-5379-1

Wang, Z., Yuan, B., Fu, F., Huang, S., and Yang, Z. (2017). Hemoglobin enhances miRNA-144 expression and autophagic activation mediated inflammation of microglia via mTOR pathway. *Sci. Rep.* 7 (1), 11861. doi:10.1038/s41598-017-12067-2

Wiemer, E. A. C., Wozniak, A., Burger, H., Smid, M., Floris, G., Nzokirantevye, A., et al. (2017). Identification of microRNA biomarkers for response of advanced soft tissue sarcomas to eribulin: Translational results of the EORTC 62052 trial. *Eur. J. Cancer* 75, 33–40. doi:10.1016/j.ejca.2016.12.018

Wilusz, J. E. A. (2018). A 360° view of circular RNAs: From biogenesis to functions. *Wiley Interdiscip. Rev. RNA* 9 (4), e1478. doi:10.1002/wrna.1478

Wu, L., Sun, J., Su, X., Yu, Q., Yu, Q., and Zhang, P. (2016). A review about the development of fucoidan in antitumor activity: Progress and challenges. *Carbohydr. Polym.* 154, 96–111. doi:10.1016/j.carbpol.2016.08.005

Wu, L., Ye, K., Jiang, S., and Zhou, G. (2021). Marine power on cancer: Drugs, lead compounds, and mechanisms. *Mar. Drugs* 19 (9), 488. doi:10.3390/md19090488

Wu, W., Xu, C., Zhang, X., Yu, A., and Shu, L. (2020). Shrimp miR-965 induced the human melanoma stem-like cell apoptosis and inhibited their stemness by disrupting the MCL-1-ER stress-XBP1 feedback loop in a cross-species manner. *Stem Cell. Res. Ther.* 11 (1), 248. doi:10.1186/s13287-020-01734-3

Xiao, R., Li, C., and Chai, B. (2015). miRNA-144 suppresses proliferation and migration of colorectal cancer cells through GSPT1. *Biomed. Pharmacother.* 74, 138–144. doi:10.1016/j.biopha.2015.08.006

Xie, G., Zhu, X., Li, Q., Gu, M., He, Z., Wu, J., et al. (2010). SZ-685C, a marine anthraquinone, is a potent inducer of apoptosis with anticancer activity by suppression of the Akt/FOXO pathway. *Br. J. Pharmacol.* 159 (3), 689–697. doi:10.1111/j.1476-5381.2009.00577.x

Xu, Q., Zhang, Q., Dong, M., and Yu, Y. (2021). MicroRNA-638 inhibits the progression of breast cancer through targeting HOXA9 and suppressing Wnt/β-cadherin pathway. *World J. Surg. Oncol.* 19 (1), 247. doi:10.1186/s12957-021-02363-7

Xu, W., Hua, Y., Deng, F., Wang, D., Wu, Y., Zhang, W., et al. (2020). MiR-145 in cancer therapy resistance and sensitivity: A comprehensive review. *Cancer Sci.* 111 (9), 3122–3131. doi:10.1111/cas.14517

Yan, M. D., Hong, C. C., Lai, G. M., Cheng, A. L., Lin, Y. W., and Chuang, S. E. (2005). Identification and characterization of a novel gene Saf transcribed from the opposite strand of Fas. *Hum. Mol. Genet.* 14 (11), 1465–1474. doi:10.1093/hmg/ddi156

Yan, M. D., Lin, H. Y., and Hwang, P. A. (2019). The anti-tumor activity of Brown seaweed oligo-fucoidan via lncRNA expression modulation in HepG2 cells. *Cytotechnology* 71 (1), 363–374. doi:10.1007/s10616-019-00293-7

Yan, M. D., Yao, C. J., Chow, J. M., Chang, C. L., Hwang, P. A., Chuang, S. E., et al. (2015). Fucoidan elevates MicroRNA-29b to regulate DNMT3B-MTSS1 Axis and inhibit EMT in human hepatocellular carcinoma cells. *Mar. Drugs* 13 (10), 6099–6116. doi:10.3390/md13106099

Yan, Y., Liu, X. Y., Lu, A., Wang, X. Y., Jiang, L. X., and Wang, J. C. (2022). Non-viral vectors for RNA delivery. *J. Control. Release* 342, 241–279. doi:10.1016/j.jconrel.2022.01.008

Yang, F., Nam, S., Brown, C. E., Zhao, R., Starr, R., Ma, Y., et al. (2014). A novel berbamine derivative inhibits cell viability and induces apoptosis in cancer stem-like cells of human glioblastoma, via up-regulation of miRNA-4284 and JNK/AP-1 signaling. *PLoS One* 9 (4), e94443. doi:10.1371/journal.pone.0094443

Yang, F., Wei, J., Zhang, S., and Zhang, X. (2017). Shrimp miR-S8 suppresses the stemness of human melanoma stem-like cells by targeting the transcription factor YB-1. *Cancer Res.* 77 (20), 5543–5553. doi:10.1158/0008-5472.CAN-17-1375

Yang, G., Zhang, W., Yu, C., Ren, J., and An, Z. (2015). MicroRNA let-7: Regulation, single nucleotide polymorphism, and therapy in lung cancer. *J. Cancer Res. Ther.* 11, C1–C6. doi:10.4103/0973-1482.163830

Yingchun, L., Rong, Z., Kun, Y., Ying, Y., and Zhuogang, L. (2015). Bone marrow MicroRNA-335 level predicts the chemotherapy response and prognosis of adult acute myeloid leukemia. *Med. Baltim.* 94 (33), e0986. doi:10.1097/MD.0000000000000986

Yongchun, Z., Linwei, T., Xicai, W., Lianhua, Y., Guangqiang, Z., Ming, Y., et al. (2014). MicroRNA-195 inhibits non-small cell lung cancer cell proliferation, migration and invasion by targeting MYB. *Cancer Lett.* 347 (1), 65–74. doi:10.1016/j.canlet.2014.01.019

Yu, W., Liang, X., Li, X., Zhang, Y., Sun, Z., Liu, Y., et al. (2018). MicroRNA-195: A review of its role in cancers. *Onco. Targets. Ther.* 11, 7109–7123. doi:10.2147/OTT.S183600

Yu, X., Zhang, Y., Ma, X., and Pertsemlidis, A. (2018). miR-195 potentiates the efficacy of microtubule-targeting agents in non-small cell lung cancer. *Cancer Lett.* 427, 85–93. doi:10.1016/j.canlet.2018.04.007

Zhang, B., Pan, X., Cobb, G. P., and Anderson, T. A. (2007). microRNAs as oncogenes and tumor suppressors. *Dev. Biol.* 302 (1), 1–12. doi:10.1016/j.ydbio.2006.08.028

Zhang, H., Kang, J., Liu, L., Chen, L., Ren, S., and Tao, Y. (2020). MicroRNA-143 sensitizes acute myeloid leukemia cells to cytarabine via targeting ATG7- and ATG2B-dependent autophagy. *Aging (Albany NY)* 12 (20), 20111–20126. doi:10.18632/aging.103614

Zhang, H., Liang, H., Wu, S., Zhang, Y., and Yu, Z. (2021). MicroRNA-638 induces apoptosis and autophagy in human liver cancer cells by targeting enhancer of zeste homolog 2 (EZH2). *Environ. Toxicol. Pharmacol.* 82, 103559. doi:10.1016/j.etap.2020.103559

Zhang, H., Liu, L., Chen, L., Liu, H., Ren, S., and Tao, Y. (2021). Long noncoding RNA DANCR confers cytarabine resistance in acute myeloid leukemia by activating autophagy via the miR-874-3P/ATG16L1 axis. *Mol. Oncol.* 15 (4), 1203–1216. doi:10.1002/1878-0261.12661

Zhang, L., Liao, Y., and Tang, L. (2019). MicroRNA-34 family: A potential tumor suppressor and therapeutic candidate in cancer. *J. Exp. Clin. Cancer Res.* 38 (1), 53. doi:10.1186/s13046-019-1059-5

Zhang, S., and Zhang, X. (2019). Shrimp miRNA suppresses the stemness of human cancer stem cells via the PIN1 pathway. *Faseb. J.* 33 (10), 10767–10779. doi:10.1096/fj.201900395RR

Zhang, X., Li, Z., Ji, W., Chen, X., Gao, Q., Li, D., et al. (2020). Influence mechanism of miRNA-144 on proliferation and apoptosis of osteosarcoma cells. *Oncol. Lett.* 19 (2), 1530–1536. doi:10.3892/ol.2019.11197

Zhang, Y., Liu, X., Zhang, J., Xu, Y., Shao, J., Hu, Y., et al. (2020). Inhibition of miR-19a partially reversed the resistance of colorectal cancer to oxaliplatin via PTEN/PI3K/AKT pathway. *Aging (Albany NY)* 12 (7), 5640–5650. doi:10.18632/aging.102929

Zhang, Y. M., Wu, Q. M., Chang, L. Y., and Liu, J. C. (2020). miR-34a and miR-125a-5p inhibit proliferation and metastasis but induce apoptosis in hepatocellular carcinoma cells via repressing the MACC1-mediated PI3K/AKT/mTOR pathway. *Neoplasma* 67 (5), 1042–1053. doi:10.4149/neo_2020_191019N1062

Zheng, Y., Yang, F., Fu, L., and Liu, K. (2018). The mechanism of miR-143 inducing apoptosis of liver carcinoma cells through regulation of the NF-kappa B pathway. *Oncol. Lett.* 15 (6), 9567–9571. doi:10.3892/ol.2018.8486

Zhou, W. Y., Cai, Z. R., Liu, J., Wang, D. S., Ju, H. Q., and Xu, R. H. (2020). Circular RNA: Metabolism, functions and interactions with proteins. *Mol. Cancer* 19 (1), 172. doi:10.1186/s12943-020-01286-3

Zhou, Z., Zhang, Y., Gao, J., Hao, X., Shan, C., Li, J., et al. (2021). Circular RNAs act as regulators of autophagy in cancer. *Mol. Ther. Oncolytics* 21, 242–254. doi:10.1016/j.omto.2021.04.007

Glossary

AKT protein kinase B

AML acute myeloid leukemia

AraC cytarabine

BCL-2 B-cell lymphoma-2

BCL-XL B-cell lymphoma-extra large

BTC biliary tract cancer

CART Charge-altering releasable transporter

ceRNA competitive endogenous RNA

circRNA circular RNA

ER endoplasmic reticulum

EV extracellular vesicle

HCC hepatocellular carcinoma

K-RAS Kirsten rat sarcoma viral oncogene homolog

lncRNA long noncoding RNA

MCL-1 myeloid cell leukemia sequence 1

miRNA microRNA

mRNA messenger RNA

ncRNA noncoding RNA

mTOR mammalian target of rapamycin

NF-κB nuclear transcription factor-κB

NK natural killer

NP nanoparticle

PDCD4 programmed cell death 4

piRNA piwi-interacting RNA

PI3K phosphatidyl inositol 3-kinase

PTEN phosphatase and tensin homolog deleted from chromosome 10

RNAi RNA interference

snoRNA small nucleolar RNA

STS soft tissue sarcoma

TRAIL tumor necrosis factor related apoptosis inducing ligand

tsRNA transfer RNA-derived small RNA

VEGF vascular endothelial growth factor

Wnt wingless-type MMTV integration site family

frontiers | Frontiers in Pharmacology

TYPE Mini Review
PUBLISHED 09 January 2023
DOI 10.3389/fphar.2022.1050032

Check for updates

OPEN ACCESS

EDITED BY
Peng Qu,
National Institutes of Health (NIH),
United States

REVIEWED BY
Kun Wang,
National Institutes of Health (NIH),
United States
Guohao Wang,
National Institutes of Health (NIH),
United States

*CORRESPONDENCE
Jaewoo Hong,
jhong@cu.ac.kr

SPECIALTY SECTION
This article was submitted to
Pharmacology of Anti-Cancer Drugs,
a section of the journal
Frontiers in Pharmacology

RECEIVED 21 September 2022
ACCEPTED 22 December 2022
PUBLISHED 09 January 2023

CITATION
Sung WJ and Hong J (2023), Targeting lncRNAs of colorectal cancers with natural products.
Front. Pharmacol. 13:1050032.
doi: 10.3389/fphar.2022.1050032

COPYRIGHT
© 2023 Sung and Hong. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Targeting lncRNAs of colorectal cancers with natural products

Woo Jung Sung[1] and Jaewoo Hong[2]*

[1]Department of Pathology, Daegu Catholic University School of Medicine, Daegu, South Korea, [2]Department of Physiology, Daegu Catholic University School of Medicine, Daegu, South Korea

Non-coding RNA (ncRNA) is one of the functional classes of RNA that has a regulatory role in various cellular processes, such as modulation of disease onset, progression, and prognosis. ncRNAs, such as microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), have been actively studied in recent years. The change in ncRNA levels is being actively studied in numerous human diseases, especially auto-immune disorders and cancers; however, targeting and regulating ncRNA with natural products to cure cancer has not been fully established. Recently many groups reported the relationship between ncRNA and natural products showing promising effects to serve as additional therapeutic approaches to cure cancers. This mini-review summarizes the aspects of lncRNAs related to cancer biology focusing on colorectal cancers that natural products can target.

KEYWORDS

ncRNA, natural products, lysosome, solid tumor, colorectal cancer, lncRNA

1 Introduction

Colorectal cancer (CRC) is the third most common cancer and the fourth most cause of cancer deaths globally (Siddiqui et al., 2019; Hassen et al., 2022). CRC onset has a higher tendency in developed countries than in developing countries (Hassen et al., 2022). Several etiological factors may affect the development of CRC, such as environmental, genetic, and epigenetic factorss (Anupriya et al., 2022). Usually, CRC is developed gradually over 1–2 decades (Siddiqui et al., 2019). The most common initiation of CRC is from adenomatous polyps of colorectal glandular epithelial cells. Malignant CRC begins when adenomatous polyps have mutations in the *Adenomatous polyposis coli* gene, tumor suppressor genes, and/or oncogenes (El Zoghbi and Cummings, 2016). The mortality of CRC increases significantly after metastasis and invasion initiation to other organs and tissues (Dowli et al., 2023). So, elucidating molecular mechanisms of the development and progression of CRC and searching for new markers and therapeutic strategies are essential in both basic and clinical sciences (Yu et al., 2022; Zheng et al., 2022). Recent findings revealed that epigenetic alterations are more frequent than genetic alterations in CRC (Okugawa et al., 2015). Currently, many groups focus on epigenetic studies on CRC to discover new biomarkers for diagnosis and develop new therapies (Ullah et al., 2022). The application of natural products such as phytochemicals with anticancer effects can be considered as one of the approaches to target lncRNAs to treat CRC, which may increase the sensitivity of CRC cells additionally to the prevailing therapies. This review investigates the effect of various phytochemicals on lncRNAs of CRC and evaluates their capacity to treat or prevent CRC.

2 LncRNAs

Non-protein coding RNAs with transcripts 200 bp or longer are called lncRNA (Costa et al., 2022; Pagani et al., 2022; Razlansari et al., 2022), which were believed to be byproducts of RNA

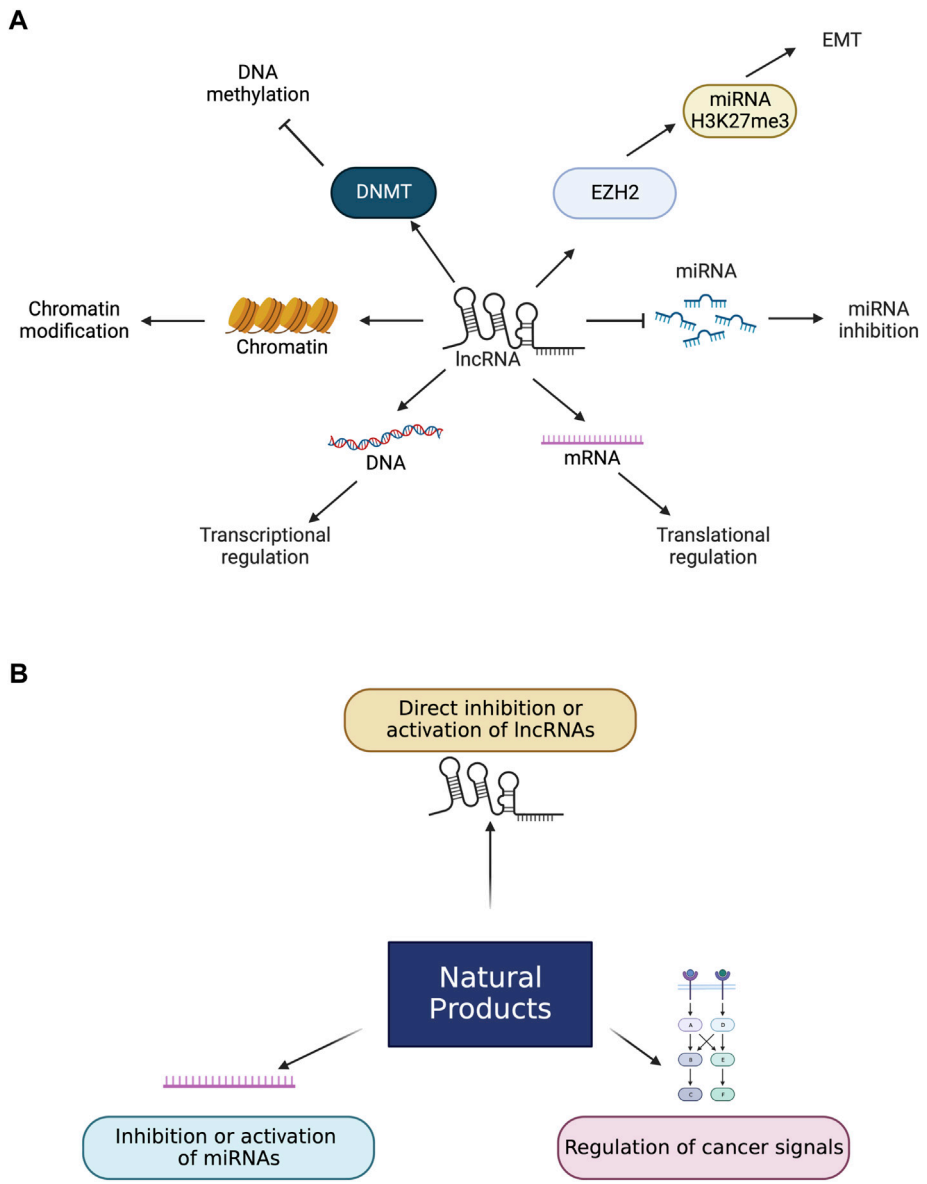


FIGURE 1
Roles of lncRNAs and natural products in cancers. **(A)** lncRNAs change the level of miRNAs which leads to apoptosis. miRNA inhibition can also lead to buffing the effect of miRNAs. LncRNAs lead to the EMT process by interacting with EZH2 and enhancing H3K27me3 levels. LncRNAs control the transcriptional and translational levels by controlling DNA and mRNA directly and are involved in chromatin modification. LncRNAs bind to DNMT and suppress DNA methylation. **(B)**. Natural products have several biological effects on lncRNAs directly and indirectly. Natural products can directly bind to lncRNAs to enhance or inactivate lncRNAs. Natural products can also control miRNAs and cancer signals that lead to activating or inactivating lncRNAs related to cancers.

polymerase II transcription without specific biological actions (Goodrich and Kugel, 2006; Wagner et al., 2013; Nojima and Proudfoot, 2022). Currently, lncRNA does not have a standardized classification. However, lncRNAs are classified by their location, such as cytoplasmic, nuclear, and cytoplasmic nuclear lncRNAs (Kerachian and Azghandi, 2022) and they have different regulatory functions where they are located (Ghafouri-Fard et al., 2022). Mainly, cytoplasmic lncRNAs act as competing endogenous RNAs (ceRNAs) against miRNAs regulating the release of target mRNAs of miRNAs. In the tumor microenvironment, lncRNAs are aberrantly expressed, breaking the balance of miRNA and target mRNA resulting in the promotion of malignant tumor progression *via* abnormal expression of tumor-promoting or tumor-suppressing genes.

The other way to classify lncRNAs is by the protein-coding gene relative location. First, the *righteous* lncRNAs overlap with exon regions. The *antisense* lncRNAs start with the reverse transcription process of exons. The *bidirectional* lncRNA begins closely with the neighboring protein-coding genes on the antisense strands. The *basal* lncRNA is from intron regions, and the *intergenic* lncRNA resides between different genes on the chromosome (Wang et al., 2021).

LncRNAs can be categorized by their molecular functions, guide, decoy, and backbone molecules. LncRNA is bound to DNA or proteins; decoy molecules inhibit the transcription of downstream genes while guide molecules enhance the transcription. Backbone molecules are scaffold molecules for protein complexes to form nucleic

acid-protein complexes involved in epigenetic functions (Han et al., 2022; Nadhan et al., 2022).

The regulatory roles of lncRNAs involve major life events and biological processes like stem cell differentiation, gene expression, development, cell proliferation, and metastasis, so they are closely correlated to the onset and development of cancer and other diseases (Figure 1) (He et al., 2022; Liu et al., 2022; Wang et al., 2022). In recent findings, the detection of lncRNAs is available from patients' blood to use lncRNAs as biomarkers over tissue lncRNAs. Circulating lncRNAs can be used as potential biomarkers to diagnose several cancers, including CRCs (Badowski et al., 2022).

3 LncRNAs and colorectal cancers

3.1 Functions of lncRNA in CRCs

In CRC, lncRNA is involved in RNA degradation, splicing, transcription, and translation (Xie et al., 2016). LncRNAs have essential roles in the carcinogenesis of CRCs, such as serving as oncogenes or tumor suppressor genes and interacting with DNAs, RNAs, and proteins (Ragusa et al., 2015). Furthermore, numerous studies revealed that lncRNAs function as endogenous miRNAs to contribute to the competitive endogenous RNA network of tumor regulation (Hashemi et al., 2022; Shen et al., 2022). Control of gene expression in developmental processes and cell differentiation has also been known as a role of lncRNAs (Cao, 2014). Indeed, lncRNAs work as gene expression regulators at epigenetic, transcriptional, and post-transcriptional levels (Xie et al., 2016). Recently, it has been revealed that lncRNAs are involved in the chemoresistance of CRCs through multiple mechanisms, including acting as structural RNAs in scaffolding ribonuclear protein complexes, interacting with other miRNAs, epigenetic modification, and regulating several gene expressions in essential cellular processes such as cell proliferation, differentiation, apoptosis, invasion, and metastasis (Lizarbe et al., 2017). Another interesting point of lncRNAs is acting as competing endogenous RNAs (ceRNAs), inhibiting targets of miRNAs. This leads to regulating miRNAs involved in CRC malignancies, such as migration, invasion, and proliferation (Li et al., 2017).

LncRNAs are commonly found in the serum or plasma of peripheral blood (Xie et al., 2016). Some blood lncRNAs are increased, working as oncogenes in tumor status. Meanwhile, others have tumor suppressor roles (Smolle et al., 2014). Some lncRNAs found in CRC cells and tissues have an increased tendency to correlate with poor prognosis and malignancy in CRC patients (Kam et al., 2014). In recent years, advanced bioinformatics, including microarray and next-generation sequencing, revealed many different lncRNAs are involved in CRC progression (Wang et al., 2015; Arun et al., 2018; Siddiqui et al., 2019). CRC shows the progression similarly to other solid tumors. In stage 0, carcinoma *in situ*, no local lymph node metastasis, and no distant metastasis are observed. In stage I, the tumor invades the submucosa or muscle layer, without local lymph node metastasis or distant metastasis. In stage II, the tumor invades the serosa layer or the large intestine and surrounding tissues through the muscular layer, without peritoneal coverage, local lymph node metastasis or distant metastasis. In stage III, the tumor directly invades other organs, with local lymph node metastasis and no distant metastasis. In stage IV, the tumor directly invades other organs, with local lymph node metastasis and distant metastasis (Fabian et al., 2023). We discuss some of the CRC-related lncRNAs, which can be good candidates to develop new approaches to target CRC, especially using natural products.

3.2 LncRNAs related to CRC

More than 80 different lncRNAs have been reported to be directly or indirectly associated with colorectal cancer. The functions and mechanisms are still studied actively to utilize them as prognostic markers or therapeutic targets. Among them, we discuss ten representative lncRNAs, which have been actively studied with obvious evidence affecting both positively and negatively in CRCs in different aspects (Table 1).

3.2.1 Cancer susceptibility candidate 11 (CASC11)

CASC11 is located on chromosome 8q24. CASC11 lncRNA has been reported to be increased in CRC cells and tissues. Furthermore, the tumor size correlates with the expression level of CASC11 (Zhang et al., 2016). When CASC11 is inhibited in CRC, proliferation and metastasis are suppressed in tumor cells by interacting with heterogeneous ribonucleoprotein. This leads to the protection of β-catenin degradation and increases the transcription activity (Shen et al., 2017). CASC11 has been reported to suppress Wnt signaling in colorectal cancer (Javed et al., 2020).

3.2.2 Colon cancer-associated transcript 1 (CCAT1)

CCAT1 has been recently identified as a lncRNA correlated with colorectal adenomas and adenocarcinomas at any stage (Ye et al., 2015). CCAT1 is a good target for real-time *in vivo* imaging techniques (Kam et al., 2014). CCAT1 has an oncogenic role in activating Myc, the target of miR-155, and promotes cell proliferation and invasion through direct interaction with the promoter region (Wang et al., 2015). A study reported that CCAT1 is increased in gallbladder cancer tissues, and this is through knocking-down miRNA that is related to tumor cell invasion and proliferation (Ma et al., 2015).

3.2.3 CCAT2

CCAT2 interacts with TCF7L2 and leads to the enhanced expression of Myc. Then, Myc regulates miR-17p and miR-20a functions. The output of this process is genomic instability and the promotion of cancer malignancy (Wu et al., 2016). Since CCAT2 has shown the modification of clinical outcomes, CCAT2 is considered an excellent target for lncRNA therapies and a diagnostic marker of CRC (Catana et al., 2017). Moreover, CCAT2 is critical in the loop formation between genomic DNA locus rs6983267 and Myc promoter, which turns on the oncogenic activity of Myc (Xu et al., 2014).

3.2.4 Colorectal neoplasia differentially expressed (CRNDE)

CRNDE is differently upregulated in CRC tissues in 90%. Insulin and IGFs induce the Warburg effect in cancer cells by metabolic changes that regulate CRNDE (Ye et al., 2015). In a recent finding, CRNDE-h (transcript variant one of CRNDE) was highly upregulated in CRC tissues. The overexpression levels were positively correlated

TABLE 1 lncRNAs associated with colorectal cancers.

lncRNA	Characters	Level	Mechanisms in CRC	References
CASC11	Metastasis, proliferation	↑	c-Myc binding to the promoter region of CAS11 to increase histone of promoter; Interaction with hnRNP-K to activate WNT/β-catenin	Fabian et al. (2023)
CCAT1	Cancer development, invasion, metastasis, carcinogenesis	↓	Superenhancer cMyc transcribes CCAT1	Kam et al. (2014); Ye et al. (2015); Abedini et al. (2019); Xue et al. (2021)
CCAT2	Pathogenesis	↑	Regulation of miR-17-5p, miR-20a, MYC; Modification of WNT signaling	Ma et al. (2015)
CRNDE	Prognostic marker	↑	Correlation with IRX5 mRNA expression	Xu et al. (2014); Liu et al. (2016); Ding et al. (2017)
GAS5	Prognostic marker	↓	GAS5 is regulated by p53	Han et al. (2017)
H19	Prognostic marker	↑	Regulation of CDK8-β-catenin, essential Rb-E2F signaling pathway; Recruitment of eIF4A3; Mediating MTX resistance through WNT/β-catenin signal activation; Modification of EMT; Functioning as a ceRNA for miR138, miR200a	Saus et al. (2016); Schwarzenbach. (2016); Chen et al. (2017)
HOTAIR	Carcinogenesis, prognostic marker	↑	Association with PRC2 function; Modification of EMT	Cui et al. (2002); Meeran et al. (2010); Dou et al. (2016); Luo et al. (2017); Song et al. (2020); Deng et al. (2021)
MALAT1	Metastasis, proliferation	↑	Interaction with CC chemokine ligand 5; Promotion of SFSF1 phosphorylation to enhance AKAP-9	Yang et al. (2015); Schmitt and Chang (2016)
PCAT-1	Prognostic marker, proliferation	↑	Promotion of PRC2	Ge et al. (2013); Zhao et al. (2015); Zhao et al. (2016)
UCA1	Carcinogenesis; chemoresistance; prognostic marker	↑	Inhibition of miR-204-5p; Regulation of glucose metabolism	Han et al. (2014); Bian et al. (2016); Qiao et al. (2017)

with the degree of malignancy, such as tumor size, lymph node metastasis, distant metastasis, and survival rate (Liu et al., 2016; Ding et al., 2017). The knockdown study of CRNDE showed the apoptosis of CRC cells *in vitro* and *in vivo* (Ding et al., 2017). Furthermore, the knockdown of CRNDE with miR-181a-5p showed the inhibition of cell proliferation and the reduction of chemoresistance *via* the downregulation of Wnt/β-catenin signaling (Han et al., 2017).

3.2.5 Growth arrest-specific transcript 5 (GAS5)

GAS5 is a lncRNA with tumor suppressor function. GAS5 interacts with the intracellular glucocorticoid receptor and regulates cellular metabolism and survival (Kino et al., 2010). Recent findings revealed that GAS5 is suppressed in several different cancers, and the downregulation of GAS5 was accompanied by the advanced TNM stage and large tumor size in CRC (Saus et al., 2016).

3.2.6 H19

H19 is a lncRNA enhanced in the early stages of embryogenesis and suppressed after birth (Ariel et al., 1998). H19 was first identified from the transcript of a gene cluster, H19/insulin-like growth factor 2 (IGF2), more from the maternal allele than the paternal (Chen et al., 2017). H19 regulates several cancer-associated proteins, including ubiquitin E3 ligase family, a retinoblastoma tumor suppressor, and calneuron 1 (Schwarzenbach, 2016). Furthermore, the methylated region of H19 and the upstream of IGF2 exon three were hypomethylated (Cui et al., 2002). The upregulation of H19 is correlated with the high TNM stage and poor prognosis (Chen et al., 2017).

3.2.7 HOX transcript antisense intergenic RNA (HOTAIR)

HOTAIR binds with polycomb repressive complex 2 (PRC2) in trans and changes cellular gene expression and epigenetics (Dou et al., 2016; Xie et al., 2016). HOTAIR is upregulated in epithelial cancer cells, inducing histone methylation and cancer cell invasion (Svoboda et al., 2014). Furthermore, HOTAIR upregulation is closely related to the proteins associated with the malignancy of CRCs, such as angiogenesis, invasion, metastasis, and high tumor stage, i.e., E-cadherin, vimentin, and matrix metalloproteinase (Luo et al., 2017). The correlation of HOTAIR with cancer malignancy and poor prognosis is related to CRC and several cancers like pancreatic cancer, epithelial ovarian cancer, mammary cancer, and hepatocellular carcinoma (Deng et al., 2017). A study showed the correlation between HOTAIR and poor prognosis using CRC blood, and tissue samples suggested this lncRNA as a prognostic marker for sporadic CRC (Svoboda et al., 2014).

3.2.8 Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1)

MALAT1 regulates alternative splicing through pre-mRNA binding to localize transcriptionally active genes in chromatin with serine/arginine splicing factor (Schmitt and Chang, 2016). MALAT1 activates AKAP-9, which leads to the malignancy of several cancers, such as melanoma, breast cancer, thyroid cancer,

TABLE 2 Regulation of lncRNA by natural products in CRC.

Natural compound	Target lncRNA	Effect	Targeting mechanisms	References
Berberine	HOTAIR	↓	Inhibition of EMT	Song et al. (2020); Zhong et al. (2022)
Calycosin	HOTAIR	↓	Induction of apoptosis	Wu et al. (2019); Deng et al. (2021)
Curcumin	H19	↓	Inhibition of EMT, transcriptional regulation	Ashrafizadeh et al. (2020)
	GAS5	↑	Transcriptional regulation	Grynkiewicz and Slifirski, (2012)
	HOTAIR	↓	Inhibition of migration	Ashrafizadeh et al. (2020)
DIM	HOTAIR	↓	Inhibition of autophagy	Zhang et al. (2014)
Gambogic acid	GAS5	↑	Transcriptional regulation	Lee et al. (2015); Che Hassan et al. (2018); Gao et al. (2021)
Genistein	HOTAIR	↓	Transcriptional regulation, chromatin remodeling	Meeran et al. (2010); Ravishankar et al. (2013)
Ginsenoside	HOTAIR	↓	Inhibition of proliferation and invasion	Abedini et al. (2019); Xue et al. (2021)
	H19	↓	Inhibition of proliferation and invasion	Li and Qi, (2019)
Quercetin	MALAT1	↓	Transcriptional regulation	Reyes-Farias and Carrasco-Pozo (2019); Zhang et al. (2019)
Resveratrol	HOTAIR	↓	Transcriptional regulation, chromatin remodeling	Cimino et al. (2012)
	MALAT1	↓	Induction of apoptosis	Vallino et al. (2020)
	GAS5	↓	Inhibition of proliferation and invasion	Cimino et al. (2012)
	UCA1	↓	Transcriptional regulation	Cimino et al. (2012)

oral cancer, lung cancer, and colorectal cancer, through enhanced cell proliferation, migration, invasion, and metastasis (Yang et al., 2015). In CRC cells, MALAT1 promotes SPRK1 expression and SRSF1 phosphorylation, which leads to the upregulation of AKAP-9 expression (Hu et al., 2016).

3.2.9 Prostate cancer-associated ncRNA transcript 1 (PCAT-1)

As the terminology, PCAT-1 was first identified in prostate cancer, but this lncRNA has also been reported to be related to CRCs' metastasis (Zhao et al., 2016). PCAT-1 promotes the expression of PRC2, which induces cell proliferation in cancer cells *in vitro* (Smolle et al., 2014). Additionally, PCAT-1 is involved in non-small cell lung cancer to upregulate cancer cell proliferation, invasion, and migration (Zhao et al., 2015). In CRC, PCAT-1 expression is highly correlated with distant metastasis, patient survival, and prognosis (Ge et al., 2013). In a recent study, PCAT upregulation in CRC enhanced c-myc signaling. At the same time, CRC deficiency decreased proliferation and blockage of the cell cycle *via* the suppression of c-myc and cyclins (Qiao et al., 2017).

3.2.10 Urothelial carcinoma-associated 1 (UCA1)

UCA1 is a lncRNA with the character of oncofetal genes that are involved in embryonic development (Han et al., 2014). However, bladder cancer is where UCA1 is highly expressed; UCA1 has been reported to be upregulated in CRC cells to inhibit apoptosis and develop tumorigenesis (Bian et al., 2016). UCA1 has a critical role in cancer biologies, such as cell transformation, proliferation, invasion, mortality, and chemoresistance (Wang et al., 2008). Furthermore, the UCA1 expression level is correlated with the tumor size. Meanwhile, CRC tumor size is reduced when UCA1 is deficient (Han et al., 2014).

4 Targeting lncRNAs with natural products for potential CRC treatment

Natural products and their derivatives have been widely studied and applied as anticancer agents for several decades (da Rocha et al., 2001). Natural products and their derivatives have various potent biological activities such as anticancer, anti-inflammatory, pro-apoptotic, and antioxidant characteristics, with the potential for chemotherapies and chemo-preventions for several cancers. They show anticancer effects primarily through epigenetic change, regulation of signaling pathways, and miRNA regulation in cancer cells or tissues (Homayoonfal et al., 2021). Below we introduce several practical natural products employed in cancer treatment targeting lncRNAs introduced previously (Table 2).

4.1 Berberine

Berberine is a pentacyclic isoquinoline alkaloid compound isolated from *Berberis* genus plants. The broad pharmacological application of berberine includes anticancer, antidiabetic, anti-obesity, and cardioprotective effects (Zhong et al., 2022). This compound interacts with specific receptors, ligands, and biological enzymes leading to anti-inflammatory and antioxidant activities (Song et al., 2020). Like other natural products, berberine modulates lncRNAs to inhibit cancer progression. In a recent study, the combination treatment of berberine with gefitinib downregulated HOTAIR function to enhance miR-34a-5p. The upregulation of miR-34a-5pupregulatess E-cadherin, to the arrest of EMT, invasion, and migration by SNAIL-mediated E-cadherin increase in lung cancer cells (Zheng et al., 2020). As the effect of miR-34a-5p is involved in colorectal cancer, berberine can be a supportive candidate to treat colorectal cancer.

4.2 Calycosin

Calycosin ($C_{16}H_{12}O_5$) is an isoflavone phytoestrogen isolated from the dried roots of Radix astragali with several biological effects (Wu et al., 2019). The anticancer effect of calycosin has been vigorously studied in several different cancers, such as breast cancer, liver cancer, colorectal cancer, and osteosarcoma (Deng et al., 2021). In breast cancer, calycosin downregulated phosphorylation of Akt and its downstream lncRNA, HOTAIR. This effect strongly decreased cancer development (Chen et al., 2015). This result was from breast cancer cell line MCF-7 downregulating EGFR and ERK1/2 with suppressed proliferation and enhanced apoptosis. Although MCF-7 is not a colorectal cancer cell line, the onset of colorectal cancer shares the effect of the EGFR signaling pathway, and calycosin can be an excellent synergetic candidate for current therapies.

4.3 Curcumin

The chemical formulation of curcumin (diferuloylmethane) is $C_{21}H_{20}O_6$. This is a polyphenol compound with bright yellow color isolated from Curcuma longa (the rhizome of turmeric) (Ashrafizadeh et al., 2020). This compound has been utilized as a traditional herbal medicine in Eastern society for a long time. The unique structure of curcumin enables suppression of ROS generation and several different pharmacological properties such as anticancer, neuroprotective, cardioprotective, hepatoprotective, anti-analgesic, and anti-inflammatory effects. Since it has been reported that curcumin targets lncRNAs, many groups have focused on this compound and are being actively studied (Grynkiewicz and Slifirski, 2012). In a recent study, As mentioned previously, the curcumin-pretreated cancer cells showed the activation of GAS5 promotors, while GAS5 is downregulated in CRC (Zheng et al., 2021). Further studies regarding the effect of curcumin targeting GAS5 have not been studied rigorously, but this can be an extraordinary therapeutic approach when more preclinical and clinical studies are fulfilled.

4.4 3,3′-diinodolymethane (DIM)

DIM ($C_{17}H_{14}N_2$) is a phytochemical in several cruciferous vegetables like cabbage, broccoli, lettuce, and kale (Licznerska and Baer-Dubowska, 2016). DIM modulates various signaling pathways to induce proliferation, cell survival, apoptosis, and angiogenesis (Zhang et al., 2014). DIM, directly and indirectly, downregulates Akt/FOXM1 signaling pathway and suppresses cancer progression and metastasis (Cai et al., 2015). The downregulation of Akt/FOXM1 leads to the decreased expression of lncRNAs, such as HOTAIR and CCAT1-L, that are highly involved in colorectal cancers, and cancer malignancy has been regulated through this pathway (Zinovieva et al., 2017). More vigorous studies about DIM are required to develop CRCs, but this is a very hopeful candidate for future therapeutics.

4.5 Gambogic acid (GA)

GA is a brownish resin and the most potent compound of gambose, isolated from Garcinia hanburyi (Che Hassan et al., 2018). GA has been used as a traditional medicine with various biological activities such as anticancer, anti-inflammatory, and antiviral effects with extremely minimal toxicity (Lee et al., 2015; Gao et al., 2021; Xu et al., 2022). When cancer cells are treated with GA, GAS5 expression is increased, which leads to the downregulation of EZH2 by binding E2F4. The downregulation of EZH2 enhances miR-101. miR-101 has a pro-apoptotic property that consequently suppresses cancer cell invasion and progression in preclinical stages.

4.6 Genistein

Genistein ($C_{15}H_{10}O_5$) is a phytoestrogen-originated isoflavone derived from soy. Phytoestrogens are non-steroidal herbal components with structures like estrogen functioning estrogen-like or anti-estrogenically (Ravishankar et al., 2013). The biological activities of genistein include tyrosine kinase inhibition, anticancer, and antioxidants. The anticancer function of genistein affects various cellular processes such as angiogenesis, apoptosis, and cell cycle (Meeran et al., 2010). One of the targets of genistein to have an anticancer effect is epigenetic changes affecting cancer-associated genes, including lncRNAs (Imai-Sumida et al., 2020). Genistein downregulates EED levels in PRC2, followed by the inhibition of the interaction between HOTAIR and PRC2. The suppression of HOTAIR/PRC2 recruitment to the promoter region of ZO-1 leads to the increased transcription of ZO-1. The other effect of genistein is the inhibition of SNAIL transcription by suppressing the interaction between HOTAIR and SMARCB1. The reduced HOTAIR interaction with chromatin remodeling factors leads to the repression of HOTAIR/chromatin remodeling pathways, followed by the downregulation of cancer malignancy (Imai-Sumida et al., 2020).

4.7 Ginsenoside

Ginsenoside is one of the steroid glycoside fractions, triterpene saponin from ginseng roots (Nakhjavani et al., 2019). Ginsenoside is not a single compound, but more than ten molecules have been identified. According to the number of hydroxyl groups in its chemical structure, ginsenoside is subcategorized into two major classes. The first group is protopanaxatriol (PPT), with six positions occupied by hydroxyl groups, and the next group is protopanaxadiol (PPD), with six positions not occupied by hydroxyl groups. The members of PPT are Re, G1, Rg2, and Rh1 and of PPD are Rb1, Rb2, Rb3, Rc, Rd, Rg3, and Rh2. Ginsenoside molecules have various biological activities showing slight differences between each molecule (Xue et al., 2021). It has been studied that CCAT1 is highly expressed in several CRCs affecting cell proliferation, invasion, and migration (Abedini et al., 2019). Especially CCAT1 suppression by ginsenoside-Rg3 reduced the signaling of the PI3K/Akt pathway, followed by suppression of CRC development (Li and Qi, 2019). In addition, ginsenoside Rg3 suppressed cancer development by suppressing HOTAIR in hepatoma (Pu et al., 2021).

4.8 Quercetin

Quercetin (3,5,7,30,40-pentahydroxyflavone) is a natural flavanol ubiquitously found in fruits and vegetables, which can be found as one of the easiest in the western diet (Hertog and Hollman, 1996; Boots et al., 2008). Various biological activities of quercetin include anticancer, antidiabetic, anti-inflammatory, and antioxidant effects

(Carullo et al., 2017). Arresting cell cycle, anti-proliferation, and apoptosis functions are remarkable anticancer effects of quercetin. Furthermore, it has been reported that various lncRNAs are affected by quercetin in cancer cells and tissues (Reyes-Farias and Carrasco-Pozo, 2019). A study reported quercetin could alter the expression of 240 lncRNAs along with 1,415 mRNAs, 83 miRNAs, and 131 circRNAs through the analysis of HCT-116 colorectal cancer cell line with MTS assay and flow cytometry (Zhang et al., 2019). Additionally, quercetin suppresses the expression of MALAT1 and MIAT, followed by decreased cell survival (Esteghlal et al., 2021). So, in experimental studies, quercetin may be applied to CRCs to inactivate PI3K/Akt signaling pathway by reducing the expression of lncRNAs, MALAT1, and MIAT.

4.9 Resveratrol

Resveratrol ($C_{14}H_{12}O_3$, trans-3,5,4′-trihydroxystilbene) is a natural polyphenolic phytoalexin isolated from various foods, including red wine, berries, grapes, nuts, and else (Bishayee, 2009). Resveratrol shows anticancer effects targeting multiple signaling molecules leading to the suppression of cancer cell viability and growth with minimal toxicity (Cimino et al., 2012). Resveratrol increases tumor suppressive lncRNAs such as GAS5, HULC, UCA1, and PVT1 in several cancers (Vallino et al., 2020). Furthermore, resveratrol decreased MALAT1 expression, followed by the Wnt/β-catenin signaling pathway reducing tumor progression in CRC, showing a reduced transformation, invasion, and metastasis, and further studies may lead to the development of a new therapeutic candidate (Ji et al., 2013).

5 Conclusion and future perspectives

Discovery and studying novel therapeutic reagents are extremely difficult and time-consuming. Many methodologies and strategies have been applied in cancer biology for a long time, which will continue forever as long as humans exist on earth. These difficulties are from various cancer types, locations, oncogenic mechanisms, and others. People have already developed various effective chemo-reagents to treat and prevent cancers, but most are partially effective. Furthermore, the mediocre effect and inappropriate potential of chemotherapies could be the reason for cancer recurrence. The paradigm shift from sole chemotherapy to chemoprevention with chemotherapy was first elected in breast cancer. Further desperate trials of chemoprevention and chemotherapies have been made to understand detailed signaling molecules and pathways as the target of effective natural products.

LncRNAs are one of the non-protein coding RNA classes that affect several cancer-related cellular processes such as proliferation, differentiation, and apoptosis. After many lncRNAs have been known as tumor suppressive or oncogenic, many groups started focusing on small molecules such as phytochemicals and natural compounds to treat cancer targeting those lncRNAs. We discussed several lncRNAs related to CRC and possible natural products to regulate those lncRNAs. The biggest hurdles to applying these natural compounds for chemotherapy are experiments' challenging time and effort to confirm their activity and clinical challenges. Neither laboratory experiment nor clinical exam is enough to develop a new chemotherapeutic natural compound. Proper animal experiments should follow up for *in vivo* analysis to prove and support the preliminary *in vitro* data for establishment. Many of the lncRNAs and natural products discussed in this review have not been clearly studied in CRC; however, considering the many sharing mechanisms of CRC with other cancers, the lncRNAs and natural products have a high chance of being one of the critical factors in onset and progression in CRCs.

Additionally, low bioactivity, short availability, poor solubility, and a delivery method must be considered not to restrict the efficacy of natural products in clinical studies and *in vivo* experiments. Successful collaborative studies by nanotechnologists, chemists, biologists, and physicians will promise to overcome the hurdles to developing natural products for applicable chemotherapeutics. Another recommended method to optimize the stated approaches is electing the combination method. Various mixture therapies have been studied and tried in current cancer biology to treat cancer, and the combination of various natural products or with other anticancer agents as well as adjuvants with proved to have low toxicity. Since most of the studies were carried out experimentally and they show discrepancies in results by groups, it is important to study more about lncRNAs and natural products to standardize as a diagnostic marker and therapeutic purpose.

The concept of transitioning chemotherapy to chemoprevention has been first suggested in treating breast cancer to prevent recurrent cancers. Afterward, several studies have been made for a while to detect proper signaling molecules as a target of functional natural compounds. More detailed biochemical studies should be conducted to reveal the correct mechanisms to prevent unwanted actions of natural products in the future.

Natural products are highly available, inexpensive, and low toxic, with minimal side effects. These phytochemicals can be regarded as an innovative and promising field for developing new therapeutic strategies to overcome colorectal cancer and other cancers with minimal recurrence after treatment.

Author contributions

WJS and JH wrote the original draft. JH supervised and finished the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (2021R1I1A3043909).

Acknowledgments

The authors would like to thank Daegu Catholic University School of Medicine for providing generous support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Abedini, P., Fattahi, A., Agah, S., Talebi, A., Beygi, A. H., Amini, S. M., et al. (2019). Expression analysis of circulating plasma long noncoding RNAs in colorectal cancer: The relevance of lncRNAs ATB and CCAT1 as potential clinical hallmarks. *J. Cell Physiol.* 234, 22028–22033. doi:10.1002/jcp.28765

Anupriya, S., Chakraborty, A., and Patnaik, S. (2022). Clonal evolution and expansion associated with therapy resistance and relapse of colorectal cancer. *Mutat. Res. Rev. Mutat. Res.* 790, 108445. doi:10.1016/j.mrrev.2022.108445

Ariel, I., Miao, H. Q., Ji, X. R., Schneider, T., Roll, D., de Groot, N., et al. (1998). Imprinted H19 oncofetal RNA is a candidate tumour marker for hepatocellular carcinoma. *Mol. Pathol.* 51, 21–25. doi:10.1136/mp.51.1.21

Arun, G., Diermeier, S. D., and Spector, D. L. (2018). Therapeutic targeting of long non-coding RNAs in cancer. *Trends Mol. Med.* 24, 257–277. doi:10.1016/j.molmed.2018.01.001

Ashrafizadeh, M., Najafi, M., Makvandi, P., Zarrabi, A., Farkhondeh, T., and Samarghandian, S. (2020). Versatile role of curcumin and its derivatives in lung cancer therapy. *J. Cell Physiol.* 235, 9241–9268. doi:10.1002/jcp.29819

Badowski, C., He, B., and Garmire, L. X. (2022). Blood-derived lncRNAs as biomarkers for cancer diagnosis: The good, the bad and the beauty. *NPJ Precis. Oncol.* 6, 40. doi:10.1038/s41698-022-00283-7

Bian, Z., Jin, L., Zhang, J., Yin, Y., Quan, C., Hu, Y., et al. (2016). LncRNA-UCA1 enhances cell proliferation and 5-fluorouracil resistance in colorectal cancer by inhibiting miR-204-5p. *Sci. Rep.* 6, 23892. doi:10.1038/srep23892

Bishayee, A. (2009). Cancer prevention and treatment with resveratrol: From rodent studies to clinical trials. *Cancer Prev. Res. (Phila)* 2, 409–418. doi:10.1158/1940-6207.CAPR-08-0160

Boots, A. W., Haenen, G. R., and Bast, A. (2008). Health effects of quercetin: From antioxidant to nutraceutical. *Eur. J. Pharmacol.* 585, 325–337. doi:10.1016/j.ejphar.2008.03.008

Cai, H., Chen, J., He, B., Li, Q., Li, Y., and Gao, Y. (2015). A FOXM1 related long non-coding RNA contributes to gastric cancer cell migration. *Mol. Cell Biochem.* 406, 31–41. doi:10.1007/s11010-015-2421-3

Cao, J. (2014). The functional role of long non-coding RNAs and epigenetics. *Biol. Proced. Online* 16, 11. doi:10.1186/1480-9222-16-11

Carullo, G., Cappello, A. R., Frattaruolo, L., Badolato, M., Armentano, B., and Aiello, F. (2017). Quercetin and derivatives: Useful tools in inflammation and pain management. *Future Med. Chem.* 9, 79–93. doi:10.4155/fmc-2016-0186

Catana, C. S., Pichler, M., Giannelli, G., Mader, R. M., and Berindan-Neagoe, I. (2017). Non-coding RNAs, the Trojan horse in two-way communication between tumor and stroma in colorectal and hepatocellular carcinoma. *Oncotarget* 8, 29519–29534. doi:10.18632/oncotarget.15706

Che Hassan, N. K. N., Taher, M., and Susanti, D. (2018). Phytochemical constituents and pharmacological properties of Garcinia xanthochymus-a review. *Biomed. Pharmacother.* 106, 1378–1389. doi:10.1016/j.biopha.2018.07.087

Chen, J., Lin, C., Yong, W., Ye, Y., and Huang, Z. (2015). Calycosin and genistein induce apoptosis by inactivation of HOTAIR/p-Akt signaling pathway in human breast cancer MCF-7 cells. *Cell Physiol. Biochem.* 35, 722–728. doi:10.1159/000369732

Chen, T., Yang, P., Wang, H., and He, Z. Y. (2017). Silence of long noncoding RNA PANDAR switches low-dose curcumin-induced senescence to apoptosis in colorectal cancer cells. *Onco Targets Ther.* 10, 483–491. doi:10.2147/OTT.S127547

Cimino, S., Sortino, G., Favilla, V., Castelli, T., Madonia, M., Sansalone, S., et al. (2012). Polyphenols: Key issues involved in chemoprevention of prostate cancer. *Oxid. Med. Cell Longev.* 2012, 632959. doi:10.1155/2012/632959

Costa, V. G., Costa, S. M., Saramago, M., Cunha, M. V., Arraiano, C. M., Viegas, S. C., et al. (2022). Developing new tools to fight human pathogens: A journey through the advances in RNA technologies. *Microorganisms* 10, 2303. doi:10.3390/microorganisms10112303

Cui, H., Onyango, P., Brandenburg, S., Wu, Y., Hsieh, C. L., and Feinberg, A. P. (2002). Loss of imprinting in colorectal cancer linked to hypomethylation of H19 and IGF2. *Cancer Res.* 62, 6442–6446.

da Rocha, A. B., Lopes, R. M., and Schwartsmann, G. (2001). Natural products in anticancer therapy. *Curr. Opin. Pharmacol.* 1, 364–369. doi:10.1016/s1471-4892(01)00063-7

Deng, J., Yang, M., Jiang, R., An, N., Wang, X., and Liu, B. (2017). Long non-coding RNA HOTAIR regulates the proliferation, self-renewal capacity, tumor formation and migration of the cancer stem-like cell (CSC) subpopulation enriched from breast cancer cells. *PLoS One* 12, e0170860. doi:10.1371/journal.pone.0170860

Deng, M., Chen, H., Long, J., Song, J., Xie, L., and Li, X. (2021). Calycosin: A review of its pharmacological effects and application prospects. *Expert Rev. Anti Infect. Ther.* 19, 911–925. doi:10.1080/14787210.2021.1863145

Ding, J., Li, J., Wang, H., Tian, Y., Xie, M., He, X., et al. (2017). Long noncoding RNA CRNDE promotes colorectal cancer cell proliferation via epigenetically silencing DUSP5/CDKN1A expression. *Cell Death Dis.* 8, e2997. doi:10.1038/cddis.2017.328

Dou, J., Ni, Y., He, X., Wu, D., Li, M., Wu, S., et al. (2016). Decreasing lncRNA HOTAIR expression inhibits human colorectal cancer stem cells. *Am. J. Transl. Res.* 8, 98–108.

Dowli, A., Fichera, A., and Fleshman, J. (2023). Primary colorectal cancer. *Surg. Oncol. Clin. N. Am.* 32, 153–168. doi:10.1016/j.soc.2022.08.002

El Zoghbi, M., and Cummings, L. C. (2016). New era of colorectal cancer screening. *World J. Gastrointest. Endosc.* 8, 252–258. doi:10.4253/wjge.v8.i5.252

Esteghlal, S., Mokhtari, M. J., and Beyzaei, Z. (2021). Quercetin can inhibit angiogenesis via the down regulation of MALAT1 and MIAT LncRNAs in human umbilical vein endothelial cells. *Int. J. Prev. Med.* 12, 59. doi:10.4103/ijpvm.IJPVM_103_20

Fabian, A., Bor, R., and Szepes, Z. (2023). The use of ultrasound in colonic and perianal diseases. *Curr. Opin. Gastroenterol.* 39, 50–56. doi:10.1097/MOG.0000000000000891

Gao, X., Dai, J., Li, G., and Dai, X. (2021). Gambogic acid protects LPS-induced apoptosis and inflammation in a cell model of neonatal pneumonia through the regulation of TrkA/Akt signaling pathway. *BMC Pharmacol. Toxicol.* 22, 28. doi:10.1186/s40360-021-00496-9

Ge, X., Chen, Y., Liao, X., Liu, D., Li, F., Ruan, H., et al. (2013). Overexpression of long noncoding RNA PCAT-1 is a novel biomarker of poor prognosis in patients with colorectal cancer. *Med. Oncol.* 30, 588. doi:10.1007/s12032-013-0588-6

Ghafouri-Fard, S., Khoshbakht, T., Hussen, B. M., Baniahmad, A., Taheri, M., and Hajiesmaeili, M. (2022). A concise review on dysregulation of LINC00665 in cancers. *Cells* 11, 3575. doi:10.3390/cells11223575

Goodrich, J. A., and Kugel, J. F. (2006). Non-coding-RNA regulators of RNA polymerase II transcription. *Nat. Rev. Mol. Cell Biol.* 7, 612–616. doi:10.1038/nrm1946

Grynkiewicz, G., and Slifirski, P. (2012). Curcumin and curcuminoids in quest for medicinal status. *Acta Biochim. Pol.* 59, 201–212. doi:10.18388/abp.2012_2139

Han, P., Li, J. W., Zhang, B. M., Lv, J. C., Li, Y. M., Gu, X. Y., et al. (2017). The lncRNA CRNDE promotes colorectal cancer cell proliferation and chemoresistance via miR-181a-5p-mediated regulation of Wnt/β-catenin signaling. *Mol. Cancer* 16, 9. doi:10.1186/s12943-017-0583-1

Han, Y., Yang, Y. N., Yuan, H. H., Zhang, T. T., Sui, H., Wei, X. L., et al. (2014). UCA1, a long non-coding RNA up-regulated in colorectal cancer influences cell proliferation, apoptosis and cell cycle distribution. *Pathology* 46, 396–401. doi:10.1097/PAT.0000000000000125

Han, Y., Zhao, G., Shi, X., Wang, Y., Wen, X., Zhang, L., et al. (2022). The emerging role of long non-coding RNAs in esophageal cancer: Functions in tumorigenesis and clinical implications. *Front. Pharmacol.* 13, 885075. doi:10.3389/fphar.2022.885075

Hashemi, M., Mirdamadi, M. S. A., Talebi, Y., Khaniabad, N., Banaei, G., Daneii, P., et al. (2022). Pre-clinical and clinical importance of miR-21 in human cancers: Tumorigenesis, therapy response, delivery approaches and targeting agents. *Pharmacol. Res.* 187, 106568. doi:10.1016/j.phrs.2022.106568

Hassen, H. Y., Hussien, F. M., Hassen, A. M., Dewau, R., Amsalu, E. T., Limenih, M. A., et al. (2022). Survival pattern of colorectal cancer in sub-saharan africa: A systematic review and meta-analysis. *Cancer Epidemiol.* 81, 102276. doi:10.1016/j.canep.2022.102276

He, X., Chen, J., Zhou, J., Mao, A., Xu, W., Zhu, H., et al. (2022). LncRNA-EWSAT1 promotes hepatocellular carcinoma metastasis via activation of the Src-YAP signaling axis. *FASEB J.* 36, e22663. doi:10.1096/fj.202200825R

Hertog, M. G., and Hollman, P. C. (1996). Potential health effects of the dietary flavonol quercetin. *Eur. J. Clin. Nutr.* 50, 63–71.

Homayoonfal, M., Asemi, Z., and Yousefi, B. (2021). Targeting long non coding RNA by natural products: Implications for cancer therapy. *Crit. Rev. Food Sci. Nutr.*, 1–29. doi:10.1080/10408398.2021.2001785

Hu, Z. Y., Wang, X. Y., Guo, W. B., Xie, L. Y., Huang, Y. Q., Liu, Y. P., et al. (2016). Long non-coding RNA MALAT1 increases AKAP-9 expression by promoting SRPK1-catalyzed SRSF1 phosphorylation in colorectal cancer cells. *Oncotarget* 7, 11733–11743. doi:10.18632/oncotarget.7367

Imai-Sumida, M., Dasgupta, P., Kulkarni, P., Shiina, M., Hashimoto, Y., Shahryari, V., et al. (2020). Genistein represses HOTAIR/chromatin remodeling pathways to suppress kidney cancer. *Cell Physiol. Biochem.* 54, 53–70. doi:10.33594/000000205

Javed, Z., Khan, K., Sadia, H., Raza, S., Salehi, B., Sharifi-Rad, J., et al. (2020). LncRNA & Wnt signaling in colorectal cancer. *Cancer Cell Int.* 20, 326. doi:10.1186/s12935-020-01412-7

Ji, Q., Liu, X., Fu, X., Zhang, L., Sui, H., Zhou, L., et al. (2013). Resveratrol inhibits invasion and metastasis of colorectal cancer cells via MALAT1 mediated Wnt/β-catenin signal pathway. *PLoS One* 8, e78700. doi:10.1371/journal.pone.0078700

Kam, Y., Rubinstein, A., Naik, S., Djavsarov, I., Halle, D., Ariel, I., et al. (2014). Detection of a long non-coding RNA (CCAT1) in living cells and human adenocarcinoma of colon tissues using FIT-PNA molecular beacons. *Cancer Lett.* 352, 90–96. doi:10.1016/j.canlet.2013.02.014

Kerachian, M. A., and Azghandi, M. (2022). Identification of long non-coding RNA using single nucleotide epimutation analysis: A novel gene discovery approach. *Cancer Cell Int.* 22, 337. doi:10.1186/s12935-022-02752-2

Kino, T., Hurt, D. E., Ichijo, T., Nader, N., and Chrousos, G. P. (2010). Noncoding RNA gas5 is a growth arrest- and starvation-associated repressor of the glucocorticoid receptor. *Sci. Signal* 3, ra8. doi:10.1126/scisignal.2000568

Lee, J. Y., Lee, B. H., and Lee, J. Y. (2015). Gambogic acid disrupts toll-like Receptor4 activation by blocking lipopolysaccharides binding to myeloid differentiation factor 2. *Toxicol. Res.* 31, 11–16. doi:10.5487/TR.2015.31.1.011

Li, H., Ma, S. Q., Huang, J., Chen, X. P., and Zhou, H. H. (2017). Roles of long noncoding RNAs in colorectal cancer metastasis. *Oncotarget* 8, 39859–39876. doi:10.18632/oncotarget.16339

Li, J., and Qi, Y. (2019). Ginsenoside Rg3 inhibits cell growth, migration and invasion in Caco-2 cells by downregulation of lncRNA CCAT1. *Exp. Mol. Pathol.* 106, 131–138. doi:10.1016/j.yexmp.2019.01.003

Licznerska, B., and Baer-Dubowska, W. (2016). Indole-3-Carbinol and its role in chronic diseases. *Adv. Exp. Med. Biol.* 928, 131–154. doi:10.1007/978-3-319-41334-1_6

Liu, M., Zhang, L., Hu, J., Yan, C., Zhang, Y., and Yan, Z. (2022). LINC01311 exerts an inhibitory effect in thyroid cancer progression by targeting the miR-146b-5p/IMPA2 axis. *Transl. Oncol.* 27, 101588. doi:10.1016/j.tranon.2022.101588

Liu, T., Zhang, X., Yang, Y. M., Du, L. T., and Wang, C. X. (2016). Increased expression of the long noncoding RNA CRNDE-h indicates a poor prognosis in colorectal cancer, and is positively correlated with IRX5 mRNA expression. *Onco Targets Ther.* 9, 1437–1448. doi:10.2147/OTT.S98268

Lizarbe, M. A., Calle-Espinosa, J., Fernandez-Lizarbe, E., Fernandez-Lizarbe, S., Robles, M. A., Olmo, N., et al. (2017). Colorectal cancer: From the genetic model to posttranscriptional regulation by noncoding RNAs. *Biomed. Res. Int.* 2017, 7354260. doi:10.1155/2017/7354260

Luo, J., Qu, J., Wu, D. K., Lu, Z. L., Sun, Y. S., and Qu, Q. (2017). Long non-coding RNAs: A rising biotarget in colorectal cancer. *Oncotarget* 8, 22187–22202. doi:10.18632/oncotarget.14728

Ma, M. Z., Chu, B. F., Zhang, Y., Weng, M. Z., Qin, Y. Y., Gong, W., et al. (2015). Long non-coding RNA CCAT1 promotes gallbladder cancer development via negative modulation of miRNA-218-5p. *Cell Death Dis.* 6, e1583. doi:10.1038/cddis.2014.541

Meeran, S. M., Ahmed, A., and Tollefsbol, T. O. (2010). Epigenetic targets of bioactive dietary components for cancer prevention and therapy. *Clin. Epigenetics* 1, 101–116. doi:10.1007/s13148-010-0011-5

Nadhan, R., Isidoro, C., Song, Y. S., and Dhanasekaran, D. N. (2022). Signaling by LncRNAs: Structure, cellular homeostasis, and disease pathology. *Cells* 11, 2517. doi:10.3390/cells11162517

Nakhjavani, M., Hardingham, J. E., Palethorpe, H. M., Tomita, Y., Smith, E., Price, T. J., et al. (2019). Ginsenoside Rg3: Potential molecular targets and therapeutic indication in metastatic breast cancer. *Med. (Basel)* 6, 17. doi:10.3390/medicines6010017

Nojima, T., and Proudfoot, N. J. (2022). Mechanisms of lncRNA biogenesis as revealed by nascent transcriptomics. *Nat. Rev. Mol. Cell Biol.* 23, 389–406. doi:10.1038/s41580-021-00447-6

Okugawa, Y., Grady, W. M., and Goel, A. (2015). Epigenetic alterations in colorectal cancer: Emerging biomarkers. *Gastroenterology* 149, 1204–1225. doi:10.1053/j.gastro.2015.07.011

Pagani, G., Pandini, C., and Gandellini, P. (2022). Navigating the multiverse of antisense RNAs: The transcription- and RNA-dependent dimension. *Noncoding RNA* 8, 74. doi:10.3390/ncrna8060074

Pu, Z., Ge, F., Wang, Y., Jiang, Z., Zhu, S., Qin, S., et al. (2021). Ginsenoside-Rg3 inhibits the proliferation and invasion of hepatoma carcinoma cells via regulating long non-coding RNA HOX antisense intergenic. *Bioengineered* 12, 2398–2409. doi:10.1080/21655979.2021.1932211

Qiao, L., Liu, X., Tang, Y., Zhao, Z., Zhang, J., and Feng, Y. (2017). Down regulation of the long non-coding RNA PCAT-1 induced growth arrest and apoptosis of colorectal cancer cells. *Life Sci.* 188, 37–44. doi:10.1016/j.lfs.2017.08.024

Ragusa, M., Barbagallo, C., Statello, L., Condorelli, A. G., Battaglia, R., Tamburello, L., et al. (2015). Non-coding landscapes of colorectal cancer. *World J. Gastroenterol.* 21, 11709–11739. doi:10.3748/wjg.v21.i41.11709

Ravishankar, D., Rajora, A. K., Greco, F., and Osborn, H. M. (2013). Flavonoids as prospective compounds for anti-cancer therapy. *Int. J. Biochem. Cell Biol.* 45, 2821–2831. doi:10.1016/j.biocel.2013.10.004

Razlansari, M., Jafarinejad, S., Rahdar, A., Shirvaliloo, M., Arshad, R., Fathi-Karkan, S., et al. (2022). Development and classification of RNA aptamers for therapeutic purposes: An updated review with emphasis on cancer. *Mol. Cell Biochem.* doi:10.1007/s11010-022-04614-x

Reyes-Farias, M., and Carrasco-Pozo, C. (2019). The anti-cancer effect of quercetin: Molecular implications in cancer metabolism. *Int. J. Mol. Sci.* 20, 3177. doi:10.3390/ijms20133177

Saus, E., Brunet-Vega, A., Iraola-Guzman, S., Pegueroles, C., Gabaldon, T., and Pericay, C. (2016). Long non-coding RNAs as potential novel prognostic biomarkers in colorectal cancer. *Front. Genet.* 7, 54. doi:10.3389/fgene.2016.00054

Schmitt, A. M., and Chang, H. Y. (2016). Long noncoding RNAs in cancer pathways. *Cancer Cell* 29, 452–463. doi:10.1016/j.ccell.2016.03.010

Schwarzenbach, H. (2016). Biological and clinical relevance of H19 in colorectal cancer patients. *EBioMedicine* 13, 9–10. doi:10.1016/j.ebiom.2016.11.001

Shen, P., Pichler, M., Chen, M., Calin, G. A., and Ling, H. (2017). To Wnt or lose: The missing non-coding linc in colorectal cancer. *Int. J. Mol. Sci.* 18, 2003. doi:10.3390/ijms18092003

Shen, W., Pu, J., Gu, S., Sun, J., Wang, L., Tan, B., et al. (2022). LINC01635, a long non-coding RNA with a cancer/testis expression pattern, promotes lung cancer progression by sponging miR-455-5p. *Oncol. Lett.* 24, 438. doi:10.3892/ol.2022.13558

Siddiqui, H., Al-Ghafari, A., Choudhry, H., and Al Doghaither, H. (2019). Roles of long non-coding RNAs in colorectal cancer tumorigenesis: A review. *Mol. Clin. Oncol.* 11, 167–172. doi:10.3892/mco.2019.1872

Smolle, M., Uranitsch, S., Gerger, A., Pichler, M., and Haybaeck, J. (2014). Current status of long non-coding RNAs in human cancer with specific focus on colorectal cancer. *Int. J. Mol. Sci.* 15, 13993–14013. doi:10.3390/ijms150813993

Song, D., Hao, J., and Fan, D. (2020). Biological properties and clinical applications of berberine. *Front. Med.* 14, 564–582. doi:10.1007/s11684-019-0724-6

Svoboda, M., Slyskova, J., Schneiderova, M., Makovicky, P., Bielik, L., Levy, M., et al. (2014). HOTAIR long non-coding RNA is a negative prognostic factor not only in primary tumors, but also in the blood of colorectal cancer patients. *Carcinogenesis* 35, 1510–1515. doi:10.1093/carcin/bgu055

Ullah, I., Yang, L., Yin, F. T., Sun, Y., Li, X. H., Li, J., et al. (2022). Multi-omics approaches in colorectal cancer screening and diagnosis, recent updates and future perspectives. *Cancers (Basel)* 14, 5545. doi:10.3390/cancers14225545

Vallino, L., Ferraresi, A., Vidoni, C., Secomandi, E., Esposito, A., Dhanasekaran, D. N., et al. (2020). Modulation of non-coding RNAs by resveratrol in ovarian cancer cells: *In silico* analysis and literature review of the anti-cancer pathways involved. *J. Tradit. Complement. Med.* 10, 217–229. doi:10.1016/j.jtcme.2020.02.006

Wagner, S. D., Yakovchuk, P., Gilman, B., Ponicsan, S. L., Drullinger, L. F., Kugel, J. F., et al. (2013). RNA polymerase II acts as an RNA-dependent RNA polymerase to extend and destabilize a non-coding RNA. *EMBO J.* 32, 781–790. doi:10.1038/emboj.2013.18

Wang, F., Li, X., Xie, X., Zhao, L., and Chen, W. (2008). UCA1, a non-protein-coding RNA up-regulated in bladder carcinoma and embryo, influencing cell growth and promoting invasion. *FEBS Lett.* 582, 1919–1927. doi:10.1016/j.febslet.2008.05.012

Wang, J., Song, Y. X., Ma, B., Wang, J. J., Sun, J. X., Chen, X. W., et al. (2015). Regulatory roles of non-coding RNAs in colorectal cancer. *Int. J. Mol. Sci.* 16, 19886–19919. doi:10.3390/ijms160819886

Wang, J., Wang, N., Zheng, Z., Che, Y., Suzuki, M., Kano, S., et al. (2022). Exosomal lncRNA HOTAIR induce macrophages to M2 polarization via PI3K/p-AKT/AKT pathway and promote EMT and metastasis in laryngeal squamous cell carcinoma. *BMC Cancer* 22, 1208. doi:10.1186/s12885-022-10210-5

Wang, W., Min, L., Qiu, X., Wu, X., Liu, C., Ma, J., et al. (2021). Biological function of long non-coding RNA (LncRNA) xist. *Front. Cell Dev. Biol.* 9, 645647. doi:10.3389/fcell.2021.645647

Wu, G., Niu, M., Qin, J., Wang, Y., and Tian, J. (2019). Inactivation of Rab27B-dependent signaling pathway by calycosin inhibits migration and invasion of ER-negative breast cancer cells. *Gene* 709, 48–55. doi:10.1016/j.gene.2019.04.005

Wu, L., Jin, L., Zhang, W., and Zhang, L. (2016). Roles of long non-coding RNA CCAT2 in cervical cancer cell growth and apoptosis. *Med. Sci. Monit.* 22, 875–879. doi:10.12659/msm.897754

Xie, X., Tang, B., Xiao, Y. F., Xie, R., Li, B. S., Dong, H., et al. (2016). Long non-coding RNAs in colorectal cancer. *Oncotarget* 7, 5226–5239. doi:10.18632/oncotarget.6446

Xu, H., Zhang, D., Wei, R., Zhou, Y., Dai, G., Li, J., et al. (2022). Gambogic acid induces pyroptosis of colorectal cancer cells through the GSDME-dependent pathway and elicits an antitumor immune response. *Cancers (Basel)* 14, 5505. doi:10.3390/cancers14225505

Xu, M. D., Qi, P., and Du, X. (2014). Long non-coding RNAs in colorectal cancer: Implications for pathogenesis and clinical application. *Mod. Pathol.* 27, 1310–1320. doi:10.1038/modpathol.2014.33

Xue, Q., He, N., Wang, Z., Fu, X., Aung, L. H. H., Liu, Y., et al. (2021). Functional roles and mechanisms of ginsenosides from Panax ginseng in atherosclerosis. *J. Ginseng Res.* 45, 22–31. doi:10.1016/j.jgr.2020.07.002

Yang, M. H., Hu, Z. Y., Xu, C., Xie, L. Y., Wang, X. Y., Chen, S. Y., et al. (2015). MALAT1 promotes colorectal cancer cell proliferation/migration/invasion via PRKA kinase anchor protein 9. *Biochim. Biophys. Acta* 1852, 166–174. doi:10.1016/j.bbadis.2014.11.013

Ye, L. C., Zhu, X., Qiu, J. J., Xu, J., and Wei, Y. (2015). Involvement of long non-coding RNA in colorectal cancer: From benchtop to bedside (Review). *Oncol. Lett.* 9, 1039–1045. doi:10.3892/ol.2015.2846

Yu, I., Wu, R., Tokumaru, Y., Terracina, K. P., and Takabe, K. (2022). The role of the microbiome on the pathogenesis and treatment of colorectal cancer. *Cancers (Basel)* 14, 5685. doi:10.3390/cancers14225685

Zhang, W. W., Feng, Z., and Narod, S. A. (2014). Multiple therapeutic and preventive effects of 3, 3'-diindolylmethane on cancers including prostate cancer and high grade prostatic intraepithelial neoplasia. *J. Biomed. Res.* 28, 339–348. doi:10.7555/JBR.28.20140008

Zhang, Z., Li, B., Xu, P., and Yang, B. (2019). Integrated whole transcriptome profiling and bioinformatics analysis for revealing regulatory pathways associated with quercetin-induced apoptosis in HCT-116 cells. *Front. Pharmacol.* 10, 798. doi:10.3389/fphar.2019.00798

Zhang, Z., Zhou, C., Chang, Y., Zhang, Z., Hu, Y., Zhang, F., et al. (2016). Long non-coding RNA CASC11 interacts with hnRNP-K and activates the WNT/β-catenin pathway to promote growth and metastasis in colorectal cancer. *Cancer Lett.* 376, 62–73. doi:10.1016/j.canlet.2016.03.022

Zhao, B., Hou, X., and Zhan, H. (2015). Long non-coding RNA PCAT-1 over-expression promotes proliferation and metastasis in non-small cell lung cancer cells. *Int. J. Clin. Exp. Med.* 8, 18482–18487.

Zhao, B., Lu, M., Wang, D., Li, H., and He, X. (2016). Genome-wide identification of long noncoding RNAs in human intervertebral disc degeneration by RNA sequencing. *Biomed. Res. Int.* 2016, 3684875. doi:10.1155/2016/3684875

Zheng, F., Li, J., Ma, C., Tang, X., Tang, Q., Wu, J., et al. (2020). Novel regulation of miR-34a-5p and HOTAIR by the combination of berberine and gefitinib leading to inhibition of EMT in human lung cancer. *J. Cell Mol. Med.* 24, 5578–5592. doi:10.1111/jcmm.15214

Zheng, Z., Bian, C., Wang, H., Su, J., Meng, L., Xin, Y., et al. (2022). Prediction of immunotherapy efficacy and immunomodulatory role of hypoxia in colorectal cancer. *Ther. Adv. Med. Oncol.* 14, 17588359221138383. doi:10.1177/17588359221138383

Zheng, Z. H., You, H. Y., Feng, Y. J., and Zhang, Z. T. (2021). LncRNA KCNQ1OT1 is a key factor in the reversal effect of curcumin on cisplatin resistance in the colorectal cancer cells. *Mol. Cell Biochem.* 476, 2575–2585. doi:10.1007/s11010-020-03856-x

Zhong, X. D., Chen, L. J., Xu, X. Y., Liu, Y. J., Tao, F., Zhu, M. H., et al. (2022). Berberine as a potential agent for breast cancer therapy. *Front. Oncol.* 12, 993775. doi:10.3389/fonc.2022.993775

Zinovieva, O. L., Grineva, E. N., Prokofjeva, M. M., Karpov, D. S., Krasnov, G. S., Prassolov, V. S., et al. (2017). [Treatment with anti-cancer agents results in profound changes in lncRNA expression in colon cancer cells]. *Mol. Biol. Mosk.* 51, 841–848. doi:10.7868/S0026898417050123

Frontiers in Pharmacology

Explores the interactions between chemicals and living beings

The most cited journal in its field, which advances access to pharmacological discoveries to prevent and treat human disease.

Discover the latest Research Topics

See more →

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact