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Reviews in cancer genetics

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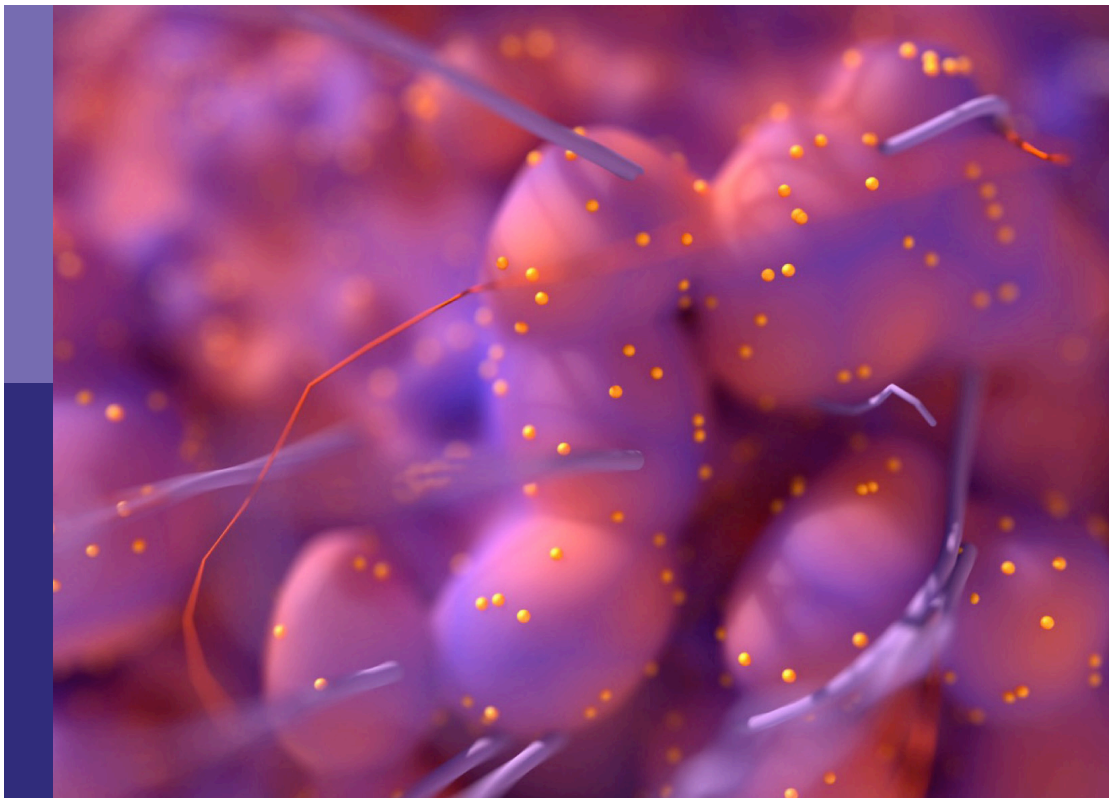

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Reviews in cancer genetics

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Editorial: Reviews in cancer genetics

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Editorial on the Research Topic
Reviews in cancer genetics

Li et al. conduced a systematic review and meta-analysis to evaluate the association between Matrix Metalloproteinase 9 (MMP-9) expression and endometrial cancer (EC) risk, clinical features, and prognosis. MMP-9 is involved in many biological processes such as proteolytic degradation of extracellular matrix (ECM), cleavage of cell surface proteins, and alteration of cell-cell or cell-ECM interactions (1). Endometrial carcinoma can invade the basement membrane and myometrium through gelatinase, penetrating the lymphatic vascular lumen and spreading (2). MMP-9 gene was located at chromosome 20q13.12, which encoded Gelatinase B. Gelatinase B degraded gelatin, collagen, and elastin through proteolytic cleavage to regulate extracellular matrix (ECM) remodeling (3). A total of 28 eligible studies were acquired. MMP-9 overexpression was found to be significantly associated with the risk, tumor grade, FIGO stage, lymph node metastasis, and myometrium invasion of EC. In addition, the overall results showed that MMP-9 overexpression predicted a worse prognosis of EC (OR = 1.82, 95% CI = 1.01-2.62, $P < 0.05$).

Ding et al. reviewed the formation and regulatory mechanism of circRNAs, their biological function, and their relationship with gastric cancer. Differential expressions of sixty reviewed circRNAs were found to be upregulated in 35 cases of gastric cancer, mostly analyzed by RT-PCR. Studies have found that some circRNAs may act as oncogenes to promote, or as tumor suppressors to inhibit the occurrence and development of gastric cancer. Diagnostic markers, therapeutic targets, and prognostic markers for gastric cancer were deeply reviewed.

Montella et al. began their review article on genetic alterations in Glioblastoma (GBM) with the question “Is Genomics the Right Path?” In this review, the authors examined the most relevant molecular drivers of GBM from an interesting point of view, emphasizing the frustrating gap between translational research and its success in clinical applications. The article reviewed different up-to-date targeting strategies and clinical trial successes of important genetic alterations found in GBM: the telomerase reverse transcriptase gene, receptor tyrosine kinases, epidermal growth factor signaling, RAS, as well as the downstream cascade of kinases (mitogen-activated protein kinase (MAPK) and extracellular-regulated kinase (ERK)), neurofibromatosis 1 gene, mesenchymal–epithelial

transition, fibroblast growth factor receptor, and the neurotrophic tyrosine receptor kinase family.

Zhang et al. reviewed the current understanding of the inhibitor of the apoptosis protein-related-like protein 2 (ILP-2) structure and function, as well as its potential application in cancer therapy. *BIRC8* (ILP-2 coding gene) is overexpressed in several tumors and can contribute to tumor immune evasion due to its role in apoptosis inhibition. The authors discussed its role as a biomarker for early tumor detection and the possibility of targeting ILP-2, associated with other cancer treatments, which can expand the options for cancer patients.

Yang et al. systematically searched for publications before 25 August 2021 to analyze the connections between CHRNA SNPs and lung cancer (LC) or chronic obstructive pulmonary disease (COPD). A total of 70,960 cases and 124,838 controls from 29 publications were identified for meta-analysis based on at least three data sources. Eight CHRNA SNPs (rs1051730, rs12914385, rs578776, rs6495309, rs8042374, and rs938682 in CHRNA3, as well as rs16969968 and rs588765 in CHRNA5) were identified to be associated with LC or COPD risk. Of them, rs1051730, rs6495309, and rs16969968 were significantly associated with COPD susceptibility, whereas rs1051730, rs578776, rs6495309, rs938682, rs16969968, and rs588765 were significantly associated with LC risk. By constructing functional annotations with the ENCODE project and other public databases, the authors found that these six SNPs may locate in several putative regulatory areas. In brief, this study found the variants of CHRNA genes associated with the risk of LC or COPD.

Author contributions

All authors made direct and intellectual contributions to this article and provided their approval for the publication.

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REVIEW
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Long Noncoding RNA: Shining Stars in the Immune Microenvironment of Gastric Cancer

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Gastric cancer (GC) is a kind of malignant tumor disease that poses a serious threat to human health. The GC immune microenvironment (TIME) is a very complex tumor microenvironment, mainly composed of infiltrating immune cells, extracellular matrix, tumor-associated fibroblasts, cytokines and chemokines, all of which play a key role in inhibiting or promoting tumor development and affecting tumor prognosis. Long non-coding RNA (lncRNA) is a non-coding RNA with a transcript length is more than 200 nucleotides. LncRNAs are expressed in various infiltrating immune cells in TIME and are involved in innate and adaptive immune regulation, which is closely related to immune escape, migration and invasion of tumor cells. LncRNA-targeted therapeutic effect prediction for GC immunotherapy provides a new approach for clinical research on the disease.

Keywords: lncRNA, immune microenvironment, gastric cancer, targeted therapeutic, tumor mircroenvironment

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INTRODUCTION

Gastric cancer (GC) is a kind of malignant tumor that develops from the gastric mucosa. According to the most recent International Agency for Research on Cancer (IARC) statistics, there were 1,089,000 new cases of GC and 776,000 deaths globally in 2020, making it the fourth leading cause of cancer mortality worldwide (1, 2). The pathogenesis of GC is very complex. At present, the role of Helicobacter pylori (HP) infection in the pathogenesis of GC has gradually been widely recognized. In addition, dietary influence, oncogene activation mutation and/or amplification, tumor suppressor gene mutation and/or inhibition, abnormal expression of cell cycle regulatory factors and signal molecules are all closely related to the occurrence and development of GC (3, 4). The screening and diagnostic procedures for middle and early GC include barium meal fluoroscopy, electronic gastroscopy, and serum pepsinogen (5). However, due to the hidden onset of early GC or the high cost of screening, the majority of patients with GC have been diagnosed as advanced stage (6). The main therapeutic strategies for GC include surgery, chemotherapy, and targeted therapy, however due to a lack of targets and drug resistance, these therapeutic strategies have not demonstrated promising results, particularly in patients with advanced GC. Immunotherapy for GC has received increasing attention in recent years as immune checkpoint research has developed, although various subtypes of GC patients respond differently to immunotherapy (7). Therefore, it is

very important to find biomarkers for GC that are convenient for screening, diagnosis, prediction of drug efficacy and prognosis to guide the formulation of treatment strategies.

Long non-coding RNA (lncRNA) is a kind of non-coding RNA that has a transcript length more than 200 nucleotides (8). LncRNA is involved in a wide range of cell processes, including cell proliferation (9), differentiation (10), apoptosis (11) and immune response (12), all of which are closely related to the evolution of tumors. According to preliminary estimations from the human ENCODE project, the human genome encodes more than 28,000 distinct length lncRNA (13). Clarifying the functions of all lncRNAs is an unsolved and difficult task, although great advances have been done in recent studies on their mechanism of action. Current studies have proved that lncRNAs can regulate gene expression at the transcriptional level, post-transcriptional level and epigenetic level. Abnormal expression of lncRNAs can influence selective gene splicing, miRNA binding to mRNA, chromosome remodeling, and promoter activation through interactions with DNA, RNA, and protein, therefore impacting almost every link in gene expression (**Figure 1**) (14). Thus far, many abnormally expressed lncRNAs have been found in GC tissues. These genes can be used as oncogenes or tumor suppressor genes to regulate cell pathways, affect cell functions and participate in the generation and development of tumors (**Table 1**). Since some lncRNAs were found to be tissue-specific (34), they have been used as biomarkers for early diagnosis and prognosis of tumors by an increasing number of researchers in recent years.

The internal environment in which tumor cells formed and survive is known as the tumor microenvironment (TME). It plays an important role in tumor genesis and evolution. TME is mainly composed of tumor cells themselves and their surrounding fibroblasts, immune and inflammatory cells, glial cells and other stromal cells. The tumor immune microenvironment (TIME), which is composed of immune cells, is particularly important. Various immune cell components of the immune microenvironment interact closely with cancer cells during the recruitment of cytokines and tumor-related signals, and then evolve with each other to jointly promote tumor invasion and metastasis (35, 36). The components of interstitial cells involved in the regulation of TIME are complex and variable, which promote each other to form a cascade effect and jointly promote the evolution of tumor cells. The main components include tumor-associated macrophages, tumor-infiltrating lymphocytes, neutrophils, tumor-associated fibroblasts, extracellular matrix, cytokines and so on (37–40). LncRNAs molecules have an important role in tumor cell remodeling TIME and regulation of tumor cell immune escape. For example, Lnc-Tim interacts with Tim-3 to induce Bat3 release and promote $CD8^+$T cell failure, resulting in hepatocellular carcinoma immune evasion (41). Lnc-sox5 promotes colorectal cancer by increasing IDO1 expression, which inhibits $CD8^+$T infiltration and cytotoxicity (42). Nifk-as1 inhibits macrophage M2 polarization and endometrial cancer cell malignant phenotype by targeting miR-146a (43). The main focus of this paper was on the basic characteristics and functional roles of lncRNAs in GC TIME, as well as the immunotherapeutic potential of lncRNAs in GC treatment.

LncRNA IS A REGULATOR OF IMMUNE CELLS IN GC TIME

LncRNAs and GC-Associated Innate Immune Cell

GC-associated **i**nnate immune cells are mainly composed of GC-associated macrophages (CAFs), followed by dendritic cells (DCs) and natural killer cells (NK cells), etc. Through autophagocytosis, antigen recognition, cytokine synthesis and secretion, these cells play a significant role in the GC TIME.

LncRNAs and GC-Associated Macrophages

GC-associated macrophages infiltrated by bone marrow monocyte differentiation in TME are an important component of TIME. In TIME, macrophages are polarized into two different subtypes of macrophages by different stimuli: conventionally activated macrophages (M1 phenotype macrophages) and alternatively activated macrophages (M2 phenotype macrophages) (44, 45). M1-phenotype macrophages are activated by IFN-γ (interferon-γ), LPS (lipopolysaccharide), TNF-α (tumor necrosis factor-α), etc. After activation, immune stimulators are secreted to induce adaptive responses, as well as the secretion of reactive oxygen species and nitrogen intermediates. It is classified as anti-tumor or "good" macrophages since it is primarily involved in Th1 type immune response, monitoring tumor lesions, and resisting pathogen invasion (46). Meanwhile, M2 phenotype macrophages are usually activated in response to stimulation such as IL-4, IL-10 and IL-13. Activated M2 macrophages can release VEGF, PDGF, bFGF and other angiogenic factors as well as growth factors and matrix metalloproteinase, which can stimulate the formation of blood vessels in tumor and activate epithelial-mesenchymal transformation, invasion and metastasis of tumor cells (47). At the same time, it can also promote the formation and maintenance of tumor stem cells by increasing the expressions of IL-10 and TGF-β (transforming growth factor-β) in TIME, and reduce the expressions of IL-1, IL-6, IL-12 and TNF-α (transforming growth factor-α) (48, 49). Therefore, it is regarded as a "bad" macrophage promoting tumor.

So far, it has been proven that a variety of LncRNAs play a role in the polarization of GC-associated macrophages, hence influencing GC progression. Xie et al. found that highly expressed LncRNA ANCR in GC tissues down-regulated FoxO1 expression by promoting FoxO1 ubiquitination and degradation, and reduced IL-1β and IL-6 secretion, facilitating GC cell invasion and metastasis (50). Nie et al. found that lncRNA HCG18 up-regulated KLF4 expression by decreasing miR-875-3p in macrophages mediated by GC derived exosomes, thereby promoting polarization of M2 macrophages (51). Furthermore, a bioinformatics analysis revealed that H19, which is significantly expressed in GC, can regulate the expression of COL1A2 in sponge tissue miR-29A-3p. In GC,

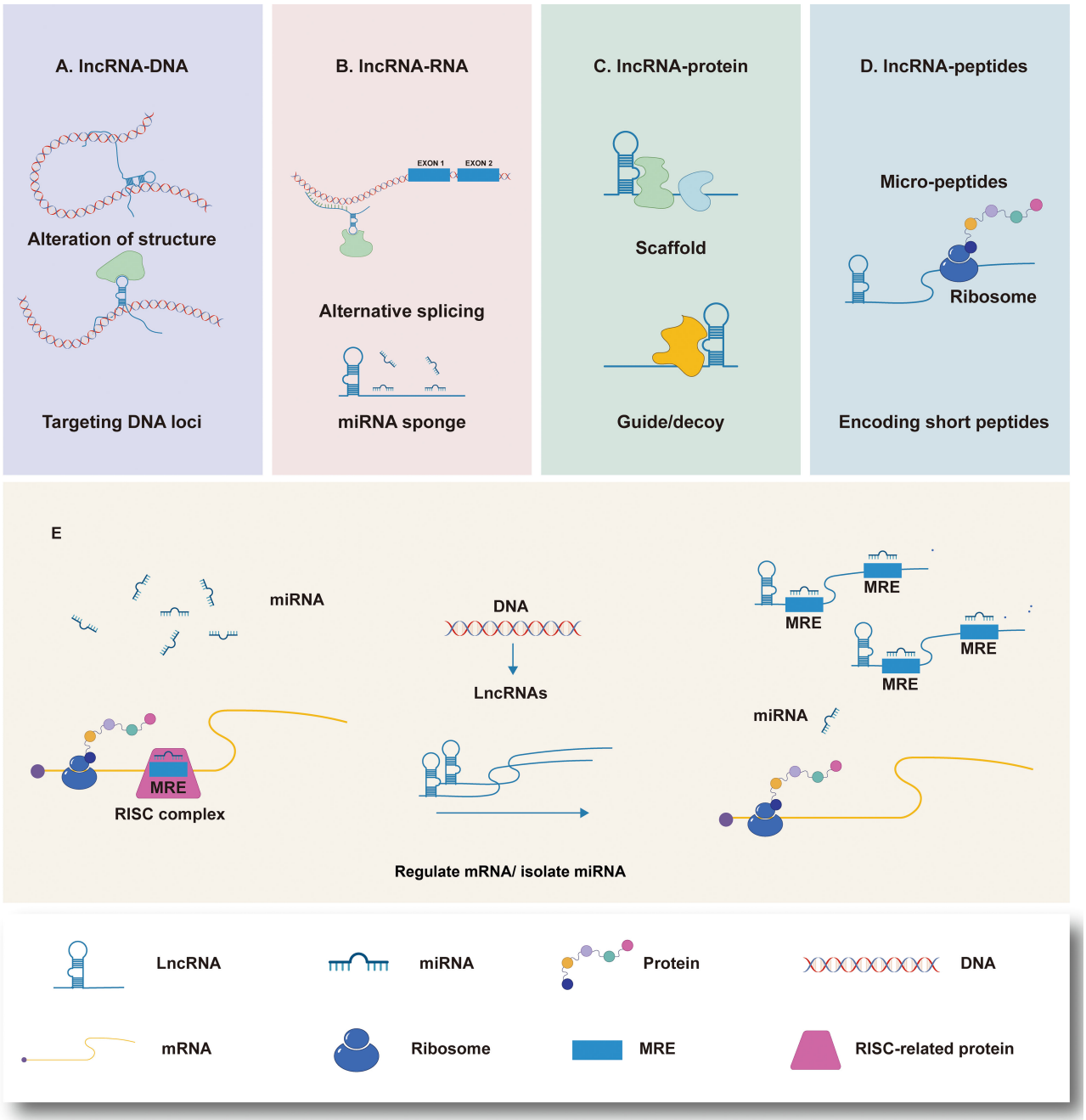

FIGURE 1 | LncRNAs interactions and functions, and the mechanism of lncRNAs acting as molecular sponge. **(A)** LncRNAs regulate gene expression by affecting local chromatin structure or recruiting regulatory proteins to specific loci. **(B)** LncRNAs facilitate RNA inhibition and degradation through interacting with mRNA and miRNA to control splicing or acting as a ceRNA of miRNA. **(C)** LncRNAs can serve as molecular scaffolds, guides, or decoys for regulatory proteins to regulate protein. **(D)** A part of lncRNAs are able to encode short peptides. **(E)** MiRNAs are capable of directly binding to the matched regions of mRNAs by specific identification in a base-pairing manner, and thus inducing mRNA degradation at the post-transcriptional level by forming RNA-induced silencing complex (RISC) with related proteins such as Argonaute 2 (AGO2). LncRNAs own the miRNA response elements (MREs) which have complementary miRNA binding sites that can competitively bind to miRNAs. Therefore, lncRNAs are able to exert its biological functions by regulating the expression of mRNAs or sequestering corresponding miRNA molecules.

the H19-miR-29A-3p-COL1A2 axis can induce macrophage polarization from M1 to M2 (52). In summary, lncRNAs expressed by GC-associated macrophages or secreted by tumor cells regulate the function of GC-associated macrophages through a variety of mechanisms, further affecting the occurrence and metastasis of tumors, implying that targeting these lncRNAs in GC-associated macrophages or tumor cells may be a potential anti-tumor strategy.

TABLE 1 | LncRNAs involved in GC TME.

LncRNA	Chromosomal position	Expression	Signaling pathways	Stromal cells	Clinical Significance	Reference
LINC00342	2q11.1	Upregulated in GC tissues and cell lines.	miR-545-5p/CNPY2 axis	GC	—	(15)
NCRNA00072	12q13.13	Upregulated in GC tissues and cell lines	Targeting miR-126 to active CXCR4 and RhoA	GC	—	(16)
LINC00008	11p15.5	Upregulated in GC tissues and cell lines	miR-138/E2F2 Axis	GC	—	(17)
LINC00047	11q13.1	Upregulated in GC tissues and cell lines	PI3K/AKT pathway	GC	—	(18)
LINC00256A	9q32	Upregulated in GC tissues and cell lines	FAM225A-miR-206-ADAM12 axis	GC	—	(19)
RP11-357H14.17	—	Upregulated in GC tissues and cell lines	Activating ATF2 Signaling and Enhancing Treg Cells	GC	OS	(20)
SUMO1P3	1q23.2	Upregulated in GC tissues and cell lines	Wnt/β-catenin signaling pathway	GC	—	(21)
LSINCT5	5p15.33	Upregulated in GC tissues and cell lines	Affecting the epithelial-mesenchymal transition	GC	—	(22)
LINC00001	Xq13.2	Upregulated in GC tissues and cell lines	Regulating miR-497/MACC1 axis	GC	—	(23)
LINC01540	18p11.31	Upregulated in GC tissues and cell lines	Acting as a molecular sponge of miR-378 to modulate MAPK1 expression	GC	—	(24)
HOXA-AS2	7p15.2	Upregulated in GC tissues and cell lines	Epigenetically silencing P21/PLK3/DDIT3 expression	GC	—	(25)
LINC00152	2p11.2	Upregulated in GC tissues and cell lines	EGFR-dependent pathway	GC	—	(26)
LOC554202	9p21.3	Downregulated in GC tissues	Regulate E2F1 and P15 expression	GC	—	(27)
RMRP	9p13.3	Downregulated in GC tissues	Acts as a miR-206 sponge to modulate cell cycle through regulating the expression of Cyclin D2	GC	—	(28)
GAS5	1q25.1	Downregulated in GC tissues	regulating E2F1 and P21 expression.	GC	OS, DFS	(29)
WT1-AS	11p13	Downregulated in GC tissues	Inhibit cell proliferation, migration and invasion, and increase the proportion of G0/G1 cells	GC	—	(30)
LINC00902	3q13.31	Downregulated in GC tissues	Tumor suppressors regulated by p53 and play a role by inhibiting mIR-23b	GC	DFS, DDS	(31)
LINC00023	14q32.2	Downregulated in GC tissues	p53 signaling pathway	GC	—	(32)
LINC -POU3F3	2q12.1	Upregulated in T-reg from peripheral blood of GC patients.	TGF-beta signal pathway	T-reg	—	(33)

LncRNAs and GC-Associated NK Cells

Natural killer (NK) cells, in addition to T cells, have pan-specific natural immune recognition and a rapid killing mechanism, making them an useful tool in anti-tumor therapy. Different from T cells, NK cells do not rely on the activation of antigen presenting cells to detect early signs of tumor transformation in time and respond immediately, making them the first line of host defense against tumor (53). It is worth noting that NK cells are not only killer cells, but also immunomodulatory cells. T cells and dendritic cells can be modulated by NK cells to have positive or negative impacts on tumor response in a variety of ways (54). For example, NK cells produce cytokines and chemokines, recruit dendritic cells (DCs), promote the maturation of DCs, and enhance adaptive immune response (55). Previous clinical studies have shown that NK cell killing activity and the number of intratomatous invasion are negatively correlated with GC risk and prognosis (56). This may be closely related to the effect of NK cell infiltration in maintaining tumor cell dormancy and inhibiting tumor metastasis (57).

Many LncRNAs are involved in the differentiation of NK cells, with the most well-known being the research of lnc-CD56.The expression of lnc-CD56, also known as AB128931, is significantly up-regulated in human NK cells and is closely related to the expression of typical NK cell surface marker CD56, which is involved in NK cell development (58). Tumor-infiltrating CD3+CD56+ NKT-like cells and impaired effector function in GC have been linked to immune escape and tumor progression. This may be related to the downregulation of lnc-CD56 in GC, although further research is needed to confirm this (59). In addition, Wei et al. found that lncRNA GAS5 in GC also enhanced the secretion of IFN-γ and TNF-α by regulating miR-18a, as well as the cytotoxicity of NK cells to GC, and the up-regulation of GAS5 expression may provide a new idea for anti-tumor therapy (60). Therefore, the importance of LncRNA in regulating NK cell infiltration in GC TIME cannot be ignored, and more exciting studies are expected to further confirm it.

LncRNAs and GC-Associated DCs Cells

DCs play an important role in antigen presentation. They are considered to be the most powerful professional antigen-presenting cells, with antigen presentation capability 100-1000 times that of macrophages and B cells (61). DCs and NK cells are both referred to be "former sentinels" of the immune response. In the immature state of DCs, they have a strong ability to

devour. After phagocytic antigen, mature under the stimulation of cytokines, and then express CD80/86/40 and other costimulatory molecules, presenting the antigen to T cells to activate the downstream specific immune response (62). During tumor growth, DCs present antigen to naive T cells and memory T cells under the influence of the inflammatory environment and costimulatory signals, which leads to antigen tolerance or initiates and triggers effector T cell response (63). According to their origins and degrees of differentiation, DC cells can be classified as DC1 (myeloid DC, mDC) or DC2 (plasmacytoid DC, pDC) (64). Studies have shown that adequate density of mature DC in the tumor can prolong the survival of GC patients, and higher CD1/CD2 ratio and lower DC2 cell level are negatively correlated with the degree of tumor differentiation, degree of $Foxp3^+$ Treg cells invasion and the risk of lymphatic metastasis (65, 66).

It is worth noting that studies on the regulation of lncRNAs on DCs mainly focused on HOTAIRM1 and lnc-DC genes. LncRNA HOTAIRM1 (HOX Antisense intergenicRNA myeloid 1, HOTAIRM1) was located between human HOXA1 and HOXA2 and played a functional role in regulating the expression of adjacent genes at the 3 end of HOXA cluster (67, 68). LncRNA HOTAIRM1 was found to be down-regulated during differentiation from monocytes to dendritic cells, and upregulation of HOTAIRM1 appeared to inhibit DCs maturation (69). Conversely, Lu et al. showed that the LncRNA HORAIRM1 suppressed the PI3K/AKT pathway and inhibited the development of GC by acting as a competing endogenous RNA of miR-17-5p and mediating the expression of PTEN (70). Because the outcomes of these two studies may be contradictory, more research into the specific mechanism of HOTAIRM1 in GC TIME is required. High-throughput screening analysis showed that lnc-DC was a specific regulatory gene for DC differentiation and development. Further mechanism studies showed that lnc-DC could promote DC cell maturation by activating STAT3 signaling pathway, positively regulate $CD4^+$T cell differentiation to Th1 cell, and then regulate immune inflammatory response (71, 72). Unfortunately, the regulatory role of lnc-DC in immune system diseases such as Sjogren's syndrome, multiple sclerosis, and systemic lupus erythematosus has been confirmed (73–75), but there is no report on the anti-tumor effect. We expect that future studies can further explore the role of lnc-DC in TIME. Recently, Zhu et al. found that LINC00963, which is highly expressed in GC tissues, regulates CDC5L expression and mediates DCs related anti-tumor immune response through competitive binding with miR-612, thus promoting GC progression. Therefore, targeting LINC00963 may be a promising GC treatment strategy (76).

LncRNAs and GC-Associated Adaptive Immune Cell

Compared with innate immunity, adaptive immunity is relatively slow, but it has high specificity and memory function. Adaptive immunity consists of cellular immunity mediated by T cells and humoral immunity mediated by B cells. Nevertheless, since humoral immunity is rarely engaged in GC TIME, no studies on the role of B cells in GC TIME are currently available. Here, we principally focus on reviewing the role of T cells in GC TIME.

LncRNAs and GC-Associated T Cells

T cells, which are the second most common type of immune cell in tumors after macrophages, play a dual role in tumor development. Immune escape of tumor cells is usually closely related to the activation of immunosuppressive properties of T cells and the weakening of anti-tumor properties (77).

$CD8^+$ T Cell

$CD8^+$ T cells are the main T cell population in TIME and have effective anti-tumor attack effect (78). Activated $CD8^+$ T differentiates into cytotoxic T lymphocytes (CTL), which have an effective anti-tumor effect by releasing perforin or promoting apoptosis, leading to direct destruction of target cells (79). In general, high levels of CD8+ T cell infiltration are linked to favorable therapeutic response and clinical outcomes in a variety of tumor tissues (80). Similarly, Lu et al. found that GC patients with a high density of $CD8^+$ T cells in MSI-High GC had a higher overall survival rate than patients with low density (81). LncRNAs are currently regarded to be an important regulator of CD8+ T cell activity. LINC0152, which is up-regulated in tumor tissues and perimeters of GC patients, has been considered as an oncogene. Ou et al. found that LINC00152 inhibits the production of Th1-type chemokines CXCL9 and CXCL10 by binding to the enzymatic subunit EZH2 of PPC2, reducing the number of tumor-infiltrating $CD8^+$ T cells and thereby contributing to tumor progression (82).

$CD4^+$ T Cell

CD4+ T cells are activated primarily by MHC class II antigen recognition and serve an important regulatory role in anti-tumor immune response. It has been found that in tumor immunity, $CD4^+$ T cells can activate $CD8^+$ T cells through a variety of mechanisms, allowing them to differentiate into CTL while maintaining and enhancing the anti-tumor response of CTL. On the other hand, CD4+ T cells can kill tumor cells directly through the IFN- γ mechanism even in the absence of CD8+ T cells (83). Therefore, scientists regard it as a non-negligible "supporting role" in TIME.

To adapt to varied developmental and environmental conditions, naive CD4+ T cells have high plasticity and can differentiate into multi-seed cells (84). Th1, Th2 and Th17 are part of helper T (Th) cells, which are differentiated from antigen-stimulated primitive CD4+ T cells and play different anti-tumor immune functions. Th1 cells mainly secrete IFN-γ and IL-2, which activate CD8+ T cells and natural killer (NK) cells, promoting cellular immunity. To mediate humoral immunity, Th2 cells mainly secrete IL-4, IL-10, and IL-13. Th17 cells differentiate from Naïve $CD4^+$ T cells induced by both TGF-β and IL-6, and they affect inflammation and progression of tumor diseases (85). LncRNA is also involved in the regulation of Th cells. According to Yao et al., high expression of lncRNAs (A2M-AS1, C2orf27A, and ZNF667-AS1) in GC tissues may act on hub ferroptosis-related genes, impair the activation of $CD4^+$ T cells

and Th cell infiltration, and ultimately lead to poor prognosis of GC (86). Lnc-SGK1 was shown to be significantly upregulated in GC tissue and peripheral blood, and it was linked to HP infection and a high salt diet. On another study, Yao et al. found that Lnc-SGK1 induces Th2 and Th17 differentiation while reducing Th1 differentiation through the SGK1/JunB signaling pathway, which is closely related to the poor prognosis of GC (87).

Treg Cells

Treg cells are a subset of $CD4^+$ T cells with a significant immunosuppressive effect. At present, the most studied cells are $CD4^+CD25^+$ Treg cells, which express the transcription factor Foxp3 in their cytoplasm. Most scholars identify $CD4^+CD25^+Foxp3^+$ T cells as Treg cells. Numerous investigations have revealed that immunosuppressive regulation of Foxp3+ Treg cells is an essential mechanism of tumor immune escape (88). Deng et al. used TGF-1 signaling to induce Foxp3+Treg cells in a hypoxic environment, which could allow dominant selection in GC to evade immune surveillance (89). Some studiesshowed that the absolute number of $Foxp3^+$Treg cells in peripheral blood of patients with GC was significantly lower than that of normal controls, especially in patients with lymph node metastasis (90). Generally, LncRNA serves as an oncogene in the regulation of Treg cells. High-throughput sequencing revealed that Lnc-POU3F3 could promote the proliferation of GC cells by recruiting TGF-β protein, activating TGF-β signaling pathway and promoting the distribution of $Foxp3^+$ Treg in peripheral blood T cells (33). Tang et al. found through ssGSEA analysis that LncRNA RP11-357H14.17 enhanced differentiation of Treg cells by activating the ATF2 signaling pathway, and thus played a carcinogenic role in GC (20).

It can be seen that lncRNAs plays a assignable role in the immune cells in TIME during the whole process of GC generation and development (**Figure 2**). However, due to the variety of lncRNA and the limited number of existing studies, further exploration is necessary.

LncRNA IS A REGULATOR OF EXTRACELLULAR MATRIX IN GC TIME

Extracellular matrix (ECM) is a macromolecular substance synthesized by cells that is secreted and distributed on the cell surface or between cells. ECM is composed of basement

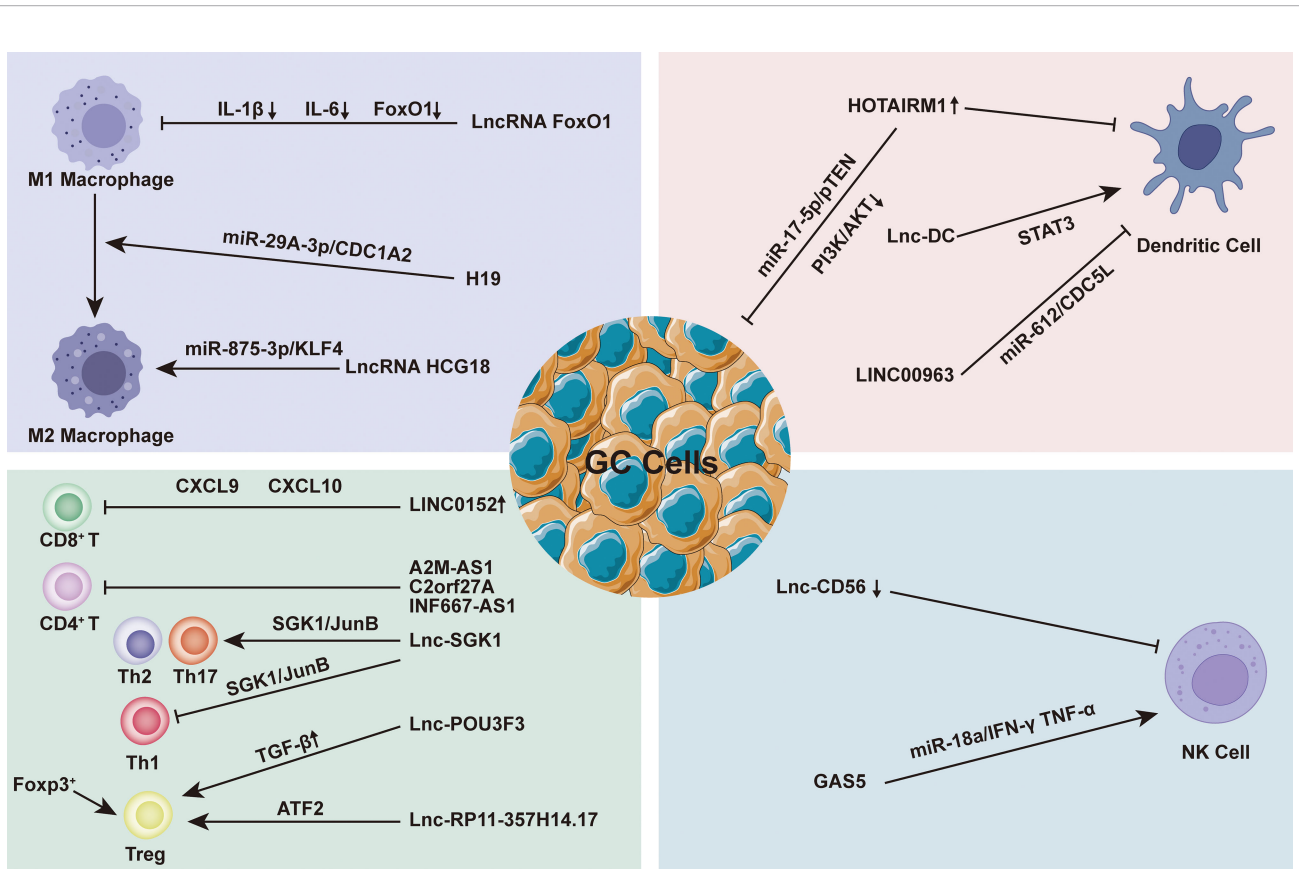

FIGURE 2 | Regulation of Long non-coding RNA (lncRNAs) on immune cells in the immune microenvironment of gastric cancer (TIME). At the microenvironmental level, lncRNAs are involved in mediating and controlling various immune-cancer cell interactions. Abnormal anti-tumor immune cells [such as macrophages, dendritic cells (DC), natural killer cells (NK), and T cells and regulatory T cells (Tregs) induce the formation of immunosuppressive microenvironments, thus contributing to tumor cell metastasis.

membrane (BM) and intercellular matrix, and it serves as an important tissue barrier to prevent tumor cell metastasis. Its main components include glycosaminoglycans, proteoglycans, collagen and elastin, fibronectin (FN) and laminin (LN), the precise composition of which varies from tissue to tissue (91). ECM utilizes collagen and proteoglycans as the basic skeleton and produces a fibrous network complex on the cell surface by FN or LN directly to the cell surface membrane integrin receptor and to the cytoskeleton proteins. Through membrane integration proteins, ECM connects the inside and outside of cells, contributes in cell survival and apoptosis, affects cell shape, and regulates cell differentiation and migration. Increasing experimental and clinical observational data shows that ECM remodeling plays an important role in the precancerous cascade of GC, enhancing GC proliferation, survival, migration, invasion, and metastasis (92). For example, tenonin expression is increased in precancerous and malignant gastric epithelium, while collagen is shown to be dysregulated at more advanced stages (93, 94). ECM components and interactions are considered to have better clinical potential as prognostic biomarkers and pharmacological targets for GC.

LncRNAs play a considerable role in EMC regulation by regulating multiple targets including miRNA to achieve tissue-specific modification of ECM. Based on the evidence, we hypothesized that the modification of ECM by lncRNAs in GC is primarily focused on the regulation of matrix metalloproteinases (MMPs) and the epithelial-mesenchymal transition (EMT). MMPs are a family of Zn^{2+} and Ca^{2+} dependent endogenous proteolytic enzymes, which can be synthesized and secreted by fibroblasts, neutrophils, macrophages and tumor cells (95). The primary condition for tumor cell invasion and metastasis is degradation of ECM and destruction of BM. MMPs is the most important protease for degradation of ECM. Currently, MMPs has been found to be involved in multiple steps of tumor genesis, invasion and metastasis (95). The evolution and metastasis of GC mainly focus on MMP-2, MMP-9 and MMP-14. EMT is the biological process through which epithelial cells undergo a particular transformation into mesenchymal phenotypes. It is characterized by decreased expression of adhesion molecules (such as e-cadherin), transformation of cytoskeleton from keratin to vimentin, and mesenchymal cell morphology (96). EMT caused epithelial cells to lose their polarity, their connection to the basement membrane, and other epithelial characteristics, as well as the capacity to degrade the extracellular matrix, allowing for further migration and invasion (97). Sun et al. found that the lncRNA VIM AS1 up-regulated the expression of MMP-2 and MMP-9 proteins by regulating FDZ1 and activating the Wnt/β-catenin pathway, promoting cell proliferation, migration, invasion and epithelial-mesenchymal transformation (98). Meanwhile, LINC01296 is defined as an oncogene because it can sponge out miR-122 and then up-regulate the expression of MMP-9 protein, leading to the progression of GC (99). Moreover, Li et al. found that lncRNA CASC2 with high expression in GC tissues could reverse the regulatory effect of E2F6 gene on MMP-2, down-regulate MMP-2 expression and increase caspase-3 activity. The E2F6/CASC2 axis is expected to become a potential therapeutic target (100). Xu et al. discovered that by silencing the lncRNA ZFAS1, they could block the Wnt/-catenin signaling pathway, down-regulate the expression of MMP-2 and MMP-14 proteins, and inhibit the growth, proliferation, migration, invasion and EMT of GC cells (101). When Wei studied the SOX2OT/miR-194-5p axis in GC, they showed that the expression of miR -194-5p was negatively regulated by lnc-SOX2OT expression in GC cells. Downregulation of SOX2OT inhibited the growth of GC and the expression of MMP-2 and MMP-9 by inhibiting EMT, and it also played an effective role in anti-tumor cell metastasis (102). In addition, analysis of gene data showed that the high expression of LINC00473 in GC tissues was associated with poor histological type, advanced clinical stage, more lymph node metastasis and distant metastasis. Silencing LINC00473 can effectively regulate the expression of MMP2 and MMP9 and inhibit the migration and invasion of GC cells (103) (**Figure 3**).

Aside from the MMPs family and EMT, some ECM-related proteins have also attracted the attentions of researchers. As a collagen family protein, COL5A1 is involved in ECM formation. Bioinformatics identification showed that COL5A1 may be a key factor in many cancers, including breast cancer, ovarian cancer, lung cancer and so on (104–106). Wei et al. proved that COL5A1 may mediate the regulation of the occurrence and development of GC through its effect on ECM. Lnc-HOTAIR overexpression in GC tissues upregulated COL5A1 by sponging miR-1277-5p. ECM1 (extracellular matrix protein 1) is a glycoprotein that is involved in a variety of biological processes. A great number of studies have indicated that ECM1 can accelerate cancer development and invasion, and ECM1 overexpression has been identified as a poor prognosis indicator (107, 108). Mechanism studies have shown that ECM1 is positively correlated with the expression of lnc-FALEC in GC, and high level of ECM1 predicts shorter survival time in GC patients. Downregulation of lnc-FALEC and disruption of ECM1 expression, which significantly inhibits GC cell migration and invasion, may become potential novel therapeutic strategies (**Figure 3**).

LncRNA IS A REGULATOR OF CANCER ASSOCIATED FIBROBLASTS IN GC TIME

Cancer associated fibroblasts (CAFs) are the most common stromal cells in the TIME, accounting for around half of the total amount of tumor tissue cells (109). Studies in recent years have shown that CAFs mainly originate from different cells through various mechanisms, and there are three main sources of CAFs: transformation from fibroblasts (110), bone marrow mesenchymal stem cells (111), and epithelial tumor cells after EMT (112). CAFs can secrete a variety of cytokines and metabolites with tumor cells through direct contact or paracrine mode, assisting tumor cells in immune escape, promoting tumor angiogenesis, inducing tumor cells to undergo epithelial-mesenchymal transformation, promoting tumor extracellular matrix remodeling, and making the microenvironment more conducive to tumor growth (113). It has been proved that CAFs play an undeniable regulatory role in

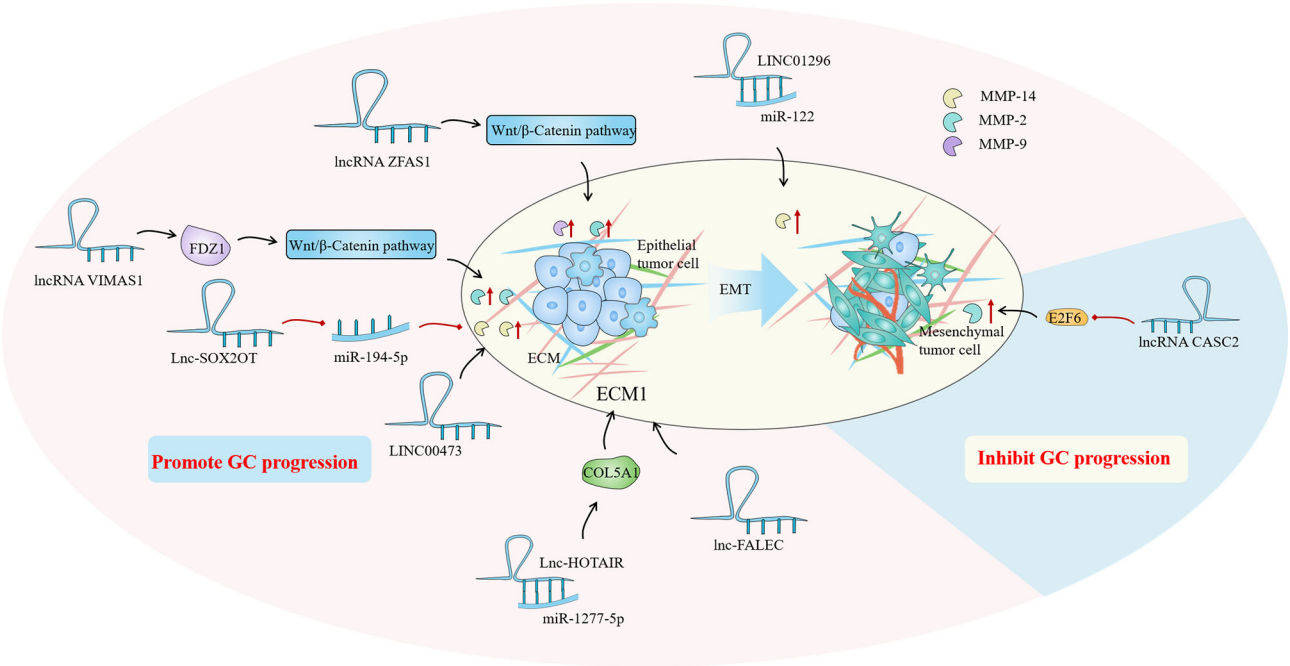

FIGURE 3 | In the gastric cancer (GC) immune microenvironment (TIME), lncRNAs mainly regulate by regulating matrix metalloproteinases (MMPs) and epithelial-mesenchymal transition (EMT) to achieve the regulation of extracellular matrix (ECM), and then play a role in promoting or inhibiting GC progression.

the whole process of the occurrence and evolution of GC. An analysis of the relationship between cell expression profile and clinicopathological features in TIME of 1524 patients with GC showed that the higher the number of CAFs infiltrates in TIME, the worse clinical prognosis (114). A large number of studies have shown that CAFs can directly or indirectly promote the migration and invasion of GC cells by releasing growth factors or cytokines. GC CAFs exhibit high levels of miRNA-106B, 143, and 145 expression and down-regulate miRNA-200 expression, all of which can enhance GC invasion and metastasis by various cascade pathways (115). Besides, CAFs also play a role in ECM remodeling, metabolism, and immune reprogramming. The signature function of CAFs are known for producing ECM components (such as collagen, fibronectin, proteoglycan, periostein, and tenonosin-C), which disrupt the structure of cancer tissues (116). Simultaneously, CAFs are another major source of MMPs in addition to cancer cells. All of these factors contribute to the probability of GC cell metastasis and diffusion (117).

Until now, the regulation of lncRNA in GC-related CAFs is mainly manifested as the regulation of autophagy of tumor cells and the expression of HIF family genes, fibroblast growth factor and inflammatory factor interleukin. Autophagy is an intracellular process that has evolved that relies on lysosomes to degrade intracellular macromolecules in bulk (118). CAFs autophagy participates in the complex metabolic and nutritional networks of tumor cells, influencing tumor progression and resistance to treatment through interactions with a variety of TIME (119). Wang et al. found that lncRNA can be used as a new regulator of autophagy, and the up-regulated lncRNA MALAT1 in GC tissues can lead to the overexpression of metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), which leads to autophagy inhibition and increased IL-6 expression, thereby activating the AKT/mTOR pathway and ultimately leading to the progression of GC (120). Members of the hypoxia-inducible factor (HIF) family play a crucial part in cell hypoxia metabolism. The promotion of HIF1A and HIF2A on angiogenesis, cell metabolism, proliferation, and extracellular matrix remodeling have been demonstrated (121). By comparing the differences between GC cancer tissues and adjacent tissues, Bahramian et al. found that lnc-CAF was significantly down-regulated in cancer tissues, while the expression of HIF1A was significantly increased, which may be related to the regulation of HIF1A expression by lnc-CAF. Lnc-CAF might be one of the potential targets for cancer-targeted gene therapy (122). Additionally, Liu et al. reported that LINC00342 regulates the expression of canopy fibroblast growth factor signaling regulator 2 (CNPY2) as ceRNA by direct sponge adsorption of miR545-5P and promotes cell proliferation, colony formation, migration, and invasion *in vitro* (15). Furthermore, noncoding RNA activated by DNA damage (NORAD) is a novel lncRNA derived from segment q11.23 of chromosome 20. Huang confirmed that NORAD could enhance the promoting effect of CAFs in GCTIME by upregulating IL-33 and targeting miR-496 (123). Overall, the regulation of lncRNAs on CAFs affects tumor progression, implying that targeting lncRNAs in CAFs and tumor cells might be a novel cancer therapy strategy (**Figure 4**).

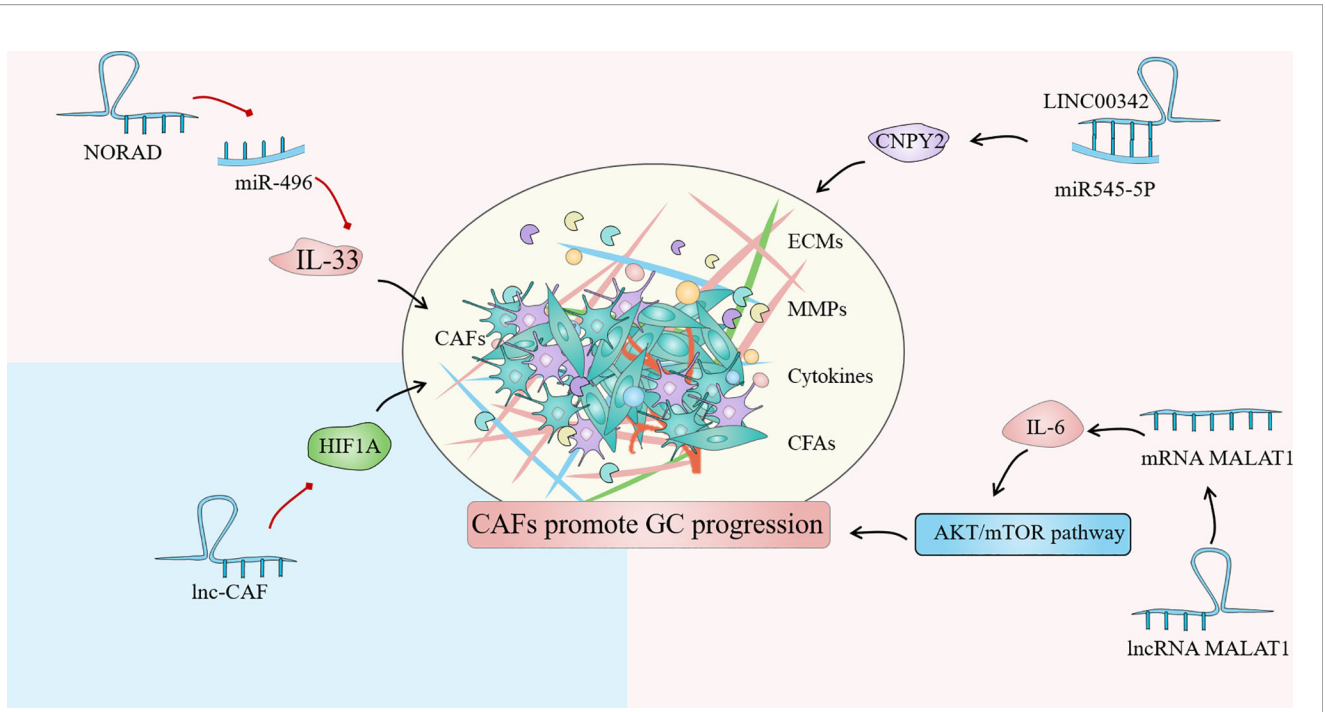

FIGURE 4 | Cancer associated fibroblasts (CAFs) are one of the most common stromal cells in the gastric cancer (GC) immune microenvironment (TIME). The degree of invasion of CAFs in tissues is closely related to poor clinical prognosis. The regulation of lncRNA in GC-related CAFs is mainly manifested in the regulation of tumor cell autophagy, expression of HIF family genes, fibroblast growth factor and inflammatory factor interleukin.

LncRNA IS A REGULATOR OF CANCER ASSOCIATED CYTOKINES IN GC TIME

Cytokines are derived from immune cells and tumor cells in TIME and have diverse roles in tumor evolution and transformation *in vivo*, exerting either synergistic or antagonistic effects. They serve as a bridge for information exchange between TIME and tumor cells, despite the fact that they have no definite anti-tumor ability. The main cytokines include interleukin (IL), tumor necrosis factor (TNF), tumor growth factor (TGF), chemokine and so on (124).

The term interleukin (IL) refers to a group of soluble proteins secreted by white blood cells that can influence the functioning of other white blood cells and tissue cells. It is mainly responsible for immune cell activation and regulation, T and B cell proliferation and differentiation, and inflammatory responses *in vivo* (125). At the moment, at least 38 IL have been identified, although there haven't been many investigations on lncRNA-related IL. IL-21, a member of the IL-2 family, is involved in tumor biological activity and autoimmunity by binding to its receptor IL-21R (126). The IL-21/IL-21R axis has been shown to have a role in the pathogenesis and lymph node metastasis of malignant tumors by activating the JAK/STAT signaling pathway (127). Yan et al. found that IL-21R overexpression was associated with inhibition of the tumor suppressor gene miR-125a. LncRNA MALAT1 acts as a sponge for miR-125a in GC cells, and the maladjustment of the lncRNA MALAT1/miR-125a axis increaseed the risk of survival and recurrence in GC patients (128). Zhou et al. found that OLC8, a new LncRNA, was associated with IL-11 transcription. The binding of OLC8 to IL-11 greatly impaired the degradation of IL-11 mRNA. Unsurprisingly, higher IL-11 expression increased STAT3 activation and therefore contributed in the development of GC (129).

TGF mainly includes TGF-α and TGF-β, among which there are few reports on the correlation between TGF-α polymorphism and GC. The TGF-β signaling pathway plays a vital role in the genesis and development of various tumors, and this pathway has become one of the hot spots in tumor research. TGF-β1 and TGF-β2 are the core genes of this pathway, and their genetic variation has been proved to be closely related to the strength and normal down transmission of TGF-β signal, which is involved in the occurrence and development of a variety of tumors including GC (130). Zhang et al. found that the expression of LINC00665 was correlated with tumor depth, lymph node metastasis and TNM stage, and TGF-β1 was significantly reduced after LINC00665 was knocked out, which may be related to the regulation of TGF-β1 by LINC00665 (131). TGF-β1 expression is inversely linked with miR-185 expression, and the newly discovered lncRNA-XIST can reduce TGF-β1 expression by up-regulating miR-185. Therefore, the XIST/miR-185/TGF-β1 axis is also one of the primary culprits leading to the progression of GC cells (132). Likewise, several studies have found that the TGF family has a regulatory effect on LncRNA. For instance, Saito et al. discovered that TGF can activate lncRNA-ATB, promoting infiltration and metastasis in EMT

through TGF-β/miR-200s/ZEB axis, leading to poor prognosis of GC (133).

Chemokines belong to the family of small molecule cytokine proteins, and nearly 50 chemokines have been discovered so far. All chemokine protein sequences in basic have four conservative cysteine; according to the first two cysteine differences in the relative position, it can be divided into CXC, CC, C and CX3C 4 subtypes. These chemokines are not only important in tissue differentiation and wound healing, but they are also implicated in tumor occurrence, development, invasion, and metastasis. Many investigations have currently discovered that CXC, CC, and CX3C are directly connected to GC invasion and metastasis (134). Dong et al. found that frequent up-regulation of lncRNA COL1A1-014 in GC tissues and cells increased the mRNA expression of chemokines ligand (CXCL12) in GC cells and increased the expression of CXCL12 and CXCR4 proteins through sponge absorption of miR-1273H-5p (135). Furthermore, inhibition of LINC00152 may increase the number of tumor-infiltrating CD8+ T cells and promote the expression of CXCL9, CXCL10, and C-X-C Motif chemokine receptor 3 (CXCR3) in xenograft tumors, thereby achieving the goal of tumor suppression. Collectively, LncRNAs have a significant role in tumor cytokine regulation, with complex mechanisms and various targets (**Figure 5**). Discovering effective targets of LncRNA may provide new light on targeted cancer therapy.

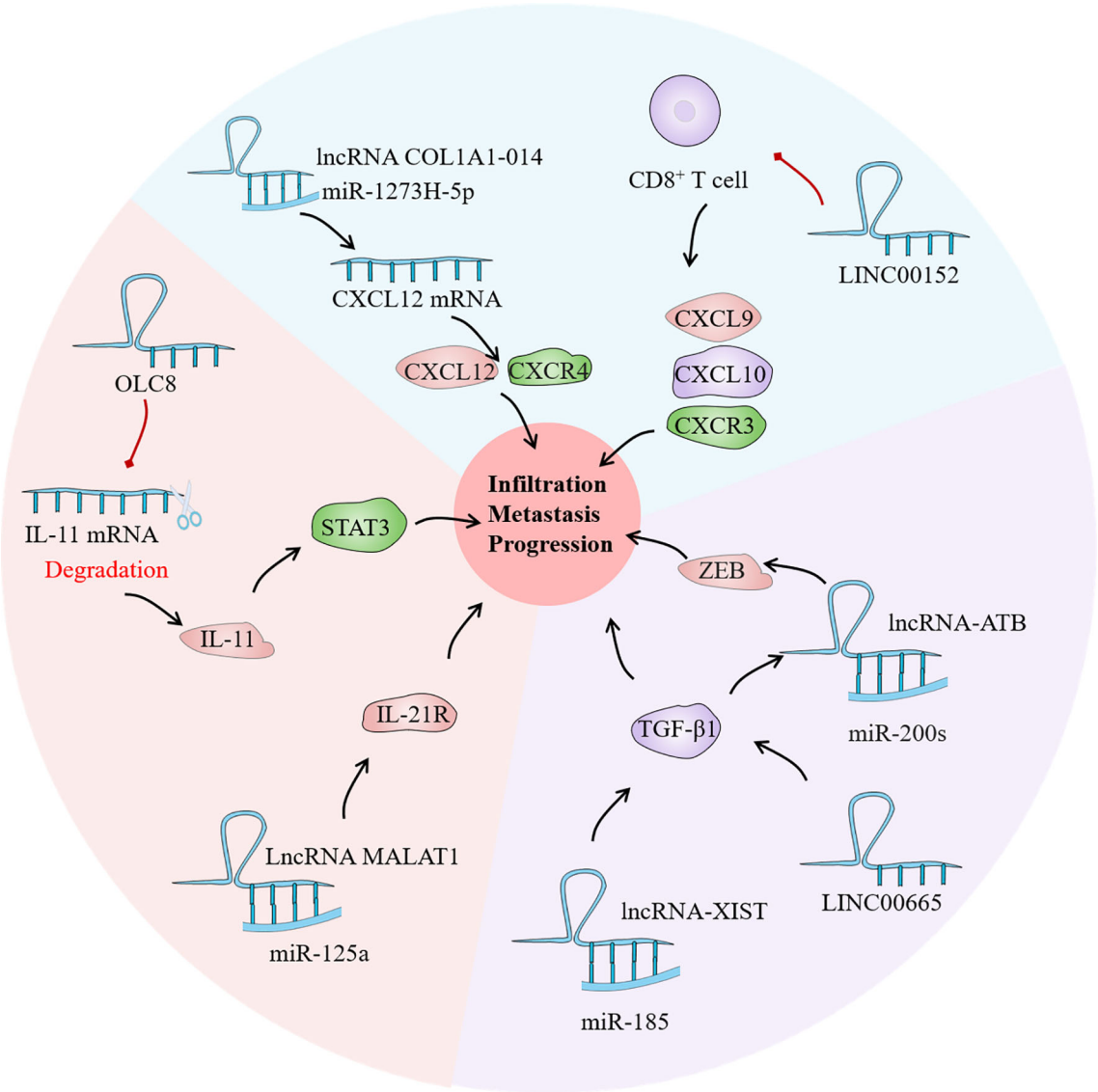

FIGURE 5 | As a bridge of information exchange between gastric cancer (GC) immune microenvironment (TIME) and tumor cells, cytokines play an important role in the evolution of GC. Current studies have confirmed that lncRNA has regulatory effects on the interleukin (IL) family, tumor growth factor (TGF) and chemokines in the GC TIME, which may become a potential tumor therapeutic target.

CONCLUSION

There are interactions between cancer cells and TIME: On the one hand, cancer cells constantly secrete factors to regulate TIME, making it become a microenvironment conducive to tumor development, making TIME become a "hotbed" for cancer diffusion; on the other hand, in response to changes in environmental conditions and carcinogenic signals of tumors, TIME constantly changes during cancer development and regulates cancer progression, leading to abnormal growth, angiogenesis, metastasis and drug resistance of cancer. LncRNAs plays an important role in this process. This paper reviews the research progress of lncRNAs in GC TIME. There are several types of lncRNAs, each with a specific set of functions. LncRNAs regulate TIME cells in several ways to either inhibit or promote tumor growth and progression. LncRNAs targeting cancer immunotherapy have a wide range of potential applications. Although the application of lncRNA-based therapies has been challenging, as research advances and improves, the use of lncRNAs as therapeutic targets will contribute to the development of novel cancer treatment strategies.

AUTHOR CONTRIBUTIONS

XX, WC, and GZ designed the manuscript. XX wrote the manuscript. CZ, BS, and FK drew the figures and tables. YJ revised the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Circular RNA and Its Roles in the Occurrence, Development, Diagnosis of Cancer

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Circular RNAs (circRNAs) are non-coding single-stranded covalently closed circular RNA, mainly produced by reverse splicing of exons of precursor mRNAs (pre-mRNAs). The characteristics of high abundance, strong specificity, and good stability of circRNAs have been discovered. A large number of studies have reported its various functions and mechanisms in biological events, such as the occurrence and development of cancer. In this review, we focus on the classification, characterization, biogenesis, functions of circRNAs, and the latest advances in cancer research. The development of circRNAs as biomarkers in cancer diagnosis and treatment also provides new ideas for studying circRNAs research.

Keywords: circRNAs, biogenesis, cancer, biomarkers, mechanisms

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1 INTRODUCTION

CircRNAs were first discovered in RNA viruses in 1976 (1). Subsequently, it was discovered in eukaryotic cells and humans (2–4). CircRNAs are covalently closed ring structures with 5 'and 3' ends directly linked together, which makes them more stable than linear RNA. They were originally thought to be the product of splicing errors during low abundance transcription. With the development of high-throughput RNA sequencing technology and bioinformatics algorithms, a new understanding of circRNAs has emerged. The functions and mechanisms of new types of circRNAs during biogenesis have been identified. CircRNAs have been found to act as ceRNA or miRNA sponges and bind to proteins. As well as some newly discovered functions, such as regulating parental gene expression, regulating pre-RNA splicing and potential translation templates for proteins (5). Many circular RNAs have been discovered to be biomarkers that impact the onset and growth of malignancies in recent years, attracting a lot of attention. They have been identified in lung cancer (6), hepatocellular carcinoma (7), gastric cancer (8), colorectal cancer (9), and so on. However, the molecular mechanisms and early diagnosis of cancer are not well understood. And, diagnosis and treatment based on circRNAs are still lacking. Therefore, it is urgent to explore new molecular mechanisms and effective biomarkers for the diagnosis of cancer.

In this review, we focused on the biological characteristics, functions, mechanisms, and detection techniques of circRNAs associated with cancer, and discussed their potential application as biomarkers and therapeutic targets. Thus, provide valuable clinical information for the diagnosis and timely treatment of cancer in the future.

2 CircRNAs

2.1 Classification and Properties of CircRNAs

According to the formed sequence, circRNAs can be divided into six categories: exonic circular RNAs (ecircRNAs), circular intronic RNAs (ciRNAs), exon-intron circular RNAs (EIciRNAs), intergenic circRNAs, anti-sense circRNAs, and tRNA intronic circRNAs (tricRNAs) (10).

CircRNAs have no ends so it is highly stable and have specific spatiotemporal expression patterns. Numerous circRNAs usually express in specific tissues and specific developmental stages (11). CircRNAs were found to be evolutionarily conserved in diversity and the conservation is different in different tissues, among which the most conservative in the brain (12). Jeck et al. identified over 25,000 different circRNAs in human fibroblasts (13). In addition, circRNAs are widely distributed and have been reported in thousands of animal cells, such as humans, mice, and nematodes, and expressed in high abundance (11, 14). Rybak-wolf et al. found that circRNAs are abnormally enriched in the mammalian brain and are specifically and dynamically expressed in neuronal differentiation (15). New classification methods such as differences in length, stability, function, and characteristics of circRNAs still need to be continuously explored.

2.2 Biogenesis of CircRNAs

CircRNAs are derived from the reverse splicing mechanism of pre-mRNAs to form a single-stranded closed loop (16). However, the biogenesis mechanism has not been fully elucidated, circRNAs can be derived from exons, introns, 3'UTR, 5'UTR, intergenic regions, or antisense sequences (**Figure 1**). As early as 2013, Jeck et al. proposed two circRNAs cyclization models: Lariat-driven

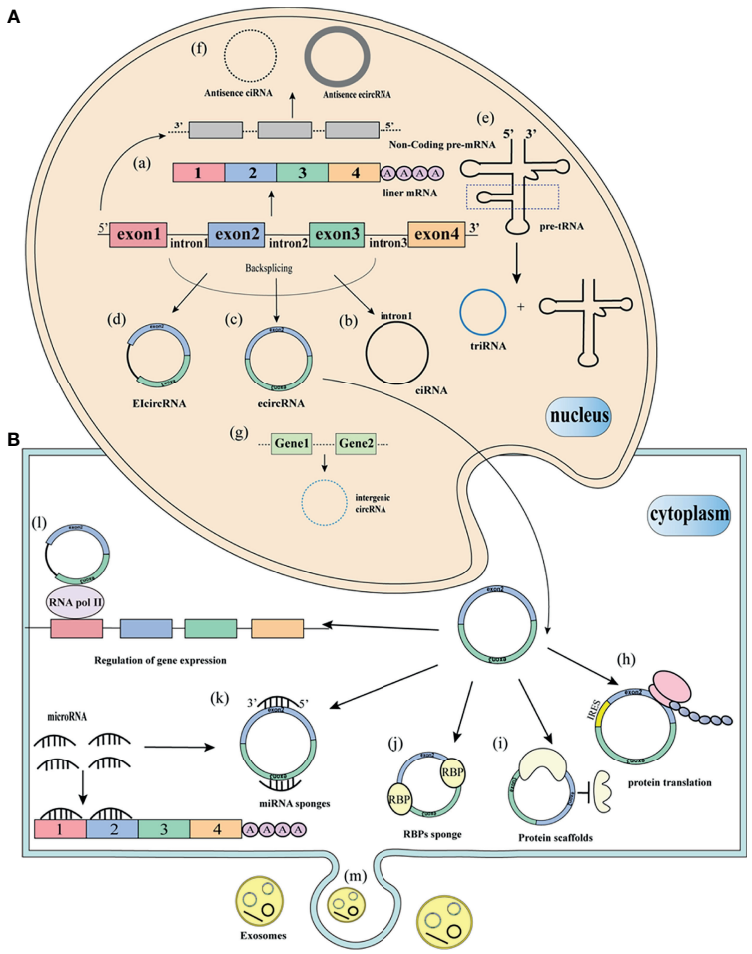

FIGURE 1 | Biogenesis and function of circRNAs **(A)** Biogenesis of circRNAs. (a) Pre-mRNA splicing removes introns to form mature linear mRNA. (b) The introns removed by pre-mRNA splicing form circularization to form a stable ciRNA. (c) Circularization of pre-mRNA exons into ecircRNA. (d) pre-mRNA exons and introns are circularized into ecircRNAs. (e) removal of pre-tRNA introns, release to form triRNA and tRNA. (f) form antisense circRNAs from non-coding regions of pre-mRNA. (g) from two different intergenic sequences to form intergenic circRNAs. **(B)** Functions and of circRNAs. (h) circRNAs translation protein with similar IRES sequence. (i) circRNAs as protein scaffolds. (j) circRNAs bind to RBPs. (k) circRNAs as sponges for miRNA. (l) circRNAs interact with RNA polymerase II to regulate gene expression. (m) circRNAs are packaged into vesicles and released outside the cell to perform biological functions.

circularization and intron-pairing-driven circularization (13). Lariat-driven circularization, also known as exon skipping, is connected by non-adjacent exons as donors and acceptors to form a lasso structure. Then the introns in the lasso are removed, resulting in ecircRNAs. The biogenesis of circRNAs is mainly studied in ecircRNAs, other types of circRNAs are rarely studied. EcircRNAs account for 80% of identified circRNAs (17). The biogenesis of eicircRNAs is similar to ecircRNAs. In some cases, the intron portion is completely sheared to form eicircRNAs. Another model is intron-pairing-driven circularization, also known as direct reverse splicing. This model is based on the direct base pairing of the flanking introns, and then the introns are removed to form a ring structure (17). Zhang et al. reported a class of intron-derived circRNAs, namely CiRNAs (5). The formation of ciRNAs depends on the 7 nucleotides GU enrichment element near the 5' splicing site and the 11 nucleotides C-rich element near the branch site. Zhang et al. believed that the exon cycle depends on the complementary sequences of the flanking introns. Intergenic circRNAs are formed from genes outside known genetic loci (11). TricRNA is formed by intron excision of the pre-tRNA by the tRNA splicing mechanism, followed by intron release and binding into tRNA and TricRNA (18).

In addition, multiple factors are involved in the biogenesis of circRNAs. Zhang et al. reported that the exon cycle depends on the complementary sequences of the flanking introns (19). Some RBPs play an important role in reverse splicings, such as MBL (splicing factor muscleblind), QKI(Quaking), and FUS(fused in sarcoma), binding to both sides of the flanking intron sequence enhances exon cycling by tightly linking the 3 'and 5' ends of circRNAs. Thereby promoting exon circulation. Muscleblind is a splicing factor for MBL-derived genes and MBL in Drosophila promotes the production of circRNAs from the second exon of its own pre-mRNA by binding to flanking introns (20). The QKI of the STAR family is a tumor suppressor protein with three isoforms, all of which have the same KH domain but have different 3'UTRs. Among them, QKI-5, the most abundant nuclear isoform, acts on circRNAs during splicing. QKI dimerizes through its N-terminal Qua1 domain and binds to two-part sequence motifs that can be located on the same or separate RNA molecules (21). The investigation of PAR-CLIP cross-linking in human embryonic kidney cells (HEK293T) indicated that the majority of QKI binding occurs within introns and is responsible for circRNA synthesis, which limits proliferation and EMT during human cancer (22). In addition, Conn et al. also introduced consensus binding sequences for QKI in flanking introns to enable circRNAs to be generated from exons that normally only undergo canonical linear splicing (21). A recent study showed that overexpression of circ-SHPRH in cadmium-transformed BEAS-2B cells promoted the expression of QKI and significantly inhibited cell proliferation, EMT, invasion, migration, and non-anchored growth. Conclusions of Conn et al. (23). FUS was first reported to be involved in circRNA generation in the nervous system in 2017 (24). Cao et al. recently discovered that the nematode homologous gene FUST-1 promotes the creation of numerous circRNAs while having no effect on the analogous linear mRNA, regulating exon skipping and reverse splicing, surprisingly, CLIP-seq results suggest that FUS attaches to stem-loop secondary structure rather than particular sequences (25).

Besides, negative regulators destroy the stability of intron interactions, thereby reducing the cyclization efficiency, such as adenosine deaminases acting on RNA 1 (ADAR1) reduces the efficiency of cyclization by disrupting the base pairing between flanking introns through the A to I RNA editing mechanism (15). ADAR is an adenosine deaminase that is widely expressed in humans and can be applied to RNA modification. ADAR systems are used for programmable RNA editing *in vitro* and *in vivo* by recruiting ADARs to target RNA sequences using ADAR recruitment guide RNAs (adRNAs). Two recent studies have designed circRNAs that can recruit ADARs to improve RNA editing efficiency. Katrekar et al. engineered a highly stable circular ADAR-recruiting guide RNA (cadRNA) to recruit endogenous ADARs, improving the efficiency and durability of RNA editing (26). The engineered circ-arRNAs designed by Yi et al.'s LEAPER2.0 system have much higher editing efficiency than the corresponding linear arRNAs, which greatly improves the efficiency and robustness of RNA editing (27).

Interestingly, UAP56 and URH49 proteins can assist the transport of circRNAs from the nucleus to the cytoplasm (28). This discovery is novel, however, many of the regulatory factors involved in circRNAs biogenesis remain unclear and require more research. A better understanding of the biogenesis mechanism of circRNAs will lead to a better understanding of their specific roles in cancer development. Future studies can explore the levels of specific key factors that regulate the biogenesis of circRNAs, which will also provide innovative strategies for cancer treatment and prevention.

2.3 Biological Functions of CircRNAs

In addition to its unique way of formation, how circRNAs participate in the process of biogenesis has also deepened our understanding of circRNAs. However, the functions of most circRNAs are still unknown. Recent studies show that the functions of circRNAs are mainly ceRNA or miRNA sponging, binding with proteins, regulation of pre-RNA splicing, regulation of parental gene expression, and potential translation templates for proteins or peptides (**Figure 1**).

2.3.1 Acting as CeRNA or MiRNA Sponging

MicroRNAs (miRNAs) are a class of small non-coding RNAs, which play a regulatory role in various cellular activities including cancer by pairing regulatory genes with mRNAs target bases. Hansen et al. first proposed the concept of miRNAs sponges in 2013 (29). There are miRNAs response elements on circRNAs, which can competitively bind to miRNAs, eliminate the inhibitory effect of miRNAs on target genes, and regulate the expression of related genes. They demonstrated for the first time that circCIRS-7 (CDR1as) can be a sponge of miRNAs. CIRS-7 promotes the progression of a variety of tumors. There are more than 70 miR-7 sponge binding sites on CIRS-7. CIRS-7 inhibits miR-7 and participates in various events in tumorigenesis, such as cell proliferation,

migration, invasion, and differentiation (30). With the development of scientific research, more and more circRNAs have been found to act as sponges for miRNAs. This mechanism affects cell proliferation, migration, invasion, and blood vessel formation, and has been widely reported in the cancer field. For example, the combination of circSATB2 and miR-326 regulates the expression of FSCN1 and further promotes the proliferation, migration, and invasion of NSCLC cells (31). In addition, some circRNAs have also been found to combine multiple miRNAs to act on different systems. For example, cirMAT2B can be combined with miR-515-5p to increase the expression of HIF-1α and promote the growth of gastric cancer (32). Moreover, circMTO1 can also combine with miR-541-5p to inhibit the progression of liver cancer (33).

2.3.2 Interaction With Proteins

Another function of circRNAs is to directly bind proteins to participate in physiological and pathological processes (**Figure 1**). More than 800 RNA-binding proteins (RBPs) have been identified in the human genome (34). RBPs play a role in circRNAs splicing, processing, folding, stabilization, and positioning. For example, CircFoxo3 is formed by Foxo3 exon 2 and has a wide range of complex biological functions, which are related to cell differentiation, apoptosis, and cycle. It has been reported that in the cytoplasm, the senescence-related proteins ID-1, E2F1, FAK, and HIF1α interact with circFoxo3 and no longer exert their anti-aging and anti-stress effects, leading to the promotion of cell senescence (35). CircFoxo3 can also bind to cyclin cells cyclin-dependent-kinase 2 (CDK2) and cyclin-dependent kinase inhibitor 1 (p21) to form a ternary complex to inhibit the binding of CDK2 and p21, and in the G1 phase inhibits the cell cycle progression (36). In addition, circMBL can bind to mannose-binding lectin (MBL) protein to control excess MBL protein (20). Two circRNAs, KIRKOS-73 and KIRKOS-71, are able to regulate the exosomal metastasis of p53 expression in recipient cells, and p53 plays a key role in metastasis and tumorigenesis (37). CircAgo2 transfers HuR protein from the nucleus to the cytoplasm, stabilizing the binding of mRNA and AU-rich elements in UTR (38). CircPABPN1 competitively binds to HuR, prevents HuR from binding to PABPN1 mRNA, and subsequently inhibits the translation of PABPN1 (39). The above studies have proved that the interaction between circRNAs and proteins plays an important role.

2.3.3 Regulation of Pre-RNA Splicing

CircRNAs may affect the splicing of pre-RNA and can compete with pre-RNA for splicing sites. For example, circUBR5 may be involved in the RNA splicing regulation process, it can be combined with the splicing regulator QKI in the nucleus, NOVA alternative splicing regulator 1 (NOVA1), and U1 small nuclear RNA (snRNA) (40). CircSMARCA5 regulates VEGFA mRNA splicing and angiogenesis in glioblastoma multiforme through the binding of SRSF1 (41).

2.3.4 Regulation of Gene Expression

EIciRNAs and ciRNAs are circRNAs with intron sequences, which are mainly located in the nucleus. Experiments have shown that EIciRNAs and ciRNAs can regulate gene expression. For example, the knockdown of circEIF3J and circPAIP2 can cause the transcription level of EIF3J and PAIP2 to decrease (42). EIciRNAs can promote the transcription of their parental genes in cis by interacting with U1 snRNA, revealing a new regulatory strategy for gene expression in RNA-RNA interactions (43). Li et al. found that ci-ankrd52 shows a different open structure conformation from pre-mRNA with the same sequence, which can replace pre-mRNA to form more stable R-loops (44). It can be seen that ci-ankrd52 plays a potential role in promoting transcription elongation. However, the effect of EIciRNAs and ciRNAs regulation still need to study in-depth.

2.3.5 Translation Templates for Proteins or Peptides

Although circRNAs have long been considered non-coding RNAs that cannot translate proteins, recent studies have shown that circRNAs do not rely on conventional translation modes and have translation potential. Previous studies have found that some circRNAs have internal ribosome entry (IRE) site sequences or open reading frame (ORF) translatable proteins such as circMAPK1 and circMBL3. CircMAPK1 encodes a new protein with a length of 109 amino acids that competitively binds to MEK1 to inhibit the phosphorylation of MAPK1 (45). CircMBI translates to a small peptide in the head of a fly (46). However, recent studies have found that circRNAs lacking this sequence can also translate proteins. The N6-methyladenosine (M6A) modification allows circRNAs to be translated in a cap-independent manner (47). In addition, bioinformatics tools have been developed to predict translation potential, but the accuracy needs to be verified. Although these new discoveries are exciting, the function and efficiency of these translated proteins or peptides need further research (48, 49).

2.4 Identification of CircRNAs

Early RNA sequencing did not identify circular RNAs without a ployA tail, and non-linear fragments were often considered errors and were ignored. This section introduces some traditional circRNAs detection techniques and emerging technical methods (**Table 1**). Northern blot, qRT-PCR, RNA-seq, and Microarrays are examples of traditional circRNA detection techniques (63). However, previous RNA analysis methods are also difficult to study circRNAs. RNA-seq detection of RNA detection efficiency is low, so many low abundance circRNAs may be missed. And microarray technology has been used to detect linear RNA for a long time. The detection efficiency of circRNAs microarray is much higher than RNA-seq because it contains probes that target the head-to-tail connection (51). But it produces data that is difficult to compare between studies. Therefore, there is an urgent need to develop simple, effective, and sensitive new methods to study circRNAs.

Recently, a newly reported exome capture RNA sequencing technology can detect and characterize circRNAs in more than 2000 cancer samples (64). In addition, Zhang et al. developed a new method for quantitative detection of circular RNA with high sensitivity and specificity (56). The two cleverly designed DNA probes can be precisely connected by using ligase at the connection site of circular RNA. Distinguish circular RNA

TABLE 1 | Detection of circRNAs.

Methods	Mechanism	Strengths	Weaknesses	Refs
Northern blot	Oligonucleotide probe capture	Distinguished between circRNAs and linear RNAs Estimated the circRNAs size	Low sensitivity and required large sample	(50)
Microarrays	Nucleic acid hybridization	High detection efficiency	Identified only known circRNAs	(51)
FISH	DNA probe hybridization	Subcellular localization of circRNAs	Expensive cost and time-consuming	(52)
RNA-seq	Transcript map	High sensitivity and specificity	Expensive equipment and reagents Complex operation process and data process	(53)
qRT-PCR	PCR amplification	High sensitivity and quantitative detection	Linear RNAs residue Amplified rolling loop error	(54)
ddqRT-PCR	PCR amplification based on Poisson distribution algorithm	High sensitivity and accuracy Simplified experimental process	Expensive equipment	(55)
Ligation-based PCR	PCR amplified DNA probe	Exonuclease and reverse transcription steps are not required Simple, high sensitivity, and specificity	Identified only one circRNA at a time	(56)
RT-RCA	Reverse transcriptase rolling cycle amplification	Simple operation, low cost, and high sensitivity	Complex process and time-consuming	(57)
LAMP	SLP induced doubleexponential amplification	High amplification efficiency, high sensitivity, and specificity Distinguished between circRNAs and linear RNAs	Complex process and expensive cost	(58)
circFL-seq	Rolling-cycle reverse transcription (RCRT) and nanopore sequencing	Identified and quantified full-length circular RNAs and isomer level Suitable for mass screening accurately	Identified fewer circRNAs isoforms	(59)
CIRI-long	RCRT	Resisted interference from residual linear RNAs	Less sensitive than full-length reads	(60)
isoCirc	RCA	Longer readings (up to 50 KB) High sensitivity	Expensive cost and false-positive	(61)
Electrochemical method	Back-splice junction (BSJ) and duplex-specific nuclease (DSN)	Avoided errors caused by additional RNase R process Super sensitivity and repeatability	Identified only specific circRNAs	(62)

from corresponding linear RNA. Liu et al. proposed a reverse transcription-rolling cycle amplification (RT-RCA) process that can selectively amplify target circular RNA (57). Zhang et al. designed a pair of Stem-loop primers (SLPs) based on loop-mediated isothermal amplification (LAMP), an excellent nucleic acid amplification method, that could accurately recognize circRNA junction sequences, thereby establishing an SLP-induced dual amplification system (58).The exponential amplification method makes circRNA detection simple and accurate. Additionally, Li et al. designed an electrochemical method for the recognition and capture of circRNAs with hairpin probes, avoiding additional RNase R treatment, and the method exhibited good sensitivity and selectivity (62).

According to the characteristics of circRNAs, some detection methods for circRNAs have been developed, including traditional detection methods and newly developed methods, which have their own advantages and disadvantages. However, the way forward is clear, and the detection method of circRNAs still needs to be developed or improved, so that its sensitivity, specificity, rapidity, and convenience can be applied to biomedical research and clinical detection. All these make it possible for circRNAs to become diagnostic tools and therapeutic targets.

2.5 Online Database for CircRNAs Research

In recent years, with the intensive study of circRNAs, researchers have developed many high-quality online databases. This section introduces some databases that can be used for circRNAs research in **Table 2**. These artificially established databases are of great significance to the study of the biological functions of circRNAs. In addition to the online database listed in the table, in 2022, the University of Padova developed a circRNA function prediction software CRAFT, which can predict circRNA sequences and molecular interactions with miRNAs and RBPs, as well as their coding potential (81). Nevertheless, the existing circRNAs collection is largely limited to certain well-studied species, such as humans and mice. In addition, the current annotations and naming are rather incomplete. Most databases only use one or two resources for annotations. Searching for the same circRNAs has different naming methods in different databases, which increases the difficulty of studying circRNAs.

3 ROLES OF CircRNAs IN CANCER

To date, a large number of reports have found that circRNAs are abnormally expressed in tumor tissues, and more and more evidence shows that circRNAs play a critical role in the occurrence and development of tumors (82, 83). Most of the abnormal circRNAs discovered are the sponges or proteins of miRNAs (**Table 2**). In addition to affecting cancer cell proliferation, migration, invasion, and escape from apoptosis and angiogenesis. CircRNAs can also regulate these cancer markers by regulating signal pathways such as Wnt/β-catenin (7), PIK3/AKT (84), and MAPK/ERK pathways (85). Among

TABLE 2 | Database for circRNAs research.

Database	URL	Specie	Function	Refs
CircBase	http://www.circbase.org/	Human, Mouse, Caenorhabditis elegan, Latimeria	Searched for circRNAs sequence	(14)
Circbank	http://www.circbank.cn/help.html	Human	Organized human circular RNA data in the CircBase database, and performed protein-coding potential and miRNA interaction prediction analysis based on sequence information	(48)
CircAtlas	http://circatlas.biols.ac.cn/	Human, Macaque, Mouse, Rat, Pig, Chicken	Fully annotated circRNAs and Assessed the relevance of circRNAs to various diseases	(49)
MiOncoCirc	https://mioncocirc.github.io/	Human	Associated circRNAs with cancer clinical symptoms and diseases	(64)
CircInteractome	http://circinteractome.nia.nih.gov/	Human, Fruitfly	Predicted the binding of circRNAs to RBP or miRNA and designed PCR primers and circRNAs specific siRNA	(65)
CircRNADb	http://reprod.njmu.edu.cn/circrnadb	Human	Predicted the binding of circRNAs to RBP or miRNA	(66)
StarBase	http://starbase.sysu.edu.cn/	Human, Mouse, Elegan	Analyzed miRNAs-circRNAs interactions to find potential microRNA targets	(67)
Circ2Traits	http://gyanxet-beta.com/circdb/	Human, Mouse, Elegan	Collected circRNAs related to human diseases and predicted the interaction between miRNA and human protein-coding genes, lncRNA and circRNAs	(68)
CircRNA disease	http://cgga.org.cn:9091/circRNADisease/	Human	Retrieved disease-related circRNAs information	(69)
CirclncRNAnet	http://app.cgu.edu.tw/circlnc/	Human	Annotated the multi-line function-related information of CircRNAs/LncRNAs	(70)
CSCD2	http://geneyun.net/CSCD2 or http://gb.whu.edu.cn/CSCD2	Human	An abundant circRNAs data volume, focusing on tumor-specific circRNAs expression and predicted potential full-length and open reading frame sequences of circRNAs	(71)
Deepbase	http://rna.sysu.edu.cn/deepBase/	Human, Mouse, Chicken, Pan troglodytes, Gorilla, Macaca mulatta, Bos Taurus	Annotated and identified circRNAs/miRNAs/piRNAs, etc. and their expression patterns	(72)
CIRCpedia	http://www.picb.ac.cn/rnomics/circpedia/	Human, Mouse, Rat, Fruitfly, Worm, zebrafish	Annotated circRNAs	(73)
TRCirc	http://www.licpathway.net/TRCirc/view/index	Human	Searched for the TFBS of circRNAs can help discover the transcriptional regulation mechanism of circRNAs	(74)
ExoRBase	http://www.exoRBase.org	Human	Retrieved circRNAs information expressed in peripheral blood exosomes	(75)
CircNet2.0	https://awi.cuhk.edu.cn/~CircNet.	Human	Identified new circRNAs and integrated the circRNAs-miRNAs-mRNAs interaction network	(76)
CircR2Disease	http://bioinfo.snnu.edu.cn/CircR2Disease/	Human, Mouse, Rat	Searched for the relationship between circRNAs and disease in the literature	(77)
CirComPara2	https://github.com/egaffo/CirComPara2			(78)
circMine	http://hpcc.siat.ac.cn/circmine http://www.biomedical-web.com/circmine/	Human	Assessed the clinical and biological significance of circRNAs and predicted circRNA-miRNA interactions and circRNAs translatability	(79)
ViroidDB	https://viroids.org	viroids	Collected viroid-like circular RNA sequences	(80)

them, CIRS-7 is widely studied in cancer and is usually up-regulated in cancer cells. It has been described to be expressed in liver cancer, lung cancer, gastric cancer, colorectal cancer, breast cancer, and other cancers (30). The miRNA regulated by CIRS-7 are miR-7 (86), miR-135a-5p (87, 88), miR-1270 (89), miR-26a-5p (90) and so on.

In addition to the circRNAs described above, some circRNAs expressed in common cancers have recently been discovered, as shown in **Table 3** and **Figure 2**.

3.1 Lung Cancer

Lung cancer is the malignant tumor with the highest mortality rate in the world (199). Circ0003222 sponges miR-527 to down-regulate the expression of PHF21B and its downstream β-catenin. Thereby promoting the proliferation, migration, and invasion of tumor cells. Yu et al. (91) discovered a new type of circHMGA2 (hsa_circ_0027446) molecule through microarray, which is highly expressed in lung adenocarcinoma (LUAD). Mechanically, circHMGA2 promotes LUAD cell metastasis through the miR-1236-3p/ZEB1 axis. Yao et al. (99) found that circ_0018414 was down-regulated in LUAD tissues and cells, and inhibited the progression of LUAD through the Wnt/β-catenin pathway of miR-6807-3p/DKK1 axis inactivation. Some circRNAs have been described as binding to proteins in lung cancer. For example, circNDUFB2 (101), which is down-regulated in non-small cell lung cancer, acts as a scaffold to enhance the interaction between TRIM25 and IGF2BPs. It inhibits the growth and metastasis of NSCLC cells by regulating protein ubiquitination and degradation and cellular immune responses. In addition, Huang et al. (103)found that circXPO1 is highly expressed in LUAD through RNA sequencing. In terms of mechanism, circXPO1 can bind to IGF2BP1 to enhance the stability of CTNNB1 mRNA, thereby promoting the progress of LUAD.

The development of new NSCLC-specific biomarkers to aid in diagnosis and clinical decision-making has always been a pressing concern. Li et al. found that circ0003222 is related to the staging, metastasis, and survival rate of patients with non-small cell lung cancer (NSCLC) (6). Additionally, high expression of circ 0070354 was demonstrated to be substantially linked to advanced TNM staging and poor differentiation in NSCLC and was an independent predictor of poor prognosis. CEA, SCC, and Cyfra21-1 are the acronyms for CEA, SCC, and Cyfra21-1, respectively. The AUC of circ0070354, when combined with the other three mature tumor markers, was 0.730, which was much higher than the solitary diagnosis (200). According to the findings, some circRNAs potentially outperform traditional tumor markers in terms of diagnosis, and the combined diagnosis has higher sensitivity and specificity for lung cancer diagnosis and treatment.

3.2 Colorectal Cancer

Colorectal cancer (CRC) is the second leading cause of death from cancer worldwide (199). Jian et al. (110) tested the gene expression in 42 pairs of colorectal cancer tissues and normal tissues adjacent to cancer. The results showed that circ001680 was overexpressed in 71.4% of colorectal cancer tissues. In terms of mechanism, circ001680 promotes the proliferation and migration of colorectal cancer cells by targeting miR-340. Yang et al. discovered a new circRNA, circPTK2, and found that circPTK2 binds to the Ser38, Ser55, and Ser82 sites of vimentin protein to promote EMT of CRC cells *in vivo* and *in vitro (*112). In addition to interacting with proteins to regulate the expression of target genes, circRNAs encoding proteins or peptides have also been found in colorectal cancer, such as circ0006401 (116), circPLCE1 (117), and circFNDC3B (118). Among them, circ000641 encoding peptide fragment promotes the proliferation and migration of CRC and promotes the stability of the host gene col6a3 mRNA, and thus promotes the proliferation and metastasis of CRC. The circPLCE1-411 protein encoded by circPLCE1 combined with the HSP90α/RPS3 complex plays a key role in the NF-κB activation of CRC and ultimately inhibits tumor proliferation and metastasis in CRC cells (117). The tumor suppressor circFNDC3B is mainly located in the cytoplasm and encodes a new protein circFNDC3B-218aa, thereby inhibiting the proliferation, invasion, and migration of colon cancer cells (118).

Wang et al. (108) found that circSPARC is highly expressed in the tissues and plasma of CRC patients, is associated with advanced TNM staging, lymph node metastasis, and a low survival rate of CRC. Mechanistically, circSPARC can upregulate the expression of JAK2 by sponge miR-485-3p, and ultimately promote the accumulation of phosphorylated p-STAT3, thereby promoting the proliferation and migration of cancer cells. The most commonly used colorectal tumor marker CEA has limited sensitivity in early CRC (201, 202). While circRNAs can be employed as reliable biomarker complements for CEA in CRC early diagnosis and treatment monitoring. According to the ROC curve analysis of Hu et al., the AUC (0.831 vs 0.657), sensitivity (0.677 vs 0.532), and specificity (0.915 vs 0.675) values of circ 001659 in the early diagnosis of CRC were higher than those of CEA as a novel biomarker of successful treatment and response for cancer tracking thing (203). These findings indicate that circRNAs can become potential diagnostic and prognostic biomarkers and therapeutic targets for the treatment of CRC.

3.3 Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths. There have been multiple reports that a variety of circRNAs inhibit or promote tumor progression in liver cancer. Hu et al. (120) found that circASAP1 promotes the proliferation and invasion of liver cancer cells by regulating the miR-326/miR-532-5p-MAPK1 signaling pathway, and then mediates tumor-associated macrophages by regulating the miR-326/miR-532-5p-CSF-1 pathway Cell infiltration. The circRNAs array analyzes the expression of circRNAs in tumor tissues and normal tissues. In a study by Dong et al. (125), it was found that 28 up-regulated and 18 down-regulated circRNAs were found in liver cancer tissues. circMEMO1 is significantly down-regulated in HCC samples and can act as a sponge of miR-106b-5p to regulate TCF21 promoter methylation and gene expression, thereby regulating HCC progression. Li et al. (128) found that circMRPS35 was highly expressed in 35 pairs of HCC

TABLE 3 | Dysregulated circRNAs in common cancer.

Cancer type	CircRNA	CircBase ID	Mechanism	Target	Expression in cancer	Function	Refs
Lung cancer	circ0003222	hsa_circ_0003222	MiRNA sponge	miR-527/PHF21B/β-catenin	up-regulated	Promote cell proliferation, invasion, and migration	(6)
	circHMGA2	hsa_circ_0027446	MiRNA sponge	miR-1236-3p/ZEB1	up-regulated	Promote cell metastasis and EMT	(91)
	circSATB2	hsa_circ_0118551	MiRNA sponge	miR-326/FSCN1	up-regulated	Promote cell proliferation, migration, and invasion	(31)
	circPVT1	hsa_circ_0085536	MiRNA sponge	miR-30d and miR-30e/cyclin F (CCNF)	up-regulated	Promotes LUSC progression	(92)
	circCPA4	hsa_circ_0082369	MiRNA sponge	miRNA let-7/PD-L1	up-regulated	Promote cell proliferation, mobility, and EMT	(93)
	circ0000326	hsa_circ_0000326	MiRNA sponge	miR-338-3/RAB14	up-regulated	Promote cell proliferation and migration Inhibit apoptosis	(94)
	circFOXM1		MiRNA sponge	miR-614/FAM83D	up-regulated	Promoted cell proliferation and cell cycle progression	(95)
	circ100146		MiRNA sponge	miR-361-3p and miR-615-5p/ SF3B3	up-regulated	Promote cell proliferation and invasion Inhibit apoptosis	(96)
	circ0000190	hsa_circ_0000190	MiRNA sponge Regulation of gene expression	miR-142-5p/CDKs EGFR-MAPK-ERK	up-regulated	Promote cell proliferation, migration, and tumor growth	(97)
	circ103820	hsa_circ_0072309	MiRNA sponge	miR-200b-3p/LATS2 and SOCS6	down-regulated	Inhibit cell proliferation, migration, and invasion	(98)
	circ0018414	hsa_circ_0018414	MiRNA sponge	miR-6807-3p/DKK1	down-regulated	Inhibit cell proliferation Promote apoptosis	(99)
	circHIPK3	hsa_circ_0021592	MiRNA sponge	miR-124-3p-STAT3-PRKAA/ AMPKa	down-regulated	Inhibit cell proliferation, migration, invasion, and autophagy	(100)
	circNDUFB2	hsa_circ_0082730	Protein scaffolds	RIM25/IGF2BPs	down-regulated	Inhibit cell proliferation and migration	(101)
	circDCUN1D4	hsa_circ_0126569	Protein scaffolds	HuR/TXNIP	down-regulated	Inhibit cell invasion and migration	(102)
	circXPO1	hsa_circ_0054899	Protein binding	IGF2BP1/CTNNB1	up-regulated	Promote tumor growth	(103)
	circMMP2	hsa_circ_0039411	Protein binding	IGF2BP3/FOXM1	up-regulated	Promote cell proliferation, migration, and EMT	(104)
Colorectal cancer	circCSPP1	hsa_circ_0001806	MiRNA sponge	miR-431/ROCK1/ZEB1	up-regulated	Promote cell proliferation, migration, and invasion	(105)
	circ001971		MiRNA sponge	miR-29c-3p	up-regulated	Promote cell proliferation, invasion, and angiogenesis	(106)
	circ3823		MiRNA sponge	miR-30c-5p/TCF7	up-regulated	Promote cell proliferation, metastasis, and angiogenesis	(107)
	circSPARC		MiRNA sponge	miR-485-3p/JAK2/STAT3	up-regulated	Promote cell migration and proliferation	(108)
	circCAMSAP1	hsa_circ_0001900	MiRNA sponge	miR-328-5p/E2F1	up-regulated	Promote tumor growth	(109)
	circ001680		MiRNA sponge	miR-340/BMI1	up-regulated	Promote cell proliferation and migration	(110)
	circCUL2		MiRNA sponge	miR-208a-3p/PPP6C	down-regulated	Inhibit cell proliferation Promote apoptosis and autophagy	(9)
	circPTEN1		Protein binding	Smad4/TGF-β/Smad	down-regulated	Inhibit cell metastasis and invasion	(111)
	circPTK2	hsa_circ_0005273	Protein binding	vimentin	up-regulated	Promote cell proliferation, metastasis, and EMT	(112)
	circMYH9		Protein scaffolds	hnRNPA2B1/p53	up-regulated	Promote cell proliferation	(113)
	circPPP1R12A	hsa_circ_0000423	Protein code	circPPP1R12A-73aa/hippoyap	up-regulated	Promote cell proliferation, migration, and invasion	(114)
	circRHOBTB3	hsa_circ_0073431	Protein binding	HuR/PTBP1	down-regulated	Inhibit cell metastasis, and invasion	(115)
	circ0006401	hsa_circ_0006401	Protein code	col6a3	up-regulated	Promote cell proliferation and migration	(116)
	circPLCE1		Protein code	circPLCE1-411/HSP90α/RPS3/ NF-κB	down-regulated	Inhibit cell proliferation and metastasis	(117)
	circFNDC3B		Protein code	circFNDC3B-218aa	down-regulated	Inhibit cell proliferation, migration, and invasion	(118)
	circLONP2	hsa_circ_0008558	Transcriptional regulation	DGCR8/Drosha/miR-17	up-regulated	Promote cell invasion and metastasis	(119)

(Continued)

TABLE 3 | Continued

Cancer type	CircRNA	CircBase ID	Mechanism	Target	Expression in cancer	Function	Refs
Hepatocellular carcinoma	circ104348		MiRNA sponge	miR-187-3p/RTKN2/Wnt/β-catenin	up-regulated	Promote cell proliferation, migration, and invasion Inhibit cell apoptosis	(7)
	circASAP1	hsa_circ_0085616	MiRNA sponge	miR-326/miR-532-5p-MAPK1/ CSF-1	up-regulated	Promote cell proliferation, colony formation, migration, and invasion	(120)
	circMET	hsa_circ_0082002	MiRNA sponge	miR-30-5p/Snail/DPP4/CXCL10	up-regulated	Promote cell invasion and EMT	(121)
	circSOD2		MiRNA sponge	miR-502-5p/DNMT3a/JAK2/ STAT3/	up-regulated	Promote cell proliferation and invasion	(122)
	circRASGRF2	hsa_circ_0073181	MiRNA sponge	miR-1224/FAK	up-regulated	Promote cell proliferation and migration	(123)
	circ0003998	hsa_circ_0003998	MiRNA sponge	miR-143-3p/FOSL2	up-regulated	Promote invasion	(124)
	circMEMO1		MiRNA sponge	miR-106b-5p/TCF21	down-regulated	Inhibit invasion and metastasis	(125)
	circMTO1		MiRNA sponge	miR-541-5p/ZIC1/Wnt/β-catenin	down-regulated	Inhibit cell proliferation, migration, and invasion	(33)
	circSETD3	hsa_circRNA_0000567 /hsa_circRNA_101436	MiRNA sponge	miR-421/MAPK14	down-regulated	Inhibit cell proliferation	(126)
	circ0003410	hsa_circ_0003410	MiRNA sponge	miR-1393p/CCL5	up-regulated	Promote cell proliferation and migration	(127)
	circMRPS35	hsa_circ_0000384	MiRNA sponge Protein code	miR-148a -3p/STX3/PTEN circMRPS35-168aa	up-regulated	Promote cell proliferation, migration, invasion, clone formation, and cell cycle	(128)
	circLRIG3	hsa_circ_0027345	Protein scaffolds	EZH2/STAT3	up-regulated	Promote cell proliferation, migration, and invasion Inhibit apoptosis	(129)
	circDLC1		Protein binding	HuR/MMP1	down-regulated	Inhibit cell proliferation and motility	(130)
Gastric cancer	circLMO7	hsa_circ_0008259	MiRNA sponge	miR-30a-3p/WNT2/β-Catenin	up-regulated	Promote cell proliferation, migration, invasion, and metastasis	(131)
	circFAM73A		MiRNA sponge	miR-490-3p/HMGA2	up-regulated	Promote cell proliferation, migration	(132)
	circHIPK3	hsa_circ_0021592	MiRNA sponge	miR-637/AKT1	up-regulated	Promote cell proliferation	(8)
	circ0110389	hsa_circ_0110389	MiRNA sponge	miR-127-5p/miR-136-5p-SORT1	up-regulated	Promote cell proliferation, migration, and invasion	(133)
	circSHKBP1	hsa_circ_0000936	MiRNA sponge	miR-582-3p/HUR/VEGF	up-regulated	Promote cell proliferation, Migration, invasion, and angiogenesis.	(134)
	circRanGAP1	hsa_circ_0063535	MiRNA sponge	miR-877-3p/VEGFA	up-regulated	Promote cell invasion and metastasis	(135)
	circDUSP16	hsa_circ_0003855	MiRNA sponge	miR-145-5p/IVNS1ABP	up-regulated	Promote tumorigenesis and invasion	(136)
	circ0001829	hsa_circ_0001829	MiRNA sponge	miR-155-5p-SMAD2	up-regulated	Promote cell proliferation, migration, and invasion	(137)
	circNRIP1	hsa_circ_0061275	MiRNA sponge	miR-149-5p/AKT1/mTOR	up-regulated	Promote cell autophagy, migration, invasion, and EMT	(138)
	circRELL1	hsa_circ_0001400	MiRNA sponge	miR-637/EPHB3	down-regulated	Inhibit cell proliferation, migration, invasion, and apoptosis	(139)
	circCUL2	hsa_circ_0018193	MiRNA sponge	miR-142-3p/ROCK2	down-regulated	Promote cell autophagy Inhibit cell proliferation, migration, and invasion	(140)
	circCCDC9	hsa_circ_0051667	MiRNA sponge	miR-6792-3p/CAV1	down-regulated	Inhibit cell proliferation, migration. and invasion	(141)
	circMCTP2	hsa_circ_0000657	MiRNA sponge	miR-99a-5p/MTMR3	down-regulated	Inhibit cell proliferation, migration, invasion, and metastasis	(142)
	circPSMC3	hsa_circ_0021989	MiRNA sponge	miR-296-5p/PTEN	down-regulated	Inhibit cell proliferation and metastasis	(143)
	circMAPK1	hsa_circ_0004872	MiRNA sponge	miR-224/Smad4/ADAR1	down-regulated	Inhibit cell proliferation, migration, and invasion	(45)
	circDONSON	hsa_circ_0061550	Protein code	MAPK1-109aa/MEK1/MAPK1	down-regulated	Inhibit cell proliferation, migration	(144)
			Interaction with proteins	SNF2L/SOX4	up-regulated	Promote cell proliferation, migration, invasion, and metastasis	(145)
	circMRPS35	hsa_circ_0025733	Protein modification	KAT7/FOXO1/3a	down-regulated	Inhibit cell proliferation, invasion	(146)
	circHuR	hsa_circ_0049027	Protein binding	CNBP/HuR	down-regulated	Inhibit cell growth, invasion, and metastasis	(147)
	circURI1	hsa_circ_0050333	Transcriptional regulation	hnRNPM	down-regulated	Inhibit cell migration, invasion, and metastasis	(148)
	circDIDO1	hsa_circ_0061137	Protein code and Interaction with proteins	529aa/PARP1,PRDX2	down-regulated	Inhibit cell proliferation, migration, and invasion	(149)

(Continued)

TABLE 3 | Continued

Cancer type	CircRNA	CircBase ID	Mechanism	Target	Expression in cancer	Function	Refs
Breast cancer	circROBO1		MiRNA sponge	miR-217-5p/KLF5/FUS	up-regulated	Promote cell proliferation, migration, and invasion	(150)
	circBACH2		MiRNA sponge	miR-186-5p/miR-548c-3p/CXCR4	up-regulated	Promote cell proliferation, migration, and invasion	(151)
	circ0005273	hsa_circ_0005273	MiRNA sponge	miR-200a-3p//YAP1	up-regulated	Promote cell proliferation and migration	(152)
	circCDYL		MiRNA sponge	miR-1275-ATG7/ULK1	up-regulated	Promote autophagy and malignant progression	(153)
	circSEPT9		MiRNA sponge	miR-637/LIF/Stat3	up-regulated	Promote cell proliferation, migration, and invasion Inhibit apoptosis	(154)
	circKDM4B	hsa_circ_0002926	MiRNA sponge	miR-675/NEDD4L/PI3KCA/PI3K/AKT and VEGFA	down-regulated	Inhibit cell migration and invasion	(155)
	circNR3C2	hsa_circ_0071127	MiRNA sponge	miR-513a-3p/HRD1/Vimentin	down-regulated	Inhibit cell proliferation migration, invasion, and EMT	(156)
	circNOL10		MiRNA sponge	miR-767-5p/SOCS2/JAK2/STAT5	down-regulated	Inhibit cell proliferation, migration, invasion, and EMT	(157)
	circACTN4		Protein binding	FUBP1/MYC	up-regulated	promote cell growth, invasion, and metastasis	(158)
	circSKA3		Protein binding	Tks5/integrin β1	up-regulated	Promote cell invasion	(159)
	circEIF6		Peptide code	EIF6-224aa/MYH9/Wnt/β -catenin	up-regulated	promote cell proliferation and migration	(160)
	circHER2		Protein code	HER2-103/EGFR	up-regulated	Promote cells proliferation, invasion, and tumorigenesis	(161)
Hematopoietic cancers	circRNF220	hsa_circ_0012152	MiRNA sponge	miR-30a/MYSM1/IER2	up-regulated	Promote cell proliferation Inhibit cell apoptosis	(162)
	circSPI1		MiRNA sponge Interaction with proteins	miR-1307-3p、miR-382-5p and miR-767-5p eIF4AIII	up-regulated	Promote cell proliferation and inhibit cell apoptosis	(163)
	circ0000370	hsa_circ_0000370	MiRNA sponge	miR-1299/S100A7A	up-regulated	Increase cell viability and inhibit apoptosis	(164)
	circ0000094	hsa_circ_0000094	MiRNA sponge	miR-223-3p/FBW7	down-regulated	Inhibit cell proliferation、migration and invasion Promote cell apoptosis	(165)
	circADD2		MiRNA sponge	miR-149-5p/AKT2	down-regulated	Inhibit cell proliferation and promote cell apoptosis	(166)
	circ0009910		MiRNA sponge	miR-34a-5p/ULK1	up-regulated	Promote cell autophagy	(167)
	circRPL15	hsa_circ_0064574	MiRNA sponge	miR146b-3p/RAF1	up-regulated	Promote cell viability	(168)
	circADARB1		MiRNA sponge	miR-214-3p/p-Stat3	up-regulated	Promote cell proliferation Inhibit cell apoptosis	(169)
	circEAF2		MiRNA sponge	miR-BART19-3p/APC/β-catenin	down-regulated	Promote cell apoptosis and inhibit tumor progression	(170)
Renal carcinoma	circPRRC2A		MiRNA sponge	miR-514a-5p and miR-6776-5p/TRPM3	up-regulated	Promote EMT and invasion	(171)
	circSDHC	hsa_circ_0015004	MiRNA sponge	miR-127-3p/CDKN3/E2F1	up-regulated	Promote cells proliferation, and invasion	(172)
	circTLK1		MiRNA sponge	miR-136-5p/CBX4	up-regulated	Promote cell proliferation, migration, and invasion	(173)
	circAGAP1		MiRNA sponge	miR-15-5p/E2F	up-regulated	Promote cell proliferation, migration, and invasion	(174)
	circPTCH1		MiRNA sponge	miR-485-5p/MMP14	up-regulated	Promote RCC metastasis and EMT	(175)
	circ001287		MiRNA sponge	miR-144/CEP55	up-regulated	Promote cell proliferation, migration, and invasion	(176)
	circMET	hsa_circ_0082002	MiRNA sponge Protein binding	miR1197/SMAD3 YTHDF2/CDKN2A	up-regulated	Promote cell proliferation, and tumor progression	(177)
Bladder Cancer	circGLIS3	hsa_circ_0002874	MiRNA sponge	miR-1273f/SKP1/Cyclin D1	up-regulated	Promote cell proliferation, migration, and invasion	(178)
	circUBE2K	hsa_circ_0009154	MiRNA sponge	miR-516b-5p/ARHGAP5/RhoA	up-regulated	Promote cell proliferation, migration, and invasion	(179)
	circ0000658		MiRNA sponge	miR-498/HMGA2	up-regulated	Promote cell proliferation, migration, invasion, and EMT	(180)
	circ0001944	hsa_circ_0001944	MiRNA sponge	miR-548/PROK2	up-regulated	Promote cell invasion and proliferation	(181)
	circST6GALNAC6		MiRNA sponge	STMN1/STMN1/EMT	down-regulated	Inhibit cell proliferation, migration, invasion, and EMT	(182)
	circACVR2A	hsa_circ_0001073	MiRNA sponge	miR-626/EYA4	down-regulated	Inhibit cell proliferation and metastasis	(183)
	circSLC8A1		MiRNA sponge	miR-130b, miR-494/PTEN	down-regulated	Inhibit cell proliferation, migration, and invasion	(184)
	circZKSCAN1		MiRNA sponge	miR-1178-3p/p21	down-regulated	Inhibit cell proliferation, migration, invasion, and metastasis	(185)

(Continued)

TABLE 3 | Continued

Cancer type	CircRNA	CircBase ID	Mechanism	Target	Expression in cancer	Function	Refs
	circNR3C1		Protein binding	BRD4/C-myc/EZH2	down-regulated	Inhibit BC progression	(186)
	circNOLC1		MiRNA sponge	miR-647/PAQR4	up-regulated	Promote cell proliferation and migration	(187)
Prostate cancer	circFMN2		MiRNA sponge	miR-1238/LHX2	up-regulated	Promote cell proliferation, migration, and invasion	(188)
	circPDHX	hsa_circ_0003768	MiRNA sponge	miR-378a-3p/IGF1R	up-regulated	Promote cell proliferation and invasion	(189)
	circSOBP		MiRNA sponge	miR-141-3p/MYPT1/p-MLC2	down-regulated	Inhibit cell migration and invasion	(190)
	circ0003258	hsa_circ_0003258	MiRNA sponge	miR-653-5p/ARHGAP5	up-regulated	Promote cell migration and EMT	(191)
			Interaction with proteins	IGF2BP3/HDAC4			
Cervical cancer	circCLK3		MiRNA sponge	miR-320a/FoxM1	up-regulated	Promote cell growth, migration, invasion, and metastasis	(192)
	circAMOTL1	hsa_circ_0004214	MiRNA sponge	miR-485-5p/AMOTL1	up-regulated	Promote cell proliferation and migration	(193)
	circNRIP1	hsa_circ_0004771	MiRNA sponge	miR-629-3p/PTP4A1/ERK1/2	up-regulated	Promote cell proliferation, migration, and invasion	(194)
	circSLC26A4		MiRNA sponge	miR-1287-5p/HOXA7	up-regulated	Promote cell proliferation, migration, and invasion	(195)
	circAKT1		MiRNA sponge	miR-942-5p/AKT1	up-regulated	Promote cell proliferation, and invasion	(196)
	circEYA1		MiRNA sponge	miR-582-3p/CXCL14	down-regulated	Promote cell apoptosis	(197)
	circZFR	hsa_circ_0072088	Interaction with proteins	SSBP1/CDK2/cyclin E1	up-regulated	Promote cell proliferation, invasion, and tumor growth	(198)

patients compared with normal tissues. It is worth noting that circMRPS35 can not only adsorb miR-148a-3p, regulate the expression of Syntaxin 3 (STX3), thereby regulating the ubiquitination and degradation of phosphatase and tensin homolog (PTEN) but can also encode a peptide (circMRPS35-168aa), this peptide promotes cisplatin resistance in HCC cells. CircLRIG3 is significantly up-regulated in HCC, forming a ternary complex with EZH2 and STAT3, promoting EZH2-induced STAT3 methylation and subsequent phosphorylation, leading to the activation of STAT3 signal, thereby promoting the proliferation, migration, and invasion of liver cancer cells. Reduce cell apoptosis (129).

Wei et al. reported that the expression of circCDYL or the combined expression of HDGF and HIF1AN are independent markers for distinguishing early HCC, providing the possibility for the detection and early treatment of liver cancer (204). Yang et al. found that circFN1 promotes sorafenib resistance by regulating the miR-1205/E2F1 signaling pathway, which is a potentially valuable target for HCC resistance (205).

3.4 Gastric Cancer

Gastric cancer (GC) is the fourth leading cause of death in the world (199), especially in Asian countries, the incidence of gastric cancer is increasing year by year. Cao et al. found that circ0008259 (circLMO7) is highly expressed in GC tissues, circLMO7 sponge miR-30a-3p regulates the WNT2/β-Catenin pathway and affects the glutamine metabolism of GC cells, and ultimately promotes the growth and migration of GC (131). Peng et al. found that the level of circCUL2 in GC tissues and cells was significantly reduced, and the sponge engulfed miR-142-3p to regulate ROCK2, thereby inhibiting malignant transformation and inhibiting tumorigenicity *in vivo* (140). In addition, Yan et al. (206) found that circEVI5 was significantly down-regulated in GC tissues and cells. circEVI5 sponges swallowed miR-4793-3p and increased the expression level of FOXO1 to inhibit the proliferation of GC and delay the cell cycle. Wang et al. (148) analyzed the circRNAs of five pairs of human stomachs and corresponding non-tumor adjacent specimens and found that circURI1 was significantly highly expressed in GC and metastasized in GC. It regulates a small part of genes involved in cell movement by isolating hnRNPM protein to inhibit GC metastasis. Zhang et al. (149) found that circDIDO1 is down-regulated in gastric cancer tissues, and its low level is associated with larger tumors, distant metastasis, and poor prognosis. In mechanism, circDIDO1 encodes a new 529aa protein, which interacts with poly ADP-ribose polymerase 1 (PARP1). Effect and inhibit its activity. Interestingly, circDIDO1 also binds to peroxide reduction protein 2 (PRDX2), which promotes the ubiquitination and degradation of PRDX2 mediated by rbx1, leading to inactivation of its downstream signaling pathways.

Further, numerous research has explored the clinical utility of circRNAs as biomarkers for the early detection and prognosis of gastric cancer. For instance, Song et al. (207) detected the expression profile of circRNAs and found that hsa_circ_000780 was significantly downregulated in GC tissue samples, and its level was correlated with the level of tumor clinicopathological features. Interestingly, circ000780 was also found in gastric juice

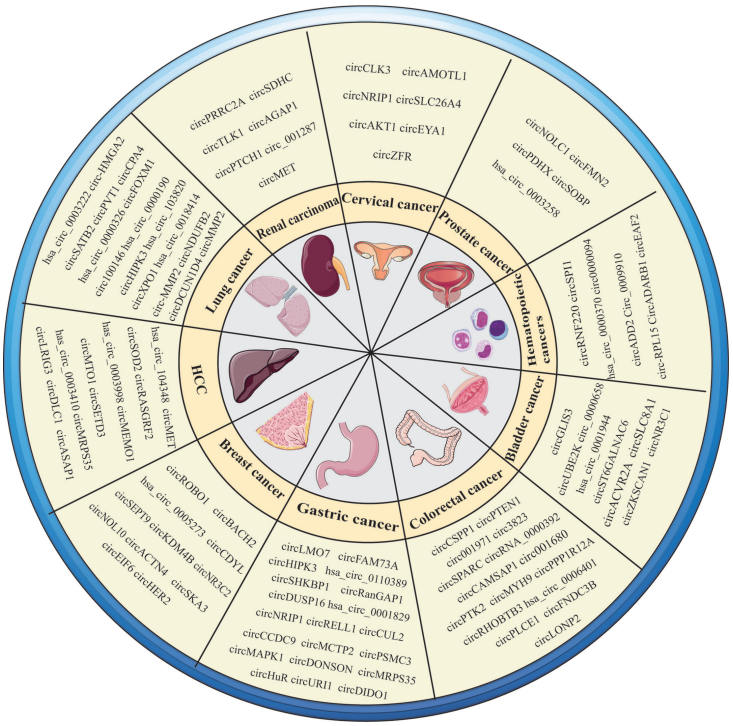

FIGURE 2 | Overview of circRNAs in various types of cancers.

of patients with early GC. In another report, circERBB2 (208) in plasma can be used as a prognostic biomarker for gastric cancer patients. CircERBB2 levels in preoperative plasma (high group) were significantly correlated with lymph node metastasis (P = 0.035), suggesting that it could be used to predict noninvasively the prognosis of GC.

3.5 Breast Cancer

Breast cancer (BC) is the main cause of cancer in women and the main cause of death in women. Wang et al. (151) used the circRNAs microarray data set and found that four circRNAs were abnormally expressed in TNBC. Among them, circBACH2 is most significantly elevated in BC tissues, and its high expression promotes epithelial-mesenchymal transition and cell proliferation and is positively correlated with the malignant progression of BC patients. Mechanistically, circBACH2 sponges miR-186-5p and miR-548c-3p, thereby releasing the expression of C-X-C chemokine receptor type 4 (CXCR4). Li et al. (160) discovered that circ-EIF6 encodes a new peptide called EIF6-224 amino acid (aa). EIF6-224aa directly interacts with the oncogene MYH9 in BC and inhibits the ubiquitin-proteasome pathway and subsequently activates the Wnt/β-catenin pathway to reduce the degradation of MYH9, thereby playing a carcinogenic effect. In addition, Wang et al. reported that circACTN4 can competitively bind to far upstream element-binding protein 1 (FUBP1) to prevent FUBP1 from binding to FIR, thereby activating MYC transcription and promoting tumor progression in breast cancer (158).

Some potential circRNAs biomarkers for early diagnosis of BC and prediction of recurrence and metastasis have emerged from the detection of clinical samples in the tissues and peripheral blood of BC patients and healthy controls, combined with the correlation analysis of clinicopathological factors and the analysis of prognosis and survival. For example, CircSMARCA5 can form an R-loop with its parental locus, causing a transcriptional pause at SMARCA5 exon 15, and SMARCA5 DNA is involved in chromatin remodeling in damaged regions.circSMARCA5 may serve as a therapeutic target for patients with drug-resistant BC (209). The above studies have provided new insights into the role of circRNAs in BC.

3.6 Hematopoietic Cancers

According to recent research findings, the involvement of circRNAs in hematological malignancies is becoming more widely recognized (210). Among them, aberrant circRNAs expression might upset the balance between self-renewal and differentiation of hematopoietic stem cells (HSCs), resulting in bone marrow failure or hematological malignancies.

Acute myeloid leukemia (AML), the most common leukemia in adults, is uncommon in children, but has a poor prognosis and is prone to relapse (211). Liu et al. discovered that circRNF220 is abundantly and precisely expressed in children's peripheral blood and bone marrow using microarray technology. CircRNF220 knockout can reduce the proliferation of AML cell lines and primary cells while also promoting cell death

(162). FLT3-ITD+ AML is a significant subtype of AML, and Zhang et al. observed that the effect of circ0000370 on the development of FLT3-ITD-positive AML may be directly connected to miR-1299 and S100A7A (164). Acute lymphoblastic leukemia (ALL) is the most prevalent malignant tumor in children, and circ0000094 has been demonstrated to be a molecular sponge of miR-223-3p, which can upregulate the expression of FBW7 by limiting the expression of miR-223-3p, hence preventing ALL progression (165). Zhu et al. found that circADD2, as a tumor suppressor gene in ALL, inhibited cell proliferation and promoted cell apoptosis both *in vitro* and *in vivo*. Mechanistically, circADD2, which can sponge miR-149-5p, may serve as a potential biomarker for ALL diagnosis or treatment (166). Interestingly, circRNAs expression profiles can also clearly distinguish Acute leukemia (AL). For example, Guo et al. reported circ0001857 and circ0012152 ALL and AML (212). The recently discovered Circ0009910 can regulate ULK1-induces autophagy by targeting miR-34a-5p and accelerating the resistance of CML cells to imatinib (167). High expression of circ-RPL15 was positively correlated with IGHV mutation status, which is crucial for evaluating CLL prognosis. MiR-146b-3p-mediated RAS/RAF1/MEK/ERK pathway inhibition could be alleviated by circRPL15 overexpression in CLL. CircRPL15 may represent a promising novel plasma biomarker for the diagnosis of CLL (168). Mei et al. found that the relative expression of circADARB1 was significantly increased in the plasma of Natural killer/T-cell lymphoma (NKTCL), which binds to miR-214-3p and regulates p-Stat3, promotes the proliferation of NKTCL cells, and inhibits apoptosis (169). Furthermore, Zhao et al. reported that CircEAF2 inhibited Epstein-Barr infection positive diffuse large B cell expansion and advanced apoptosis *via* the miR-BART19-3p/APC/-catenin axis (170).

Some of the circRNAs reported above play a regulatory role in the occurrence and development of hematological tumors through various molecular mechanisms, suggesting some potentials of circRNAs in the research of hematological malignancies in the future. These findings also aid in the diagnosis and prediction of hematological malignancies.

3.7 Other Types of Cancer

The abnormal expression of many circRNAs has been verified in many cancers. In renal cell carcinoma (RCC), Cen et al. found that circSDHC competitively binds to miR-127-3p, preventing it from inhibiting the downstream genes CDKN3 and E2F1 pathways, leading to RCC Malignant progress (172). NONO-TFE3 TRCC (Xp11.2 translocation/NONO-TFE3 fusion renal cell carcinoma) is a subgroup of renal cell carcinoma. Yang et al. found that highly expressed circMET accelerates the decay of CDKN2A mRNA by recruiting YTHDF2, while competitively binding miR- 1197, Regulates SMAD3 expression (177). In bladder cancer (BC), Yang et al. (179) used high-throughput sequencing and RT-qPCR to verify the abnormally high expression of circUBE2K BC tissue. As a ceRNA, the expression of ARHGAP5 was regulated by sponge miR-516b-5p to promote tumor development. The down-regulated circZKSCAN1 in BC tissues and cell lines up-regulates the expression of p21 through sponge miR-1178-3p, which inhibits the proliferation, migration, and invasion of bladder cancer (185). In addition, some circRNAs have also been found to play an important role in prostate cancer and cervical cancer, as shown in **Table 3**.

In short, various studies have shown that circRNAs are involved in the occurrence and development of various cancers. However, the role of circRNAs in the diagnosis and treatment of cancers needs to be further studied.

4 CircRNAs AS LIQUID BIOPSY BIOMARKERS

There are RNase in human body fluids, and circRNAs can resist this enzyme, thus being a stable biomarker for the detection of body fluids such as blood, exosomes, saliva, and urine (213). In addition, the half-life of circRNAs in the blood is longer than that of mRNA. Coupled with the high abundance and specificity of circRNAs, circRNAs are expected to become an excellent non-invasive biomarker for tumor diagnosis and prognosis. Xu et al. reported that compared with breast cancer and adjacent normal tissues, the expression of circRNAs in peripheral blood was significantly higher than that of host genes (209). This discovery helps to explore diagnostic biomarkers for breast cancer.

CircRNAs may be used as biomarkers for cancer diagnosis and prognosis. It is worth noting that exosomes can protect RNA RNases from degradation, so circRNAs are also enriched and stably expressed in exosomes (214). Exosomes derived from cancer cells can target specific organs to promote the formation of pre-metastasis niches (215) and tumor microenvironment (216). Exosomal circRNAs participate in cell proliferation, invasion, EMT, and metastasis through intercellular communication. Shang et al. (217) discovered a new circRNA in colorectal cancer exosomes, circPACRGL, which acts as a sponge for miR-142-3p/miR-506-3p and promotes the expression of transforming growth factor-β1 (TGF-β1). It has been reported that exosomal circSHKBP1 inhibits HSP90 degradation and promotes GC progress through miR-582-3p/HUR/VEGF pathway (134). Recently, Li et al. used circRNA deep sequencing and bioinformatics methods to build a circRNA repertoire, and 3 up-regulated serum exosomal circRNAs (circ0075828, circ0003828, and circ0002976) could be used to screen for high-grade astrocytoma (HGA). Five highly expressed exosomal circRNAs (circ0005019, circ0000880, circ0051680, and circ0006365) were used as HGA prognostic markers. revealed that circular RNAs in HGA exosomes are targets for HGA liquid biopsy and prognostic monitoring (218). At present, progress has been made in the research of exosomal circRNAs, but the mechanism of circRNAs entering exosomes and the role of circRNAs in exosomes are still unclear.

In summary, the prospects of circRNAs as biomarkers for liquid biopsy and therapeutic targets are promising, but there are few studies at present.

5 CircRNAs AND FUTURE THERAPEUTIC OPPORTUNITIES

CircRNAs are attractive targets for cancer therapy and offer novel cancer treatment techniques. In this section, we will discuss some future perspectives on the usage of circRNAs in cancer therapy.

As mentioned above, more and more studies have demonstrated that dysregulation of circRNAs in cancer can promote or inhibit cancer (**Table 3**). While the up-or down-regulation of certain circRNAs is linked to clinical aspects such as TNM and other related phases, differentiation, or survival (142, 219). This shows that circRNAs actions are context-dependent, making it difficult to categorize circRNAs as oncogenic or tumor suppressors.

CircRNAs rely on the sponge action of miRNAs to promote cancer progression. In recent years, small molecule inhibitors (SMIs) and small molecule degradants (SMDs) of miRNAs have been reported for drug therapy, so whether it is possible to develop blockers targeting miRNAs to reduce the cancer-promoting activity of circRNAs (220). Of course, the specificity of the drug requires other biotechnological validation and the safety of the drug also needs to be assessed. In addition, when cancer develops, some critical circRNAs are greatly up-regulated. Can it decrease cancers by reducing the number of cancer-promoting circRNAs without influencing the expression of their parental genes? It may be able to regulate the occurrence of back-splicing events by focusing on the splicing mechanisms that affect circRNAs. For example, Tassinari et al. demonstrated that downregulation of the RBP splicing factor ADAR1, which controls circular RNA biogenesis, is sufficient to strongly inhibit glioblastoma growth *in vivo* (221). This inspires the prospect of a technique that modulates RBP to suppress circular RNA expression.

Finally, gene editing techniques such as CRISPR/Cas13 has been applied to RNA editing (222), Whether circRNAs can also be edited to reduce or increase activity. Recently, Ishola et al. found that CRISPR/Cas13a-mediated knockdown of circ0000190 reduced the proliferation and migration of non-small cell lung cancer cells *in vitro* and inhibited tumor growth *in vivo* (223). This also confirms the potential of the novel CRISPR/Cas13a system as a cancer therapy tool.

6 CHALLENGES AND PERSPECTIVES

CircRNAs have been considered splicing errors before, but they have attracted widespread attention in recent years. A lot of innovative research has emerged in the field of circRNAs, but there are still many challenges and problems that need to be solved. From the above-mentioned large number of retrospective reports, it can be seen that the importance of circRNAs is beyond doubt. However, the function of most circRNAs is still unclear, whether there are new undiscovered functions. In addition, the coding potential of circRNAs is often overlooked. And whether the proteins encoded by circRNAs have the functions of conventional proteins. Thousands of circRNAs have been detected, some of them are highly abundant in cancer and some are low in abundance. The detection method for low-abundance circRNAs is not yet mature, and their use as non-invasive biomarkers requires a large number of clinical sample collections. Moreover, their sensitivity and specificity are controversial. In addition, packaging circRNAs into cells to regulate cell activities also requires a lot of research and exploration, so that these studies can truly produce clinical application value. Standardization is needed in many aspects, such as the extraction of differences between detection technologies and the standardization of naming.

In summary, circRNAs play an important role in cancer and provide new insights for cancer management, but the mechanism of action is still in its infancy. The research of circRNAs still has a long way to go.

AUTHOR CONTRIBUTIONS

ZL, HQ, and WX contributed to conception and design of this review. YZ wrote the first draft of the manuscript. XZ, YX, and SF wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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REVIEW
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Homologous Recombination Deficiency in Ovarian, Breast, Colorectal, Pancreatic, Non-Small Cell Lung and Prostate Cancers, and the Mechanisms of Resistance to PARP Inhibitors

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Homologous recombination (HR) is a highly conserved DNA repair mechanism that protects cells from exogenous and endogenous DNA damage. Breast cancer 1 (BRCA1) and breast cancer 2 (BRCA2) play an important role in the HR repair pathway by interacting with other DNA repair proteins such as Fanconi anemia (FA) proteins, ATM, RAD51, PALB2, MRE11A, RAD50, and NBN. These pathways are frequently aberrant in cancer, leading to the accumulation of DNA damage and genomic instability known as homologous recombination deficiency (HRD). HRD can be caused by chromosomal and subchromosomal aberrations, as well as by epigenetic inactivation of tumor suppressor gene promoters. Deficiency in one or more HR genes increases the risk of many malignancies. Another conserved mechanism involved in the repair of DNA single-strand breaks (SSBs) is base excision repair, in which poly (ADP-ribose) polymerase (PARP) enzymes play an important role. PARP inhibitors (PARPIs) convert SSBs to more cytotoxic double-strand breaks, which are repaired in HR-proficient cells, but remain unrepaired in HRD. The blockade of both HR and base excision repair pathways is the basis of PARPI therapy. The use of PARPIs can be expanded to sporadic cancers displaying the "BRCAness" phenotype. Although PARPIs are effective in many cancers, their efficacy is limited by the development of resistance. In this review, we summarize the prevalence of HRD due to mutation, loss of heterozygosity, and promoter hypermethylation of 35 DNA repair genes in ovarian, breast, colorectal, pancreatic, non-small cell lung cancer, and prostate cancer. The underlying mechanisms and strategies to overcome PARPI resistance are also discussed.

Keywords: pathogenic mutation, loss of heterozygosity, promoter hypermethylation, DNA repair genes, hereditary and familial cancer, PARP inhibitor, base excision repair

1 INTRODUCTION

Homologous recombination (HR) is one of the major pathways for the repair of DNA double-strand breaks (DSBs) in eukaryotic cells. Pathogenic mutations in genes encoding HR-related proteins are associated with the development of certain malignancies, including breast, ovarian, and other cancers (1). Normal and cancer cells rely on multiple DNA damage response pathways that specifically repair different forms of DNA damage. Key pathways include homologous recombination repair (HRR), base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), nonhomologous end-joining (NHEJ), translesion synthesis (TLS), and interstrand crosslink (ICL) repair (2, 3). However, these repair pathways are not equally effective in DNA repair, and some mechanisms are error-prone. For instance, non-canonical DNA repair systems such as NHEJ, single-strand annealing, and TLS are activated when the canonical pathways are deficient (4, 5). In response to DSBs, cells activate the HRR pathway, which relies on the undamaged sister chromatid as a template for repair. Because of this reliance on sister chromatids, HRR is active during the S and G2 phases of the cell cycle and is a high fidelity and error-free DNA repair pathway (3). By contrast, in NHEJ, the break ends are directly ligated without a homologous template, resulting in an error-prone repair pathway that can predispose to genetic instability (6, 7).

Genomic scars in relation to HR are caused by chromosomal and sub chromosomal aberrations. Genomic aberrations arise from mutation, structural copy number aberrations, or structural rearrangements. Mutations are substitutions (transversion & transition) or indel (insertion & deletion) mutations, and can inactivate tumor suppressor genes (TSGs). Structural copy number alterations can be copy number gain (leading to allelic imbalance) or copy number loss [leading to deletion, loss of heterozygosity (LOH), or haplo-insufficiency (one copy of a gene is deleted or contain loss of function mutation leading to insufficient level of proteins)]. Structural rearrangements can be inversion (paracentric), translocation (reciprocal), or recombination leading to copy neutral LOH events (8). Frequent copy number alterations are the hallmarks of homologous recombination deficiency (HRD) and can occur at the regional or whole chromosome level. Quantification of large-scale structural variants is used as an indicator of the HRD phenotype (presence of HRD in sporadic cancers other than BRCA1 and 2 inactivation), including telomeric allelic imbalance (TAI: large allelic imbalances extending to the telomere), large scale transition (LST: number of transitions between large regions of different allelic states or chromosomal breaks between adjacent regions of >10 MB), and LOH (large regions displaying somatic loss of one haplotype, which can be copy variable as in deletion or copy neutral LOH). Genes involved in HR, including tumor suppressor genes, can also be repressed by aberrant promoter hypermethylation, an epigenetic mechanism that contributes to HRD (9).

HR is a DNA repair pathway of clinical interest because of the sensitivity of HRD cells to poly-ADP-ribose polymerase (PARP) inhibitors (PARPIs) (10). DNA repair targeting therapies exploit DNA repair defects in cancer cells to generate synthetic lethality, and DNA repair defects vary according to cancer type. For example, approximately 50% of ovarian carcinomas exhibit dysfunctional HRR, whereas the rate of HRR dysfunction is lower in other cancer types such as colorectal cancer (CRC) (<5%) (11, 12). Hereditary mutations in one copy of the *BRCA* gene predispose patients to female breast cancer (85% lifetime risk), ovarian cancer (10%–40%), male breast cancer, pancreatic cancer (PC), prostate cancer, non-small cell lung cancer (NSCLC), CRC, and other cancer types. Precancerous cells deficient in BRCA1 and 2 cannot repair DSBs properly, resulting in genomic instability that eventually leads to cancer (8). These tumors are intrinsically sensitive to DNA damage response inhibitors (PARPIs), which induce synthetic lethality. Synthetic lethality can arise from the combined inactivation of HRR genes by (mutation, LOH and promoter hypermethylation) and PARP inhibition (13). The use of PARPIs in non-*BRCA* mutation carrier patients can be expanded to sporadic cancers that display "BRCAness" (cancers that have defective HR without germline *BRCA1* and *2* mutations). Findings showed that TSGs with BRCAness phenotypes are often inactivated, for example, *ATM*, *ATR*, *PALB2*, *RAD51*, *RAD51B*, *RAD51C*, *RAD51D*, *BARD1*, and *FANCM* among others (14–17).

The use of PARPIs was recently expanded to other cancers in addition to breast and ovarian cancer, such as prostate and PC (18). Although PARPIs have shown beneficial effects in many other cancer types, the frequent development of resistance is challenging. For instance, in a phase II clinical trial, secondary resistance mutations were detected in circulating free tumor DNA in two patients with a germline *BRCA2* mutation. These mutations were predicted to lead to the reversal of a somatic mutation (19). A comprehensive investigation of the underlying mechanisms is necessary to design strategies for overcoming PARPI resistance.

Another challenging issue is the development of effective biomarkers to identify patients who are more likely to respond to specific targeted therapy by using companion diagnosis (CDx). CDx is an *in vitro* medical device that uses biomarkers to provide information on the safe and effective use of drugs or biologicals. FDA-approved CDx includes BRACAnalysis CDx® and Myriad myChoice® CDx developed by Myriad Genetic Laboratories, and FoundationOne® CDx [F1CDx] and FoundationOne® Liquid CDx developed by Foundation medicine. The HRDetect test utilizes machine learning algorithm (20).

BRACAnalysis CDx® is an *in vitro* diagnostic method used for the detection and classification of DNA sequence variants in the protein-coding regions, intron or exon boundaries of the germline *BRCA1* and *2* genes from whole blood sample. PCR and Sanger sequencing are used to detect small insertions and deletions (indels), and single nucleotide variants (SNVs). Large deletions and duplications are detected by multiplex PCR. The test results used as an aid to identify eligible patients for PARPIs in breast, ovarian, pancreatic and prostate cancers treatment (21). Myriad myChoice® CDx is NGS-based *in vitro* diagnostic test that evaluates the qualitative detection and classification of

SNVs, indels and large rearrangements (LRs) in protein-coding regions and intron/exon boundaries of the *BRCA1* and *2* genes, and determine Genomic Instability Score (GIS) by measuring [LOH, TAI, and LST] using DNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissue. The test used to select eligible patients for ovarian cancer with positive HRD status for the treatment with Zejula® (niraparib) (22).

FoundationOne®CDx (F1CDx) is a qualitative NGS- and high throughput hybridization-based capture test for the detection of indels, substitutions and copy number alterations (CNAs) in 324 genes. It detects gene rearrangements, genomic signatures including microsatellite instability (MSI), tumor mutational burden (TMB) and positive HRD status (somatic BRCA-positive and/or LOH high) using DNA isolated from FFPE tumor tissue. It provides definite information for the identification of eligible patients for specific treatments of different class using specific biomarkers for many solid tumors (23).

FoundationOne® Liquid CDx is a qualitative NGS based test, which can identify indels, and substitutions in 311 genes, rearrangements in 4 genes and CNAs in 3 genes. It utilizes circulating cell-free DNA (cfDNA) isolated from plasma-driven peripheral whole blood collected in anti-coagulants. The test identifies patients that can benefit from different targeted treatments for NSCLC, breast, ovarian and prostate cancers based on specific biomarkers detected in each cancer. Negative result does not rule out the presence of an alteration in the patient's tumor, in this case patients can opt for another tumor tissue-based CDx. The test analytical accuracy is not well demonstrated in all genes e.g., the test does not detect heterozygous deletions, and copy number losses/homozygous deletion in ATM (24).

HRDetect is a whole genome sequencing (WGS)-based classifier of HRD that can predict *BRCA1* and *2* deficiency based on six mutational signatures (the HRD index [LOH + TAI + LST], microhomology-mediated indels, base-substitution signature 3 and 8, and rearrangement signature 3 and 5). It can also identify HRD in sporadic cancers (BRCAness) with and without any single detectable defect in HR genes (20, 25). HRDetect was shown highly sensitive method as compared to other HRD detection CDxs (20), but require clinical validation in independent set to avoid overfitting issue.

In this review, we did not classify mutation and hypermethylation data as bi-allelic or mono-allelic inactivation. Oftentimes, cases of pathogenic mutation in tumor suppressor genes lead to bi-allelic inactivation. Whole-exome sequencing analysis of breast cancer cases by Mutter et al. (26) revealed that 89% of bi-allelic inactivation results in HRD, whereas in cases of mono-allelic inactivation significant association existed between RAD51 functional status and LST. In a study by Li et al. (27) mono-allelic germline pathogenic mutation of *PALB2* had predisposed to a high-risk breast cancer development, underscoring the role of PALB2 in HR repair. Moreover, protein-truncating variants and rare missense variants of DNA repair genes were significantly associated with the risk of breast cancer (16). Many findings confirmed the importance of haplo-insufficiency in tissue and gene specific manner; for instance, PTEN hypermorphic mice expressing 80% normal levels of PTEN protein was sufficient to predispose for different cancers development (28). Mono-allelic inactivation of TSGs (e.g., p53 and PTEN) leads to the inability to perform normal cellular functions which contributed to cancer development (29).

Here, we investigated the potential implications of pathogenic mutations, LOH, and promoter hypermethylation of HR-related genes using recent data in ovarian, breast, colorectal, pancreatic, non-small cell lung, and prostate cancers. Additionally, the mechanisms underlying PARPI resistance and possible strategies to overcome PARPI resistance are discussed.

1.1 BRCA1 and BRCA2 in Homologous Recombination Repair

BRCA1 and 2 interacts with a number of other DNA repair proteins to form a complex system for DNA damage repair, including ATM, RAD51, PALB2, MRE11A, RAD50, NBN, and the Fanconi anemia proteins (30). BRCA1 and BRCA2 are potential biomarkers for HRD in ovarian and breast cancer. In the presence of DNA DSBs, BRCA1 and 2 collaborate with other HR proteins to maintain the breaks. For instance, ATM is specifically activated in response to DSBs and is essential for phosphorylating many proteins involved in controlling cell cycle checkpoints and DNA repair. Three proteins are involved in recruiting ATM to DSBs, meiotic recombination 11 (MRE11A), RAD50, and NBS1 or MRN complex. Cells deficient in ATM and NBS1 are thus sensitive to PARPIs, similar to BRCA1- and BRCA2-deficient cells (7, 31). Germline pathogenic mutations of *BRCA1* and *BRCA2* suppress the HR mechanism and cause hereditary breast and ovarian cancer (HBOC) syndrome (32, 33). The functions of 35 HR-related genes are described briefly in **Supplementary Table S1**.

1. 2 Interaction Between *FANC* and *BRCA* Genes in Homologous Recombination Repair

Fanconi anemia (FA) is a clinically and genetically heterogeneous syndrome involving bone marrow failure (BMF), developmental/congenital abnormalities that may affect all organ systems (renal dysplasia, craniofacial malformations, endocrine dysfunction, developmental delay, VACTERL association, radial ray malformations, osteoporosis, progressive BMF, skin abnormalities, short stature, cardiac defects, decreased fertility, and genitourinary and gastrointestinal malformations), and cancer predisposition (34). This review focused only on the role of five FA genes in cancer predisposition. FA is a congenital defect that results from loss of function of any of 21 genes, which indicates their essential role in maintaining the chromosomal stability of hematopoietic stem cells. The main cause of FA Complementation Group (*FANC*) gene abnormality is mutation (95%). The unique clinical phenotype associated with *FANC* gene mutations implies that proteins encoded by these genes function in a common cellular pathway. This pathway, known as the FA/BRCA DNA repair pathway (**Figure 1**), preserves genomic homeostasis in response to specific types of DNA

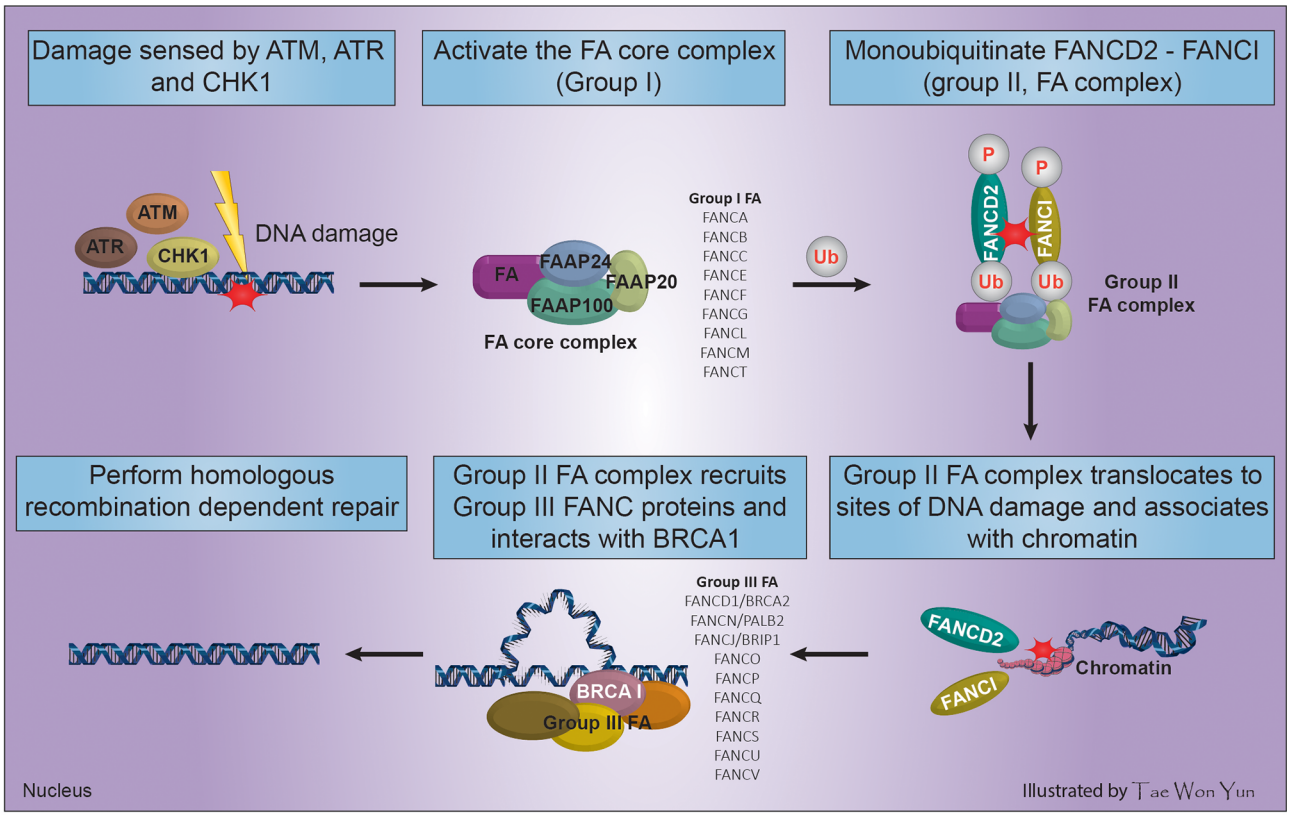

FIGURE 1 | Process of FA complex formation and DNA interstrand cross-linking maintenance through the interaction of *FANC* and *BRCA* genes.

damage (35). The FA pathway serves to remove ICLs and shares components, such as BRCA2 and PALB2, with the HR and NER pathways (2).

The main genome housekeeping function of the FA pathway in the DNA damage response necessitates multifactorial activation of HR. Cells use the error-prone NHEJ repair mechanism when FA-based repair systems are deficient, which may negatively affect genomic stability (36). In response to DNA damage caused by radiation, tobacco smoke, alcohol, or reactive oxygen species among others, FA proteins and three other Fanconi-associated proteins (FAAP100, FAAP24, and FAAP20) are activated by DNA damage response sensors such as ATM, ATR, and cell cycle check point (CHK1) to arrest the cell cycle, forming the FA core complex (group I) (34). The assembled FA core complex (group I) binds to the ubiquitin-conjugating enzyme UBE2T *via* the FANCL subunit and activates the FANCD2-FANCI complex (group II FA complex) through mono-ubiquitination and phosphorylation of FANCD2/FANCI (2). The ubiquitinated FANCD2-FANCI complex translocates to sites of DNA damage, associates with chromatin, and co-localizes with/recruits the downstream FA effector proteins (group III FA complex) including FANCD1/BRCA2, FANCN/PALB2, and FANCJ/BRIP1. Then, the group III FA complex perform HR-dependent DNA repair by interacting with BRCA1 (**Figure 1**) (35, 37).

2 HOMOLOGOUS RECOMBINATION DEFICIENCY IN OVARIAN CANCER

The International Agency for Research on Cancer (IARC) (38) reports that ovarian cancer is the 8th and 9th cause of incidence and mortality worldwide in females, respectively. In 2020, the incidence and mortality of ovarian cancer were estimated at 3.4% (313,959 of all cancer types) and 4.68% (207,252 of all cancer types), respectively. Approximately 75% of epithelial ovarian cancer patients are diagnosed with advanced disease, which is curable in only a minority of cases, resulting in a modest 5-year survival rate of 20–30% (39). According to the cancer genome atlas (TCGA), high grade serous ovarian cancers (HGSOCs) are characterized by frequent genetic and epigenetic alterations of HR pathway genes, most commonly *BRCA1* and *BRCA2*. In addition, approximately 50% of patients with HGSOC exhibit genetic and epigenetic alterations in the FANC-BRCA (**Figure 1**) pathway (11). Germline mutations in the *BRCA1* and *BRCA2* genes are well-known mechanisms of HRD, and loss of BRCA1 or BRCA2 thus poses a significant risk to genome integrity, leading not only to cancer predisposition, but also affecting the sensitivity to DNA-damaging agents and thus therapeutic approaches (40). Pathogenic variants of *BRCA1* and *BRCA2* only explain the genetic cause of approximately 10% of hereditary breast and ovarian cancers (transmitted to

offspring), underscoring the clinical importance of testing other DNA repair genes (41). Findings showed that BRCA1 and 2 inactivation frequently led to higher HRD score in ovarian and breast cancers. This high HRD score has positive prognostic significance for platinum and PARPI therapy. In unclassified ovarian cancer patients who undertook germline *BRCA1* and *2* test, 19% (44/235) were carriers of germline mutations, and somatic mutation test was done on 28 specimens, 42.9% (9/21) and 28.6% (2/7) were found to be *BRCA1* and *2* positives, respectively (42). In another study by Pennington et al. (43), among 367 ovarian carcinomas tested for somatic mutation, 2.5% (19/367) and 1.63% (6/367) were positive for *BRCA1* and *2*, respectively. These carriers of somatic mutation have shown a positive impact on overall survival and platinum responsiveness as germline *BRCA1* and *2* mutation carriers. Other factors such as germline and somatic mutations in HR genes (**Table 1**) and epigenetic alterations (promoter hypermethylation) are implicated in HRD (84).

3 HOMOLOGOUS RECOMBINATION DEFICIENCY IN BREAST CANCER

Breast cancer is the most common cancer among women and ranks first and fifth among causes of incidence and mortality, respectively, compared with all other cancers in both sexes in 2020 worldwide. According to the IARC (38), an estimated 2,261,419 (11.72% of all cancer types) new incidences and 684,996 (6.88% of all cancer types) deaths from breast cancer were recorded worldwide in 2020. Similar to ovarian cancer, DNA repair pathways are frequently anomalous in breast cancer, leading to the accumulation of DNA damage and genomic instability. The *BRCA1* and *BRCA2* genes are associated with hereditary breast and ovarian cancer (85). BRCA1 and BRCA2 play a significant role in DNA repair, especially as components of the FANC/BRCA DNA damage response pathway (**Figure 1**). This DNA repair pathway is a highly conserved system involved in the DSB response *via* HR and in the BER pathway for the repair of DNA single-strand breaks (SSBs) (9). The PARP enzyme plays a decisive role in this pathway and is critical for resolving the stalled replication forks. Inhibition of PARP during the base excision process requires BRCA-dependent HRR to resolve it. Targeting HR-related genes (**Table 2**) has potential for destabilizing tumor genomic integrity (13). Clinical trials confirmed that HRD is necessary for the sensitivity to DNA-damaging agents (e.g., cisplatin) and PARPIs (106).

Although *BRCA1* and *BRCA2* have been studied extensively, other genes are also involved in the occurrence of breast cancer. In 3,388 breast cancer patients who underwent genetic testing for 25 genes, nearly half of the pathogenic variants were in the *BRCA1* (24%) and *BRCA2* (24.5%) genes. The remaining 51.5% of pathogenic variants were detected in other genes tested including *CHEK2* (11.7%), *ATM* (9.7%), *PALB2* (9.3%), and Lynch syndrome genes (7%); other genes accounted for the remaining 13.8%. The same study showed that pathogenic mutations in *BRCA1*, *PALB2*, *BARD1*, *BRIP1*, and *RAD51C* are significantly more prevalent than those of other genes in triple negative breast cancer (TNBC) (85). In a study by Lang et al. (144) using NGS based sequencing, the prevalence of somatic *BRCA1* and *2* mutation in sporadic breast cancer cases which carries germline-*BRCA* (g*BRCA*) mutations was 3.5% (15/416). Among these, 1.9% (8/416) and 1.7% (7/416) were *BRCA1* and *2*, respectively. In the same study, somatic *BRCA* mutation in g*BRCA*-negative cases was not detected; indicating somatic *BRCA* mutation in g*BRCA*-negative cases is rare. The prevalence of pathogenic mutations, LOH, and promoter hypermethylation in breast cancer is summarized in **Table 2** based on recently published data.

4 HOMOLOGOUS RECOMBINATION DEFICIENCY IN COLORECTAL CANCER

CRC is the third most common cancer worldwide. In 2020 alone, the incidence of CRC was estimated at 1,931,590 (10% of all other cancers) worldwide, and it is the second most common cause of cancer-related deaths 935,173 (9.39% of all other cancers) in both sexes after lung cancer (38). Most CRC occurrences are sporadic and are not related to genetic predisposition or family history; however, 20–30% of patients with CRC have a positive family history, and 5% of these tumors arise from genetic predisposition (145). Mutation is a frequent event in CRC. According to TCGA (146), 16% of colorectal carcinomas are hyper-mutated; of these, 75% have high microsatellite instability, typically with hypermethylation and MLH1 silencing, and 25% have somatic mismatch repair gene and polymerase ϵ (POLE) mutations. The prevalence of mutations in *APC*, *TP53*, *SMAD4*, and *PIK3CA* was also reported, as well as *KRAS* mutations. Recurrent copy number alterations (e.g., *ERBB2* amplification), chromosomal translocations, such as the fusion of NAV2 and the WNT pathway member *TCF7L1*, and biallelic inactivation of *APC* were among the common features leading to LOH in CRC. These and other findings confirmed the differences in both oncogenes and tumor suppressor genes involved in CRC compared with those in breast and ovarian cancers. However, a recent study from Japan revealed moderate genomic alterations in DNA repair genes including *BRCA2*, *ATM*, and *NBN* in CRC patients (147). High penetrance genes in HBOC differ from those in CRC; high penetrance genes in CRC are *APC*, *MLH1*, *MSH2*, *MSH2/MUTYH*, *SMAD4*, *MAP*, and *APC/PMS2*; moderate penetrance genes are *MSH6* and *PMS2*; and low penetrance genes are *APC* p.I1307K and *MUTYH* mono-allelic (148).

The methylation profile of CRC differs from that of other cancers; it is characterized by global hypomethylation and promoter-specific DNA hypermethylation. At the whole genome level, CRC has 10–40% lower levels of absolute methylation than normal colonic tissue (149, 150). Global DNA hypomethylation, which is accompanied by genomic instability and tumor initiation, is primarily due to loss of methylation within repetitive elements such as long interspersed nuclear element-1 (*LINE-1*) and Arthrobacter

TABLE 1 | Prevalence of mutation, LOH, and promoter hypermethylation in ovarian cancer.

Genes	Mutation [%(proportion)]	LOH [%(proportion)]	Promoter Methylation [%(proportion)]
BRCA1	12.2% (31/255) (44); 5% (15/300) (45); 18% (60/333) (46); 15.5% (81/523) (47); 16.5% (26/158) (48)	88% (36/41) (49); 44% (4/9) (50); 67% (6/9) (50); 81.5% (123/151) (51); 60% (60/100) (52); 97% (30/31) (53); 10.13% (16/158) (48)	20% (22/112) (54); 14% (2/14) (50); 14% (5/35) (55); 9.6% (32/332) (56); 14% (38/257) (57); 35% (15/42) (58); 9.34% (45/482) (59); 73.7% (56/76) (60)
BRCA2	9.8% (25/255) (44); 2% (6/300) (45); 3.3% (11/333) (46); 5.5% (29/523) (47); 5.06% (8/158) (48)	58% (24/41) (49); 50% (3/6) (50); 67% (4/6)] (50); 68.9% (104/151) (51); 73% (75/103) (52); 53% (16.5/31) (53); 0.63% (1/158) (48)	21% (3/14) (50); 44% (22/50) (61)
RAD50	7.7% (29/380) (62); 60% (12/20) (63); 2.94% (2/68) (64); 0.63% (1/158) (48)	0.63% (1/158) (48)	–
RAD51	0.3% (1/316) (11)	2% (10/489) (11)	–
RAD51B	2.1% (3/142) (65); 0.06% (2/3.429) (66)	0.8% (4/489) (11)	–
RAD51C	0.7% (1/141) (67); 2.5% (13/523) (47); 0.41% (14/3,429) (66)	97% (30/31) (53); 0.5 (2/429) (68)	1.45% (7/482) (59); 2.7% (9/332) (69); 2.67% (14/524) (70); 3% (9/316) (11)
RAD51D	1.3% (1/77) (67); 2.6% (10/380) (62); 0.35% (12/3429) (66)	0.7% (3/429) (68); 1.2% (6/489) (11)	–
PALB2	3% (9/299) (71); 3.03% (2/66) (72); 0.6% (2/333) (46); 0.63% (12/1915) (73); 2.9% (2/69) (74); 1.1% (6/523) (47); 1.9% (3/158) (48)	0.23% (1/429) (75); 0.7% (3/429) (68); 10.8% (17/158) (48)	3.08% (4/130) (76)
FANCA	4.35% (1/23) (45)	56.45% (17.5/31) (53); 1.16% (1/86) (77); 0.7% (3/429) (75)	–
FANCD2	0.3% (1/316) (11)	32.25% (10/31) (53); 0.23% (1/429) (75)	–
FANCF	0.3% (1/300) (45)	0.2% (1/572) (78)	32.14% (36/112) (79);13.2% (7/53) (80)
FANCI	0.6% (92/300) (45)	1.16% (1/86) (77)	–
FANCM	4.35% (1/23) (45); 2.1% (5/235) (81); 0.96% (5/523) (47)	0.2% (1/489) (11)	–
NBN/NBS1	1.8% (6/333) (46); 0.28% (9/3236) (82); 0.42% (1/235) (81); 0.38% (2/523) (47)	0.6% (3/489) (11)	–
BARD1	0.12% (4/3,236) (82); 1.6% (4/255) (83); 0.63% (1/158) (48)	0.63% (1/158) (48)	–
ATM	1.78% (7/392) (121); 0.3% (1/333) (46); 16.7% (8/48) (292); 0.82% (3/367 (43); 3.2% (5/158) (48)	29% (9/31) (53); 1.86% (8/429) (75); 1.9% (3/158) (48)	–
ATR	6% (3/50) (293); 69.7% (23/33) (294);; 4.8% (12/141) (295)	29% (9/31) (53) (75);	–
MRE11A	5.92% (17/287) (296); 0.4% (2/523) (47); 0.22% (1/466) (297)		–
BRIP1	7.7% (29/380) (62); 1.47% (1/68) (64); 0.4% (2/523) (47); 1.7% (8/466) (297); 0.52% (1/192) (131); 0.63% (1/158) (48)	0.7% (3/429) (68); 1.3% (2/158) (48)	–
ERCC1	2.6% 10/380) (62); 0.2% (1/523) (78)	0.4% (2/489) (11)	–
CHEK2	20.3% (77/380) (62); 45% (9/20) (63); 1.47% (1/68) (64); 4.2% (12/287) (296); 0.4% (2/523) (47); 1.72% (10/581) (298); 0.43% (2/466) (297); 0.52% (1/192) (131); 0.63% (1/158) (48)	10% (1/10) (298); 7.6% (12/158) (48)	–
EMSY	3.8% (14/380) (62); 8% (25/316) (11); 1.5% (8/523) (78)	0.2% (1/489) (11)	–
TP53	1.47% (1/68) (64); 3.83% (11/287) (296); 0.3% (2/581) (298); 1.04% (2/192) (131); 96% (312/316) (11); 57% (90/158) (48); 71.3% (375/523) (78)	0.63% (1/158) (78)	–
STK11	4.2% (12/287) (296); 1.3% (2/158) (48)	1.6% (8/489) (11)	–
PTEN	5.23% (15/287) (296); 0.43% (2/466) (297); 11.4% (18/158) (48)	6.7% (21/316) (11); 1.9% (3/158) (48); 6.1% (30/489) (11)	16.9% (21/124) (299)
CDH1	7.32% (21/287) (296); 0.52% (1/192) (131)	2.3% (11/489) (11)	–
BLM	0.4% (9/2561) (300); 1.27% (4/316) (11)	0.6% (3/489) (11)	–
RBBP8	1.04% (2/192) (131); 0.32% (1/316) (11); 1.9% (3/158) (48)	0.2% (1/489) (11)	–
CDK12	2.9% (9/316) (11); 4% (21/523) (11)	0.4% (2/489) (11)	–
TP53BP1	1.27% (4/316) (11); 0.8% (4/523) (78)	1.4% (7/489) (11)	–
XRCC1	0.6% (2/316) (11); 0.8% (4/523) (78)	0.4% (2/489) (11)	–
MAD2L2/REV7	0.3% (1/316) (11)	0.3% (2/572) (78)	–
XRCC5/Ku80	0.2% (1/523) (78)	–	–

(Continued)

TABLE 1 | Continued

Genes	Mutation [%(proportion)]	LOH [%(proportion)]	Promoter Methylation [%(proportion)]
XRCC6/Ku70	0.3% (1/316) (11); 0.8% (4/523) (78)	0.2% (1/489) (11)	–
SLFN11	0.6% (3/523) (78)	0.8% (4/489) (11)	39% (16/41) (209)

"–" no report found, "LOH" loss of heterozygosity.

TABLE 2 | Prevalence of mutation, LOH, and promoter hypermethylation in breast cancer.

Genes	Mutation [%(proportion)]	LOH [%(proportion)]	Methylation [%(proportion)]
BRCA1	5% (15/300) (45); 16.67% (20/120) (86); 7.07% (7/99) (87); 10.6% (409/3,844) (88); 7.35% (201/2733) (89); 9.7% (97/999) (90); 14.7% (118/802) (91)	17.1% (12/70) (92); 44% (16/36) (50); 6.8% (10/147) (93); 41.6% (69/166) (94)	63.9% (46/72) and 27.5% (22/80) (95); 13.33% (8/60) (96)
BRCA2	2% (6/300) (45); 5.83% (7/120) (86); 11.11% (11/99) (87); 5.2% (157/3,024) (88); 5.01% (137/2733) (89); 3.5% (35/999) (90); 1.1% (9/802) (91)	14.3% (10/70) (92); 50% (4/20) (50); 31.3% (52/166) (94); 93% (27/29) (97)	69.4% (50/72) and 12.5% (10/80) (95); 15.52% (9/58) (96)
RAD50	1.03% (1/97) (98)	32.4% (44/136) (99)	–
RAD51	0.2% (2/999) (100)	55% (20/36) (101); 24.41% (31/127) (102); 29.4% (40/136) (99)	5.26% (2/38) (96)
RAD51B	1.41% (2/142) (65)	47.1% (64/136) (99)	–
RAD51C	1.41% (2/142) (65); 5.25% (15/286) (103); 0.53% (23/4309) (104)	0.13% (1/770) (68); 33.8% (46/136) (99)	8% (4/50) (96); 14.46% (23/159) (105); 3.64% (2/55) (106)
RAD51D	0.1% (1/894) (107)	36.8% (50/136) (99)	–
PALB2	5.7% (404/7093) (108); 5.9% (34/571) (104); 3.4% (33/972) (109); 0.8% (241/30,025) (110); 1.2% (11/937) (111)	2.66% (2/77) (112); 0.72% (7/972) (109); 0.13% (7/770) (68); 14.7% (20/136) (99)	16.7% (8/48) (96); 4.6% (6/130) (76); 95.35% (41/43) (113)
FANCA	0.4% (1/255) (44); 0.81% (1.124) (114)	60.3% (82/136) (99)	–
FANCD2	1.01% (1/99) (87); 1.2% (3/247) (115); 0.9% (2/216) (116)	22.1% (30/136) (99)	60% (71/118) (117)
FANCF	0.5% (4/817) (118); 0.9% (2/216) (116)	17.7% (24/136) (99)	4.04% (4/99) (119); 0.8% (91/120) (120)
FANCI	0.4% (1/255) (44); 0.26% (1/392) (121), 2.32% (28/1207) (41); 1.01% (1/99) (87); 5.52% (9/163) (122); 0.81% (1.124) (114)	0.3% (2/816) (118)	–
FANCM	0.4% (1/255) (44); 1.5% (6/392) (121); 7.97% (13/163) (122); 0.81% (1.124) (114); 1.4% (6/427) (123); 0.7% (2/286) (124); 1.03% (16/1547) (125)	0.8% (6/770) (68); 35.3% (48/136) (99)	2.33% (1/43) (113); 2.7% (113)
NBN/NBS1	1.74% (59/3388) (85); 1.03% (1/97) (126); 0.22% (8/3617) (104); 0.16% (14/8612) (127); 1.2% (53/4566) (128); 1.7% (4/235) (129)	3.33% (1/30) (130); 1.5% (2/136) (99)	8.93% (5/56) (96)
BARD1	2% (68/3,388) (85); 0.2% (7/3,667) (104); 0.52% (1/192) (131); 0.18% (52/28,536) (110); 1.67% (2/120) (132); 0.53% (5/937) (111); 0.33% (7/2134) (133)	0.3% (1/330) (134)	10.34% (6/58) (96)
ATM	7.1% (5/70) (92); 2.42% (3/124) (114); 1.56% (3/192) (131); 4.6% (55/1207) (41); 1.5% (81/5589) (135); 0.98% (329/33409) (85); 0.94% (274/29229) (110); 0.4% (30/7,657) (136); 5% (3/60) (137)	67% (14/23) (138); 40% (298/745) (138); 19% (13/70) (92); 0.4% (3/770) (68); 48.5% (66/136) (99)	13.79% (8/58) (96); 91.4% (174/190) (139); 53.2% (33/62) (140); 97.4% (223/229) (141); 81% (102/126) (142); 58% (29/51) (143); 25.58% (11/43) (113)

luteus restriction endonucleases (Alu), and it is supposed to contribute to CRC initiation by enhancing genomic instability. On the other hand, genes methylated in CRC are established tumor suppressor genes, and 50% of these are also methylated in the normal colonic epithelium. As a result, methylation in CRC can have three phenotypes according to the CpG-island methylator phenotype (CIMP), i.e., CIMP-high, CIMP-low, and non-CIMP tumors (151). Similar to breast and ovarian cancers, the prevalence of mutation, LOH, and promoter hypermethylation in 35 HR-related genes is described based on recently published data (**Table 3**).

5 HOMOLOGOUS RECOMBINATION DEFICIENCY IN PANCREATIC CANCER

Pancreatic cancer (PC) is one of the most devastating types of cancer. PC has a low 5-year survival rate of 9%, and the development of new therapeutics is urgent. The global incidence and mortality of PC in 2020 were 2.6% (495,773 of all cancer types) and 4.68% (466,003 of all cancer types), respectively. PC is the 12th and 6th leading cause of cancer-related incidence and deaths in the world, respectively (38). Although most PC cases occur sporadically, familial (individual

TABLE 3 | Prevalence of mutation, LOH, and promoter hypermethylation in colorectal cancer.

Genes	Mutation [%(proportion)]	LOH [%(proportion)]	Methylation [%(proportion)]
BRCA1	0.29% (16/5481) (152); 0.28% (3/1058) (153); 0.4% (2/450) (148); 4% (25/619) (154); 3.2% (17/534) (78)	—	—
BRCA2	0.34% (5/1474) (152); 0.76% (8/1058) (153); 0.9% (4/450) (148); 6.8% (40/619) (154); 7.1% (38/534) (78)	0.2% (1/592) (78)	10.6% (8/78) (155)
RAD50	3.2% (18/619) (154)	0.5% (3/592) (78)	—
RAD51	0.5% (3/619) (154)	1.7% (10/592) (78)	—
RAD51B	0.8% (5/619) (154)	0.5% (3/592) (78)	—
RAD51C	0.08% (1/1260) (156); 0.8% (5/619) (154)	–	—
RAD51D	1% (6/619) (154)	0.2% (1/592) (78)	—
PALB2	0.44% (3/680) (12); 0.19% (2/1058) (153); 0.4% (2/450) (148); 2.6% (16/619) (154);	0.3 (3/592) (78)	—
FANCA	3.1% (19/619) (154)	0.7% (4/592) (78)	—
FANCD2	2% (1/50) (157); 4% (25/619) (154)	—	—
FANCF	1.3% (8/619 (154)	0.2% (1/592) (78)	—
FANCI	2.1% (13/619) (154)	0.2% (1/592) (78)	—
FANCM	2% (1/50) (158); 5% (31/619) (154)	2% (1/50) (158)	—
NBN/NBS1	0.2% (2/1058) (153); 3.2% (20/619) (154)	—	—
BARD1	0.1% (1/1058) (153); 2.33% (1/43) (159); 0.08% (1/1260) (153); 1.1% (7/619) (154)	—	—
ATM	0.74% (5/680) (12); 0.95% (10/1058) (153); 0.9% (4/450) (148); 10.3% (64/619) (154)	0.74% (5/680) (12); 2.33% (1/43) (159)	16.67% (13/78) (155)
ATR	18.8% (9/48) (160); 4.5% (34/619) (154)	—	—
MRE11A	0.3% (3/1006) (161); 2.6% (16/619) (154)	0.2% (1/592) (78)	—
BRIP1	0.16% (2/1260) (156); 2.6% (16/619 (154)	—	—
XRCC1	1.8% (11/619) (154)	—	—
CHEK2	0.23% (1/430) (162); 0.4% (5/1260) (156); 2.65% (4/151) (163); 5.8% (36/619) (154)	0.5% (3/592) (78)	—
EMSY	3.2% (20/619) (154);	—	—
TP53	0.23% (1/430) (162); 0.1% (1/1058) (153); 0.66% (1/151) (163); 51.7% (320/619) (154)	1.4% (8/292) (78)	—
STK11	0.08% (1/1260) (156); 0.8% (5/619) (154)	0.5% (3/292) (78)	—
PTEN	60% (87/146) (164); 8.2% (51/619) (154)	23% (6/26) (164)	11.86% (10 (165) (164)
CDH1	1.33% (2/151) (163); 2.9% (18/619) (154)	0.2% (1/592) (78)	17.7% (3/17) (166); 87% (53/61) (167)
CDK12	5.2% (32/619) (154)	—	—
BLM	1.62% (3/185) (168); 1.9% (12/619) (154)	50% (1/2) (168)	—
TP53BP1	6.3% (5/124) (169); 6.5% (40/619) (154)	1.5% (9/592) (78)	—
ERCC1	0.8% (5/619) (154)	28.3% (43/152) (158)	—
RBBP8	1.6% (6/619) (154)	0.2% (1/592) (78)	—
MAD2L2/REV7	1% (6/619) (154)	0.5% (3/592) (78)	—
XRCC5/Ku80	1.4% (9/619) (154)	—	—
XRCC6/Ku70	2.1% (13/619) (154)	—	—
SLFN11	2.4% (15/619) (154)	0.2% (1/592) (78)	55.47% (71/128) (170)

"—" no report found, "LOH" loss of heterozygosity.

having two or more first-degree relatives diagnosed with PC) and hereditary syndrome PC account for 10% of cases. Hereditary cancer syndromes associated with increased risk of PC include Lynch syndrome, familial adenomatous polyposis, Peutz-Jeghers syndrome, atypical multiple mole melanoma, and familial and hereditary breast and ovarian cancer syndrome. Hereditary cancer syndrome accounts for 3% of PC cases, whereas familial PC accounts for 4–10% of cases (171, 172). Familial PC includes individuals with two or more affected first-degree relatives with PC excluding patients with hereditary syndrome. The risk of PC increases with the number of affected first-degree relatives. A study of 838 families including 5,179 individuals showed that the relative PC risk was 4.5 [95% confidence interval (CI): 0.5–16.3) among 1,253 cases with one affected first-degree relative, 6.4 (95% CI: 1.8–16.4) among 634 cases with two affected first-degree relatives, and 32 (95% CI: 10.4–74.7) in 106 cases with three or more affected first-degree relatives (173).

The specific hereditary pancreatic susceptibility genes are *PRSS1*, *SPINK1*, *GGT1*, *CTRC*, and *CFTR*, and mutations in these genes cause early onset PC. However, mutations in these genes are rare and account for a small proportion of PC cases, although the cumulative risk at 70 years age reaches 7–40% with early onset PC (174). Familial PC susceptibility genes include *BRCA2*, *ATM*, *PALB2*, *CDKN2A*, *PRSS1*, *STK11*, *MLH1*, and *MSH2* (175). Recent findings showed the association of PC with genetic alterations in *BRCA2*, *BRCA1*, *ATM*, *CHECK2*, *PALB2*, *FANCC*, and *CDKN2A* genes (176–178). Mutations in *BRCA2* are among the most common genetic mutations involved in familial pancreatic ductal carcinoma. *BRCA* mutation predisposes to PC, and PC more frequently affects *BRCA2* mutation carriers than *BRCA1* carriers. Among 204 *BRCA* mutation carriers with PC, 42.7% (87/204) had *BRCA1* mutations and 57.3% (117/204) had *BRCA2* mutations (179). The prevalence of mutation, LOH, and promoter

hypermethylation of 35 HR genes is described according to recently published data in PC (**Table 4**).

6 HOMOLOGOUS RECOMBINATION DEFICIENCY IN NON-SMALL CELL LUNG CARCINOMA

Lung cancer is the leading cause of cancer-related morbidity and mortality in both genders worldwide. According to the IARC, the estimated number of incidences and deaths from lung cancer was 2,206,771 (11.44%) and 1,796,144 (18.04%) of all cancers worldwide in 2020, respectively (38). The majority of lung cancer cases are associated with smoking or the use of different tobacco products, although other factors such as asbestos, air pollution, radon gas exposure, and chronic infection also contribute to lung carcinogenesis (194). Inherited and acquired mechanisms of lung cancer susceptibility have been proposed, although they are rare. For instance, germline T790M mutation predisposes to a unique hereditary lung cancer syndrome that affects never-smokers and accounts for 31% of the estimated risks for lung cancer in never-smoker carriers (195, 196). Lung cancer is highly invasive, rapidly metastasizing, and broadly categorized into two histological groups, small-cell lung carcinomas (SCLCs) and NSCLCs, which grow and spread differently. NSCLCs account for 87% of cases and can be subdivided into three or four subtypes (adenocarcinoma, squamous cell carcinoma, large cell carcinoma, and undifferentiated NSCLCs), whereas SCLCs account for 12% of lung cancer cases (194).

Generally, lung adenocarcinoma is characterized by recurrent aberrations in multiple key pathways, including activation of RTK/RAS/RAF; activation of PI3K-mTOR; alterations of p53, cell cycle regulators, and the oxidative stress pathway; and mutation of various chromatin and RNA splicing factors. The research network of TCGA demonstrated the activation of oncogenes including *KRAS* (32%), *EGFR* (11%), *MET* (7%), *BRAF* (7%), *MDM2* (8%), *CDK4* (7%), *PIK3C4* (4%), and *CCND1* (4%) and the inactivation of tumor suppressor genes such as *TP53* (46%), *CDKN2A* (43%), *KEAP1* (19%), *STK11* (17%), *NF1* (11%), *ATM* (9%), *RBM10* (9%), *ARID1* (7%), *ARID2* (7%), and *RB1* (7%) in lung cancer (197). Analysis of DNA repair genes associated with squamous cell carcinoma showed a correlation between pathogenic mutations of DNA repair genes and tumor mutation burden. Among DNA repair genes *BRCA1* and *BRCA2* showed the greatest mutation frequency and the tumor burden increased in correlation with the number of affected DNA repair genes (198). A study analyzing mutations in the *BRCA 1* and *2* genes in different cancers showed that *BRCA* mutation is associated with increased incidence of non-breast and ovarian cancers in first- and second-degree relatives of high-risk breast cancer patients. Among 337 *BRCA* mutation carriers, the second highest *BRCA* mutation rate was recorded in lung cancer [8.8% (33/337)] after stomach cancer [13.8% (52/337)] (199). In this review, we displayed the prevalence of mutation, LOH, and promoter hypermethylation of 35 HR genes in NSCLC (**Table 5**).

7 HOMOLOGOUS RECOMBINATION DEFICIENCY IN PROSTATE CANCER

Prostate cancer is the most prevalent cancer in men next to lung cancer, according to the IARC in 2020 (38), it ranked 2nd and 5th in its incidence and mortality among men, respectively. The estimated number of incidences and deaths from prostate cancer in 2020 were 1,414,259 (14.05%) and 375,304 (6.79%) worldwide, respectively. Prostate cancer is characterized by a high degree of heritability, and genetic components contribute significantly to disease incidence (210). A large cohort study conducted in the Nordic region that analyzed the different cancer heritability risks in monozygotic and dizygotic twins identified a risk of prostate cancer of 57% (95% CI: 51–63), which was higher than that of other cancer types such as ovarian cancer at 39% (95% CI: 23–55) and breast cancer at 31% (95% CI: 11–51) (210). Although the high rate of heritability of prostate cancer has been demonstrated in patients with a positive family history, candidate genes that contribute to prostate cancer heritability have not been identified except *HOXB13* (211). Recurrent mutation of the *HOXB13* gene at G84E was identified in many families. The *HOXB13* G84E allele accounts for approximately 5% of hereditary prostate cancer (211). A study including 2,443 prostate cancer families of European descent detected at least one *HOXB13* G84E mutation carrier, among 112 prostate cancer families (4.6%) (212). Moreover, a study conducted in unrelated subjects of European descent revealed, *HOXB13* G84E mutation was detected in 1.4% (72/5083) and 0.07% (1/1401) of participants with- and without prostate cancer, respectively ($P<0.05$) (213). Another study comprising 9,012 men diagnosed with different cancers showed a rate of 0.54% (49/9012) of *HOXB13* G84E mutation carriers, of whom 1.4% (19/1362) were positive for prostate cancer compared with 0.4%(23/5,898) of *HOXB13* G84E mutation carriers without prostate cancer ($p < 0.05$) (214). Prostate cancer has a genetic origin in <5% of cases, and this risk becomes higher when high penetrance genes such as *HOXB13* are involved (215). Recent gene linkage studies identified additional prostate cancer susceptibility genes such as *HPC1*, *HPC2/ELAC2*, *MSR1*, *BRCA1*, *BRCA2*, and *BRIP1* (216).

An estimated 20% of patients with prostate cancer have a positive family history, which can be attributed not only to shared genes, but also to a shared pattern of exposure to environmental carcinogens and common lifestyle habits (216). Additional challenges in the management of prostate cancer include its genetic heterogeneity and a high rate of sporadic cases; many common genetic variants are associated with prostate cancer, explaining the familial clustering of the disease rather than hereditary causes (211). The importance of both germline and somatic alterations in DNA repair genes is suggested by the fact that carriers of mutations in these genes are at a high risk of developing aggressive or metastatic prostate cancer (211). Deleterious mutations of *BRCA1* and *BRCA2* are associated with increased risk of prostate cancer and experienced very aggressive course of the disease (215). However, studies focusing on families with only prostate cancer failed to

TABLE 4 | Prevalence of mutation, LOH, and promoter hypermethylation in pancreatic cancer.

Gene	Mutation [%(proportion)]	LOH [%(proportion)]	Methylation [%(proportion)]
BRCA1	42.7% (87/204) (179); 2.4% (1/42) (180); 0.3% (1/332) (181); 2.4% (15/615) (177); 0.6% (18/3,030) (178); 1.34% (4/298) (176); 0.4% (3/854) (182); 1.3% (5/456) (183)	20% (10/50) (177); 50% (2/4) (184); 2% (2/100) (185)	8.3% (1/12) (186); 70.6% (12/17) (186); 34.3% (12/35) (186); 60.3% (35/58) (186); 46% (22/48) (187)
BRCA2	57.3% (117/204) (179); 26.2% (11/42) (180); 2.11% (7/332) (181); 5.7% (35/615) (177); 1.9% (59/3,030) (178); 1.34% (4/298) (176); 1.41% (12/854) (182); 5.56% (3/54) (188); 0.8% (5/638) (189); 2.1 (8/456) (183)	40% (20/50) (177); 75% (3/4) (184); 6% (6/100) (185)	—
RAD50	0.32% (2/615) (177)	3.7% (4/109) (115); 0.32% (2/615) (177); 0.6% (1/183) (78)	—
RAD51	—	0.9% (1/109) (115)	—
RAD51B	—	1.8% (2/109) (115)	—
RAD51C	0.1% (3/3030) (178)	0.1% (3/3030) (178); 0.34% (1/289) (190); 2.8% (3/109) (115)	—
RAD51D	0.16% (1/615) (177)		—
PALB2	2.4% (1/42) (180); 0.16% (1/615) (177); 0.4% (12/3030) (178); 0.34% (1/298) (176); 0.23% (2/854) (182); 3.7% (2/54) (188); 0.8% (5/638) (191)	0.16% (1/615) (177); 2% (2/100) (185)	—
FANCA	0.3% (1/456) (183)	—	—
FANCD2	1% (4/456) (183)	3.7% (4/109) (115)	—
FANCF	2.8 (3/109) (192)	0.9% (1/109) (115)	—
FANCI	—	—	—
FANCM	0.47% (3/638) (191); 1.8% (7/456) (183); 1.8% (7/456) (183)	2.8% (3/109) (115)	—
NBN/NBS1	0.16% (1/615) (177); 0.13% (4/3030) (178)	0.9% (1/109) (115)	—
BARD1	0.16% (1/615) (177); 0.13% (4/3030) (178); 0.34% (1/298) (176)	0.49% (1/615) (177); 0.9% (1/109) (115)	—
ATM	0.3% (1/332) (181); 1.8% (11/615) (177); 2.28% (69/3030) (178); 3.36% (10/298) (176); 1.17% (10/854) (182); 3.7% (2/54) (188); 2.98% (19/638) (191); 3.7% (14/456) (183)	72.73% (8/11) (177); 5% (5/100) (185); 4.6% (5/109) (115)	—
ATR	0.5% (2/456) (183); 0.9% (1/109) (192)	1.8% (2/109) (115)	—
MRE11A	0.07% (2/3030) (178)	0.9% (1/109) (115)	—
BRIP1	0.17% (5/3030) (178); 1.04% (3/289) (190)	0.34% (1/289) (190); 2.8% (3/109) (115)	—
XRCC1	0.6% (1/179) (78)	—	—
CHEK2	2.28% (14/615) (177); 1.09% (33/3030) (178); 1.68% (5/298) (176)	1.95% (12/615) (177); 2.8% (3/109) (115)	—
EMSY	0.5% (2/456) (183); 0.9% (1/109) (192)	—	—
TP53	89.8% (344/456) (183); 50.5% (55/109) (192); 0.35% (1/289) (190); 0.2% (6/3030) (178)	0.34 (1/289) (190); 5.5% (6/109) (115)	—
STK11	0.16% (1/615) (177)	4.6% (5/109) (115)	—
PTEN	0.3% (1/456) (183); 0.9% (1/109) (192)	0.6% (1/183) (78); 1.8% (2/109) (115)	–
CDH1	0.03% (1/3030) (178); 0.8% (3/456) (183)	—	10.5% (6/57) (186); 50% (1/2) (166); 38% (19/50) (193)
CDK12	0.5% (2/456) (183)	—	—
BLM	0.49% (3/615) (177)	0.33% (2/615) (177); 1.8% (2/109) (115)	—
TP53BP1	0.5% (2/456) (183); 0.9% (1/109) (192)	—	—
ERCC1	0.6% (1/179) (78)	0.9% (1/109) (115)	—
RBBP8	0.9% (1/109) (192)	2.8% (3/109) (115)	—
MAD2L2/REV7	—	1.1% (2/183) (78)	—
XRCC5/Ku80	0.6% (1/179) (78)	1.8% (2/109) (115)	—
XRCC6/Ku70	0.3% (1/456) (183); 0.6% (1/179) (78)	—	—
SLFN11	—	—	

"—"no report found, "LOH" loss of heterozygosity.

TABLE 5 | Prevalence of mutation, LOH, and promoter hypermethylation in NSCLC.

Genes	Mutation [%(proportion)]	LOH [%(proportion)]	Methylation [%(proportion)]
BRCA1	4.5% (8/178) (198); 2.9% (7/240) (200); 4.2% (48/1114) (201)	0.15% (1/655) (68); 0.2% (2/1114) (201)	3.8% (6/158) (202)
BRCA2	3.9% (7/178) (198); 3.9% (9/240) (200); 5.2 (60/1114) (201)	0.3% (2/655) (68); 0.36% (4/1114) (201)	—
RAD50	1.1% (2/178) (198); 11.11% (2/18) (203); 0.8% (2/240) (200); 1.7% (19/1114) (201)	0.6% (7/1114) (201)	—
RAD51	0.56% (1/178) (198); 0.4% (1/240) (200); 0.3% (4/1114) (201)	1.2% (14/1114) (201)	—
RAD51B	5.56% (1/18) (203); 0.8% (2/240) (200); 0.8% (9/1114) (201)	0.2% (2/1114) (201)	—
RAD51C	0.4% (1/240) (200); 1% (11/1114) (201)	0.09% (1/114) (201)	—
RAD51D	0.4% (1/240) (200); 0.6% (7/1114) (201)	0.4% (5/1114) (201)	—
PALB2	2.25% (4/178) (198); 1.7% (4/240) (200); 2.3% (26/1114) (201)	0.09% (1/1114) (201)	—
FANCA	2.25% (4/178) (198); 11.11% (2/18) (203); 2.5% (6/240) (200); 1.5% (17/1114) (201)	1.1% (12/1114) (201)	—
FANCD2	1.2% (14/1114) (201)	0.3% (3/1114) (201)	—
FANCF	0.9% (9/1114) (201)	0.15% (1/655) (68); 0.2% (2/1114) (201)	14% (22/126) (202)
FANCI	1.8% (19/1114) (201)	—	—
FANCM	5.6% (64/1114) (201)	0.5% (6/1114) (201)	—
NBN/NBS1	3.75% (17/453) (204); 1.7% (4/240) (200); 1.4% (16/1114) (201)	—	—
BARD1	1.1% (2/178) (198); 3.9% (9/240) (200); 1.9% (22/1114) (201)	0.36% (4/1114) (201)	—
ATM	4.5% (8/178) (198); 5.56% (1/18) (203); 7.9% (19/240) (200); 7.6% (87/1114) (201); 11.9% (12/101) (205)	0.61% (4/655) (68)	—
ATR	5.6% (10/178) (198); 5.56% (1/18) (203); 3.3% (8/240) (200); 4.5% (52/1114) (201)	0.2% (2/1114) (201)	—
MRE11A	1.7% (4/240) (200); 1.6% (18/1114) (201)	0.15% (1/655) (68); 0.27% (3/1114) (201)	—
BRIP1	4.6% (11/240) (200); 2.5% (28/1114) (201)	0.5% (3/655) (68)	—
XRCC1	1% (11/1114) (201)	—	—
CHEK2	1.7% (3/178) (198); 1.3% (3/240) (200); 1.9% (22/1114) (201);	0.09 (1/1114) (201)	—
EMSY	2.8% (32/1114) (201)	0.2% (2/1114) (201)	—
TP53	66.7% (4/6) (206); 20% (46/230) (197); 39.4% (20/1078) (205); 27.8% (5/18) (203); 59.2% (150/240) (200); 67.7% (775/1114) (201)	0.9% (10/1114) (201)	—
STK11	7.4% (17/230) (197); 1.8% (20/1078) (205); 27.8% (5/18) (203); 23.3% (56/240) (200); 9.7% (111/1114) (201)	65% (80/124) and 11% (7/62) (207); 0.4% (5/1114) (201)	—
PTEN	1.8% (20/1078) (205); 3.3% (8/240) (200); 5.9% (67/1114) (201)	3.1% (36/1114) (201)	—
CDH1	1.3% (3/240) (200); 1.8% (20/1114) (201)	0.09% (1/1114) (201)	20% (4/20) (166); 48% (11/23) and 76% (32/42) (208)
CDK12	11.11% (2/18) (203); 1.3% (3/240) (200); 3.2% (37/1114) (201)	0.09% (1/1114) (201)	—
BLM	2.9% (7/240) (200); 1.8% (20/1114) (201)	—	—
TP53BP1	2.9% (7/240) (200); 2.6% (30/1114) (201)	1.4% (16/1114) (201)	—
ERCC1	0.2% (2/1114) (201)	—	—
RBBP8	1.1% (12/1114) (201)	—	—
MAD2L2/ REV7	0.3% (3/1114) (201)	0.27% (3/1114) (201)	—
XRCC5/Ku80	1.6% (18/1114) (201)	0.36% (4/1114) (201)	—
XRCC6/Ku70	1.3% (15/1114) (201)	0.09% (1/1114) (201)	—
SLFN11	2.3% (26/1114) (201)	0.3% (3/1114) (201)	13.6% (3/22) (209)

"—" no report found, "LOH" loss of heterozygosity

identify a significant number of *BRCA1* or *BRCA2* mutations, indicating their minimal role in hereditary prostate cancer predisposition (211). A comprehensive genomic analysis of 1,013 prostate cancer patients revealed the indispensable role of alterations in DNA repair genes (78). Here, the prevalence of mutation, LOH, and promoter hypermethylation in 35 DNA repair genes in prostate cancer was described based on recently published data (**Table 6**).

8 POLY (ADP-RIBOSE) POLYMERASE INHIBITOR RESISTANCE MECHANISM

8.1 The Role of PARP in DNA Repair

BER, HR, NHEJ, and micro homology-mediated end-joining (MMEJ) repair SSBs and DSBs. PARP1 is involved in all DNA repair mechanisms. SSBs are primarily repaired by BER (high fidelity DNA repair) using PARP1. DSBs are repaired by three

TABLE 6 | Prevalence of mutation, LOH, and promoter hypermethylation in prostate cancer.

Genes	Mutation [%(proportion)]	LOH [%(proportion)]	Methylation [%(proportion)]
BRCA1	0.6% (6/1013) (38); 0.3% (3/494) (78); 1.2% (4/333) (217); 1% (5/504) (218); 1.8% (8/444) (219)	1.3% (13/1013) (38); 1.2% (6/489) (78);	25-75% (220)
BRCA2	2.9% (6/1013) (38); 1.6% (8/494) (78); 2.7% (9/333) (217); 5.2% (26/504) (218); 8.3% (37/444) (219)	2.5% (25/1013) (38); 3.5% (17/489) (78); 0.6% (2/333) (217); 3% (15/501) (218); 2.9% (13/444) (219)	—
RAD50	0.4% (4/1013) (38); 0.2% (1/494) (78); 0.6% (3/504) (218); 0.7% (3/444) (219)	1.2% (12/1013) (38); 0.8% (4/489) (78); 1.2% (4/333) (217); 0.4% (2/501) (218); 1.1% (5/444) (219)	—
RAD51	0.1% (1/1013) (38); 0.2% (1/494) (78);	0.8% (4/489) (78); 1.8% (6/333) (217); 0.4% (2/501) (218); 1.4% (6/444) (219)	—
RAD51B	0.6% (3/494) (78)	0.6% (3/489) (78); 1.2% (4/333) (217); 0.4% (2/501) (218); 1.1% (5/444) (219)	—
RAD51C	0.2% (1/504) (218)	1.3% (13/1013) (38); 0.6% (3/489) (78); 0.3% (1/333) (217); 0.5% (2/444) (219)	—
RAD51D	0.3% (1/333) (217); 0.2% (1/504) (218); 0.7% (3/444) (219)	0.6% (6/1013) (38); 0.6% (3/489) (78); 1.2% (4/333) (217); 0.2% (1/444) (219)	—
PALB2	1% (10/1013) (38); 0.6% (3/494) (78); 0.3% (1/333) (217); 1.2% (6/504) (218); 1.4% (6/444) (219)	0.3% (1/333) (217); 0.6% (3/501) (218); 0.2% (1/444) (219)	—
FANCA	0.3% (3/1013) (38); 0.3% (1/333) (217); 0.6% (3/504) (218); 0.9% (4/444) (219)	2% (20/1013) (38); 4.7% (23/489) (78); 7.8% (26/333) (217); 2.4% (12/501) (218); 0.2% (1/444) (219)	—
FANCD2	0.3% (3/1013) (38); 0.4% (2/494) (78); 0.3% (1/333) (217); 0.7% (3/444) (219)	1.5% (15/1013) (38); 2% (10/489) (78); 0.6% (2/333) (217); 1.1% (5/444) (219)	—
FANCF	0.2% (2/1013) (38); 0.2% (1/494) (78); 0.3% (1/333) (217); 0.5% (2/444) (219)	0.5% (2/444) (219)	—
FANCI	0.3% (3/1013) (38); 0.4% (2/494) (78); 0.3% (1/333) (217);	0.7% (3/444) (219)	—
FANCM	0.6% (6/1013) (38); 0.4% (2/494) (78); 0.3% (1/333) (217); 1.6% (7/444) (219)	0.2% (489) (78)	—
NBN/ NBS1	0.6% (6/1013) (38); 0.6%(3/494) (78); 0.3% (1/333) (217); 0.4% (2/504) (218); 0.7% (3/444) (219)	0.2% (2/1013) (38); 0.4% (2/489) (78); 0.2% (1/444) (219)	—
BARD1	0.6% (6/1013) (38); 0.8% (4/494) (78); 0.6% (3/504) (218); 0.9% (4/444) (219)	0.5% (5/1013) (38); 0.2% (1/489) (78); 0.3% (1/333) (217); 0.2% (1/501) (218); 0.9% (4/444) (219)	—
ATM	3.8% (38/1013) (38); 4.3% (21/494) (78); 3.9% (13/333) (217); 3.6% (18/504) (218); 6.1% (27/444) (219)	0.8% (8/1013) (38); 1.2% (6/489) (78); 2.1% (7/333) (217); 1.2% (6/501) (218); 1.6% (7/444) (219)	—
ATR	1% (10/1013) (38); 1% (5/494) (78); 1.4% (7/504) (218); 1.4% (7/444) (219)	0.5% (5/1013) (38); 0.4% (2/489) (78); 0.3% (1/333) (217); 0.2% (1/444) (219)	—
MRE11A	0.5% (5/1013) (38); 0.6% (3/504) (218); 0.9% (4/444) (219)	0.7% (7/1013) (38); 0.4% (2/489) (78); 0.6% (2/333) (217); 0.2% (1/501) (218)	—
BRIP1	0.6% (6/1013) (38); 0.6% (3/494) (78); 0.4% (2/504) (218); 0.9% (4/444) (219)	–	—
ERCC1	0.3% (3/1013) (38); 0.2% (1/494) (78); 0.5% (2/444) (219)	0.2% (1/489) (78); 0.3% (1/333) (217); 0.7% (3/444) (219)	—
CHEK2	0.4% (4/1013) (38); 0.4% (2/504) (218); 1.4% (6/444) (219)	1.2% (12/1013) (38); 1.4% (7/489) (78); 3% (10/333) (217); 0.4% (2/501) (218); 1.4% (6/444) (219)	—
EMSY	0.8% (8/1013) (38); 0.4% (2/494) (78); 0.3% (1/333) (217); 1.1% (5/444) (219)	0.4% (4/1013) (38); 0.2% (1.444) (219)	—
TP53	18.7% (189/1013) (38); 12.3% (61/494) (78); 6.9% (23/333) (217); 33.5% (169/504) (218); 36.7% (163/444) (219)	2% (20/1013) (38); 4.3% (21/489) (78); 0.6% (2/333) (217); 1.8% (9/501) (218); 3.4% (15/444) (219)	—
STK11	0.2% (2/1013) (38); 0.2% (1/494) (78); 0.4% (2/504) (218); 0.2% (1/444) (219)	3.4% (34/1013) (38); 0.2% (1/501) (218); 2.9% (13/444) (219)	—
PTEN	4.3% (44/1013) (38); 5.5% (27/494) (78); 2.7% (9/333) (217); 6% (30/504) (218); 6.3% (28/444) (219)	12.2% (124/1013) (38); 17.4% (85/489) (78); 15%(50/333) (217); 12.4% (62/501) (218); 25.7% (114/444) (219)	7.8% (221)
CDH1	0.9% (9/1013) (38); 0.8% (4/494) (78); 0.6% (2/333) (217); 1.2% (6/504) (218); 0.9% (4/444) (219)	1.7% (17/1013) (38); 2.9% (14/489) (78); 4.5% (15/333) (217); 0.4% (2/501) (218); 2% (9/444) (219)	69% (70/101) (222); 61% (49/81) (223); 27% (27/101) (224)
BLM	0.2% (2/1013) (38); 0.4% (2/504) (218); 0.5% (2/444) (219)	0.1% (1/1013) (38); 0.7% (3/444) (219)	—
RBBP8	0.2% (2/1013) (38); 0.2% (1/444) (219)	–	—
CDK12	3.3% (33/1013) (38); 2.2% (12/494) (78); 1.8% (6/333) (217); 5.6% (28/504) (218); 5.9% (26/444) (219)	0.7% (7/1013) (38); 0.4% (2/489) (78); 0.6% (2/333) (217); 0.4% (2/501) (218); 0.5% (2/444) (219)	—
TP53BP1	0.9% (9/1013) (38); 1.4% (7/494) (78); 0.9% (3/333) (217); 0.5% (2/444) (219)	1.7% (17/1013) (38); 0.8% (4/489) (78); 1.8% (6/333) (217); 1.4% (6/444) (219)	—

(Continued)

TABLE 6 | Continued

Genes	Mutation [%(proportion)]	LOH [%(proportion)]	Methylation [% (proportion)]
XRCC1	0.2% (2/1013) (38); 0.3% (1/333) (217); 0.5% (2/444) (219)	0.8% (4/489) (78); 1.5% (5/333) (217); 1.4% (6/444) (219)	–
MAD2L2/ REV7	0.1% (1/1013) (38); 0.2% (1/494) (78);	0.6% (6/1013) (38); 0.2% (1/489) (78); 0.3% (1/333) (217); 0.5% (2/444) (219)	—
XRCC5/ Ku80	0.2% (2/1013) (38); 0.2% (1/494) (78); 0.3% (1/333) (217); 0.2% (1/444) (219)	0.5% (5/1013) (38); 0.4% (2.489) (78); 1.2% (4/333) (217); 0.2% (1/444) (219)	—
XRCC6/ Ku70	0.5% (5/1013) (38); 0.6% (4/494) (78); 0.3% (1/333) (217); 0.2%(1/ 444) (219)	0.2% (1/489) (78); 0.6% (2/333) (217); 0.7% (3/444) (219)	—
SLFN11	0.3% (3/1013) (38); 0.2% (1/494) (78); 0.6% (2/333) (217); 0.2% (1/444)	0.4% (2/489) (78); 0.9% (3/333) (217); 0.2% (1/444) (219)	—

"—" no report found, "LOH" loss of heterozygosity.

mechanisms: HR, NHEJ, and MMEJ. HRR (high fidelity DNA repair mechanism) of DSBs is performed by recruiting BRCA1 and 2, RAD51, the MRN complex, and ATM, here PARP1 contributes to HR by recruiting MRE11 and NBS1 or by ribosylating BRCA. NHEJ (error-prone DNA repair mechanism) repair of DSBs involves the recruitment of Ku70, Ku80, and DNA-dependent protein kinase catalytic subunit (PKcs); PARP1 prevents the binding of Ku proteins to free DNA ends (first step of NHEJ) and thus inhibits NHEJ. MMEJ (error-prone DNA repair mechanism) repairs DSBs by recruiting Flap Structure-Specific Endonuclease-1 and NBN; PARP1 prevents binding of Ku proteins and directs DSBs to an alternate end-joining (MMEJ) repair pathway (225–227).

8.2 Mechanisms of PARP Inhibition

In the presence of SSBs, PARP1 binds to the SSB site and undergoes poly (ADP- ribosyl) ation, an important step for PARP1 activation; then, the poly(ADP-ribosyl)ated PARP1 recruits the DNA repair complexes BARD1-BRCA1 and MRN, which restore the integrity of DNA through a high fidelity DNA repair mechanism, resulting in cell survival. Inhibition of PARP by PARPIs drive to change the repair mechanism from SSBs to DSBs. PARPIs bind to PARP1 and inhibit its poly (ADP-ribosyl) ation as well as inhibiting BER. In addition, PARPIs prevent the release of PARP from the polymer form, thereby inhibiting the recruitment and binding of DNA damage repair proteins (PARP trapping), which further aggravates the inhibition of BER. Once BER is inhibited by PARPI, SSBs are converted into DSBs, forcing cells to opt for HRR. However, HR can only be used if cells are HR-proficient. HR defects (mutation, LOH, and hypermethylation) in HR-related genes such as *BRCA1* and *2*, *RAD51* and its paralogs (*RAD51B*, *RAD51C*, and *RAD51D*), *FA* genes, *PALB2*, the MRN complex, *BARD1*, *ATM*, *ATR*, and *BRIP1* cause cells to become HR-deficient and unable to repair DSBs. This causes the persistence of DNA DSBs, which leads to genomic instability and cell death. Cells need to activate alternative DNA repair mechanisms such as NHEJ and MMEJ, the only remaining repair mechanisms. Thus, cells are forced to use the two error-prone DSB repair mechanisms, which results in genomic instability and cell death (225–227). PARP1 and PARP2 are constitutively expressed enzymes that peak during the S-phase of the cell cycle and are activated by binding to DNA damage sites. PARPIs are particularly effective in the treatment of high grade ovarian and breast cancers with HR defects, which are characterized by frequent replication of tumor cells, and PARP expression and DNA damage recognition are highest during S-phase. However, the use of PARPIs is not limited to these two cancers. The concept of synthetic lethality or the BRCAness phenotype is wider, and most cancers with HR repair defects benefit from PARPI treatment including pancreatic and prostate cancers (227).

8.3 Mechanisms of Resistance to PARP Inhibitors

Despite the introduction of new drugs, the emergence of resistance to PARPIs remains a limiting factor (227, 228). Several mechanisms of PARPI resistance have been identified, including restoration of HRR proficiency, switching to alternate repair mechanisms such as NHEJ, replication fork stabilization, drug efflux, decreased PARP expression and binding, secondary mutations in HR-related genes and *RAD51*, regulation by microRNAs, phosphorylation of PARP by c-MET, loss of end resection regulation by 53BP1, epigenetic reversion of methylated promoters, and mutations in the shielding complex among others (**Figure 2**).

8.3.1 Restoration of Homologous Recombination Proficiency

HR proficiency can be restored directly by reverse mutation of *BRCA1* and *BRCA2* mutants (229). Reverse mutation might be elicited by genomic instability due to *BRCA* loss. In addition, the presence of hypomorphic (partial loss of gene function) *BRCA1* mutation leads to selection of cells with restored BRCA function, which confer resistance to PARPIs (230). In a recent study of high-grade ovarian carcinoma, *BRCA* reversion mutation was identified in 18% (2/11) and 13% (5/38) of pretreatment cell-free DNA extracts from platinum refractory and resistant cancers, respectively, compared with 2% (1/48) of platinum-sensitive cancers ($p < 0.05$) (231). Patients without *BRCA* reversion mutation detected in pretreatment circulation cell-free DNA extracts had significantly ($p < 0.05$) longer progression free survival than those with reversion mutation after treatment with rucaparib (9 versus 1.8 months), which decreased the clinical benefit from rucaparib (231). Although genetic reversion of *BRCA1* and *BRCA2* is one of the underlying mechanisms, it does not explain PARPI resistance in all cases. For example, loss of *REV7* (*MAD2L2*) re-establishes CTIP-dependent end resection of DSBs in *BRCA1*-deficient cells, leading to HR restoration and PARPI resistance (232).

Secondary somatic mutations that restore *BRCA1/2* in carcinomas from women with germline *BRCA1/2* mutations predict the resistance to platinum and PARPIs. In cohorts of 64 primary and 46 recurrent ovarian carcinoma patients, secondary somatic mutation was detected in 3.1% (2/64) of primary carcinomas and in 28.3% (13/46) of secondary carcinomas ($p < 0.05$) due to secondary mutations in *BRCA1/2*. In the same study, 46.2% (12/26) of the platinum resistance recurrence cases had secondary mutations that restored BRCA1/2 function compared with 5.3% (1/19) of platinum-sensitive recurrence cases ($p < 0.05$) (165). Furthermore, the reversion mutations were not only detected in *BRCA1/2*, but also in other HRR pathway genes including *RAD51C*, *RAD51D*, and *PALB2* in ovarian, prostate, and breast carcinomas as a mechanism of acquired resistance to platinum-based chemotherapies and PARPIs. Therefore, primary mutations of HR genes cause sensitivity to platinum and PARPI therapy, whereas secondary mutations cause resistance (231, 233). *BRCA2* reversion mutations confer resistance to olaparib and talazoparib in prostate cancer patients. Analysis of circulating cell-free DNA provides information on reversion mutation heterogeneity that is not distinct from that of single solid tumor biopsy, as well as potential indications for monitoring the emergence of PARPI resistance (234).

8.3.2 RING Domain-Deficient BRCA1 and Intragenic Deletion in BRCA2

High expression levels of RING domain-deficient BRCA1 proteins promote cisplatin and PARPI resistance by reducing the DNA repair capacity of BRCA1 in breast cancer cell lines. The BRCA1 185delAG hypomorphic allele, a common inherited mutation located close to the protein translation start site, produces a shortened and nonfunctional peptide. In contrast to the full length BRCA1, the translation start site for the RING domain-deficient BRCA1 protein is located downstream of the frameshift mutation at the BRCA1-Met-297 codon and does not require interaction with BARD1 for stability unlike the full length BRCA1. Functionally, the RING domain-deficient BRCA1 supports RAD51 foci formation, which increases HRR and confers partial PARPI and cisplatin resistance (235). A recent case control association study and functional analysis of BRCA2 identified a hypomorphic missense variant (Y3035S)

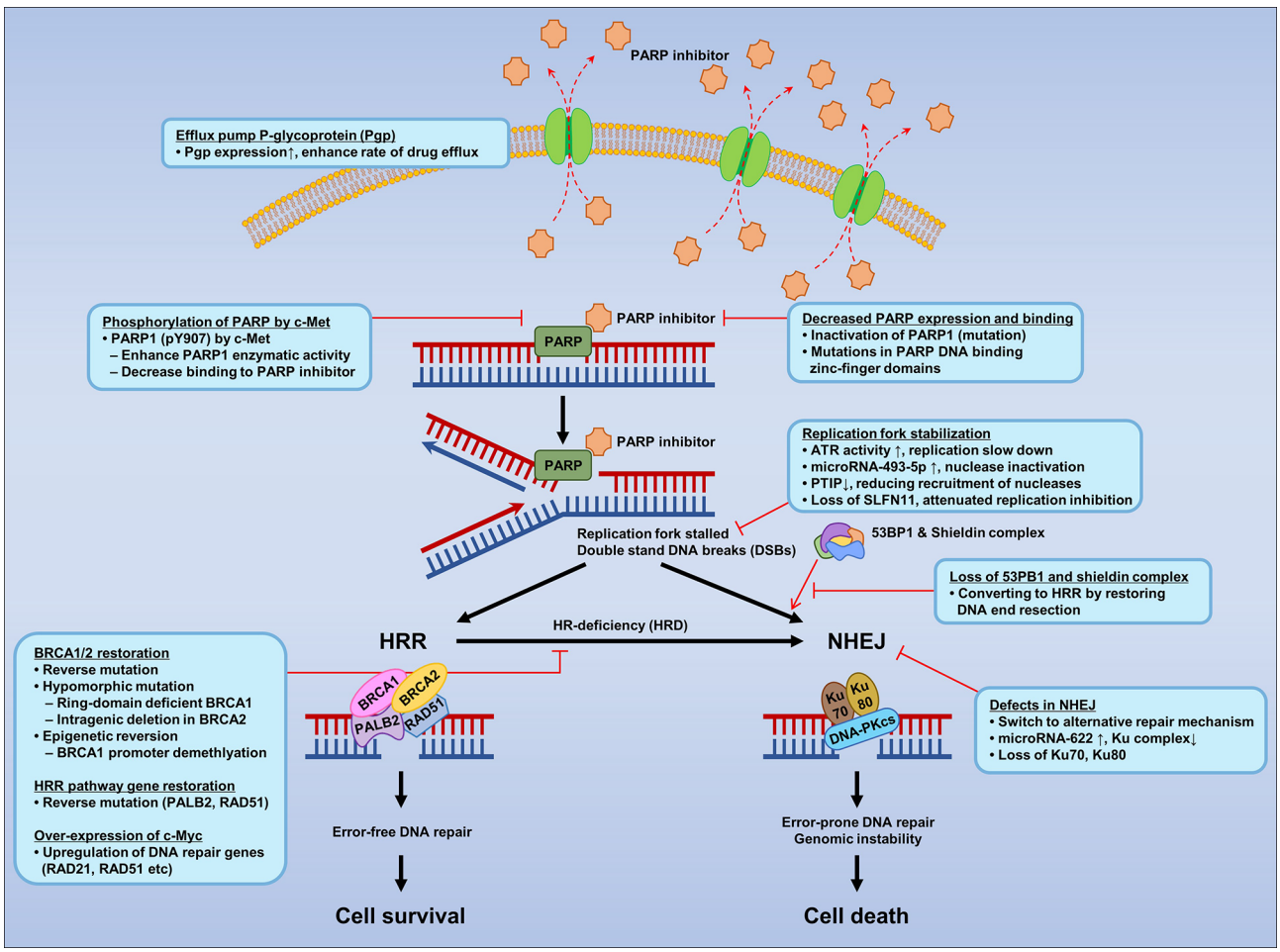

FIGURE 2 | Mechanisms of resistance to PARP inhibitors according to mechanisms of PARPI in DNA repair.

associated with a moderate risk of breast cancer. However, the role of this hypomorphic missense variant in the resistance to PARPIs and cisplatin remains to be investigated (236). Another study identified new BRCA2 isoforms that are expressed in resistant cell lines as a result of intragenic deletion of the c.6174delT mutation and restoration of the open reading frame. Reconstitution of BRCA2-deficient cells with these reverting BRCA2 alleles made resistant to PARPI and HR proficient (237). This finding indicates that resistance to PARPIs can arise by intragenic deletion mutations in BRCA2.

8.3.3 Epigenetic Reversion of Methylated Promoters

Epigenetic silencing of the promoter region of tumor suppressor genes is one of the mechanisms underlying HRD. For instance, *BRCA1* promoter methylation is an important somatic driver in high grade serious ovarian carcinoma (238, 239). A patient with *BRCA1* promoter methylation who was initially sensitive to PARPIs became resistant after loss of *BRCA1* promoter methylation in the relapsed sample, and the gene was expressed at comparable levels to those in HR-proficient tumors (238). In the same study, analysis of the global methylation status of the primary and reverted samples revealed loss of *BRCA1* promoter methylation. Another study showed *BRCA1* promoter demethylation in therapy-resistant patients and patient-derived xenograft (PDX) tumors. Among 103 TNBC patients, 26 (25.24%) showed *BRCA1* promoter methylation before treatment. Of these, 17 showed pathologic complete response and nine showed partial/no response; the three partial responders underwent post-treatment surgery. Post-treatment *BRCA1* promoter methylation was 2.66-fold lower than that in pretreatment samples, and the mRNA expression of *BRCA1* increased by 12–28-fold. Loss of *BRCA1* promoter methylation was observed in 69.6% (16/23) of therapy-resistant PDX tumors with BRCA1 re-expression (240).

8.3.4 Switch to Alternate Repair Mechanisms

As discussed earlier, DNA DSBs in homologous recombination-deficient cancer cells can be repaired by alternative DNA repair mechanisms such as NHEJ and MMEJ. Thus, the shift from the canonical DNA repair mechanism to alternate repair mechanisms can affect the therapeutic efficacy of PARPIs (228). NHEJ functions throughout the cell cycle, and defects in NHEJ contribute to genomic instability and are associated with the development of chemo-resistance. NHEJ is crucial for determining the sensitivity to PARPIs, as confirmed recently (241). For example, ovarian cancer cells with a 40% deficiency in the NHEJ DSB repair pathway are resistant to PARP inhibition irrespective of HR status. Only NHEJ-competent and HRD cells are sensitive to the PARPI rucaparib, confirming the resistance observed in HRD tumors (241). Therefore defects in NHEJ, the lack of error-prone repair results in resistance to PARPIs. The role of NHEJ in PARPI resistance is related to the error proneness of NHEJ. The errors in repair cause lethal defects in DNA, and the absence of HR results in apoptosis, which is required for PARPI sensitivity (242).

8.3.5 Replication Fork Stabilization

HR-deficient cells are susceptible to replication fork degradation and are sensitive to PARPIs. However, cancer cells possess a mechanism to protect against replication fork degradation known as fork stabilization. Replication fork stabilization is a compensatory mechanism that protects the replication fork, which results in PARPI resistance in the absence of HR competency (105). Another mechanism to stabilize the replication fork is ATR activation in response to SSBs. In this mechanism, CHK1 is phosphorylated by ATR, and activated CHK1 phosphorylates WEE1 and inactivates the sCDC25A and CDC25C phosphatases. Activated WEE1 activates CDK1 and CDK2 to promote G1/S and G2/M cell cycle arrest (243, 244). Another mechanism of replication fork protection was identified by Meghani et al. (245). In this mechanism, miR-493-5p overexpression protects the replication fork from nuclease degradation, subsequently inducing PARPI and platinum resistance in *BRCA2*-mutated carcinomas. In addition, Pax2 transactivation domain-interacting protein (PTIP), which forms nuclear foci for DSBs, can destabilize the MRE11 nuclease installed replication forks. By contrast, loss of PTIP stabilizes nascent DNA strands by blocking degradation in *BRCA1/2* deficient cells, a mechanism that rescues the stalled replication fork and causes PARPI and cisplatin resistance (246).

8.3.6 Decreased PARP Expression and Binding

Deletion of PARP1 using CRISPR/Cas9 gene editing tools in two ovarian cancer cells (one with *BRCA1* mutation and one with *BRCA1* promoter methylation) shows >90% reduction of PARP1 expression in *BRCA1* mutant and promoter methylated cells as measured by immunofluorescence and western blot analysis. Therefore, loss of PARP1 by different mechanisms (e.g., mutation) results in resistance to PARPIs (247). Although *BRCA1* mutant and promoter methylated ovarian cancer cells are synthetically lethal with PARPI, the loss of the target (PARP) results in PARPI resistance. Point mutations that interfere with the PARP1 DNA binding zinc-finger domains cause PARPI resistance and affect PARP1 trapping. PARP1 p.R591C mutation (c.1771C>T) was detected in ovarian cancer patient who showed resistant to olaparib (248). Other mutations that occur outside of the zinc-finger domain of PARP1 also reduce PARP trapping.

8.3.7 Efflux Pump P-Glycoprotein

The multidrug efflux pump P-glycoprotein (Pgp) contributes markedly to chemotherapy resistance by increasing rate of drug efflux. Long-term administration of PARPIs causes selective pressure-induced PARPI resistance mediated by the upregulation of a gene-encoding efflux pump. Pgp recognizes and transports a variety of chemical substrates with hydrophobic in nature (249). The expression of abcb1a and abcb1b, encoding murine Pgp were increased by 2 to 15 fold in 73.3% (11/15) of mice treated with AZD2281 (currently, Olaparib) (249). Upregulation of Pgp expression is considered a mechanism of resistance in *BRCA1* and *BRCA2* mutant cancers treated with PARPIs (250). Upregulation of multidrug resistance gene 1 increases the expression of Pgp and the rate of drug efflux, which decreases the therapeutic effect of PARPIs (251).

8.3.8 Loss of End Resection Regulation by 53BP1 and Mutation in the Shieldin Complex

The 53BP1 is an important regulator of the cellular response to DSBs, and it suppresses HR by stimulating NHEJ of the distal DNA end. Deletion of 53BP1 converts processing of damaged DNA ends into recombination of single-stranded DNA competent for HR. Loss of 53BP1 partially restores the error-free HR and reduces the sensitivity of BRCA1 mutant tumors to PARPIs (7, 252). 53BP1 is crucial for the control of DSB repair, as its presence promotes NHEJ and its loss promotes HR. Inhibiting 5′ end resection is necessary for HRD, and 53BP1 uses Rif1 to impair 5′ end resection. Rif1 inhibits end resection by recruiting CtIP, BLM, and Exo1, which restricts buildup of BRCA1/BARD1 complexes at sites of DNA damage. These mechanisms underlie the effect of 53BP1 on inducing chromosomal aberrations in *BRCA1*-deficient cells. Therefore, loss of 53BP1 favors HR and thus leads to PARPI resistance (253, 254).

The 53BP1 effector complex (shieldin) includes SHLD1, SHLD2, SHLD3, and REV7. Shieldin functions as a downstream effector of 53BP1-RIF1 in preventive DNA DSB repair, whereas deletion of the shieldin complex confers resistance to PARPIs in *BRCA1*-deficient cells. Binding of single-stranded DNA by SHLD2 is critical for shieldin function (255). *BRCA1* mutant cancers show minimal resection of DSBs, which renders them deficient in homology-directed repair and sensitive to inhibitors of PARP1. In *BRCA1* mutants, the resection of DSBs is inhibited by 53BP1, RIF1, and the shieldin complex, and loss/deletion of these factors reduces sensitivity to PARP1 inhibitors (256). Mutations in genes that encode shieldin subunits also cause resistance to PARPIs in *BRCA1*-deficient cells and tumors, resulting in restoration of HR (257). Silencing of shieldin components increases end resection, as extreme resection would make DNA ends unsuitable for repair by NHEJ; this may explain the defective NHEJ in shieldin-depleted cells. Downregulation of 53BP1, as well as that of REV7, confers resistance to PARPIs in *BRCA1* mutant cells. An experiment in *BRCA1*-deficient and shieldin complex knockout cells confirmed the important role of the shieldin complex in controlling PARPI (olaparib) sensitivity. *BRCA1*-depleted cells are highly sensitive to olaparib; however, simultaneous depletion of shieldin components rescues cell viability similar to the effects of depletion of BRCA1 and 53BP1 (258). Therefore, loss of 53BP1, RIF1, and shieldin components is sufficient to bypass the HR function of BRCA1 and confer PARPI resistance.

8.3.9 Overexpression of MicroRNAs

Overexpression of miR-622 is implicated in the development of resistance to PARPIs and cisplatin by restoring HR and impairing NHEJ in *BRCA1*-deficient ovarian cancer. miR-622 suppresses NHEJ by downregulating the Ku complex, thus promoting HR-mediated repair of DNA DSBs in the S-phase of the cell cycle. In addition, overexpression of miRNA-622 in HGSOC patients is correlated with worse survival after platinum chemotherapy, associating miRNA-mediated resistance by rescuing HRR (259). Overexpression of miR-493-5p induces resistance to platinum and PARPIs in *BRCA2* mutant patient-derived cells by targeting DNA repair pathways involved in genomic stability. MiR-493-5p induces resistance by downregulating R-loop processing genes, which increases the R-loop and decreases the single-strand repair pathway, and by downregulating nucleases, which protects the replication fork. HRR is not restored in relation to miR-493-5p mediated cisplatin and PARPI resistance. Overexpression of miR-493-5p is negatively correlated with disease-free survival, especially in *BRCA2* mutant patients and specifically in platinum resistant or refractory disease (245).

8.3.10 Phosphorylation of PARP by C-Met

Phosphorylation of PARP1 at Tyr907 by the receptor tyrosine kinase c-Met causes PARPI resistance. The phosphorylation of PARP1 by c-Met (pY907) enhances PARP1 enzymatic activity and decreases binding to PARPI, resulting in resistance of cancer cells to PARPIs. PARPIs and c-Met inhibitors act synergistically in suppressing the growth of breast and lung cancer cells *in vitro* and in a xenograft model. Detection of pY907 is an indicator of PARPI resistance in combination with a poor response to PARPIs and high c-Met expression (260). PARPIs are commonly used for the treatment of ovarian and breast cancers. A recent study investigating the therapeutic efficacy of PARPIs against hepatocellular carcinoma (HCC) showed discouraging results. The mechanisms underlying the poor efficacy of PARPIs in HCC involve the formation of EGFR and MET heterodimer that interacts with and phosphorylates Y907 of PARP1 in the nucleus, which contributes to PARPI resistance. However, inhibition of both c-Met and EGFR sensitizes HCC cells to PARPIs, although both EGFR and c-Met are usually overexpressed in HCC (261). The use of c-Met and EGFR inhibitors in combination with PARPIs is a potential strategy for the treatment of HCC.

8.3.11 Overexpression of C-Myc

Overexpression of c-Myc increases cisplatin and PARPI resistance by reducing the production of the c-Myc inhibitor BIN1 (bridging integrator 1) which restores the intrinsic PARP-1 activity. Suppression of BIN1 releases the automodification domain of PARP1, which increases its intrinsic catalytic activity for DNA repair, thereby increasing resistance to PARPIs and cisplatin. Conversely, inhibition of c-Myc increases BIN1 abundance, which decreases PARP1 activity and reverses cisplatin and PARPI resistance (262). *Myc* amplification is accompanied by the upregulation of several DNA repair genes, including *RAD21*, *RAD54L*, and *RAD51*, in both breast and ovarian cancer. *RAD51* is the third most significant DNA repair gene associated with Myc expression in TNBC tumor samples. c-Myc regulates PARPI resistance by upregulating RAD51 paralogs, which are important in HRR of damaged DNA. A recent study using TNBC cell lines confirmed that PARPI-resistant cells have increased RAD51 foci, whereas PARPI-sensitive cells show impaired RAD51 foci independent of *BRCA* mutation status. In the same study, pharmacological

inhibition of c-Myc by dinaciclib reversed the resistance to PARPIs, confirming the induction of synthetic lethality and the role of c-Myc in drug resistance (263). *RAD51C*-deficient cancer cells are sensitive to the PARPI olaparib and undergo cell death by inducing G2/M cell cycle arrest and apoptosis. By contrast, silencing of *RAD51C* in resistant cancer cell lines increases the sensitivity to olaparib and decreases RAD51 foci (264).

8.3.12 Loss of SLFN11

High SLFN11 expression is associated with the response to DNA-damaging agents and the overall survival of patients with colorectal and ovarian cancer (265). Conversely, SLFN11 inactivation is a determinant of PARPI resistance. Cells that express SLFN11 are more sensitive to talazoparib and olaparib than cells with low SLFN11 expression. Genomic analysis confirmed the high correlation between treatment response and SLFN11, which is considered a biomarker of the response to PARPI treatment (266). PDXs and SCLC cell lines treated with cisplatin/PARPIs show down-regulation of SLFN11 associated with therapeutic resistance. This was confirmed by silencing SLFN11, which reduced the *in vitro* sensitivity to cisplatin and PARPIs as well as drug-induced DNA damage (267). SLFN11 was identified as a relevant predictive biomarker of sensitivity to PARPI monotherapy in SCLC, and loss of SLFN11 confers resistance to PARPIs. SCLC cell lines were treated with the PARPIs olaparib, rucaparib, and veliparib, and gene expression and the HRD genomic scar score were analyzed. SLFN11 was correlated with the response to olaparib, rucaparib, and veliparib treatment but not to the HRD genomic score scar. An *in vivo* PDX model and immunohistochemical staining confirmed that loss of SLNF11 confers resistance to PARPIs (268).

8.3.13 Loss of XRCC5 (Ku80) and XRCC6 (Ku70)

Loss of PARP activity leads to accumulation of SSBs, which are converted to DSBs by the cellular replication and/or transcription machinery. These DSBs can be repaired by HR in BRCA-proficient cells, whereas they accumulate in BRCA-deficient cells leading to cell death. NHEJ is initiated when free DNA ends are bound by XRCC5/Ku80 and XRCC6/Ku70 through the catalytic subunit of DNA-dependent protein kinases (DNA-PKcs). The DNA-PKc complex phosphorylates downstream targets and activates the DNA damage response, thereby initiating NHEJ (269). The NHEJ-mediated repair of DNA DSBs requires the formation of a Ku70/Ku80/DNA-PKc complex at the DSB sites. Simultaneous loss of HR and PARP1 activity results in deregulated/increased NHEJ activity, which increases the activation of DNA-PKcs leading to increased genomic instability (resulting from this error-prone pathway) (270). PARP1 plays a crucial role in suppressing NHEJ, which serves as a target of PARPI-induced lethality in HR-deficient cells. Conversely, inhibition or loss of multiple components of NHEJ such as XRCC5/Ku80, XRCC6/Ku70, and DNA-PK confer HR-deficient cells resistance to PARPIs by reducing NHEJ activity (242, 261). The activity of the error-prone NHEJ DSB repair pathway that causes genomic instability is required for PARPI sensitivity.

8.4 Strategies to Overcome PARP Inhibitor Resistance

Although PARPIs are likely to be beneficial for a large fraction of ovarian and breast cancer patients, the development of PARPI resistance brings challenges to their utility. As mentioned in this review, there are many mechanisms that can reverse HR deficiency to HR proficiency. Many strategies have been designed to reverse PARPI resistance (13, 105). For instance, replication fork stabilization is a compensatory mechanism for PARPI resistance. Cell cycle checkpoint (ATR, CHK1, WEE1) proteins that contribute to replication fork stabilization may be potential targets for combination therapy with PARPI by limiting the time for tumor cells to repair damaged DNA. The three proteins, ATR1, CHK1 and WEE1, play different roles in replication fork stabilization, indicating that different combination regimen may be effective for combating resistance. For example, ATR inhibitor AZD6738 sensitized *BRCA2* mutant, *BRCA2* reversion mutation, and *BRCA1* wild-type ovarian cancer cells to olaparib more effectively than the CHK1 inhibitor MK8776 (271). WEE1 may have a critical role in cell cycle arrest compared to ATR and CHK1 because WEE1 is required to maintain ATR and CHK1 activity (243). WEE1 inhibitor AZD1775 had synergistic effect with olaparib in TNBC cells (272). Even inhibition of ATR, CHK1 and WEE1 proteins effectively abrogated G2 arrest, but not sufficient to overcome PARPI resistance caused by other mechanisms such as HR pathway.

Many strategies have been designed to selectively convert HR-proficient cells to HR-deficient status. The combined use of PARPIs with CDK1 inhibitors induces HRD in HR-proficient cells by inhibiting the phosphorylation of BRCA1 by CDK1. The reduction of CDK1 compromises the capacity of cells to repair DNA using HR because BRCA1-deficient cells do not efficiently form RAD51 foci (an essential component of HRR). In addition to checkpoint activation, CDK1-mediated phosphorylation of BRCA1 is required for HR (273). The PI3K/AKT/mTOR pathway is aberrantly dysregulated in certain cancers such as TNBC; therefore, direct inhibition of the PI3K/AKT/mTOR pathway in combination with PARPIs could be an effective strategy to overcome PARPI resistance. Under normal conditions, PI3K stabilizes and conserves DSB repair by interacting with the HR complex (274). mTOR inhibitors and PARPIs show strong synergism when used in combination, as indicated by the effect of mTOR inhibitors on suppressing HRR in BRCA-proficient TNBC cell lines (275). The combined use of PARPIs with histone deacetylase inhibitors (HDIs) can sensitize cancer cells to PARPIs because HDIs block the deacetylation of heatshock protein 90 (HSP90), which leads to the degradation of several proteins such as BRCA1, RAD52, ATR, and CHK1. Direct inactivation of HSP90 is another approach to the induction of BRCAness (276, 277).

Another mechanism to induce BRCAness is the combined use of PARP and EGFR inhibitors, which alters the DSB repair capacity and activates the intrinsic pathway of apoptosis. *In vitro* and *in vivo* findings show that inhibition of EGFR1 and 2 induces a transient DNA repair deficit and alters the interaction of EGFR

with BRCA1 by increasing cytosolic BRCA1 and EGFR, pulling them away from their nuclear DNA repair substrates (277, 278). Another study showed that ATM depletion sensitizes breast cancer cell lines to the PARPI olaparib (279). PARPIs in combination with androgen receptor inhibitors promote DNA damage-induced cell death, which inhibits prostate cancer cell proliferation and the growth of tumor xenografts in mice, suggesting a potentially effective treatment combination for androgen-expressing breast cancers (280).

Recently, Johnson et al. (281) reported that *BRCA*-mutant TNBC cells with acquired PARPI resistance are resensitized to PARP inhibition by dinaciclib, a potent CDK12 inhibitor that disrupts HR. In *BRCA*-mutated cancer, *de novo* resistance to PARPIs is caused by residual HR. In addition, dinaciclib compromises HR repair and sensitizes *BRCA* wild-type TBNC cells to PARP inhibition. This study also showed that dinaciclib amplifies the response to PARPIs in HR-deficient cancers. MYC inhibitors induce PARPI sensitivity. The downstream oncogenic role of MYC relies on its heterodimerization with the basic loop helix protein MAX, which is essential in causing the transcriptional initiation of targets. For instance, the small molecule 10058-F4 inhibits MYC-MAX binding (282), resulting in the supression of RAD51 in MDA-MB-231 and SUM149 cell lines (263). CDK12 inhibitor dinaciclib which downregulates MYC expression resensitizes PARPI-resistant cells to PARP inhibition when used in combination with niraparib; the synergistic effect was observed in *BRCA* wild-type and mutant TNBC cell lines in associtaion with the down-regulation of the HR gene RAD51 (263). This finding indicates that targeting the c-*Myc* oncogene could be an effective strategy to induce synthetic lethality and reverse PARPI resistance in MYC-driven cancers.

Recent preclinical and clinical studies demonstrated that the efficacy of PARPI could be enhanced in combination with immune checkpoint inhibitors (ICIs) *via* a synergistic effect. In cancers with defective DNA repair, such as HRD, accumulated DNA damage by PARPI leaded to high tumor mutational burden resulting in neoantigen formation and an increased anti-cancer immune response (283, 284). In addition, these DNA damages might increase the exposure of double-strand DNA (dsDNA) in the cytoplasm and activate the stimulator of interferon genes (STING) pathway which upregulates cytokines like type I interferon, thereby promoting immune response and recruiting tumor-infiltrating lymphocytes (TILs), especially $CD8^+$ T cells (285, 286). PARPI also increases PD-L1 expression, a biomarker for ICI response, through the STING pathway (287), the ATM-ATR-CHK1 pathway (288), and inactivation of glycogen synthase kinase 3-beta (GSK3β) (289). Upregulation of PD-L1 may be a resistance mechanism of PARPI. For these reasons, subsequent immune checkpoint blockade could sensitize PARPI-treated tumor regression. Clinical trials investigating combined regimen of PARPI and ICIs such as anti-PD1 (BGB-A317, nivolumab, pembrolizumab, TSR-042), anti-PD-L1 (atezolizumab, avelumab, durvalumab), and anti-CTLA4 (ipilimumab, tremelimumab) demonstrated promising results in patient outcomes in solid tumors (290, 291).

9 CONCLUSION

HRR is the guardian of the genome because of its role in repairing DSBs with high fidelity. Defects in HR due to mutation, LOH, and promoter hypermethylation of certain HR genes result in HRD, which confers sensitivity to DNA-damaging agents and PARPIs. This review demonstrated that HRD is higher in ovarian and breast cancers than in other cancer types such as CRC, PC, NSCLC, and prostate cancer. HRD is not limited to *BRCA1* and *2*, and comprises many DNA repair genes. The fundamental vulnerability of HRD has led to the design of a wide range of HRD-directed therapies. DNA repair targeted therapies exploit DNA repair defects because HR-deficient tumors are intrinsically sensitive to PARPIs. This highlights the concept of synthetic lethality associated with the concurrent inactivation of two or more HRR genes. The use of PARPIs in non-*BRCA* mutation carriers can be expanded to sporadic cancers that display DNA repair defects. PARPIs are essential for the treatment of ovarian and breast cancers. Recently, FDA approved olaparib for the treatment of prostate and pancreatic cancers characterized by HRD. However, the benefits of PARPIs are limited by the development of resistance, especially when used as monotherapy. Many mechanisms of resistance to PARPI have been identified in HR-deficient cancers, which are challenges to overcome. Numerous preclinical and clinical studies revealed that combination therapy of PARPI with targeted chemotherapy or ICIs improved the efficacy by overcoming PARPI resistance. Understanding the mechanisms of PARPIs resistance will be useful for designing strategies to overcome PARPI resistance as summarized in this review. Based on accumulated research, more potential PARPIs and more effective combined regimens targeting HR-deficient cancers would be developed in the future.

AUTHOR CONTRIBUTIONS

NM, collected data, sketched the initial version, and reviewed the final manuscript. HY, reviewed and drafted the final version of the manuscript. YS, conceived the idea, guided the write up, and reviewed the final manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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ALDH1A1 in Cancers: Bidirectional Function, Drug Resistance, and Regulatory Mechanism

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Aldehyde dehydrogenases 1 family member A1(ALDH1A1) gene codes a cytoplasmic enzyme and shows vital physiological and pathophysiological functions in many areas. ALDH1A1 plays important roles in various diseases, especially in cancers. We reviewed and summarized representative correlative studies and found that ALDH1A1 could induce cancers *via* the maintenance of cancer stem cell properties, modification of metabolism, promotion of DNA repair. ALDH1A1 expression is regulated by several epigenetic processes. ALDH1A1 also acted as a tumor suppressor in certain cancers. The detoxification of ALDH1A1 often causes chemotherapy failure. Currently, ALDH1A1-targeted therapy is widely used in cancer treatment, but the mechanism by which ALDH1A1 regulates cancer development is not fully understood. This review will provide insight into the status of ALDH1A1 research and new viewpoint for cancer therapy.

Keywords: aldehyde dehydrogenases, cancer stem cell, carcinogenesis, drug resistance, hepatocellular carcinoma

INTRODUCTION

Aldehyde dehydrogenases (ALHDs) are a group of cytoplasmic enzymes that use nicotinamide adenine dinucleotide (NAD) as a coenzyme to oxidize aldehydes into the corresponding carboxylic acids (1, 2). The human genome is thought to contain 19 functional ALDH genes, including ALDH1 family genes. ALDH1A1 is one of the main members of the ALDH1 family. The ALDH1A1 gene is located in subregion 13 of region 21, on the long arm of human chromosome 9. This gene encodes homotetrameric cytoplasmic proteins in various tissues. ALDH1A1 has a greater affinity for the oxidation of both all-trans and 9-cis-retinal molecules than ALDH1A2 and ALDH1A3 (3). ALDH1A has an NAD+ binding pocket (amino acids 8–135 and 159–270), a catalytic site (amino acids 271–470), and an oligomerization domain (amino acids 140–158 and 486–459). ALDH1A1 can exist in monomeric, dimeric, or tetrameric forms. The tetrameric and monomeric forms of ALDH1A1 are the most abundant. ALDH1A1 exists predominantly as a tetramer at high concentrations but the activity of its monomeric form is the highest (4). According to the GenBank

database (https://www.ncbi.nlm.nih.gov/genbank/), ALDH1A1 was highly expressed in the liver, duodenum, and other tissues.

In the past decade, researchers have found that ALDH1A1 had vital physiological and pathophysiological functions in many systems, such as the central nervous system, as well as inflammatory and metabolic disorders (5–7). ALDH1A1 overexpression has been found to play an important role in obesity, diabetes, and other diseases (8–13). Because the retinoic acid (RA) signaling pathway is involved in the regulation of gene expression in cancer stem cells (CSCs), researchers have focused on the role of ALDH1A1 in cancers worldwide (14–16). Although several reviews on ALDH1A1 are available, no review has been published that discusses the roles of ALDH1A1 in all cancers (6, 17–19) (**Table 1**).

To understand the roles of ALDH1A1 in cancers, we reviewed and summarized representative correlative studies in this article. We summarized the consensus and controversies regarding the functions, regulatory mechanism, diagnostic value, and selective inhibitors of ALDH1A1. Based on the results of our experiments and bioinformatic assay, the potential uses of ALDH1A1 in hepatocellular carcinoma (HCC) will be discussed.

ALDH1A1 OVEREXPRESSION IS AN ONCOGENIC FACTOR IN MOST CANCERS

Since 2010, numerous studies have verified the fact that ALDH1A1 could promote tumor initiation and tumor progression. Several years ago, Yassin et al. had found that ALDH1A1 overexpression could reflect the poor historical subtype and advanced tumor grade in lung cancer patients (38). In a comparative study performed by Cao et al., the ALDH1A1 levels were found to be much higher in non-small cell lung cancer (NSCLC) patients at advanced stages than those with early-stage tumors (26). In contrast, triple−negative cases without ALDH1A1, CD133, and mutant p53 expression in lung adenocarcinomas were shown to have a much better prognosis than other cases (92).

The same is true for cancers of the digestive system. Li et al. found that ALDH1A1 overexpression was significantly associated with larger tumor size, deeper invasion, extensive lymph node metastasis, and advanced stage of gastric cancer. ALDH1A1 could represent an independent prognostic factor for both overall survival (OS) and recurrence-free survival (RFS) (28). ALDH1A1 overexpression was also a poor prognostic indicator of survival in patients with gastric neuroendocrine carcinoma (58). Liu et al. reported that ALDH1A1 overexpression was significantly associated with poorly differentiated histology in gastric cancer (69). Xu et al. found that ALDH1A1 overexpression had the same effect in patients with colorectal cancer as observed in gastric cancer (25, 54, 55). An analysis of the Oncomine database showed that ALDH1A1 was significantly upregulated in HCC tissues, compared to non-tumorous liver tissues (53). Peng et al. demonstrated that rs7852860 variants of the ALDH1A1 gene were associated with susceptibility to anti-tuberculosis drug-induced liver injury (96).

TABLE 1 | Representative Studies of ALDH1A1 in Cancers.

Author	Year	Origin of organ	Relative molecular	Functional role	Ref.
Adam	2012	Brain	–	Tumor-suppressor	(20)
Okudela	2013	Lung	–	Tumor-suppressor	(21)
Wang	2013	Cervix	miR-23b	Oncogenic	(22)
Wilson	2013	Liver	–	Bidirectional	(23)
Patlolla	2013	Lung	–	Oncogenic	(24)
Xu	2014	Colorectum	β-catenin	Oncogenic	(25)
Cao	2014	Lung	–	Oncogenic	(26)
Xing	2014	Thyroid	–	Oncogenic	(27)
Li	2014	Stomach	MMP9	Oncogenic	(28)
Duong	2014	Pancreas	–	Oncogenic	(29)
Pandrangi	2014	Breast	–	Oncogenic	(30)
Liu	2015	Breast	–	Tumor-suppressor	(31)
Sjoüstroüm	2015	Breast	–	Bidirectional	(32)
Gao	2015	Lung	LGR5	Oncogenic	(33)
Hoshino	2015	Pancreas	SMAD4 TGF-β	Oncogenic	(34)
Condello	2015	Ovary	β-catenin EZH2 DDB2	Oncogenic	(35)
Tanaka	2015	Liver	AFP	Tumor-suppressor	(36)
Kesharwani	2015	Breast	–	Oncogenic	(37)
Yassin	2016	Lung	–	Oncogenic	(38)
Erfani	2016	Skin	–	Oncogenic	(39)
Wang	2016	Colorectum	–	Tumor-suppressor	(40)
Kim	2016	Colorectum	–	Bidirectional	(41)
Ma	2016	Lung	NOTCH3	Oncogenic	(42)
Yokoyama	2016	Breast	BRD4	Oncogenic	(43)
Januchowski	2016	Ovary	P-gp BCRP	Ocncogenic	(44)
Croker	2017	Breast	–	Oncogenic	(45)
Kalantari	2017	Prostate	–	Oncogenic	(46)

(Continued)

TABLE 1 | Continued

Author	Year	Origin of organ	Relative molecular	Functional role	Ref.
Sun	2017	Esophagus	CPA4	Oncogenic	(47)
Lu	2017	Esophagus	DDK1 SOX2	Oncogenic	(48)
Yu	2017	Lung	TAZ	Oncogenic	(49)
Wang	2017	Pancreas	AURKA	Oncogenic	(4)
Wang	2017	nasopharynx	β-catenin TCF4 NOR1 AKT GSK-β	Oncogenic	(50)
Duong	2017	Pancreas	NRF2	Oncogenic	(51)
Allison	2017	Breast	CYP2J2	Oncogenic	(52)
Yang	2017	Liver	–	Bidirectional	(53)
van der Waals	2018	Colorectum	–	Oncogenic	(54)
Yang	2018	Colorectum	–	Oncogenic	(55)
Xia	2018	Breast	–	Oncogenic	(56)
Tulake	2018	Cervix	OCT4	Oncogenic	(57)
Ye	2018	Stomach	–	Oncogenic	(58)
Wu	2018	Tonsillar	–	Tumor-suppressor	(59)
Ciccone	2018	Breast	VEGF HIF-1α	Oncogenic	(60)
Cui	2018	Ovary	DDB2 C/EBPβ	Oncogenic	(61)
Zhao	2018	Bladder	YAP	Oncogenic	(62)
Oria	2018	Pancreas	–	Oncogenic	(63)
Wang	2018	Breast	ERα36	Oncogenic	(64)
Kalra	2018	Breast	CYP2C19	Oncogenic	(65)
Roy	2018	Ovary	–	Oncogenic	(66)
Wang	2018	Breast	CXCR4 EpCAM MUC1	Oncogenic	(67)
Kwiatkowska	2018	Skin	–	Oncogenic	(68)
Liu	2019	Stomach	–	Oncogenic	(69)
Wanandi	2019	Breast	–	Oncogenic	(70)
Gong	2019	Stomach	miR-625	Oncogenic	(71)
Świerczewska	2019	Ovary	PTPRK	Oncogenic	(72)
Wang	2019	Lung	–	Oncogenic	(73)
Charkoftaki	2019	Colorectum	–	Oncogenic	(74)
Nwani	2019	Ovary	–	Oncogenic	(75)
Althobiti	2020	Breast	CD44 CD24 TWIST SOX9 EPCAM CD133	Oncogenic	(76)
Nagare	2020	Ovary	CD9 CD24 EPHA1	Oncogenic	(77)
Szafarowski	2020	Head & neck	–	Oncogenic	(78)
Yoshino	2020	Liver	ARID1A	Oncogenic	(79)
Namekawa	2020	Bladder	TUBB3	Oncogeinc	(80)
Jiang	2020	Prostate	RARα Est1	Oncogenic	(81)
Elcheva	2020	Blood	OXB4 MYB	Oncogenic	(82)
Wang	2020	Esophagus	AKT	Oncogenic	(83)
Tieng	2020	Colorectum	–	Oncogene	(84)
Kaipio	2020	Ovary	EGFR PI3K mTOR AURKA	Oncogenic	(85)
Liu	2020	Breast	BRD4	Oncogenic	(86)
Gyan	2021	Breast	CD44 CD24	Oncogenic	(87)
Wang	2021	Esophagus	β-catenin AKT1 Slug c-Myc Vimentin	Oncogenic	(88)
Liu	2021	Breast	TKA1 GM-CSF	Oncogenic	(89)
Narendra	2021	Blood	ZEB2 EZH2 MUC1 miR-16-5p miR-26a-5p	Oncogenic	(90)
Narendra	2021	Blood	ADMET	Oncogenic	(91)
Yamashita	2022	Lung	CD133 p53	Oncogenic	(92)
Nachiyappan	2022	Lung	EHMT1 C/EBPβ	Oncogenic	(93)
Zhou	2022	Bladder	YAP	Oncogenic	(94)
Okamoto	2022	Breast	–	Oncogenic	(95)

Extensive and in-depth studies on ALDH1A1 have been performed in breast cancer patients. First, Croker et al. determined the RNA expression of ALDH1A1 in breast cancer cells and found that ALDH1A1 overexpression contributed functionally the proliferation, adhesion, migration, extravasation, and micrometastasis of breast cancer (45). Althobiti et al. found that ALDH1A1 overexpression was associated with poor prognostic features, including an increased tumor grade, poor Nottingham prognostic index, extensive lymph node metastasis, and a greater extent of luminal B and triple-negative subtypes of breast cancer (76). In the African population, Gyan et al. found that ALDH1A1 was expressed at a high level in 90% of breast cancer specimens. This study further confirmed the increased oncogenicity of the $CD44^+/CD24^-/ALDH1A1^+$ combination phenotype and its association with the increased tumor grade and clinical prognostic stage (87). Xia et al. conducted a population-based study to analyze the relationship between ALDH1A1 polymorphisms, alcohol consumption, and mortality among women diagnosed with breast cancer. They found that after adjusting all the results for multiple comparisons, rs7027604 was significantly associated with all-cause mortality in the rs1424482

CC genotype, and the rs7027604 AA genotype was positively associated with non-breast cancer mortality. Among long-term light drinkers, rs1888202 was associated with decreased all-cause mortality, while the association was not significant among non-drinkers or moderate/heavy drinkers. The increased risk of all-cause mortality associated with rs63319 was limited to women with a low level of native American ancestry (56). Furthermore, Wanandi et al. found that the expression of ALDH1A1 was higher in breast cancer stem cells than in the MCF-7 cell line, but was almost similar to that observed in the more aggressive cell line MDA-MB-231. These results suggested that ALDH1A1 overexpression might be related to the stemness and aggressiveness of breast cancers cells (70).

ALDH1A1 has been reported to promote cancers of the reproductive system. Nagare et al. found that most ALDH1A1-positive high-grade ovarian cancer cells resided in the G1 phase of the cell cycle. They also reported that the ALDH1A1-positive cells co-expressing the combination of CD9, CD24, or EPHA1 were more oncogenic and aggressive than ALDH1A1-negative cells (77). Tulake et al. found that ALDH1A1 and OCT4 were upregulated in both cervical squamous cell carcinoma and cervical intraepithelial neoplasia, as compared to healthy subjects. They found that ALDH1A1 expression levels were also increased in the peripherical blood obtained from cervical cancer patients; thus, ALDH1A1 expression could be regarded as an indicator of cervical cancer (57).

ALDH1A1 is also involved in the development of cancers that originate in other systems. A study involving Iranian prostate cancer patients by Kalantari et al. showed that the level of ALDH1A1 expression was positively correlated with tumor invasiveness (46). Among the most common cancer stem cell markers, only ALDH1A1 overexpression significantly affected the five-year OS of primary head and neck squamous cell carcinoma patients (78). ALDH1A1 levels were also higher in papillary thyroid carcinoma tissues than in normal thyroid tissues. ALDH1A1 overexpression was significantly associated with extrathyroidal extension and reflected a poorer RFS and distant recurrence-free survival (27). ALDH1A1 overexpression was also found to occur in skin cancers, particularly in melanomas (39).

ALDH1A1 ACTS AS A TUMOR SUPPRESSOR IN SOME CANCERS

Although ALDH1A1 is seen as an oncogenic factor, it also exhibits different characteristics in some cancers. In a study performed by Adam et al., ALDH1A1 was co-expressed with GFAP and S100 in mature astrocytes and was a better prognostic marker for glioblastoma patients (20). In an experiment conducted by Wang et al., both cytoplasmic and nuclear expression levels were assessed in epithelial cells. Surprisingly, in the tissue microarray and whole-tissue cohorts, univariate analysis indicated that the cytoplasmic expression of ALDH1A1 cannot be considered a prognostic marker for colorectal cancers. Furthermore, nuclear expression levels of ALDH1A1 were significantly associated with longer disease-specific survival and nuclear expression levels in low-grade adenomas, and were predominantly higher than those in high-grade adenomas, primary colorectal cancer, and the corresponding liver metastases (40). Although ALDH1A1 is ubiquitously expressed in the liver, its function in HCC is still ambiguous. Tanaka et al. found that there was no significant difference in the ALDH1A1 level between HCC and non-cancerous liver tissues. In their study, the group with high ALDH1A1 levels was significantly associated with low serum levels of AFP, a small tumor diameter, low levels of lymphovascular invasion, a more differentiated pathology, and a less advanced stage (36). Yang et al. studied the relationship between ALDH1A1 and HCC using the GEO database and found high ALDH1A1 mRNA expression levels were significantly associated with longer 57–month recurrence-free survivals (53). Okudela et al. also found that the level of ALDH1A1 expression was negatively related to carcinogenesis in NSCLC patients. ALDH1A1 was remarkably downregulated in adenocarcinomas and large cell cancers. Among adenocarcinomas, the downregulation of ALDH1A1 tended to be more significant in high-grade, poorly differentiated tumors, and tumors with a stronger proliferating activity. Moreover, the incidence of this reduction was higher in smokers than in non-smokers (21). Liu et al. suggested that the high level of expression of ALDH1A1 mRNA in tumor tissues may be an independent predictor of favorable triple-negative breast cancer, based on an analysis performed using three databases and meta-analyses (31). Wu et al. reported that ALDH1A1-positive cells were a unique component of the crypt cellular microenvironment and were not stem cells. They also found that NGFR-positive and ALDH1A1-positive cells were lost during tumorigenesis with the expression of LGR5 in the tonsillar crypt niche; this may mark the breakdown of the normal microenvironment (59).

However, several groups of researchers believe that the behavior of ALDH1A1 is complex. In a study performed by Kim et al., ALDH1A1 overexpression decreased the proliferation and invasiveness of colorectal cancer cells, while colorectal cancer liver metastasis was more likely to occur in SW480/ALDH1A1-transfected mice (41). Sjoüstroüm et al. hypothesized that in breast cancer cells, ALDH1A1 overexpression was associated with either a better or a worse prognosis, depending on the cut-off. If weakly stained cells were considered to be positively stained, ALDH1A1 overexpression was associated with a better prognosis in two cohorts. If strongly stained cells were considered to be positively stained, ALDH1A1 overexpression was associated with a worse prognosis in one of the cohorts. In addition, stromal ALDH1A1 staining was associated with improved distant disease-free survival, and gene expression analysis showed that there was a relationship between ALDH1A1 overexpression and a favorable prognosis (32).

THERAPEUTIC FAILURE IN SOME CANCERS IS ATTRIBUTABLE TO ALDH1A1-INDUCED DRUG RESISTANCE

Chemotherapy plays an important role in cancer treatment. However, many factors, including ALDH1A1, can cause chemotherapy failure. A study by Ma et al. showed that cisplatin

induced NOTCH3 expression, and NOTCH3 overexpression was a prognostic factor for shorter progression-free survival and OS in NSCLC patients. They suggested that the chemoresistance of NSCLC patients was attributable to the promotion of ALDH1A1 expression by NOTCH3 and stimulation of autophagy (42). Wang et al. performed a UPLC–MS-based metabolomics analysis and revealed the metabolic dysregulation in lung adenocarcinoma. They found that the metabolic features of lung cancer cells were altered by ALDH1A1 overexpression, and levels of most metabolites, such as glucose-6-phosphate, fructose 1,6-diphosphate, propionyl-CoA, malic acid, phosphatidylcholine, glycerol phosphatidylcholine, GMP, citrulline, and arginine succinic acid were increased. These metabolites were involved in metabolic pathways such as glycolysis, the tricarboxylic acid cycle, glycerophospholipid metabolism, nucleotide metabolism, and the urea cycle. Among these, ALDH1A1 may amplify drug resistance in tumors through nucleotide metabolic pathways (73).

ALDH1A1-induced drug resistance is also common in cancers of the digestive system. Wang et al. found that ALDH1A1 promoted the development of resistance to 5-fluorouracil in esophageal squamous cell carcinoma (83). Oria et al. found that ALDH1A1 reduced the sensitivity of pancreatic cells toward gemcitabine and chemoradiation treatment (63). Fortunately, Duong et al. suggested that a therapeutic strategy involving a combination of dasatinib and gemcitabine might overcome gemcitabine resistance, as it would decrease the level of ALDH1A1 expression in pancreatic cancer. They also found that NRF2 could promote ALDH1A1 expression and the silencing of NRF2 could enhance the anti-proliferative effects of the chemotherapeutic agent 5-fluorouracil in pancreatic cancer cells (29, 51).

ALDH1A1 is highly expressed in breast cancer cells, and the knockdown of ALDH1A1 can significantly sensitize breast cancer cells to chemotherapy and radiotherapy (45). Wang et al. found that tamoxifen could promote ERα36 binding and the activation of estrogen-responsive elements in the ALDH1A1 promoter, to increase the transcription of ALDH1A1, which accounted for the resistance to hormone therapy and metastasis of breast cancer (64). In another study of breast cancer patients on adjuvant therapy, Kalra et al. found that CYP2C19 and ALDH1A1*2 (17 bp deletion), were significantly associated with the disease outcome, including OS, recurrence, and metastasis. Both these genes were involved in the pharmacokinetics of cyclophosphamide. Allison et al. hypothesized that ALDH1A1 was activated in the MDA-MB-468 breast cancer cell line, which stably expressed CYP2J2 and attenuated caspase-3/7 activity and the production of reactive oxygen species induced by cytotoxic agents, such as paclitaxel, doxorubicin, sorafenib, and staurosporin (52).

ALDH1A1-induced drug resistance also seriously hampered ovarian cancer and leukemia treatment. Data from a study performed by Nwani et al. demonstrated that ALDH1A1 was upregulated in ovarian cancer cells that survived exposure to platinum (75). Roy et al. found that ALDH1A1 overexpression was associated with a poor response to platinum-based therapy in patients with high-grade serous ovarian cancer (66). *via* Kaipio et al. found that ALDH1A1 expression was improved after neoadjuvant chemotherapy in high-grade serous ovarian cancers and that in treatment-naive tumors, ALDH1A1 overexpression was correlated with drug resistance and a reduced duration of survival. Notably, they mentioned that EGFR, PI3K-mTOR, and the AURKA inhibitor were toxic to cancer cells in tests that assessed drug sensitivity and resistance (85). Individuals with chronic myelogenous leukemia acquired resistance to cyclophosphamide, owing to the inactivation of its active metabolite aldophosphamide *via* the overexpression of ALDH1A1 (91).

THE REGULATORY MECHANISM OF ALDH1A1 IN CANCERS IS COMPLEX

Several previous studies have reported that many molecules and signal pathways are involved in the mechanism underlying ALDH1A1 regulation in cancers (28, 33, 47, 60, 72) (**Figure 1**). Our review described comprehensively the mechanisms of ALDH1A1 in the different processes of cancers and ALDH1A1 expression regulation. ALDH1A1 could induce cancers *via* the maintenance of CSC properties, modification of metabolism and promotion of DNA repair (43, 80, 81, 86). ALDH1A1 expression is regulated by several epigenetic processes, including, phosphorylation methylation, acetylation, methylation and miRNA modification (34, 71, 82, 90, 91).

CSCs have self-renewal capacity and differentiation potential and contribute to multiple tumor malignancies, such as recurrence, metastasis, heterogeneity, multidrug resistance, and radiation resistance (97). ALDH1A1 is thought to maintain CSC properties in a variety of cancers (49, 88). Yu et al. suggested that ALDH1A1 was a critical gene involved in the mediation of TAZ-induced lung tumorigenesis and CSC phenotypes. They found that TRE1, which was localized in the -256 ~ +52 region of the ALDH1A1 promoter, was majorly responsible for the activation of TAZ (49). Lu et al. suggested that DKK1 maintained the cancer stem-like properties of esophageal cancer cells *via* the ALDH1A1/SOX2 axis (48). Another group of researchers found that ALDH1A1 could also maintain esophageal CSC properties through promoting the stability of β-catenin and activating the AKT signal pathway (83). Wang et al. reported that the ectopic overexpression of NOR1 suppressed ALDH1A1 and β-catenin expression; β-catenin/TCF4 targeted the regulation of ALDH1A1 transcription; and the silencing of ALDH1A1 reduced AKT and GSK-β expression levels and resulted in the feedback inhibition of β-catenin expression. As a result, NOR1 could suppress the tumorigenic properties of CSCs in nasopharyngeal carcinoma *via* this signal circuit (50). The most recent study by Nachiyappan et al. showed that EHMT1 could promote tumor progression and maintain the stemness of alveolar rhabdomyosarcoma *via* the stabilization of C/EBPβ, which could activate the ALDH1A1 promotor (93).

As a metabolic enzyme, the effect of ALDH1A1 on metabolism plays an important role in cancer progression.

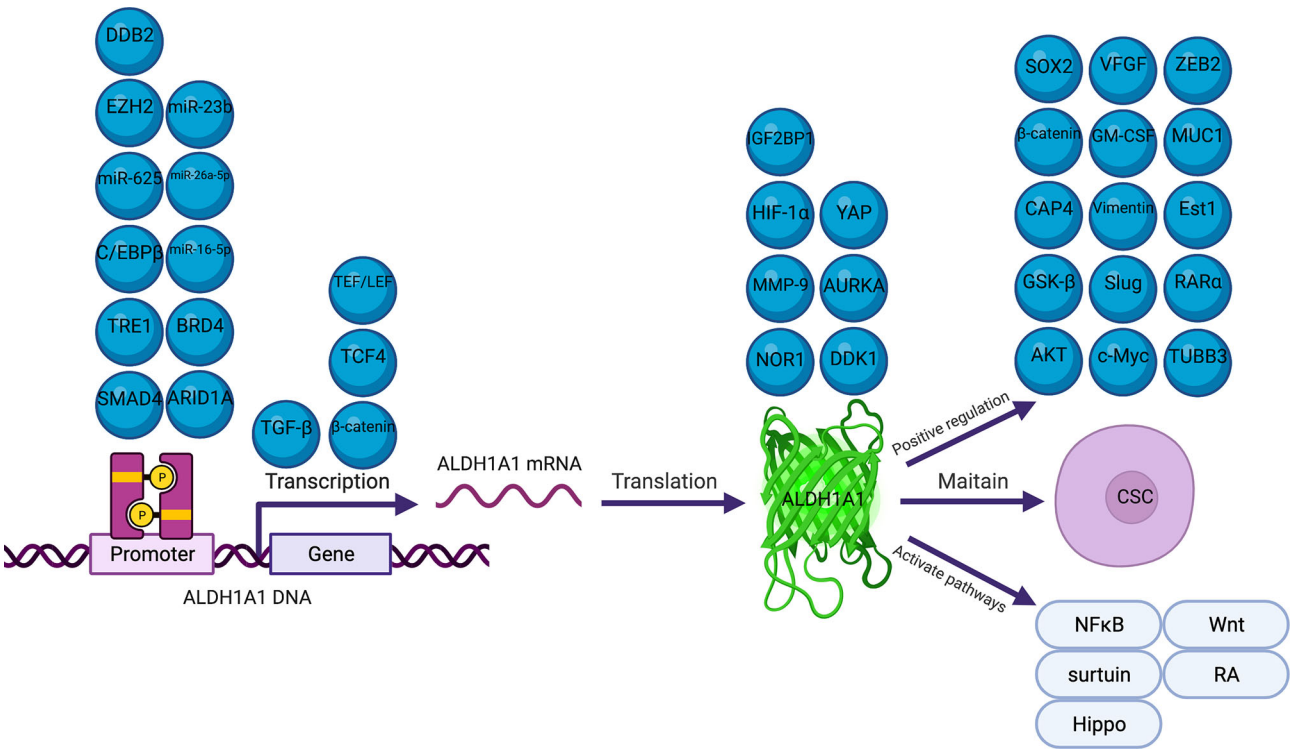


FIGURE 1 | Molecules Involved in the Regulation of ALDH1A1.

Charkoftaki et al. tried to identify the role of ALDH1A1 in lipid metabolism in colon cancer cells using an integrated multi-omics approach. They found that the suppression of ALDH1A1 could downregulate oxidative phosphorylation, mitochondrial function, the sirtuin signaling pathway, and the retinol metabolism pathway. This approach provided greater insights into the pathways through which ALDH1A1 drives the development of cancers (74). Liu et al. made a breakthrough in research regarding the mechanism by which ALDH1A1 initiates breast cancer. They found that ALDH1A1 decreased the intracellular pH in breast cancer cells, in order to promote the phosphorylation of TAK1, activate the NFκB signal pathway, and increase the secretion of GM-CSF, and this led to myeloid-derived suppressor cell expansion and immunosuppression (89).

Phosphorylation is one of the most extensively and diligently studied posttranslational modifications which orchestrates a variety of cellular functions like cell growth, differentiation and apoptosis (98). Wang et al. showed that the phosphorylation-dependent regulation of ALDH1A1 was mediated by AURKA. They found AURKA could phosphorylate ALDH1A1 at the locations T267, T422, T439, at which phosphorylation primarily regulated ALDH1A1 activity. AURKA-mediated phosphorylation could rapidly facilitate the dissociation of tetrameric ALDH1A1 into a highly active monomeric species. Surprisingly, ALDH1A1 also reciprocates and prevents the degradation of AURKA, thereby triggering a positive activation loop that drives highly aggressive phenotypes in pancreatic cancer (4).

Acetylation influence a myriad of cellular and physiological processes, including transcription, phase separation, autophagy, mitosis, differentiation and neural function (99). In a study by Yoshino et al., chromatin immunoprecipitation assay showed that the level of H3K27 acetylation was significantly increased in the promoter region of ALDH1A1, while the HDAC1 level was significantly decreased in the ARID1A knocked-out cholangiocarcinoma cell line. Therefore, ARID1A may function as a tumor suppressor in cholangiocarcinoma through the transcriptional downregulation of ALDH1A1, along with a decrease in the levels of histone H3K27 acetylation (79).

The trimethylation of H3K27 was significantly correlated with the expression of ALDH1A1. In a study regarding ovarian cancer, Condello et al. identified that β-catenin could regulate ALDH1A1 *via* the TEF/LEF transcriptional complex, which was a key element of the Wnt signaling pathway. This mechanism could enhance spheroid formation in ovarian cancer cells. They reported that DDB2 recruited EZH2 to the ALDH1A1 promoter region, thereby facilitating the trimethylation of the local histone H3 at K27, and repressed the transcription of ALDH1A1 (35). Cui et al. also found that DDB2 could bind to the ALDH1A1 gene promoter and facilitate the enrichment of histone H3K27me3, and compete with the transcription factor C/EBPβ for binding to this region, eventually inhibiting the promoter

activity of ALDH1A1. This mechanism involved the repression of ovarian cancer cell dedifferentiation (61).

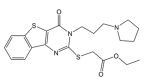
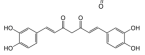
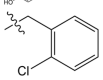
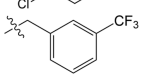
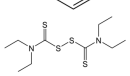
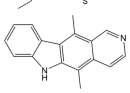
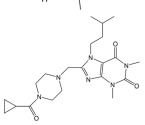
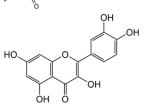
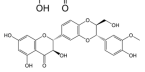
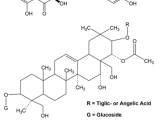
MicroRNAs are small noncoding RNAs that regulate gene expression *via* recognition of cognate sequences and interference of transcriptional, translational or epigenetic processes (100). Bioinformatics analysis identified miR-16-5p and miR-26a-5p to be hub miRNAs for ALDH1A1 (91). The regulatory mechanism of ALDH1A1 was discussed in a study by Wang et al., who found that miR-23b could directly bind to the 3′-UTR region of ALDH1A1, to cause its reduction (22).

ALDH1A1 CAN BE SEEN AS A THERAPEUTIC TARGET FOR CANCERS

Because ALDH1A1 is an oncogenic factor in many cancers, treatments targeting ALDH1A1 have become a research hotspot. During the diagnosis of NSCLC, the combined application of ALDH1A1 and carcinoembryonic antigen can significantly increase the sensitivity, compared to that observed with the use of carcinoembryonic antigen alone (26). Okamoto et al. developed a new long-acting fluorescence probe that could identify breast cancer stem cells *via* the targeting of ALDH1A1 (95).

Many researchers have developed specific ALDH1A1-targeting drugs that could be used to treat cancer (**Table 2**). Some of them believe that the combination of anti-ALDH1A1 therapy and chemotherapy can offset the ALDH1A1-induced drug resistance in cancer patients. Patlolla et al. showed that β-escin could inhibit tobacco carcinogen-induced lung tumor formation by modulating ALDH1A1-positive cells (24). Silybin could efficiently inhibit the proliferation, invasion, and metastasis of prostate cancer cells, by reducing ALDH1A1 expression levels (81). In 2015, Kesharwani et al. reported a new approach for overcoming drug resistance to breast chemotherapy *via* the targeting of synthetic curcumin analogs against ALDH1A (37). Wang et al. found that quercetin could inhibit the proliferation, clonal expansion, and mammsophere formation of CD44+/CD24- breast cancer stem cells by inhibiting ALDH1A1, CXCR4, EpCAM, and MUC1 (67). Pandrangi et al. found that ellipticine, a plant alkaloid, could inhibit mammosphere formation in ALDH1A1 overexpressed breast cancer stem cells, whereas paclitaxel enhanced mammosphere formation in the same cell lines (30). Condello et al. discovered a novel ALDH1A1 small molecule inhibitor named A37, which could moderately sensitize ovarian cancer cells to cisplatin (35). Liu et al. reported that NCT-501, an ALDH1A1 selective inhibitor, could augment the efficacy of olaparib during ovarian cancer treatment (86). Another group of researchers reported that the ALDH1A1 inhibitor disulfiram and chemotherapeutic agent gemcitabine cooperatively inhibited breast tumor growth and tumorigenesis by purging ALDH+ tumor-initiating cells and activating T-cell immunity (89). Recently, benzimidazole derivatives have been found to act as

TABLE 2 | Proven Inhibitors of ALDH1A1.

Name	Structure	MW	IC_{50} (μg)	Cancer cell type	Ref.
A37/CM37		431.6	4.6 ± 0.8	Ovarian cancer	(75, 101)
BDC		No accurate data	No accurate data	Breast cancer	(37)
Benzimidazole derivatives-61		78.34 ± 7.42	10.23 ± 0.28	-	(8)
Benzimidazole derivatives-65		82.07 ± 7.9	0.921 ± 0.19	-	(8)
Disulfiram		296.54	No accurate data	Breast cancer	(89)
Ellipticine		246.31	No accurate data	Breast cancer	(30)
NCT-501		416.52	40	Ovarian cancer	(86)
Quercetin		302.24	No accurate data	Breast cancer	(67)
Silybin		482.44	68	Prostate cancer	(81)
β-Escin		1131.26	No accurate data	Non-small lung cancer	(102)

potent and selective ALDH1A1 inhibitors (8). It has been proven that N42 could also selectively bind to and inhibit ALDH1A1 (9).

Several ALDH1A1-based cancer prevention and treatment measures have been developed. The oligomer-dependent activity of ALDH1A1 signifies that the targeting of its oligomerization state may be an efficient therapeutic approach that could counteract its protective functions in cancer (4). Januchowski et al. found that ATRA treatment could lead to the downregulation of ALDH1A1, P-gp, and BCRP proteins (44). Yokoyama et al. showed that JQ1, one of the bromodomain and extra-terminal inhibitors, suppressed BRD4-mediated ALDH1A1 expression through a super-enhancer element and its associated enhancer RNA. They also found that the combination of JQ1 and cisplatin could improve the survival of mice with ovarian cancer (43). Moreover, an allogeneic, whole-cell, genetically modified therapeutic melanoma vaccine could generate immune responses to ALDH1A1 and improve long-term survival in advanced melanoma patients (68).

CONCLUSIONS AND FUTURE PERSPECTIVES

In this review, we summarize and discuss studies that examined the roles of ALDH1A1 in cancers. Over the past decade, researchers have discovered that ALDH1A1 could induce cancers *via* the maintenance of cancer stem cell properties, modification of metabolism, promotion of DNA repair. ALDH1A1 expression is regulated by several epigenetic processes. ALDH1A1 also acted as a tumor suppressor in certain cancers. ALDH1A1 is highly expressed in the liver, and this is attributable to its bidirectional functions in HCC. The detoxification of ALDH1A1 often causes chemotherapy failure. Currently, ALDH1A1-targeted therapy is widely used in cancer treatment, but the mechanism by which ALDH1A1 regulates cancer development is not fully understood. In our previous studies, we observed that ALDH1A1 expression was significantly improved in HCC and the knockdown of ALDH1A1 weakened the proliferation and invasion of the Huh-7 cell line. Unlike in other cancers, ALDH1A1 did not maintain the properties of liver CSCs (23). Therefore, we believe that ALDH1A1 can induce HCC by mechanisms other than those involving CSCs. In clinical practice, the detoxification of ALDH1A1 may prolong the survival of HCC patients. This hypothesis needs to be tested in future studies. In the future, we will continue to explore the roles of ALDH1A1 in cancers, especially in HCC.

AUTHOR CONTRIBUTIONS

HY and ZH accomplished the manuscript and equally contributed to this study. YNZ and YW designed this study. YZ, RH and ZG provided guidance for this study. All authors contributed to the article and approved the submitted version.

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REVIEW
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ILP-2: A New Bane and Therapeutic Target for Human Cancers

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Inhibitor of apoptosis protein-related-like protein-2 (ILP-2), also known as BIRC-8, is a member of the inhibitor of apoptosis protein (IAPs) family, which mainly encodes the negative regulator of apoptosis. It is selectively overexpressed in a variety of human tumors and can help tumor cells evade apoptosis, promote tumor cell growth, increase tumor cell aggressiveness, and appears to be involved in tumor cell resistance to chemotherapeutic drugs. Several studies have shown that downregulation of ILP-2 expression increases apoptosis, inhibits metastasis, reduces cell growth potential, and sensitizes tumor cells to chemotherapeutic drugs. In addition, ILP-2 inhibits apoptosis in a unique manner; it does not directly inhibit the activity of caspases but induces apoptosis by cooperating with other apoptosis-related proteins. Here, we review the current understanding of the various roles of ILP-2 in the apoptotic cascade and explore the use of interfering ILP-2, and the combination of related anti-tumor agents, as a novel strategy for cancer therapy.

Keywords: ILP-2, IAPS, caspases, apoptosis, migration, tumor therapy

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INTRODUCTION

Cancer is the most important health problem that people in every country will face, and the most important obstacle to increasing human life expectancy (1). Tumor development is often a cytopathic process caused by a combination of high-risk factors. The characteristics of tumor cells are mainly anti-apoptosis, invasion and cell cycle disorders, and the control of this process is mainly caused by the abnormal expression of some key proteins (2). The inhibitor of apoptosis protein family (IAPs) are a class of cellular anti-apoptosis inhibitory proteins with high structural homology, highly conserved gene sequences and similar functional characteristics that have been found in yeast, invertebrates and vertebrates (3). In mammalian cells, the onset of apoptosis is tightly controlled by endogenous IAPs, and dysregulation of IAPs expression results in tumorigenesis and chemoresistance (4). The IAPs have widespread anti-apoptotic potential functions by inhibition caspases activity. Up to now, IAP-family members have been identified in humans cells: c IAP-1 (HIAP-2), c IAP-2 (HIAP-1), XIAP (ILP-1), ILP-2(BIRC-8), ML-IAP (Livin), Survivin and NAIP, and BRUCE (Apo1lon) (5, 6) (**Figure 1**). The members of the IAPs family are characterized by one or more repeats of a highly conserved 70 amino acids domain, termed the baculoviral IAP (BIR) and Ring structures (7). Notably, these structures have very important biological functions (8).

Inhibitor of apoptosis protein-related-like protein 2 (ILP-2), also known as BIRC-8, is a member of the inhibitor of apoptosis protein IAPs family, located on human chromosome 19 q13.3-13.4, and is highly conserved in genes (9). ILP-2 was first identified by RICHTER et al, while studied cDNA from RNA reverse transcription in peripheral blood cells and human genomic DNA for PCR amplification of XIPA gene was first identified as an apoptosis suppressor protein (10). Its coding sequence is very similar to that of XIAP (ILP-1 or BIRC4), with 80% identity and 95% homology at the amino acid level. In analysis the genomic organization of the BIRC4 locus by Lagacé, identified a cross-reactive band encodes a gene expresses a now 2-kb transcript homologous to ILP-1, hence the name ILP-2 (11). Initially, ILP-2 was found to be expressed only in human testicular tissue and later detected in the cytoplasm of lymphocytes. In subsequent studies, it was found that ILP-2 is highly expressed in various tumors such as nasopharyngeal carcinoma, breast cancer, liver cancer, hematological tumors, and neuroblastoma (12, 13). ILP-2 can protect cells from BAX-induced endogenous apoptosis, help tumor cells escape apoptosis, and antagonize clinical chemotherapy (14, 15). It is worth noting that ILP-2 may not operate independently and needs to cooperate with some proteins to jointly exert anti-apoptotic effects (16). At present, these key regulatory proteins are also under continuous exploration. Furthermore, since ILP-2 has multiple leukocyte Ig-like receptors, natural killer cells, ICAMs, and Fc receptors (FcRs), it appears to play a role in immune-related functions (17). This implies that the expression of ILP-2 may be closely related to the occurrence and development of tumors. In a follow-up study, it was found that by interfering with ILP-2 expression in tumor cells induced apoptosis, decreased cell migration and cell growth (18). It is confirmed that the abnormal expression of ILP-2 is closely related to the occurrence and development of tumors, indicating that ILP-2 may be a new target for human tumor therapy, and will be used as a potential new strategy for tumor clinical treatment by interfered with ILP-2 (19). This review would focus on the anti-apoptotic function of ILP-2 protein and its role in cancer development and progression, as well as ILP-2 as a potential target for cancer therapy.

BIOLOGICAL STRUCTURE AND FUNCTION OF ILP-2

Molecular Structure of ILP-2

The cDNA of *ILP-2* contains 1 exon, 2022 bases, and the entire ILP-2 gene is about 2kb (**Figure 2**). Since the coding sequence of ILP-2 is very similar to that of XIAP (ILP-1), about 80% of the sequence is identical to XIAP and about 95% of the amino acids are in the same region as XIAP, so it is called ILP-2 (11). The *ILP-2* gene produces a protein consisting of 236 amino acids with a molecular weight of 27 kDa and contains a BIR 3 domain, which is a baculovirus IAP repeat consisting of 70 amino acids (20). And also contains a UBA domain for ubiquitin binding and a RING domain with E3 ligase function (21), RING domain is a specific ring finger structure composed of 7 Cys and one His bound to 2 zinc ions (22).

Biological Functions of ILP-2

Both the N-terminal and C-terminal regions of ILP-2 protein have extremely important biological functions (23). The N-terminal domain of ILP-2, BIR 3 can specifically bind to caspase-9 to form a heterodimer, which inhibits the cleavage of the substrate by caspase-9, thereby preventing the occurrence of apoptosis; however, due to ILP Proline (Pro) 257/260 in the N-terminal domain of the -2 protein is easily mutated into Ala, and the lack of an amino acid sequence linked to BIR-3 leads to poor molecular conformational stability, so a small amount of ILP-2 The expression does not have obvious inhibitory effect on cell apoptosis (24). Only when it is overexpressed in cells or tissues and further inhibits the signaling pathway of Bax or apoptosis-promoting factor Apaf-1/caspase-9, it can exert its anti-apoptotic effect. The C-terminal domain of ILP-2 is a specific ring finger

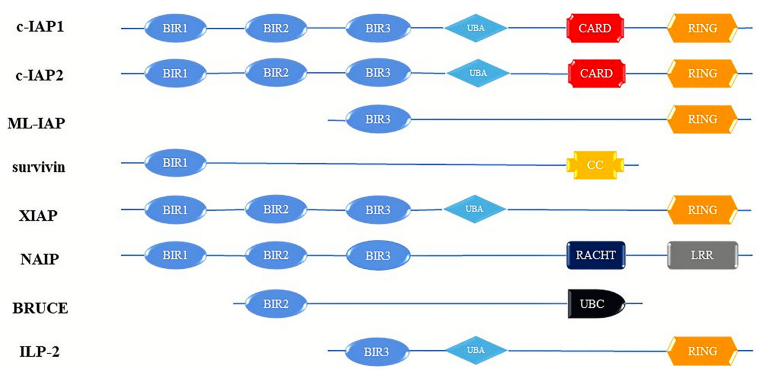

FIGURE 1 | Schematic diagram of domain structure of human IAP proteins. BIR, baculovirus IAP repeats; CARD, caspase activating and recruitment domain; RING, ring zinc-finger. c-IAP1, cellular Inhibitor of apoptosis protein; c-IAP2, cellular Inhibitor of apoptosis protein; ML-IAP, livin/melanoma-Inhibitor of apoptosis protein; XIAP, Survivin, X-linked Inhibitor of apoptosis protein; NAIP, Neuronal apoptosis inhibitory protein; BRUCE, Baculoviral IAP repeat-containing ubiquitin-conjugating enzym; ILP-2, Inhibitor of apoptosis protein-like protein-2.

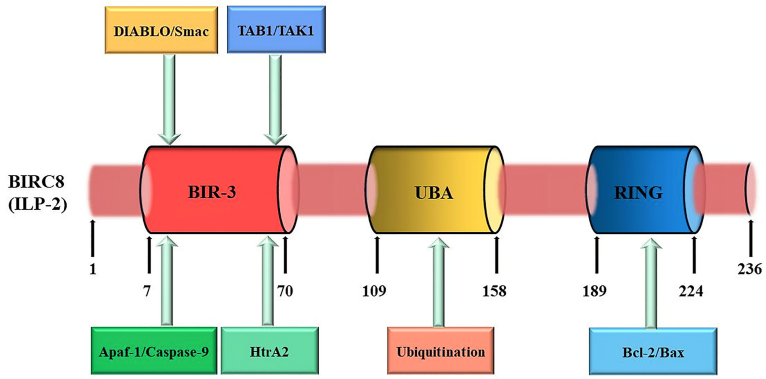

FIGURE 2 | ILP-2 protein structure and function. ILP-2 (also kwon as BIRC8) protein structure presents 236 amino acids and is composed of a Baculovirus IAP Repeat (BIR) domain, an Ubiquitin-associated (UBA) domain, and a RING finger domain. Each structural domain of ILP-2 is capable of binding to the corresponding protein to perform the relevant function. The numbers refer to amino acids.

structure formed by 7 cysteines (Cys) and 1 histidine (His) combined with 2 zinc ions (25). It can induce the ubiquitination of itself and target proteins (caspase and Smac) by the activity of ligase E3, it can also cooperate with the caspase activating and recruitment (CARD) domain to mediate the ubiquitination of other proteins involved in cell death (26). ILP-2 specifically binds apoptosis-associated proteins Caspases and Smac (Second mitochondria-derived activator of caspase) through C-terminal ubiquitination, and then activates the ubiquitination pathway to degrade caspases and Smac in the cytoplasm to reduce their relative levels in the cytoplasm, resulting in their inability to express relevant activities and thus inhibit apoptosis (27). In addition, ILP-2 has multiple leukocyte Ig-like receptors, natural killer cells, ICAMs (Intercellular adhesion molecules), and Fc receptors (FcRs) in its protein structure, which appears to play a role in immune-related functions (28).

MECHANISM OF ILP-2 ANTI-APOPTOSIS

Apoptosis is a way of programmed cell death controlled by proteins, with a high degree of autonomy and order. For mammals, apoptosis is of great significance. In normal cells, the organism uses apoptosis to remove cells under adverse conditions, such as senescent damage or genetic mutations, and to prevent excessive cell proliferation. This is important for the proliferation and differentiation of the organism and for maintaining the homeostasis of the internal environment (29). When apoptotic signaling is inhibited and mutations occur, cells will not be able to recognize and remove damaged and mutated cells, resulting in abnormal cell proliferation and eventually tumorigenesis (30). To date, only two classical apoptotic pathways have been elucidated in detail in mammalian cells. One is the extrinsic pathway, primarily triggered by the Fas death receptor, a member of the TNF (Tumor necrosis factor) receptor superfamily (31, 32). The other, the intrinsic pathway, involves the involvement of mitochondria, which respond to various noxious stimuli and release caspase-activating proteins to trigger apoptosis (33). Although generally considered to be separate pathways and capable of acting independently, cross-talk between these pathways can occur, eventually converging on downstream effector caspases (34).

At present, it has been demonstrated in humans and Drosophila that some members of the IAPs family can directly bind and inhibit caspases through their BIR domains, thereby achieving the purpose of anti-apoptosis (35). For example, in the human X chromosome, XIAP proteins bind and inhibit caspase-9 activation *via* the BIR3 domain, and at the same time combine the intramolecular region between BIR1 and BIR2 to antagonize caspase-3 and caspase-7, thereby inhibiting apoptosis (36, 37). XIAP is the only mammalian IAP that has been shown to directly inhibit caspase activity. ILP-2 as a member of the IAPs family, shares great structural similarity with XIAP, which also contains a BIR3 domain and therefore considered to be an apoptosis inhibitory protein that directly binds and inhibits caspases (38). However, it has been reported that the inhibitory effect of ILP-2 on caspase-3 and caspase-9 is much weaker than that of XIAP. Currently, the mechanism of ILP-2 anti-apoptosis is quite controversial. It has been demonstrated that although ILP-2 contains all the surface elements required for caspase inhibition, its BIR domain is inherently unstable due to the natural truncation of the amino terminus and cannot act directly as a caspase inhibitor. ILP-2 requires some binding partners to stabilize its polypeptide structure, which in turn facilitates the caspase inhibitory effect of ILP-2 (39). One possibility is that ILP-2 binds to pro-apoptotic molecules, including caspases and Smac, through their IBM (Inhibitor of apoptosis protein binding motifs) interactions and degrades them *via* the ubiquitinated proteasome pathway (40). And these are achieved through their own RING structural domains or by forming complexes with other RING-containing IAPs (41). It is thus clear that ILP-2, as an inhibitor of apoptosis, has not a direct but an indirect anti-apoptotic effect on cells, requiring

interaction with some proteins to exercise its anti-apoptotic effect. In summary, although ILP-2 contains a BIR domain, it is a poor inhibitor of caspases. ILP-2 has little direct effect on endogenous caspase activity, and the antiapoptotic effect of ILP-2 may be due to its antagonism of XIAP-Smac interaction rather than direct inhibition of caspases-9 (42). Recent studies suggest that ILP-2 may have a unique mechanism to inhibit caspases and requires some intermediate protein complex to facilitate the association between caspases and ILP-2 (**Figure 3**).

ILP-2 Regulates Bcl-2 to Block Bax Pathway-Induced Apoptosis

Studies have shown that ILP-2 cannot inhibit apoptosis induced by Fas or TNF, but can effectively inhibit apoptosis induced by Bax (Bcl-2 associated X protein) pathway (43). In tumor cells, ILP-2 can inhibit apoptosis by regulating the endogenous pathway of apoptosis mediated by Bcl-2/Bax. ILP-2 can inhibit apoptosis by inducing the expression of Bcl-2 and inhibiting the production of Bax. The Bcl-2 protein family is composed of anti-apoptotic proteins (Bcl-2, Bcl-XL, etc.) and pro-apoptotic proteins (Bax, Bad, Bid, etc.) (44). This family of proteins usually functions as heterodimers or homodimers. When cells are stimulated by various intracellular and extracellular signals, the relative ratio between intracellular Bcl-2 and Bax determines whether apoptosis occurs or not. If the ratio of Bcl-2/Bax increases, the number of homodimers Bcl-2/Bcl-2 increases and apoptosis is inhibited. On the contrary, if the ratio of Bcl-2/Bax decreases, Bax/Bax homodimers are formed and apoptosis is promoted (45). It was found that ILP-2 could inhibit Bax production by promoting Bcl-2 expression, resulting in an increase in the Bcl-2/Bax ratio in cells, which in turn inhibited Bax pathway-induced apoptosis. More interestingly, ILP-2 can also inhibit the release of Cyt-c by inducing high expression of Bcl-2, preventing its entry into the cytoplasm, which in turn inhibits the binding of ATP/d ATP and inhibits the formation of apoptotic bodies (46, 47). This inhibits the activity and expression of caspase-9, resulting in the inability of caspase-9 to further cleave and activate the apoptosis effector caspase-3, thus achieving the goal of inhibiting apoptosis. Moreover, related studies have shown that ILP-2 can also interact with caspase-9 to form a heterodimer, which leads to the loss of its catalytic activity and the inability to cleave the effector caspase-3 zymogen to produce active caspase-3, thereby inhibiting apoptosis (48). However, it is still controversial whether ILP-2 can directly act on caspase-9 to inhibit apoptosis.

Bcl-2-like protein 1 (BCL2L1) is a member of the Bcl-2 family; it inhibits the activation of caspases and is a potent inhibitor of cell death (49). BCL2L1 can participate in calcium signaling regulation by binding to voltage-dependent anion channels (VDAC), reducing mitochondrial Ca2+ uptake and preventing the release of caspase activators from the mitochondrial membrane, thereby inhibiting cell death (50, 51). The apoptosis inhibitory protein ILP-2 is known to regulate apoptosis by controlling the expression of BCL2L1 and affecting the maintenance of mitochondrial morphology. Interestingly, BCL2L1 can also bind the tumor suppressor Beclin 1 to affect autophagy (52–54). Thus, ILP-2 regulates autophagy and affects apoptosis through a number of signaling pathways. Overexpression of BCL2L1 has been shown to protect endothelial cells from TNF-mediated apoptosis and inhibit NF-κB activation, thereby suppressing the upregulation of pro-inflammatory genes to participate in the inflammatory response (55, 56). In contrast, the protein of ILP-2 is closely linked to immune function as well as the development of inflammation, and thus its possible pathway is ILP-2 through promoting the expression of BCL2L1.

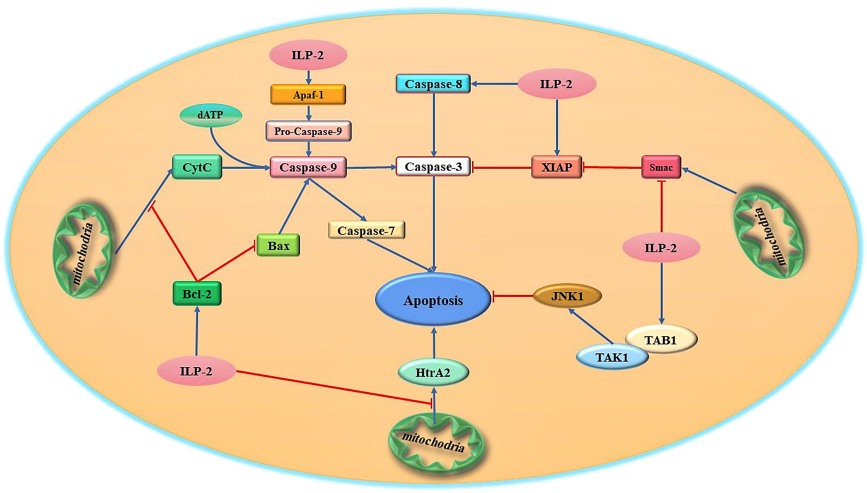

FIGURE 3 | Schematic diagram of the major apoptotic signaling pathways related to ILP-2. ILP-2, which is highly expressed in tumor cells, is one of the high-risk factors for tumor discovery and development, and can activate multiple apoptosis-inhibiting signaling pathways and regulate the expression and activity of various apoptosis-related proteins to inhibit apoptosis.

ILP-2 Inhibits Apaf-1/Caspase-9-Mediated Apoptosis Pathway

At present, studied have shown that ILP-2 protein can effectively inhibit apoptosis caused by (apoptotic protease activating factor-1) Apaf-1/caspase-9 pathway (10). Caspase-9 activation is achieved by the oligomerization of ILP-2 by the apoptotic proponent Apaf-1 in the presence of Cyt-c (cytochrome c). Cyt-c is a multifunctional protein produced by mitochondria and has the function of promoting apoptosis. In normal tissue cells, when cells receive apoptotic information, Cyt-c will be released from mitochondria into the cytoplasm if irreparable damage to DNA occurs, and binds to another apoptotic factor Apaf-1 to induce the onset of apoptosis (57). After cells receive apoptotic signals, Cyt-c is produced by mitochondria and released into the cytoplasm, where it interacts with Apaf-1 to enhance binding to ATP/d ATP, which in turn multimerizes the CARD domain of Apaf-1 protein to form a complex of protein and nucleotide containing 7-axis symmetry (apoptosome) (58). Apaf-1 is structurally altered in the apoptosome, recruits caspase-9 and causes caspase-9 to be cleaved into two segments for activation. The activated caspase-9 further cleaves and activates the activity of the apoptosis effector enzyme caspase-3, thereby promoting apoptosis. The Bcl-2 protein family plays an important role in maintaining the integrity of the mitochondrial membrane (59, 60). Studies have shown that overexpressed ILP-2 can effectively inhibit the Bax-induced apoptosis pathway and inhibit the release of Cyt-c from mitochondria. After ILP-2 binds to Apaf-1, Apaf-1 cannot recruit caspase-9, thereby inhibiting apoptosis induced by Apaf-1/caspase-9 pathway (61).

ILP-2 Binding to Smac Inhibits Apoptosis

In the endogenous pathway of apoptosis, mitochondria are at the center (62). When cells are stimulated by internal apoptotic signals, they release relevant apoptotic factors to the cytoplasm by altering the molecular structure of the mitochondrial membrane and its permeability, thus affecting the occurrence of apoptosis (63). Among them, Cyt-c and Smac (second mitochondria derived activator of caspase) are represented. Smac, also known as DIABLO (direct IAP binding protein whit low pI), is a mitochondria-derived activator of caspase (64, 65). It is also a pro-apoptotic protein produced by mitochondria that contains IAP structural domains (IBMs) and is usually found in the membrane gap of mitochondria (66). It is released from mitochondria and binds to IAP, releasing caspases closed by IAP, relieving the inhibitory effect of IAP on caspases, activating the cascade response of caspases and promoting apoptosis (67, 68). Cyt-c and Smac are considered to be endogenous apoptosis activators (69). Smac is released from the mitochondria during apoptosis together with Cyt-c and Smac can further act on caspase-9 activity in the cytoplasm, resulting in the inability of caspase-9 to activate caspase-7/3 activity (70). The amount of Cyt-c and Smac in the cytoplasm is positively correlated with apoptosis is positively correlated. The dual requirement of both ensures that the caspases cascade is activated only when the signal is sufficient (71).

Studies have reported that Smac can bind to the BIR structural domain and deprive IAPs of their inhibitory effects through physical interactions (72). ILP-2 has a high affinity for Smac, but is significantly inferior to XIAP in inhibiting caspases (73). The highly active interaction of ILP-2 with Smac is similar to that of XIAP, and this particular interaction can be completely abolished by single amino acid mutation in the amino-terminal sequence of Smac or in the BIR domain of ILP-2 (74). The study reports that the high affinity of ILP-2 for Smac would effectively compete with the XIAP-Smac interaction and regulate apoptosis by sequestering Smac and preventing its resistance to XIAP-mediated inhibition of caspases (75). Thus, ILP-2 may become an inhibitor of apoptosis rather than a direct suppressor of caspases. This study further identifies the structural basis for this difference and shows that substitution of just three residues increases the caspase-9 inhibitory activity of ILP-2 to similar levels as observed in XIAP (76, 77). Therefore, ILP-2 mainly contributes to the inhibition of caspase by blocking the ability of Smac to disrupt the XIAP-caspase interaction. Recently, studies have reported that highly expressed ILP-2 can neutralize the pro-apoptotic effect of Smac in tumor cells (78). The highly expressed ILP-2 in the cytoplasm will produce a large amount of anti-apoptotic information, resulting in changes in the conformation of mitochondrial membrane molecules, and a large amount of ILP-2 specifically binds to Smac, so that Smac cannot release the blocking effect of caspase-IAP, thereby antagonizing Smac pro-apoptotic effect (79). Moreover, ILP-2 can also reduce the relative amount of free Smac in the cytoplasm by activating the ubiquitination pathway, and the low level of Smac in the cytoplasm cannot express its caspase protein kinase activity to activate related caspases, which leads to the inhibition of apoptotic signal generation and thus inhibits apoptosis (80, 81). In addition, ILP-2 is able to further bind activated caspases, which in turn inhibits the activity of caspases, thus achieving inhibition of apoptosis.

ILP-2 Cooperates With TAB1 to Inhibit Apoptosis

IAPs seem to have a feedback effect whereby a single protein can achieve the same purpose through different biochemical mechanisms (82). Although we know that most studies on ILP-2 have focused on its role as a caspase inhibitor, there is growing evidence that it also achieves this through other mechanisms (83). It is now accepted that the selective activation of the mitogen-activated protein kinase Jun NH2-terminal kinase 1 (JNK1) is the case for XIAP as well as ILP-2 (84). Studies have shown that ILP-2 can activate JNK1 by activating TAB1, and activated JNK1 is necessary to protect cells from apoptosis induced by TNF-α and interleukin converting enzyme (85). Although it is not clear what role the BIR domain of ILP-2 plays in the mitogen-activated protein kinase pathway, these results suggest that the mechanism of BIR

domain function may also differ among IAP family members in response to different signaling molecules. In this way, it is likely that ILP-2 mediates the activation of JNK1 through the TAK1 (transforming growth factor protein kinase 1)/TAB1 (TAK1-binding protein) signaling cascade, thereby inhibiting apoptosis (86). Importantly, TAK1 and TAB1/2 act as important regulators in pyroptosis and necroptosis, which implies that ILP-2 may have a regulatory role in the immune inflammatory response induced by necroptosis and pyroptosis. Further studies of these phenomena may provide important information for disease treatment.

ILP-2 Antagonizes HTRA2 to Inhibit Apoptosis

Mitochondrial serine protease (Omi/HtrA2), released from mitochondria into cytosol during apoptosis (87). In normal cells, HtrA2 can inhibit the activity of inhibitors of apoptosis IAPs and promote caspase-independent cell death by directly binding to the BIR domain of apoptosis protein inhibitors IAPs (88). Omi/HtrA2 can directly cleave various IAPs *in vitro* and the efficiency of cleavage is determined by its IAP-binding motif AVPS (89). Cleavage of IAPs such as ILP-2 greatly reduces their ability to inhibit and ubiquitinate caspases. Compared to the stoichiometric anti-IAP activity of Smac/DIABLO, cleavage of ILP-2 by Omi/HtrA2 was catalytic and irreversible, resulting in more efficient inactivation of IAPs and promotion of caspase activity (90, 91). In conclusion, these results suggest that, unlike Smac/DIABLO, catalytic cleavage of IAPs by Omi/HtrA2 is a key mechanism for its irreversible inactivation of IAPs and promotion of apoptosis (92). Importantly, when ILP-2 is mutated or dysregulated in expression, ILP-2 can inhibit the release of HtrA2 from mitochondria in multiple ways, antagonizing the inhibitory effect of HtrA2 on IAPs proteins and thus inhibiting apoptosis (93). However, this pathway needs to be further explored.

EFFECTS OF ILP-2 ON TUMOR CELLS GROWTH, MIGRATION AND INVASION

ILP-2 Cooperates With HOXD8 to Affect Tumor Cell Growth and Invasion

It is known that some HOX (homeobox) genes play an important role in a variety of tumor diseases (94). Human Hox genes can be divided into four gene clusters, namely HOXA, HOXB, HOXC and HOXD, which exist on different chromosomes. In addition, these genes can be divided into 13 paralogous families (paralogous families), represented by numbers, HOXD8 is a member of the HOXD family. Studies have shown that HOXD8 has an important regulatory role in tumorigenesis and progression (95). Studies have reported that HOXD8 can bind to the ILP-2 promoter and regulate the expression of ILP-2 (96). When the expression of ILP-2 increased, it inhibited the expression of HOXD8 through a negative feedback pathway. It has been reported that ILP-2, which is highly expressed in breast cancer, can inhibit the expression of HOXD8 in breast cancer tissues and cell lines through certain pathways. When the expression of HOXD8 is inhibited, it can further activate the PI3K/Akt signaling pathway to promote cancer cells. proliferation, invasion and migration (97). Furthermore, ILP-2 knockout could reverse the effects of HOXD8 knockdown on breast cancer cell proliferation, invasion, and migration. Thus, it was confirmed that the synergistic effect between the two has an important regulatory role for tumor cells in growth and invasion (**Figure 4**).

Other Pathways by Which ILP-2 Promotes Tumor Cell Proliferation and Migration

One of the most remarkable characteristics of tumor cells is their high invasiveness and their ability to escape the pro-apoptotic effects of various tumor drugs by metastasizing to other tissues through various pathways (98). The invasive ability of tumor cells is

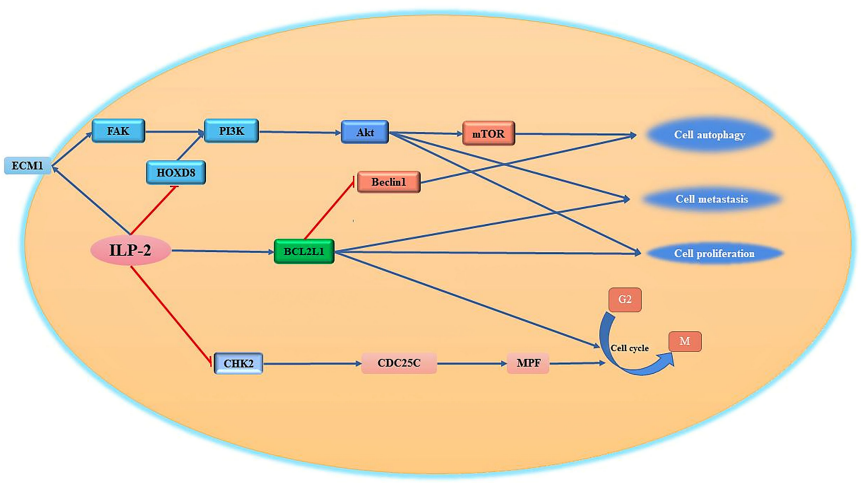

FIGURE 4 | Regulation of cell proliferation, migration and cell cycle by ILP-2. ILP-2 activates Bcl-2, ECM1-Akt signaling pathway, inhibits CHK2 expression and promotes cell proliferation, autophagy, migration as well as cell cycle transition.

closely related to the tumor microenvironment composed of extracellular matrix (ECM) (99). The extracellular matrix can regulate cell adhesion, growth, proliferation and differentiation through various signaling pathways (100). At present, it has been reported that the apoptosis inhibitory protein ILP-2 interacts with extracellular matrix protein 1 (ECM1) to regulate cell growth and cell migration (101). ECM1 can bind to integrins on the cell surface and then bind to the N-terminal domain of focal adhesion kinase (FAK) to activate FAK, which can inhibit tumor cell apoptosis by mediating the Akt pathway, promote tumor cell surface adhesion, and activate phosphatidylinositol-3-kinase (PI3K) (102–104). It was reported that the high expression of ILP-2 in breast cancer cells further activates FAK activity by promoting cellular secretion of ECM1, and the activated FAK further activates downstream protein kinase PI3K activity through the signaling pathway, and the activated PI3K further transmits external signals to the cell interior through the second messenger to further transmit signals to the downstream pathway, activating Akt to regulate cell proliferation, differentiation and metastasis (105, 106) (**Figure 4**).

BCL2L1 is a regulator of the G2 checkpoint and cytokinesis during mitosis; it has an important regulatory role in cell proliferation (107). ILP-2 can promote the high expression of BCL2L1, activate the Bcl2L1 signaling pathway, and then promote cell proliferation. Importantly, ILP-2 has been shown to promote epithelial-mesenchymal transition, migration, invasion and proliferation in a variety of tumor cells. Cell cycle checkpoint protein kinase 2 (CHK2) is an important checkpoint in the cell cycle (108). It is a tumor suppressor gene or tumor-suppressor gene similar to P53 (109, 110). It is a negative regulator in the process of normal cell proliferation (111). At the checkpoint of the cycle, it can prevent the cycle transition or promote cell apoptosis (112). If the tumor suppressor gene is mutated or loses its function, it will cause the cell cycle to go out of control and over-proliferate, eventually causing the cell to become cancerous. It has been reported that increased expression of ILP-2 prevents the inhibitory activity of CHK2 on CDC25C (113), thereby promoting the transition of the tumor cell cycle from G2 to M phase and supporting tumor cell proliferation (114, 115).

In addition, ILP-2 may also regulate the tumor microenvironment by activating ECM1, which is a major component of the extracellular microenvironment, and ILP-2 may promote its expression in tumor cells by activating ECM1, thus helping tumor cells to detach from intercellular adhesion and promoting tumor cell invasion. mediated pathways, activating NF-κB and TNF signaling pathways, linking the tumor microenvironment with tumor cells, and playing an important regulatory role in the overall tumorigenesis and development. However, the effects of ILP-2 on the tumor microenvironment are still poorly understood and need to be explored more deeply.

THE ASSOCIATION BETWEEN ILP-2 AND TUMOR IMMUNE INFLAMMATION

Inflammation, one of the several features of tumors. Non-controllable inflammation is closely related to tumorigenesis and progression, as well as invasion and metastasis. In addition, with the help of certain factors, tumor cells are able to evade immune surveillance and thus maintain their survival. ILP-2 protein, in addition to its classical anti-apoptotic role, seems to be involved in the regulation of tumor immunity and inflammation and other related functions. ILP-2 belong to immunoglobin superfamily acts as a genetic home, having many leukocyte Ig-like receptors, killer cells, several ICAMs and PSG (pregnancy-specific glycoprotein) family and Fc receptors (FcRs) (17). This family of proteins can regulate cellular physiological activity by modulating T-cell receptor signaling as well as interleukin-mediated immune inflammatory pathways. ILP-2 can also promote ubiquitination and degradation pathways of the proteasome itself with several binding ligands through its E3 ubiquitination ligase function, including NF-κB, RIPK1 (Receptor-interacting The kinase activity of RIPK1 is essential for a variety of complexes that regulate inflammation (complex I), apoptosis (complex IIa), and necroptosis (complex IIb). phosphorylation at the Ser320 locus, which fails to inhibit the caspase-8-mediated pyroptosis and necroptosis pathways, thereby promoting tumor cell growth and migration (10, 21). Importantly, ILP-2 may also promote tumor cell development and progression by regulating immune and related inflammatory responses through regulation of Smac, BCL2L1 or TAB1-mediated pyroptosis or necroptosis pathways (32). However. The mechanisms by which ILP-2 regulates tumor immunity and inflammation are unclear and need to be further explored.

EXPRESSION OF ILP-2 IN NORMAL TISSUES AND TUMORS

ILP-2 is undetectable in most normal tissues except testis, spinal cord and lymph nodes, but is present in transformed cells and some cancers including breast, liver, nasopharyngeal, neuroblastoma and hematologic tumors (116). Especially in breast cancer, nasopharyngeal cancer, hematological tumor and liver cancer cells showed overexpression of ILP-2 mRNA. In a comparison of serum samples from breast cancer patients with healthy women, women with mastopexy, women with other types of cancer or women after breast cancer surgery, ILP-2 expression was found to be increased in breast cancer patients (117). Furthermore, ILP-2 was found to be overexpressed in breast cancer tissues and breast cancer cells by immunohistochemistry and western blot (118). When the expression of ILP-2 was detected patients with chronic myeloid leukemia (CML), it was found that the expression of ILP-2 was significantly higher in CML patients than in normal hematopoietic cells. Moreover, when tyrosine kinase inhibitor resistance developed in CML patients, ILP-2 expression significantly decreased (15). Importantly, ILP-2 expression was significantly increased in bone marrow cells of patients with myelodysplastic syndrome (119). It was found that in hepatocellular carcinoma HepG2 cells were exposed to FumonisinB1 (FB1) and increased ILP-2 mRNA (5.7-fold) and

protein (2.3-fold) expression was detected, indicating that ILP-2 can promote liver tumorigenesis (120). The guanine nucleotide exchange factor 3 gene ARHGEF3 of upregulation mediates ILP-2 expression contributing to nasopharyngeal carcinogenesis and progression (121). By inhibiting ILP-2 expression in a xenograft model of adult neuroblastoma, it is able to promote radio sensitization and lead to cell death (122). In recent studies, ILP-2 was found to be possibly involved in the progression of bladder cancer and was used as a marker for early recurrence. In summary, ILP-2 highly expressed in tumor cells, is closely associated with tumorigenesis and progression. It can affect cell proliferation, apoptosis and invasion by synergizing with several key signaling proteins, triggering cellular carcinogenesis and resulting in tumorigenesis (123). In most of the investigated ILP-2-expressing tumors, high levels of the protein are a predictive marker of tumor progression, which can provide prognosis-related information.

ILP-2 AS A NEW ANTICANCER TARGET

At present, the functional mechanism of IAPs regulating tumor cell growth is continuously deepened, and various strategies for innovative therapy of IAPs have begun to emerge (124). Based on its structural characteristics and prominent role in apoptosis, ILP-2 could be a new target for tumor therapy, suitable for molecular antagonists, vaccination strategies, small molecule inhibitors and gene therapy. The following is a brief discussion of a portfolio of novel cancer therapeutic strategies targeting ILP-2 at different biological levels (**Figure 5**).

Breast Cancer

Xiang et al. (117), analyzed 400 breast cancer serum samples and 40 non-cancer serum samples (i.e., healthy controls) using bidirectional gel electrophoresis ($P<0.001$). Western blot analysis ($P<0.05$) was then performed on 10 breast cancer serum samples and 10 non-cancer serum samples. Finally, 35 serum samples from healthy controls or subjects with breast cancer, other types of cancer, galactorrhea or postoperative breast cancer were analyzed by 2DE and enzyme-linked immunosorbent assay. The results showed that ILP-2 expression was significantly higher in serum from breast cancer patients than in serum samples from other groups of women.ILP-2 is a novel biomarker for breast cancer in peripheral blood.

Zhu et al (118), further found that ILP-2 was overexpressed in breast cancer tissues and breast cancer cell lines HCC-1937, MX-1 and MCF-7 by immunohistochemistry and western blot ($P<0.01$). Then, by knockdown the expression of ILP-2 *via* RNA interference technique, it was found that inhibition of ILP-2 could induce apoptosis and inhibit breast cancer cell migration, thus confirming the survival function of ILP-2 in breast cancer cells, involved in supports the migration ability of cells. In addition, proteomic studies have been showned that in breast cancer cells, high expression of ILP-2 can synergize with multiple signaling proteins to promote the growth and invasion of breast cancer cells and assist cells to escape apoptosis. These high-risk factors, together with ILP-2, are involved in the growth and proliferation of breast cancer cells, signal transduction and regulation of the immune system (125).

Chronic Myeloid Leukeia

Glodkowska et al. (15), compared the expression of *BIRC* family genes in chronic myeloid leukemia (CML) patients cells and normal hematopoietic cells, found a significant decrease in ILP-2 expression after the development of tyrosine kinase inhibitor resistance in chronic phase CML patients. It was also hypothesized that ILP-2 does not act as a classical inhibitor of apoptosis in leukemic cells, but may actually act as a decoy for other anti-apoptotic proteins that are activated during the development of the disease. And raised the possibility of using ILP-2 expression level as a prognostic/predictive marker after further validation in clinical studies. This result suggests that ILP-2 does not display a classical apoptosis inhibitory function in leukemic cells. The potential role of ILP-2 in CML progression needs further investigation.

Myelodysplastic Syndrome

Abes et al. (119) studied myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) and found that ILP-2 expression was significantly increased in myeloid cells of patients with MDS ($P<0.05$). The experimental results show that these BIRC proteins are overexpressed in the early stage of leukemic transformation and can act as trigger factors to induce the

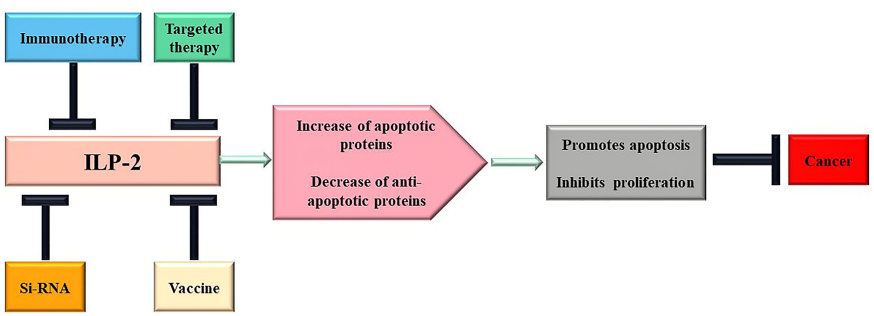

FIGURE 5 | Scheme of therapeutic approaches potentially triggered by ILP-2. Multiple changes of apoptotic proteins contribute to cancer development and progression, and treatment targeting ILP-2 can be developed to restore normal sensitivity to apoptotic stimuli and, therefore, repress cancer.

expression of other IAPs family members to trigger the occurrence of the disease.

Sun Peng et al. (71) studied acute leukemia and found that the mRNA expression level of ILP-2 in the initial treatment group and the relapsed group was significantly higher than control group (P<0.05). Concluded that the development and progression of acute leukemia are closely related to the expression levels of ILP-2, and that high expression of ILP-2 may increase the insensitivity of leukemia to chemotherapeutic drugs, thereby reducing the therapeutic effect of the drugs.

Hepatocellular Carcinoma

Chuturgoon et al. (120) studied the inhibition of apoptosis in HepG2 cells by FumonisinB1 (FB 1), they found that the mycotoxin fumonisin B1 (FB1) produced by Fusarium sp can induce apoptosis resistance in HepG2 cell lines. In the process of increasing the dose of FB1, the results of qPCR showed that the expression of *ILP-2* mRNA was significantly increased (P<0.001), while the expression of Caspase-9 and Caspase-3,7 was significantly down-regulated. Western blot results further showed that the expression of ILP-2 protein was increased, and the expression level of the pro-apoptotic protein Smac protein was significantly downregulated. The results show that the increased expression of ILP-2 can promote the growth of hepatoma cells and inhibit the apoptosis, which plays an important role in the process of hepatocarcinogenesis.

Nasopharyngeal Carcinoma

Liu et al. (121) found that upregulation of ARHGEF3 could promote ILP-2 expression and promote nasopharyngeal carcinogenesis and progression (P<0.01). In nasopharyngeal carcinoma cells, ARHGEF3 inhibits apoptosis by regulating the expression of ILP-2, which in turn inhibits the activation of caspases-3. The downregulation of ARHGEF3 by siRNA could inhibit the expression of ILP-2 and thus induce apoptosis. Thus, it is clear that the increased expression of ARHGEF3 prevents apoptosis through the upregulation of ILP-2 (P<0.01), which plays a key oncogenic role in the pathogenesis of nasopharyngeal carcinoma.

Neuroblastoma

Veeraraghavan et al. (122) found that inhibiting the expression of ILP-2 (More than 8 times) in a neuroblastoma xenograft model promoted the expression of BAK1, BAX, Caspases, and CARD, which in turn promoted radio sensitization and resulted in cell death. This effect is caused by activation of pro-apoptotic signals and inhibition of anti-apoptotic genes, including IAPs family members *NAIP* and *ILP-2* (P<0.01). Therefore, inhibiting the expression of ILP-2 may enhance the radiotherapy effect of neuroblastoma (NB).

Bladder Cancer

Chen et al. (126) used immunohistochemical analysis in bladder transitional cell carcinoma and found that there were a large number of brown-yellow fine particles in the cytoplasm, indicating that the expression of ILP-2 was high in the cytoplasm. The positive expression level of ILP-2 in bladder transitional cell carcinoma was significantly higher than that in normal bladder tissue (P<0.05). The expression level has a certain effect on the growth and proliferation of tumor cells, mainly in the aspects of cell proliferation and infiltration ability.

In conclusion, it is undeniable that ILP-2 plays an extremely important role in tumorigenesis and development. Although the therapeutic use of ILP-2 remains to be determined, its potential utility in the early diagnosis of cancer is indisputable. Expression of ILP-2 in tumors is associated with a more aggressive phenotype, shorter survival time and reduced response to chemotherapy. Recent studies suggest that it can be used as a marker for early diagnosis, a prognostic indicator to aid patient management, or to monitor disease endpoints during and after treatment. Overall, the link between ILP-2 and tumor destruction outlined here should stimulate further work to target this protein for therapeutics.

DISCUSSION AND FUTURE DIRECTIONS

At present, more and more evidences are pointing out that the abnormal expression of tumor suppressor gene ILP-2 is closely related to the occurrence of tumors, and it may be one of the main high-risk factors leading to cell carcinogenesis. In particular, abnormally high expression of ILP-2 in breast, blood and bladder cancers is closely associated with the development of these neoplastic diseases. The high expression of ILP-2 in tumor cells can help tumor cells escape apoptosis through various signaling pathways, and promote the growth, proliferation, migration and invasion of cancer cells (127). According to the related properties of ILP-2 in tumor cells, it is reasonable to speculate that ILP-2 can be used as a biomarker for early detection of tumors and is a new target for human tumor therapy. It can be seen that the in-depth study of the biological mechanism of ILP-2 in the process of tumor occurrence and development will provide new ideas for tumor treatment (128).

It is worth noting that the inhibitory effect of ILP-2 itself on apoptosis is weak. However, it is still highly expressed in most tumor cells, and cooperates with other key signaling proteins to further amplify its role to regulate the growth, proliferation, migration, apoptosis and other physiological functions of tumor cells. However, after knocking down the expression of ILP-2 by siRNA, the apoptosis rate of tumor cells was significantly increased, the migration ability was decreased, and tumor cell growth was significantly inhibited (129). In addition, many studies have also shown that the effect of radiotherapy and chemotherapy is significantly improved after combined with siRNA to knock down the expression of ILP-2. It can be seen that the use of siRNA to knock down the expression of ILP-2 has a very high potential in the clinical treatment of tumors. More interestingly, since ILP-2 protein has multiple leukocyte Ig-like receptors, natural killer cells, ICAMs and Fc receptors (FcRs), it seems to play an important role in immune-related functions (130). All these evidences directly or indirectly point to a possible close association of ILP-2 with tumorigenesis and development, which is of great research value.

Although the details of the multiple pathways from the ILP-2 network have not been fully elucidated, there is consensus that ILP-2 is an appropriate therapeutic target for the effective treatment of cancer. Today, targeted therapy for tumors is increasingly used in the clinical management of oncology patients. However, there are not many targets that can be used as targeted therapy for tumors, and there is also a certain degree of drug resistance. Therefore, it is urgent to find more effective targets for tumor therapy. We have reason to believe that with the continuous research on ILP-2, it will provide new ideas for the early clinical diagnosis and treatment of human tumors.

AUTHOR CONTRIBUTIONS

ZZ: Writing—original draft and review and editing, software, validation, and visualization. SX: Writing, data curation, and visualization. RC: Writing, formal analysis, and supervision. HP: Data curation, formal analysis, and supervision. RM: Data curation, formal analysis, and visualization. MX: Writing—review and editing, funding acquisition, investigation, and resources.

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Cumulative Evidence for Relationships Between Multiple Variants in the TERT and CLPTM1L Region and Risk of Cancer and Non-Cancer Disease

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Background: Genetic studies previously reported that variants in *TERT-CLPTM1L* genes were related to susceptibility of cancer and non-cancer diseases. However, conclusions were not always concordant.

Methods: We performed meta-analyses to assess correlations between 23 variants within *TERT-CLPTM1L* region and susceptibility to 12 cancers and 1 non-cancer disease based on data in 109 papers (involving 139,510 cases and 208,530 controls). Two approaches (false-positive report probability test and Venice criteria) were adopted for assessing the cumulative evidence of significant associations. Current study evaluated the potential role of these variants based on data in Encyclopedia of DNA Elements (ENCODE) Project.

Results: Thirteen variants were statistically associated with susceptibility to 11 cancers and 1 non-cancer disease ($p < 0.05$). Besides, 12 variants with eight cancers and one non-cancer disease were rated as strong evidence (rs2736098, rs401681, and rs402710 in bladder cancer; rs2736100, rs2853691, and rs401681 in esophageal cancer; rs10069690 in gastric cancer; rs2736100 and rs2853676 in glioma; rs2242652, rs2736098, rs2736100, rs2853677, rs31489, rs401681, rs402710, rs465498, and rs4975616 in lung cancer; rs2736100 in idiopathic pulmonary fibrosis and myeloproliferative neoplasms; and rs401681 in pancreatic and skin cancer). According

to data from ENCODE and other public databases, 12 variants with strong evidence might fall within putative functional regions.

Conclusions: This paper demonstrated that common variants of *TERT-CLPTM1L* genes were related to susceptibility to bladder, esophageal, gastric, lung, pancreatic, and skin cancer, as well as to glioma, myeloproliferative neoplasms, and idiopathic pulmonary fibrosis, and, besides, the crucial function of the *TERT-CLPTM1L* region in the genetic predisposition to human diseases is elucidated.

Keywords: *TERT*, *CLPTM1L*, genetic variant, disease, susceptibility

INTRODUCTION

Cancer as a dominating reason for death is threatening life and health of human being worldwide, with ~19.3 million newly diagnosed tumor patients along with ~10.0 million cancer-associated death cases in 2020 (1). Both environmental and genetic factors lead to cancer occurrence and progression, and 5%– 10% of cancer is resulted from variation in genes (2). Genome-wide association studies (GWAS) and genetic association research have proved multiple single-nucleotide polymorphisms (SNPs) linked with risk of human diseases (3).

The telomerase reverse transcriptase (*TERT*) gene and cleft lip and palate transmembrane 1–like (*CLPTM1L)* gene are mapped on chromosome 5p15.33. Of them, *TERT* gene, encoding the rate-limiting telomerase enzyme for catalysis, exerts a significant influence on maintaining cell immortality, telomere DNA length, and chromosomal stability (4). The protein encoded by *CLPTM1L* is a membrane protein associated with cisplatin resistance, and the overexpression of *CLPTM1L* in cisplatin-sensitive cells causes apoptosis (5). In early 1990s, researchers had made attempts to account for the existing relationships of telomeres, telomerase, aging, and cancer risk (6, 7). Wang et al. first uncovered that a novel variant (*TERT* MNS16A) had an elevated risk of lung cancer in 2003 (8). In 2006, Matsubara et al. first revealed that *TERT* rs2735940 had an elevated risk of coronary artery disease (non-cancer disease) (9). Since then, numerous genetic association studies were conducted to investigate the associations among SNPs of *TERT* and *CLPTM1L* regions with human diseases. In 2008, a GWAS was performed on the Caucasian population, and then it was found that two variants (rs402710 and rs2736100) were featured with a higher susceptibility to lung cancer (10). Subsequently, from a Japanese GWAS, it was seen that rs2736100 increased risk of idiopathic pulmonary fibrosis (11). Apart from idiopathic pulmonary fibrosis, several GWAS showed that rs2736100 could enhance lung cancer and testicular germ cell cancer susceptibility in Caucasians (12, 13) while could decrease the risk of idiopathic interstitial pneumonia and glioma in Caucasians (14, 15) and lung cancer in Asians (16). Moreover, in a GWAS conducted in multiple countries from the European ancestry, rs401681 associated with 75,000 individuals was tested, and then it was discovered that this SNP could elevate susceptibility to lung, urinary bladder, prostate, and basal cell cancer while could decrease cutaneous melanoma susceptibility (17).

Even though numerous genetic association studies investigate the association of variant in *TERT* and *CLPTM1L* regions and cancers or non-cancer disease susceptibility, the conclusions are not always consistent and the functional mechanisms remain unclear. Although, in previous published meta-analysis studies, a single SNP (for example, rs2736100 and rs2736098) with risk of individual cancer (18–20) was investigated, the results were still inconsistent. Besides, a comprehensive research synopsis with systematic functional annotation had not been performed to evaluate the epidemiological evidence of genetic correlations between *TERT* and *CLPTM1L* genes and susceptibility to cancers or non-cancer disease until now. As a result, we conducted meta-analyses to account for the relationships of SNPs in the *TERT-CLPTM1L* genes with cancers or non-cancer disease predisposition, provided the epidemiological evidence for variants with significant associations, and assessed the roles of significant SNPs using information from public databases.

MATERIAL AND METHODS

This study was conducted by following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement guidelines (**Supplementary Table 1**) and the Human Genome Epidemiology Network for systematic review of genetic association studies (21, 22).

Here, papers from PubMed, Web of science, and Embase before 30 Dec 2021 were screened using "{human telomerase reverse transcriptase} OR {hTERT} OR {TERT} OR {CLPTM1L} OR {cleft and palate transmembrane 1 like} OR {TERT-CLPTM1L region} OR {5p15.33}", and 19,425 citations were identified. Apart from that, additional articles were also collected through examining the relevant references of publications (reviews, meta-analysis studies, etc.). Finally, 109 papers were included in our study (**Figure 1**).

The included criteria were as follows: (1) assessing relationships of variations in the *TERT-CLPTM1L* region with human cancers or non-cancer disease by conducting case-control, cross-sectional, cohort studies, or GWAS; (2) providing details of genotype amount for computing the values of odds ratios (ORs) and 95% confidence intervals (95% CIs); (3) being published or full text in English, whereas the criteria of exclusion are shown as follows: (1) there is no sufficient information (especially genotype amount); (2) the interests is

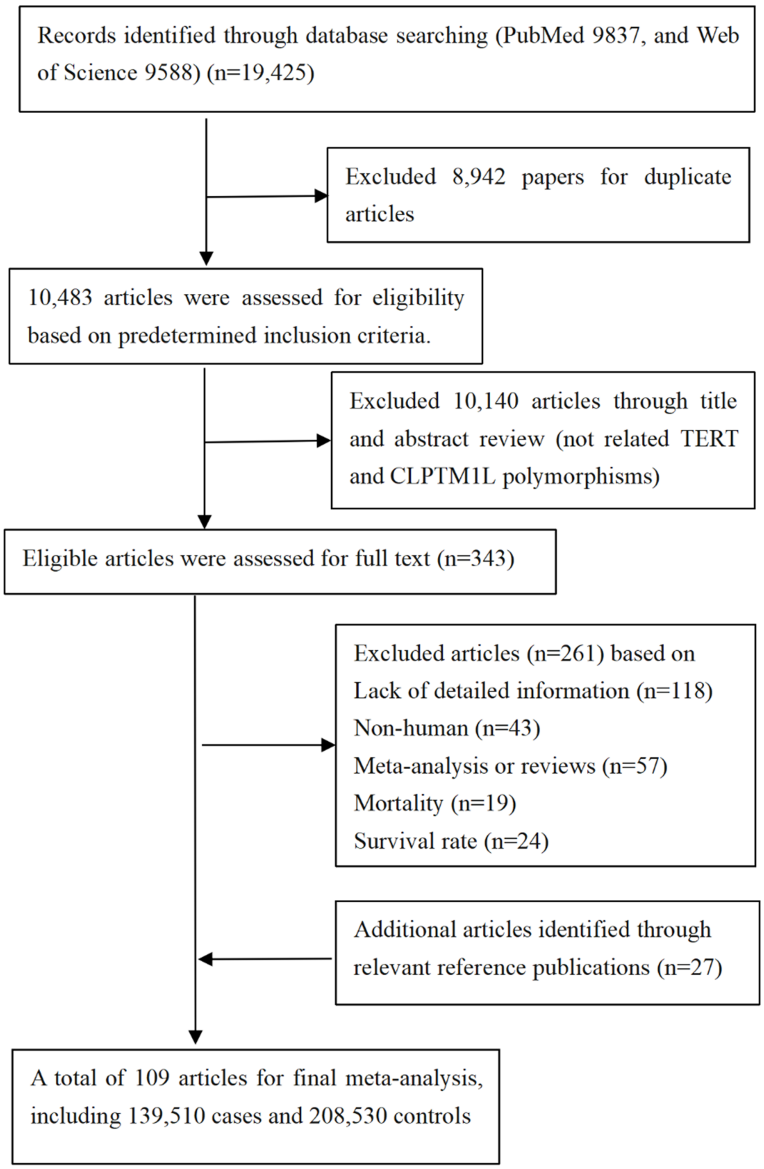

FIGURE 1 | Flow diagram of search strategy and study selection.

not *TERT-CLPTM1L* region polymorphism; (3) the study is a letter to editors or conference abstract; (iv) the interests are cancer mortality (but not incidence).

Data Extraction

Two investigators obtained data from eligible papers on their own. Any inconsistencies could be addressed by discussion with the rest of investigators. As for included variants, the information was extracted, as follows: first author, publishing year, the country or region, type of cancer and non-cancer disease, ethnicity, the gene name, the variant, amount of genotype, cases and controls, and minor allele frequency (MAF). Apart from that, ethnicity comprises four parts [Asians (East Asian descent), Caucasians (European descent), Africans (African descent), or others (Indians, Native Hawaiians, Latinos, Hispanics and the mixed, etc.)] following that more than 80% of research subjects were within the abovementioned groups; "overall populations" are composed of two or more. Furthermore, in this study, the data were extracted from publications with the largest individuals and the most comprehensive data if numerous papers had the same or overlapping data.

Statistical Analysis

$P < 0.05$ (two-sided) indicated the significance threshold, which was computed with the use of Stata, version 12 (Stata, College Station, TX, USA). As for one variant to one cancer or non-cancer disease risk, the additive genetic model with at least three

independent datasets and on the basis of the minor allele was established in meta-analyses. Moreover, the analyses performed by ethnicity and histological/pathological/clinical subtypes were also recommended if necessary. This study adopted the I^2 statistics and Cochran's Q test for investigating the heterogeneity between studies, as well as $P < 0.1$ is the significant level, as recommended (23). The values of I^2 were divided into three parts: ≤ 25%, 25%–50%, and ≥ 50% (mild heterogeneity heterogeneity, moderate heterogeneity, and large heterogeneity, respectively). We conducted sensitive analyses in order to reveal whether the significant ORs were lost by eliminating one individual study, or the first published study, or articles which have been deviated from the Hardy–Weinberg equilibrium (HWE) among controls. We investigated the probability of an excess of significant findings ($P < 0.1$ as the significant level) (24). The Begg's test and the Egger's test were used to assess potential publication bias and small-study bias, as well as $P < 0.1$ is the significant level, as recommended (25, 26).

Epidemiological Credibility of Significant Associations

The epidemiological credibility of significant relationships was evaluated combining the Venice guideline (27) and the false-positive report probability (FPRP) (28) (see **Supplementary Method**).

Functional Annotation

The underlying functional role of variants on 5p15.33 was evaluated with information from the Encyclopedia of DNA Elements (ENCODE) tool HaploReg (v4.1) (29) and the UCSC Genome browser (http://genome.ucsc.edu/). Furthermore, our work explored genome-wide cis-eQTL data in multiple tissues from the Genotype-Tissue Expression Project (30) and the Multiple Tissue Human Expression Resource Project (31) databases in order to reveal whether these genes might demonstrate the observed findings in these loci.

RESULTS

Characteristics of the Included Studies

In our study, available data from 109 papers were extracted in these meta-analyses (**Supplementary Table 2**), thus further evaluating associations between 23 variants in *TERT-CLPTM1L* region and 12 cancers and 1 non-cancer disease under an additive genetic model (**Figure 1**). In addition, the distributions of SNPs (n) with cancer and non-cancer disease were as follows: esophageal (n = 4), gastric (n = 4), glioma (n = 2), breast (n = 11), hepatocellular (n = 1), lung (n = 10), pancreatic (n = 2), skin (melanoma) (n = 1), bladder (n = 4), colorectal (n = 1), thyroid cancer (n = 1), myeloproliferative neoplasms (n = 1), and idiopathic pulmonary fibrosis (n = 1). Of these, analysis was performed with 139,510 cases and 208,530 controls (the publishing year ranged from 2007 to 2020), and then it was found that 13 SNPs were significantly associated with 11 cancers and 1 non-cancer disease risk (**Table 1** and **Supplementary Table 3**).

Associations Between TERT-CLPTM1L Variants and Risk of Cancer and Non-Cancer Diseases

We conducted meta-analyses to assess relationships of 23 variants in *TERT-CLPTM1L* region with 12 cancers and 1 non-cancer disease under an additive genetic model. Then, it was seen that 13 SNPs (rs10069690, rs2242652, rs2736098, rs2736100, rs2853676, rs2853677, rs2853691, rs31489, rs401681, rs402710, rs451360, rs465498, and rs4975616) had significantly associated with risk of 11 cancers (bladder, breast, colorectal, esophageal, gastric, glioma, lung, pancreatic, skin, thyroid, and myeloproliferative neoplasms) and 1 non-cancer disease (idiopathic pulmonary fibrosis) (**Table 1**). It is worth noting that the histological/pathological types of esophageal carcinoma and skin cancer were squamous cell carcinoma and melanoma, respectively. Apart from that, significant relationships with susceptibility to bladder cancer could be found for rs2736098 (OR = 1.193), rs2736100 (OR = 0.883), rs401681 (OR = 0.852), and rs402710 (OR = 0.863). Moreover, these associations were further assessed by ethnicity, demonstrating that the four SNPs mentioned above had significant association with bladder cancer predisposition in Asians (rs2736098: OR = 1.240; rs2736100: OR = 0.837; rs401681: OR = 0.851; rs402710: OR = 0.804), rather than the Caucasian population. In addition, the significant relationship with breast cancer predisposition was only presented for rs2736098 (OR = 0.834), whereas rs2736100 could increase colorectal cancer predisposition (OR = 1.070).

Apart from colorectal carcinoma, the A allele of rs2736100 possessed a decreased predisposition of esophageal squamous cell carcinoma (ESCC) among Asian populations (OR = 0.724). Moreover, one SNP (rs2853691) had an enhanced predisposition of ESCC (OR = 1.304) and another two SNPs (rs401681 and rs451360) were featured by the reduced predisposition of ESCC, in Asians (OR = 0.867 and OR = 0.700).

Significant relationships with gastric carcinoma predisposition were exclusively found for rs10069690 (OR = 1.317) and rs2853676 (OR = 0.675) in Asians. Besides, SNP rs2736100 was statistically associated with gastric carcinoma (OR = 0.751). Interestingly, rs2736100 remarkably associates with gastric cancer predisposition in Caucasians (OR = 0.604) but not in Asians. Moreover, two SNPs could reduce the predisposition of glioma (rs2736100: OR = 0.746; rs2853676: OR = 0.784). Noticeably, it was also uncovered that these two SNPs had a reduced susceptibility to glioma both in Asian populations and Caucasian populations.

For lung cancer, it was found that nine SNPs were significantly related to lung cancer predisposition, which also remains true in the subgroup analyses by ethnicity and pathological subtypes. Specifically, three SNPs exclusively appeared in Asians and had significant relationships with lung cancer predisposition (rs2242652: OR = 1.168; rs2853677: OR = 0.791; rs465498: OR = 0.765, respectively). Besides, additional findings from subgroup analyses by pathological subtypes for rs2853677 indicated that this SNP could decrease the predisposition of non–small cell lung cancer (NSCLC) (OR = 0.761). Interestingly, it was found that rs2853677 had no

TABLE 1 | Epidemiological evidence for associations between variants in the TERT and CLPTM1L gene with risk of cancer and non-cancerous diseases in additive model.

Gene	Variant	Alleles[a]	Group[b]	MAF[c]	Number evaluation			Disease risk		Heterogeneity		Venice criteria[d]	FPRP values[e]	Credibility of evidence[f]
					Studies	Cases	Controls	OR (95%CI)	p-value	I^2 (%)	P_Q			
Bladder cancer														
TERT	rs27360986	C/T	Overall	0.3214	5	1,863	3,381	1.193 (1.085–1.313)	< 0.001	47.8	0.104	ABA	0.006	Strong
			Caucasian	0.2534	2	948	1,649	1.132 (0.977–1.311)	< 0.001	0.0	0.886			
			Asian	0.3591	3	915	1,732	1.240 (1.094–1.405)	0.001	70.4	0.033			
TERT	rs2736100	C/A	Overall	0.5597	4	1,638	3,141	0.883 (0.803–0.970)	0.01	0.0	0.507	ABC	0.152	Weak
			Caucasian	0.5122	2	948	1,649	0.924 (0.812–1.051)	0.228	9.0	0.294			
			Asian	0.5912	2	690	1,492	0.837 (0.728–0.961)	0.012	0.0	0.674			
CLPTM1L	rs401681	C/T	Overall	0.3615	4	1,555	2,500	0.852 (0.771–0.941)	0.002	0.0	0.757	AAA	0.029	Strong
			Caucasian	0.6859	1	498	588	0.874 (0.716–1.018)	0.078	NA	NA			
			Asian	0.3376	3	1,057	1,912	0.851 (0.754–0.961)	< 0.001	0.0	0.554			
CLPTM1L	rs402710	C/T	Overall	0.3236	3	1,454	2,179	0.863 (0.772–0.965)	0.01	0.0	0.635	AAA	0.156	Strong
			Caucasian	0.3179	2	948	1,649	0.897 (0.781–1.031)	0.127	0.0	0.810			
			Asian	0.3349	1	506	530	0.804 (0.667–0.969)	0.022	NA	NA			
Breast cancer														
TERT	MNS16A	L/S	Caucasian	0.3004	6	4,591	5,159	0.834 (0.714–0.973)	0.021	72.3	0.003	ACC	0.286	Weak
TERT	rs13167280	G/A	Caucasian	0.7312	3	5,057	5,702	0.963 (0.888–1.043)	0.349	0.0	0.681			
TERT	rs2075786	A/G	Caucasian	0.6460	3	5,057	5,702	0.996 (0.942–1.054)	0.902	0.0	0.716			
TERT	rs2735940	A/G	Overall	0.5282	6	5,514	6,640	0.978 (0.913–1.048)	0.534	31.4	0.212			
			Caucasian	0.5324	5	5,446	6,049	1.006 (0.957–1.059)	0.803	83.6	< 0.001			
			Asian	0.4629	1	68	591	2.688 (1.816–3.980)	< 0.001	NA	NA			
TERT	rs2736098	C/T	Caucasian	0.3004	6	4,591	5,159	0.834 (0.714–0.973)	0.021	72.3	0.003	ACC	0.286	Weak

(Continued)

TABLE 1 | Continued

Gene	Variant	Alleles[a]	Group[b]	MAF[c]	Number evaluation			Disease risk		Heterogeneity		Venice criteria[d]	FPRP values[e]	Credibility of evidence[f]
					Studies	Cases	Controls	OR (95%CI)	p-value	I^2 (%)	P_Q			
TERT	rs2736100	C/A	Caucasian	0.4677	4	1,262	1,118	1.062 (0.946–1.192)	0.309	0.0	0.976			
TERT	rs2736109	G/A	Overall	0.4663	8	9,457	12,824	0.987 (0.922–1.056)	0.704	59.1	0.017			
			Caucasian	0.4696	7	9,130	12,257	0.995 (0.957–1.035)	0.809	64.8	0.009			
			Asian	0.3343	1	327	567	1.023 (0.841–1.246)	0.817	NA	NA			
TERT	rs2853669	T/C	Caucasian	0.4864	10	8,635	10,358	1.006 (0.942–1.074)	0.870	41.6	0.080			
TERT	rs2853677	G/A	Caucasian	0.4124	3	5,057	5,702	1.036 (0.980–1.095)	0.211	0.0	0.780			
TERT	rs2853690	G/T	Caucasian	0.1496	3	5,057	5,702	0.977 (0.905–1.054)	0.541	0.0	0.847			
TERT	rs7712562	A/C	Caucasian	0.2791	3	5,057	5,702	1.035 (0.927–1.156)	0.540	50.4	0.133			
Colorectal cancer														
TERT	rs2736100	C/A	Caucasian	0.4980	10	19,050	21,500	1.070 (1.040–1.102)	< 0.001	0.0	0.700	AAC	< 0.001	Moderate
Esophageal squamous cell carcinoma														
TERT	rs2736100	C/A	Asian	0.6055	3	2,098	2,150	0.724 (0.664–0.789)	< 0.001	0.0	0.779	AAA	< 0.001	Strong
TERT	rs2853691	A/G	Asian	0.2486	3	2,098	2,150	1.304 (1.149–1.479)	< 0.001	40.6	0.186	ABA	0.001	Strong
CLPTM1L	rs401681	C/T	Asian	0.3386	3	1,742	1,856	0.867 (0.784–0.958)	0.005	16.2	0.303	AAA	0.088	Strong
CLPTM1L	rs451360	C/A	Asian	0.1258	3	2,098	2,150	0.700 (0.610–0.904)	< 0.001	0.0	0.979	BAA	0.156	Moderate
Gastric cancer														
TERT	rs10069690	C/T	Asian	0.1816	4	2,470	2,236	1.317 (1.193–1.454)	< 0.001	28.5	0.241	ABA	< 0.001	Strong
TERT	rs2735940	A/G	Overall	0.3234	3	641	1,528	1.302 (0.689–2.460)	0.416	94.1	< 0.001			
			Caucasian	0.3684	1	104	209	0.695 (0.485–0.996)	0.047	NA	NA			
			Asian	0.3150	2	537	1,319	1.748 (0.894–3.419)	0.103	93.8	< 0.001			
TERT	rs2736100	C/A	Overall	0.6270	4	1,843	2,195	0.751 (0.568–0.993)	0.044	85.1	< 0.001	ACC	0.514	Weak
			Caucasian	0.4880	1	104	209	0.604 (0.429–0.850)	0.004	NA	NA			

(Continued)

TABLE 1 | Continued

Gene	Variant	Alleles[a]	Group[b]	MAF[c]	Number evaluation			Disease risk		Heterogeneity		Venice criteria[d]	FPRP values[e]	Credibility of evidence[f]
					Studies	Cases	Controls	OR (95%CI)	p-value	I^2 (%)	P_Q			
			Asian	0.6418	3	1,739	1,986	0.795 (0.580–1.091)	0.155	87.7	< 0.001			
TERT	rs2853676	T/C	Asian	0.7990	4	2,182	2,400	0.675 (0.484–0.942)	0.021	89.5	< 0.001	ACC	0.428	Weak
							Glioma							
TERT	rs2736100	C/A	Overall	0.5046	8	5,750	8,379	0.746 (0.666–0.835)	< 0.001	77.0	< 0.001	AAA	< 0.001	Strong
			Caucasian	0.4934	6	4,668	7,112	0.796 (0.747–0.847)	< 0.001	25.0	0.246			
			Asian	0.6575	2	1,082	1,267	0.493 (0.255–0.954)	0.036	91.4	0.001			
TERT	rs2853676	T/C	Overall	0.7477	7	5,832	8,143	0.784 (0.743–0.828)	< 0.001	0.0	0.504	AAA	< 0.001	Strong
			Caucasian	0.7263	5	4,423	6,623	0.794 (0.748–0.843)	< 0.001	0.0	0.670			
			Asian	0.8401	2	1,409	1,520	0.737 (0.645–0.843)	< 0.001	49.5	0.159			
							Hepatocellular carcinoma							
TERT	rs2736098	C/T	Asian	0.3453	3	846	867	1.211 (0.948–1.548)	0.125	65.1	0.057			
							Idiopathic pulmonary fibrosis							
TERT	rs2736100	C/A	Asian	0.5898	3	397	1,848	1.788 (1.508–2.120)	< 0.001	0.0	0.910	AAA	< 0.001	Strong
							Lung cancer							
TERT	rs2242652	G/A	Asian	0.1650	3	3,631	4,013	1.168 (1.054–1.294)	0.003	0.0	0.562	AAA	0.053	Strong
TERT	rs2736098	C/T	Overall	0.3199	10	5,350	6,115	1.212 (1.121–1.310)	< 0.001	47.0	0.049	ABA	< 0.001	Strong
			Caucasian	0.2259	1	874	1,061	1.158 (0.989–1.358)	0.069	NA	NA			
			Asian	0.3350	9	4,476	5,054	1.221 (1.118–1.334)	< 0.001	52.3	0.237			
TERT	rs2736098	C/T	NSCLC (ADC)	0.3502	3	1,131	2,203	1.401 (1.261–1.557)*	< 0.001	0.0	0.740			
TERT	rs2736098	C/T	NSCLC (SCC)[1]	0.3502	3	556	2,203	1.098 (0.954–1.263)*	0.193	0.0	0.691			
TERT	rs2736100	C/A	Overall	0.5024	27	33,918	35,844	0.856 (0.788–0.931)	< 0.001	90.9	< 0.001	AAA	0.005	Strong
			Caucasian	0.4927	5	15,861	16,515	0.874 (0.845–0.903)	< 0.001	0.0	0.754			

(Continued)

TABLE 1 | Continued

Gene	Variant	Alleles[a]	Group[b]	MAF[c]	Number evaluation			Disease risk		Heterogeneity		Venice criteria[d]	FPRP values[e]	Credibility of evidence[f]
					Studies	Cases	Controls	OR (95%CI)	p-value	I^2 (%)	P_Q			
			Asian	0.5907	21	18,017	19,289	0.847 (0.758–0.948)	0.004	91.9	< 0.001			
			African	0.0972	1	40	40	1.061 (0.541–2.078)	0.864	NA	NA			
TERT	rs2736100	C/A	Overall	0.6028	10	7,730	19,200	0.786 (0.729–0.848)	< 0.001	62.8	0.004			
			Caucasian	0.4801	1	200	553	0.714 (0.566–0.901)	0.005	NA	NA			
			Asian	0.6065	10	7,530	18,647	0.791 (0.731–0.857)	< 0.001	65.7	0.003			
TERT	rs2736100	C/A	NSCLC (ADC)	0.4962	17	8,506	30,944	0.801 (0.694–0.926)[§]	0.003	92.7	< 0.001			
			NSCLC (ADC)	0.4801	1	112	553	0.675 (0.503–0.905)[#]	0.009	NA	NA			
			NSCLC (ADC)	0.4965	16	8,394	30,391	0.809 (0.698–0.939)*	0.005	93.0	< 0.001			
TERT	rs2736100	C/A	NSCLC (SCC)[1]	0.5645	4	1,065	2,717	0.920 (0.827–1.025)[§]	0.13	0.0	0.458			
			NSCLC (SCC)[1]	0.4801	1	48	553	0.741 (0.485–1.132)[#]	0.166	NA	NA			
			NSCLC (SCC)[1]	0.5861	3	1,017	2,159	0.934 (0.836–1.044)*	0.231	0.0	0.467			
TERT	rs2853677	G/A	Asian	0.5881	3	1,123	1,340	0.791 (0.704–0.890)	< 0.001	1.5	0.362	AAA	0.002	Strong
TERT	rs2853677	G/A	NSCLC	0.6796	3	1,085	1,236	0.761 (0.672–0.861)*	< 0.001	0.0	0.639			
TERT	rs2853677	G/A	NSCLC (ADC)	0.3255	5	4,443	16,528	1.062 (0.850–1.326)*	0.596	94.1	< 0.001			
CLPTM1L	rs31489	C/A	Overall	0.3546	7	13,850	14,390	0.860 (0.813–0.909)	< 0.001	44.7	0.093	AAA	< 0.001	Strong
			Caucasian	0.4068	4	11,361	11,546	0.866 (0.817–0.919)	< 0.001	51.8	< 0.001			
			Asian	0.1496	3	2,489	2,844	0.833 (0.708–0.979)	0.027	51.7	0.126			
CLPTM1L	rs31489	C/A	NSCLC	0.2938	3	1,725	2,343	0.842 (0.603–1.070)	0.159	70.7	0.033			
			NSCLC	0.4098	1	1,154	1,137	0.884 (0.785–0.995)	0.042	NA	NA			
			NSCLC	0.1845	2	571	1206	0.826 (0.505–1.349)	0.444	82.3	0.017			

(Continued)

TABLE 1 | Continued

Gene	Variant	Alleles[a]	Group[b]	MAF[c]	Number evaluation			Disease risk		Heterogeneity		Venice criteria[d]	FPRP values[e]	Credibility of evidence[f]
					Studies	Cases	Controls	OR (95%CI)	p-value	I^2 (%)	P_Q			
CLPTM1L	rs401681	C/T	Overall	0.3600	17	11,280	12,390	0.885 (0.840–0.932)	< 0.001	35.5	0.073	AAA	< 0.001	Strong
			Caucasian	0.4284	3	4,306	4,503	0.874 (0.822–0.929)	< 0.001	0.0	0.926			
			Asian	0.3812	14	6,974	7,887	0.891 (0.830–0.957)	0.002	46.7	0.028			
CLPTM1L	rs401681	C/T	NSCLC	0.3527	10	10,109	13,800	0.846 (0.790–0.906)[§]	< 0.001	48.0	0.044			
			NSCLC	0.4425	2	4,470	2,575	0.855 (0.797–0.917)[#]	< 0.001	0.0	0.649			
			NSCLC	0.3321	8	5,639	11,225	0.853 (0.773–0.940)[*]	0.001	56.7	0.024			
CLPTM1L	rs401681	C/T	NSCLC (ADC)	0.4083	7	2,783	4,780	0.950 (0.886–1.019)[§]	0.15	0.0	0.792			
			NSCLC (ADC)	0.4425	2	1,559	2,575	0.939 (0.858–1.027)[#]	0.167	57.1	0.127			
			NSCLC (ADC)	0.3666	5	1,224	2,205	0.968 (0.865–1.082)[*]	0.565	0.0	0.960			
CLPTM1L	rs401681	C/T	NSCLC (SCC)[1]	0.4083	7	2,283	4,780	0.857 (0.787–0.934)[§]	< 0.001	4.4	0.393			
			NSCLC (SCC)[1]	0.4425	2	1,819	2,575	0.847 (0.774–0.927)[#]	< 0.001	0.0	0.510			
			NSCLC (SCC)[1]	0.3666	5	464	2,205	0.876 (0.743–1.033)[*]	0.117	23.5	0.264			
CLPTM1L	rs401681	C/T	NSCLC (SCC)[2]	0.3781	4	1,283	3,153	0.908 (0.822–1.003)[§]	0.058	0.0	0.712			
			NSCLC (SCC)[2]	0.6483	1	1,028	1,438	0.889 (0.793–0.997)[#]	0.045	NA	NA			
			NSCLC (SCC)[2]	0.3225	3	255	1,715	0.968 (0.792–1.183)[*]	0.747	0.0	0.655			
CLPTM1L	rs402710	C/T	Overall	0.3339	16	20,135	25,250	0.857 (0.832–0.883)	< 0.001	0.0	0.873	AAA	< 0.001	Strong
			Caucasian	0.3515	3	11,190	14,329	0.857 (0.822–0.893)	< 0.001	0.0	0.685			
			Asian	0.3168	11	8,945	10,921	0.858 (0.821–0.896)	< 0.001	0.0	0.747			
CLPTM1L	rs402710	C/T	NSCLC	0.3204	4	5,640	10,521	0.832 (0.785–0.882)	< 0.001	0.0	0.421			
CLPTM1L	rs402710	C/T	NSCLC (ADC)	0.3026	2	2,099	3,329	0.868 (0.796–0.947)	0.002	0.0	0.587			

(Continued)

TABLE 1 | Continued

Gene	Variant	Alleles[a]	Group[b]	MAF[c]	Number evaluation			Disease risk		Heterogeneity		Venice criteria[d]	FPRP values[e]	Credibility of evidence[f]
					Studies	Cases	Controls	OR (95%CI)	p-value	I^2 (%)	P_Q			
TERT	rs4246742	T/A	Asian	0.6252	3	3,305	3,720	1.133 (0.875–1.467)	0.343	82.0	0.004			
CLPTM1L	rs465498	A/G	Asian	0.1701	4	8,948	9,805	0.765 (0.723–0.810)	< 0.001	0.0	0.880	AAA	< 0.001	Strong
TERT/ CLPTM1L	rs4975616	G/A	Overall	0.6387	7	11,300	8,873	1.159 (1.108–1.212)	< 0.001	32.7	0.146	AAA	< 0.001	Strong
			Caucasian	0.5896	4	9,553	7,213	1.159 (1.105–1.216)	< 0.001	56.1	0.044			
			Asian	0.8517	3	1,747	1,660	1.155 (1.006–1.326)	0.041	0.0	0.577			
TERT/ CLPTM1L	rs4975616	G/A	NSCLC	0.6029	3	1,554	1,890	1.234 (0.979–1.556)[§]	0.075	66.1	0.052			
			NSCLC	0.5731	2	1,354	1,690	1.279 (0.929–1.761)[#]	0.132	82.8	0.016			
			NSCLC	0.8550	1	200	200	1.110 (0.743–1.659)*	0.609	NA	NA			
Myeloproliferative neoplasms														
TERT	rs2736100	C/A	Overall	0.5846	7	2,436	19,204	0.586 (0.538–0.637)	< 0.001	0.0	0.848	AAA	< 0.001	Strong
			Caucasian	0.4925	4	1,956	1,791	0.589 (0.532–0.654)	< 0.001	0.0	0.552			
			Asian	0.5940	3	480	17,413	0.578 (0.498–0.670)	< 0.001	0.0	0.764			
TERT	rs2736100	C/A	ET	0.5909	4	792	18,145	0.589 (0.522–0.665)[§]	< 0.001	0.0	0.707			
			ET	0.5228	2	552	833	0.561 (0.481–0.655)[#]	< 0.001	0.0	0.536			
			ET	0.5942	2	240	17,312	0.637 (0.525–0.774)*	< 0.001	0.0	0.970			
TERT	rs2736100	C/A	PV	0.5909	4	499	18,145	0.521 (0.449–0.604)[§]	< 0.001	46.7	0.131			
			PV	0.5228	2	393	833	0.562 (0.473–0.669)[#]	< 0.001	0.0	0.745			
			PV	0.5942	2	106	17,312	0.422 (0.318–0.562)*	< 0.001	64.7	0.092			
TERT	rs2736100	C/A	PMF	0.5909	4	201	18,145	0.575 (0.463–0.713)[§]	< 0.001	0.0	0.472			
			PMF	0.5228	2	168	833	0.538 (0.423–0.686)[#]	< 0.001	2.3	0.312			

(Continued)

TABLE 1 | Continued

Gene	Variant	Alleles[a]	Group[b]	MAF[c]	Number evaluation			Disease risk		Heterogeneity		Venice criteria[d]	FPRP values[e]	Credibility of evidence[f]
					Studies	Cases	Controls	OR (95%CI)	p-value	I^2 (%)	P_Q			
			PMF	0.5942	2	33	17,312	0.758 (0.464–1.237)*	0.267	0.0	0.908			
Pancreatic cancer														
CLPTM1L	rs401681	C/T	Caucasian	0.4398	3	2,591	5,383	1.173 (1.097–1.255)	< 0.001	0.0	0.974	AAA	< 0.001	Strong
TERT/ CLPTM1L	rs4635969	G/A	Caucasian	0.2057	3	2,591	5,383	1.026 (0.943–1.117)	0.547	36.3	0.208			
Skin cancer (melanoma)														
CLPTM1L	rs401681	C/T	Caucasian	0.4415	3	1,188	1,603	1.285 (1.120–1.414)	< 0.001	23.5	0.270	AAA	0.002	Strong
Thyroid cancer														
TERT	rs2736100	C/A	Asian	0.5969	4	2,752	2,752	0.762 (0.657–0.884)	< 0.001	72.2	0.013	ACC	0.007	Moderate

OR, odds ratio; A, adenine; T, thymine; G, guanine; C, cytosine; NSCLC, non–small cell lung cancer; ADC, adenocarcinoma; [1]SCC, squamous cell carcinoma. [2]SCC, small cell cancer; ET, essential thrombocythemia; PV, polycythemia vera; PMF, primary myelofibrosis; NA, not applicable.

**The association was performed in Asians.*

#The association was performed in Caucasians.

§The association was performed in overall populations.

[a]Major alleles (reference)/minor alleles.

[b]Group by ethnicity or subtype.

[c]Frequency of minor allele in controls.

[d]Strength of epidemiological evidence based on the Venice criteria.

[e]FPRP values at prior probability of 0.05 at power OR of 1.5, and the FPRP level of noteworthiness is 0.20.

[f]Degree of epidemiological credibility based on the combination of results from Venice guidelines and FPRP tests.

association with NSCLC (adenocarcinoma) risk. In addition, there were noticeable relationships between six SNPs and lung cancer predisposition among different races/pathological subtypes. Among them, rs2736098 had an elevated predisposition of lung cancer (OR = 1.212), which was shown in Asians (OR = 1.221), but not in Caucasians. Besides that, rs2736098 was distinctly associated with predisposition of NSCLC (adenocarcinoma) (OR = 1.401), rather than NSCLC (squamous cell carcinoma), whereas SNP rs2736100 had a close relationship with lung cancer predisposition (OR = 0.856), which appeared in Caucasians (OR = 0.874) and Asians (OR = 0.847) instead of Africans. Then, subgroup analyses were performed by pathological type/race, and it was uncovered that rs2736100 had a decreased risk of NSCLC (OR = 0.786) both in Caucasians (OR = 0.714) and Asians (OR = 0.791). Surprisingly, rs2736100 had a decreased risk of NSCLC (adenocarcinoma) (OR = 0.801), rather than NSCLC (squamous cell carcinoma), whereas SNP rs31489 was closely connected with lung cancer predisposition (OR = 0.860) both in Caucasians (OR = 0.866) and Asians (OR = 0.833). Noticeably, subgroup analyses by pathology type presented that rs31489 was not related to NSCLC predisposition (OR = 0.842). Additionally, SNP rs401681 was featured by the decreased lung cancer incidence (OR = 0.885) both in Caucasians (OR = 0.874) and Asians (OR = 0.891). Furthermore, the analyses also showed that rs401681 had a decreased risk of NSCLC (OR = 0.846), but not associated with small cell carcinoma (OR = 0.908). For NSCLC, it was also known that SNP rs401681 featured a reduced NSCLC (squamous cell carcinoma) incidence (OR = 0.857) but had no relationship with NSCLC (adenocarcinoma) (OR = 0.950). Other than that, SNP rs402710 could decrease predisposition of lung cancer (OR = 0.857) both in Caucasians (OR = 0.857) and Asians (OR = 0.858). Furthermore, from subgroup analyses by pathology type, it was seen that rs401681 could reduce NSCLC predisposition (OR = 0.832), especially in lung adenocarcinoma (OR = 0.868). SNP rs4975616 faced an enhanced risk of lung cancer (OR = 1.159) both in Caucasians (OR = 1.159) and Asians (OR = 1.155), while there was no relationship between rs4975616 and NSCLC predisposition (OR = 1.234).

For myeloproliferative neoplasms, SNP rs2736100 could decrease the risk of myeloproliferative neoplasms (OR = 0.586) both in Caucasians (OR = 0.589) and in Asians (OR = 0.578). Subgroup analyses by clinical subtypes indicated that rs2736100 could reduce the risk of essential thrombocythemia (OR = 0.589), polycythemia vera (OR = 0.521), and primary myelofibrosis (OR = 0.575).

Besides myeloproliferative neoplasms, SNP rs2736100 could reduce thyroid carcinoma predisposition in Asians (OR = 0.762).

Moreover, SNP rs401681 could increase risk of pancreatic cancer (OR = 1.173) and skin cancer (melanoma) (OR = 1.285) in Caucasians.

In terms of non-cancer disease, it was found that rs2736100 had an increased risk of idiopathic pulmonary fibrosis in Asian populations (OR = 1.788).

Furthermore, 13 SNPs (*TERT* MNS16A, rs13167280, rs2075786, rs2735940, rs2736100, rs2736109, rs2853669, rs2853677, rs2853690, rs7712562, rs2735940, rs2736098, rs4246742, and rs4635969) were not related to risk of five types of cancer (breast, gastric, hepatocellular, lung, and pancreatic cancer) (**Supplementary Results**). Of these, eight SNPs (rs13167280, rs2075786, rs2735940, rs2736100, rs2736109, rs2853669, rs2853677, rs2853690, and rs7712562) had no association with breast cancer with at least 10,000 individuals (**Table 2**). Beyond that, the statistical power was also calculated so as to confirm whether the large-scale sample size confirming these associations is required in the future (**Supplementary Table 4**).

Heterogeneity, Bias, and Sensitivity Analysis

The assessment of heterogeneity was performed for 29 significant correlations with 13 variants and 12 cancers and 1 non-cancer disease (**Table 1**). Of them, mild heterogeneity could be assigned to 16 (55%) associations, moderate heterogeneity fell into 7 (24%) associations, and high heterogeneity was found in 6 (21%) associations. There existed little evidence of publication bias ($p > 0.10$), except for rs2853676 with risk of glioma. Furthermore, findings from sensitivity analyses displayed that removal of some key factors did not alter the summary ORs, except for rs2736100 in bladder and colorectal cancer (low OR), rs2736100 in gastric cancer (HWE), and rs2736100 in thyroid cancer (excess of significant findings).

Cumulative Evidence of Association

Epidemiological credibility of totally 29 significant relationships was assessed using the Venice guideline. Specifically, there were 28 grades A in the amount of evidence, 21 grades A in the replication of association, and 23 grades A in the protection from bias, respectively; there were 1, 4, and 0 grades B in these three criteria, respectively; and there were 0, 4, and 6 grades C in these three criteria, respectively (**Table 1**). Therefore, strong, moderate, and weak evidence of a significant relationship with susceptibility to cancer and non-cancer disease could be found for 18, 5, and 6 associations, respectively. Subsequently, the probability for a true correlation between the 29 significant correlations was evaluated on the basis of FPRP values. Briefly, a FPRP level < 0.05 was found for 11 variants with 10 cancers and 1 non-cancer disease, whereas FPRP 0.05 to 0.2 for five variants with susceptibility to three cancers, and FPRP > 0.2 for three SNPs with risk of two cancers. Finally, strong evidence was assigned to 12 SNPs (*TERT*: rs10069690, rs2242652, rs2736098, rs2736100, rs2853676, rs2853677, and rs2853691; *CLPTM1L*: rs31489, rs401681, rs402710, and rs465498; *TERT/CLPTM1L*: rs4975616) with eight cancers and one non-cancer disease, which is presented in detail below: rs2736098, rs401681, and rs402710 in bladder cancer; rs2736100, rs2853691, and rs401681 in esophageal cancer; rs10069690 in gastric cancer; rs2736100 and rs2853676 in glioma; rs2242652, rs2736098, rs2736100, rs2853677, rs31489, rs401681, rs402710, rs465498, and rs4975616 in lung cancer; rs2736100 in idiopathic pulmonary fibrosis and myeloproliferative neoplasms; and rs401681 in pancreatic and skin cancer, whereas moderate evidence belonged to two SNPs with risk of three cancers (rs2736100 in colorectal and thyroid cancer and rs451360 in esophageal cancer), and the weak one was to three SNPs with risk of three cancers (rs2736100 in bladder cancer, rs2736098 in breast cancer, and rs2736100 and rs2853676 in gastric cancer).

TABLE 2 | Summary of functional annotations for 12 SNPs in eight cancers and one neoplastic disease (strong epidemiological credibility).

Variant	Gene	Position[a]	Annotation	Promoter histone marks[b]	Enhancer histone marks[c]	DNAse[d]	Proteins bound[e]	Motifs changed[f]
rs10069690	TERT	1279790	Intronic	4 tissues	4 tissues			BDP1, TBX5
rs2242652	TERT	1280028	Intronic	4 tissues	4 tissues			9 altered motifs
rs2736098	TERT	1294086	Synonymous	10 tissues	16 tissues	BLD		9 altered motifs
rs2736100	TERT	1286516	Intronic	ESDR, ESC	BLD			Foxa
rs2853676	TERT	1288547	Intronic	ESDR, PANC, SPLN	BLD			Pax-5
rs2853677	TERT	1287194	Intronic	ESDR, ESC	BLD			
rs2853691	TERT	1252950	Intronic	4 tissues	4 tissues			6 altered motifs
rs31489	CLPTM1L	1342714	Intronic	13 tissues	17 tissues			DMRT2, Mef2
rs401681	CLPTM1L	1322087	Intronic	5 tissues	6 tissues			Egr-1, HNF4
rs402710	CLPTM1L	13947292	Intronic	4 tissues	7 tissues			5 altered motifs
rs465498	CLPTM1L	1325803	Intronic	9 tissues	13 tissues			RXRA, Rad21
rs4975616	TERT/ CLPTM1L	1315660	2.2kb 3' of CLPTM1L	11 tissues	18 tissues	6 tissues		NF-I

[a]*The chromosome position is based on NCBI Build 37.*
[b]*Histone modification of H3K4me1 and H3K27ac (tissue types: if >3, only the number is included).*
[c]*Histone modification of H3K4me3 and H3K9ac (tissue types: if >3, only the number is included).*
[d]*Levels of DNase I hypersensitivity (tissue types: if >3, only the number is included).*
[e]*Alteration in transcription factor binding (disruptions: if >3, only the number is included).*
[f]*Alteration in regulatory motif (disruptions: if >3, only the number is included).*

Functional Annotation

The potential function roles for strong associations (12 SNPs associated with eight cancers and one non-cancer disease) were investigated with the use of the ENCODE tool HaploReg v4.1 (**Table 2**). In terms of functional annotations, 10 variants were mapped to intronic regions and *TERT* rs2736098 was mapped to synonymous regions. The total 12 SNPs might locate in a region having strong promoter and enhancer activity, DNase I hypersensitivity site, and alteration in regulatory motifs (**Table 2**). The linkage disequilibrium (LD) plots explained that the regions represented by significant SNPs had distinct genetic structures among European, Asian, and African ancestries (**Figure 2**). Besides, the Genotype-Tissue Expression Project revealed that rs2736100, rs2853676, rs2853676, rs31489, rs401681, rs402710, rs465498, and rs4975616 are eQTLs for *TERT* and *CLPTM1L*. Additionally, rs2736100, rs2853676, rs2853677, and rs4975616 are associated with an increase in *TERT* and *CLPTM1L* gene expression, whereas rs31489, rs401681, rs402710, and rs465498 are relevant to a decrease in *CLPTM1L* gene expression in skin tissues; different from that, rs31489, rs401681, rs402710, and rs465498 relate to a decrease, but rs4975616 to an increase in *CLPTM1L* gene expression in esophagus tissues; apart from that, rs31489, rs401681, and rs465498 are connected with an increase, but rs4975616 with a decrease in the *CLPTM1L* gene in stomach tissues (**Supplementary Table 5**).

DISCUSSION

Admittedly, the current large-scale research synopsis and meta-analysis comprehensively summarize and update the correlations between variants in *TERT-CLPTM1L* genes and cancer and non-cancer disease predisposition, which provides precise results for the variants and offers more SNPs and diseases that were never assessed before. To be specific, in this paper, meta-analyses were performed by employing available data from 109 papers with 139,510 cases and 208,530 controls, thus evaluating associations of 23 SNPs with risk of 12 cancers and 1 non-cancer disease; then, it was revealed that, among them, 13 SNPs had significant association with 11 cancers and 1 non-cancer disease predisposition. Besides, the Venice guidelines and FPRP tests were taken for the first time to assess these significant correlations. At last, 12 variants were rated as being strong for cumulative evidence with eight cancers and one non-cancer disease predisposition (22 significant associations: rs2736098, rs401681, and rs402710 in bladder cancer; rs2736100, rs2853691, and rs401681 in esophageal cancer; rs10069690 in gastric cancer; rs2736100 and rs2853676 in glioma; rs2242652, rs2736098, rs2736100, rs2853677, rs31489, rs401681, rs402710, rs465498, and rs4975616 in lung cancer; rs401681 in pancreatic and skin cancer; and rs2736100 in myeloproliferative neoplasms and idiopathic pulmonary fibrosis). Moreover, the study here tended to construct functional annotations for these 12 SNPs with strong evidence using information from the ENCODE Project and other public databases and subsequently revealed that these variants might fall in several putative regulatory regions. Briefly, our research offers comprehensive epidemiological evidence that common variants in the *TERT-CLPTM1L* region show association with predisposition of glioma, myeloproliferative neoplasms, idiopathic pulmonary fibrosis, esophageal cancer, gastric cancer, skin cancer, bladder cancer, lung cancer, and pancreatic cancer.

The *TERT* gene (Gene ID: 7015), encoding the enzyme of *TERT*, plays crucial roles in maintaining the telomere length (32, 33). In the previous research, it was pointed out that telomere length had linked with glioma, ovarian cancer, lung cancer, and melanoma predisposition, rather than breast and prostate cancer (34, 35). Besides, a recent meta-analysis on the GWAS revealed that the variants in *TERT-CLPTM1L* genes may affect cancer risk through a variety of different biological pathways, and telomere length is the only one of the related mechanisms (36), whereas in our study, strong evidence was given to seven SNPs (rs10069690, rs2242652, rs2736098, rs2736100, rs2853676, rs2853677, and rs2853691) in *TERT*. Four SNPs (rs2853677, rs2242652, rs2736098, and rs2736100) were related to the predisposition of lung cancer. The phase 3 of the 1000 Genomes Project (37) (**Supplementary Table 6**) presented that rs2853677 is in moderate LD with rs2736098 (in East Asians: $r^2 = 0.3070$) and rs2736100 (in East Asians: $r^2 = 0.5789$, in Europeans: $r^2 = 0.4352$), is in weak LD with rs2736098 (in Europeans: $r^2 = 0.1504$), and is uncorrelated with rs2736098 and rs2736100 in Africans ($r^2 < 0.05$). Moreover, rs2242652 is in moderate LD with rs2736098 in Europeans ($r^2 = 0.1504$) while is uncorrelated both in East Asians and Africans ($r^2 < 0.05$). According to the above findings, the functional mechanisms of the four variants related to lung cancer risk might be different in various ethnic groups, partially accounting for why some variants are demonstrated to be related to a cancer site in one ethnic group but not in others. In addition, two SNPs (rs2736100 and rs2853676) were associated with glioma risk; however, SNP rs2736100 is in weak LD with rs2853676 in East Asians ($r^2 = 0.2057$), Europeans ($r^2 = 0.2475$), and Africans ($r^2 = 0.1062$). To sum up, these data indicated that there might exist different causal variants and functional mechanisms involved associated with variants in the *TERT* gene with predisposition of glioma. Apart from that, SNP rs2736100 and SNP rs2853691 are intron variants of the *TERT* gene, and they are linked with predisposition of esophageal cancer in our study; while in our study, it was found that rs2736100 is unrelated to rs2853691 in Europeans, East Asians, and Africans ($r^2 < 0.05$ for all tests), revealing that there might exist various functional mechanisms for relationships of *TERT* variants with predisposition of esophageal cancer. Current evidence presents that the rs10069690 T allele can trigger the development of the coproduction of full-length *TERT* and an alternatively spliced by creating splice donor site in intron 4 of *TERT*, which may increase gastric cancer risk by reducing telomerase activity and telomere shortening (38). Moreover, rs2242652 allele (G > A)

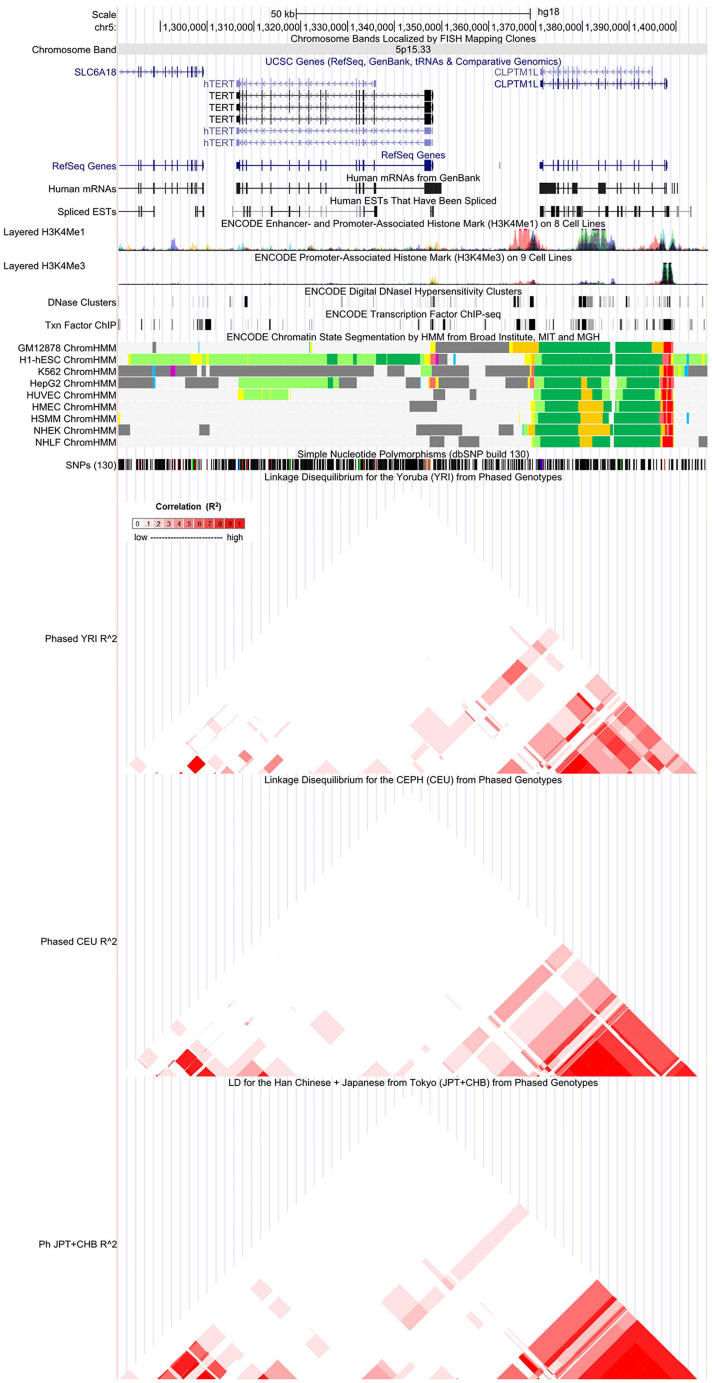

FIGURE 2 | Evidence from the Encyclopedia of DNA Elements (ENCODE) data for regulatory function of variants in 5p15.33 using the UCSC Genome Browser. The plot represents *5p15.33* within a 50-kb window centered on *TERT-CLPTM1L* gene region. Tracks (from top to bottom) in each of the plots are genome base position, chromosome bands, UCSC genes, human messenger RNAs from GenBank, human-expressed sequence tag (ESTs) that have been spliced, ENCODE enhancer and promoter-associated histone mark (H3K4Me1) on 8 Cell Lines, ENCODE promoter-associated histone mark (H3K4Me3) on 9 cell lines, ENCODE digital DNaseI hypersensitivity clusters, ENCODE transcription factor ChIP-seq, ENCODE chromatin state segmentation by hidden Markov model (HMM) from Broad Institute (bright red, active promoter; light red, weak promoter; purple, inactive/poised promoter; orange, strong enhancer; yellow, weak/poised enhancer; blue, insulator; dark green, transcriptional transition/elongation; light green, weak transcribed; gray, polycomb-repressed; light gray, heterochromatin/low signal/repetitive/copy number variation), single-nucleotide polymorphisms (dbSNP build 130), linkage disequilibrium for the Yoruba (YRI) from phased genotypes, linkage disequilibrium for the CEPH (CEU) from phased genotypes and LD for the Han Chinese + Japanese from Tokyo (JPT+CHB) from Phased Genotypes. The scale bar for the LD plot could be found in the data source (ldlink.nci.nih.gov/?tab=ldpair.).

could influence telomere length, which could increase predisposition for lung cancer in Asians (39), whereas SNP rs2736098 could cause the overexpression of *TERT* and increase telomerase activity, which regulated bladder and lung cancer development by modulating unlimited cell division, and carcinogenesis, and interacted with the activation of the glycolytic pathway (40). Previous study showed that the intron 2 segment including the rs2736100 flanking sequence proved promoter activities in ESCC cell lines and uncovered an elevated association with ESCC predisposition in carriers of rs2736100 G allele (41), which demonstrated the oncogene inherent characteristics of *TERT* in ESCC. Here, it should be noted that the T allele of rs2736100 could lead to telomere length shortening and then increase lung cancer and non-cancerous disease predisposition (like chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis) (42, 43).

The *CLPTM1L* gene (Gene ID: 81037), encoding the cleft lip and palate–associated transmembrane 1–like protein, could arouse cell apoptosis and cytokinesis (44, 45). In our study, strong evidence was assigned to four SNPs (rs31489, rs401681, rs402710, and rs465498) in *CLPTM1L* and one SNP rs4975616 in *TERT-CLPTM1L*. Of the two variants associated with bladder cancer, rs401681 is in strong LD with rs402710 in East Asians (r^2 = 0.9371) while has moderate LD in Europeans (r^2 = 0.6624) and Africans (r^2 = 0.5961). Moreover, four SNPs (rs31489, rs401681, rs402710, and rs465498) were related to lung cancer, and, in our study, it was found that rs31489 is in strong LD with rs401681 in Europeans (r^2 = 0.8145) while is in moderate LD in East Asians (r^2 = 0.4785) and Africans (r^2 = 0.6131); rs31489 is in strong LD with rs402710 in Africans (r^2 = 0.8376) while is in moderate LD in East Asians (r^2 = 0.4634) and Europeans (r^2 = 0.6624); rs31489 is in strong LD with rs465498 both in East Asians and Europeans (r^2 > 0.809) while is in moderate LD in Africans (r^2 = 0.6572). Moreover, it was revealed that rs401681 is in strong LD with rs402710 in East Asians (r^2 = 0.9371) but shows moderate LD in Europeans (r^2 = 0.6624) and Africans (r^2 = 0.5961); rs401681 is in strong LD with rs465498 both in Europeans and Africans (r^2 > 0.809) while is in moderate LD in East Asians (r^2 = 0.4839). Based on the obtained results, the functional mechanisms of the four variants associated with risk of bladder and lung cancer may be different in different ethnic groups and partly account for why some variants are discovered to be related to a cancer site in one ethnic group but not in others. Furthermore, current evidence demonstrates that rs31489, a variant in which C is changed to A in *CLPTM1L* gene, could influence the telomere length that could decrease the risk of nonsmokers' lung carcinoma (rather than in smokers), because smoking can counteract the protective role of A allele, shorten telomere length, and enhance telomerase activity (46). Interestingly, rs401681 could affect transcription regulation, result in the over-expression of the *CLPTM1L* gene, and increase risk of lung and skin carcinoma (47). Moreover, *CLPTM1L* rs402710 may affect lung tissue tumorigenesis *in vitro* by blocking DNA damage–induced apoptosis *via* enhanced accumulation of *Bcl-xL*, an antiapoptotic *Bcl2* family member (48). Beyond that, SNP rs402710 could maintain the telomere length which could decrease the risk of nonsmokers' lung cancer since the protective role of rs2736100 was counteracted in patients with bladder cancer who currently were smokers (49). In addition, seven variants in the *TERT* gene are uncorrelated or had weak LD with the four variants in the *CLPTM1L* gene in European, Asian, and African populations. According to the results, there exist different causal variants and functional mechanisms in relationships of variants in the *TERT-CLPTM1L* regions with idiopathic pulmonary fibrosis, myeloproliferative neoplasms, and glioma, as well as esophageal, gastric, bladder, lung, pancreatic, and skin cancer predisposition.

In addition, 13 SNPs had no association with five cancer risk in additive model. Of these, eight SNPs (rs13167280, rs2075786, rs2735940, rs2736100, rs2736109, rs2853669, rs2853677, rs2853690, and rs7712562) had no association with breast cancer with at least 5,000 case and 5,000 controls in additive model, which had approximately 98% statistical power to detect an OR of 1.15 for a variant with MAF 0.20 and 86% power with MAF 0.10 (type 1 error 0.05). Therefore, further research with a smaller sample size on these eight SNPs for breast cancer in

TABLE 3 | Variants in *TERT-CLPTM1L* showing no relation to breast cancer risk in meta-analyses with at least 5000 cases and 5000 controls in additive model.

Gene	Variant	Allelesa	Cancer Type	Ethnicity	MAFb	Number evaluation		Meta-analysis risk		Heterogeneity	
						Studies	Sample size (case/control)	OR (95%CI)	P_{value}	I^2(%)	$P_{(Q)}$
TERT	rs13167280	G/A	Breast cancer	Caucasian	0.7312	3	10759 (5057/5702)	0.963 (0.888-1.043)	0.349	0.0	0.681
TERT	rs2075786	A/G	Breast cancer	Caucasian	0.6460	3	10759 (5057/5702)	0.996 (0.942-1.054)	0.902	0.0	0.716
TERT	rs2735940	A/G	Breast cancer	Overall	0.5282	6	12154 (5514/6640)	0.978 (0.913-1.048)	0.534	31.4	0.212
TERT	rs2735940	A/G	Breast cancer	Caucasian	0.5324	5	11495 (5446/6049)	1.006 (0.957-1.059)	0.803	83.6	< 0.001
TERT	rs2736109	G/A	Breast cancer	Overall	0.4663	8	22281 (9457/12824)	0.987 (0.922-1.056)	0.704	59.1	0.017
TERT	rs2736109	G/A	Breast cancer	Caucasian	0.4696	7	21387 (9130/12257)	0.995 (0.957-1.035)	0.809	64.8	0.009
TERT	rs2853669	T/C	Breast cancer	Caucasian	0.4864	10	18993 (8635/10358)	1.006 (0.942-1.074)	0.870	41.6	0.080
TERT	rs2853677	G/A	Breast cancer	Caucasian	0.4124	3	10759 (5057/5702)	1.036 (0.980-1.095)	0.211	0.0	0.780
TERT	rs2853690	G/T	Breast cancer	Caucasian	0.1496	3	10759 (5057/5702)	0.977 (0.905-1.054)	0.541	0.0	0.847
TERT	rs7712562	A/C	Breast cancer	Caucasian	0.2791	3	10759 (5057/5702)	1.035 (0.927-1.156)	0.540	50.4	0.133

OR, odds ratio; A, adenine; C, cytosine; G, guanine; T, thymine.

[a]*Major alleles (reference)/minor alleles.*

[b]*Frequency of minor allele in controls.*

Caucasians will not be helpful in evaluating effects of those SNPs (**Supplementary Result**, **Table 3** and **Supplementary Table 4**).

In fact, our study has several limitations: (i) although a comprehensive research on databases was conducted, some publications may have been missed, as well as the papers with insufficient data such as the genotype amount, which might result in incomplete assessment of other malignancies (lymphoma, gallbladder cancer, cervical cancer, etc.) and non-cancer disease (chronic hepatitis B, Alzheimer's disease, diabetes mellitus, etc.); (ii) the potential publication bias might be found due to the usage of the search approach (only search for English papers); (iii) as the subgroup analyses according to ethnicity and partial pathological/clinical subtypes were only performed on lung cancer, idiopathic pulmonary fibrosis and myeloproliferative neoplasms, further analyses based on subgroups such as pathological type, gene-gene or gene-environment associations and interactions, could be required to confirm or refute the correlations with risk of cancers and non-cancer disease; (iv) potential bias for variants with cancers and non-cancer risk could be evaluated by the Venice criteria; however, the unreasonable data, like errors in genotype, could not be evaluated; and (v) meta-analyses were conducted on the basis of the minor allele of a variant; therefore, a protective association for some variants might be found because of the inherent factors in meta-analysis (for example, rs2736100 and rs2853676 could reduce the risk of glioma, myeloproliferative neoplasms, and lung cancer and rs401681 could decrease risk of bladder cancer). Given that, all genetic associations in the current work should be further confirmed and clarified by doing the molecular biology experiments.

To conclude, in our study, it was identified that 12 variants in the *TERT-CLPTM1L* genes were rated as revealing strong evidence for a significant correlation with eight cancers and one non-cancer disease risk. Moreover, our study offers foundation for further demonstrating that the variants in the *TERT-CLPTM1L* genes are related to the risk of idiopathic pulmonary fibrosis, myeloproliferative neoplasms, glioma, esophageal cancer, gastric cancer, bladder cancer, lung cancer, pancreatic cancer, and skin cancer. Apart from that, the crucial roles of the *TERT-CLPTM1L* region in the etiology of human diseases were highlighted.

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.

AUTHOR CONTRIBUTIONS

JT, YW, and GC designed this work. JT and YW integrated and analyzed the data. JT, YW, and GC wrote this manuscript. JT, YW, YD, JC, YMW, SC, and GC finished the related Tables and Figures. JT, YW, and GC edited and revised the manuscript. All authors approved this manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.946039/full#supplementary-material

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Epidemiological Evidence for Associations Between Genetic Variants and Osteosarcoma Susceptibility: A Meta-Analysis

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Background: Previous studies have showed that single nucleotide polymorphisms (SNPs) might be implicated in the pathogenesis of osteosarcoma (OS). Numerous studies involving SNPs with OS risk have been reported; these results, however, remain controversial and no comprehensive research synopsis has been performed till now.

Objective: This study seeks to clarify the relationships between SNPs and OS risk using a comprehensive meta-analysis, and assess epidemiological evidence of significant associations.

Methods: The PubMed, Web of Science, and Medline were used to screen for articles that evaluated the association between SNP and OS susceptibility in humans before 24 December 2021. Furthermore, we used Venice Criteria and a false positive report probability (FPRP) test to assess the grades of epidemiological evidence for the statistical relationships.

Results: We extracted useful data based on 43 articles, including 10,255 cases and 13,733 controls. Our results presented that 25 SNPs in 17 genes were significantly associated with OS risk. Finally, we graded strong evidence for 17 SNPs in 14 genes with OS risk (*APE1* rs1760944, *BCAS1* rs3787547, *CTLA4* rs231775, *ERCC3* rs4150506, *HOTAIR* rs7958904, *IL6* rs1800795, *IL8* rs4073, *MTAP* rs7023329 and rs7027989, *PRKCG* rs454006, *RECQL5* rs820196, *TP53* rs1042522, *VEGF* rs3025039, rs699947 and rs2010963, *VMP1* rs1295925, *XRCC3* rs861539), moderate for 14 SNPs in 12 genes and weak for 14 SNPs in 11 genes.

Conclusion: In summary, this study offered a comprehensive meta-analysis between SNPs and OS susceptibility, then evaluated the credibility of statistical relationships, and provided useful information to identify the appropriate candidate SNPs and design future studies to evaluate SNP factors for OS risk.

Keywords: meta-analysis, osteosarcoma, single nucleotide polymorphism, susceptibility, Venice criteria

INTRODUCTION

Osteosarcoma (OS) is one of the most common bone malignancies, occurring mainly in the metaphyseal area around the knee joint (1, 2). Although current treatment strategy, including neoadjuvant therapy prior to wide margin surgical resection and followed by postoperative chemotherapy, greatly improves long-term survival rate to about 70%, its outcome is not satisfactory (3). The pathogenesis of OS is a complex, multistep and multifactorial process in which interactions between genetic and environment factors are proposed to be related to the progression of OS (4, 5). Single nucleotide polymorphisms (SNPs) have been reported to be involved in DNA repair, growth regulation, antigen processing and presentation, which may be implicated in the pathogenesis of OS (6–8). Genetic variants, such as *VEGF* rs2010963, *ERCC* rs1800795, and *IL6* rs1800795, have been found to be related to the susceptibility of lung cancer, gastric cancer, and OS (9–11). Genetic variation plays crucial roles in the pathogenesis of OS and elucidating relationships between genetic variation and OS susceptibility is critical to improve the therapeutic strategies (6, 7).

The study of the association of genetic variation is widely used to filter genes susceptible to OS (6, 7). Although in previous published studies, a single SNP with the risk of OS was investigated, the results were conflicting (9, 12–14). Wang et al. reported that *VEGF* rs3025039 could increase the risk of OS in the recessive model and allelic model (14). However, a study performed by Cao et al. revealed that *VEGF* rs3025039 was not related to OS risk in different genetic models (12). Meta-analysis can assess the consistency of association and increase statistical power, as well as avert repetition and mistakes from previous studies (15). In 2018, Wang et al. summarized the relationships between genetic variants and OS susceptibility only under an allelic model without evaluating cumulative evidence (16). Besides, a comprehensive research synopsis had not been performed to evaluate the epidemiological evidence of genetic relationships between SNP and OS susceptibility till now. To classify cumulative evidence of genetic relationships with OS susceptibility, the Venice Criteria and the false positive report probability (FPRP) test were used in multiple meta-analysis studies (17, 18). Therefore, we aimed to perform an updated meta-analysis to systematically investigate all genetic variation studies of OS risk, then use Venice Criteria and FPRP test to assess the cumulative evidence of the statistical relationships.

METHODS

Literature Search

Preferred Reporting Items for Systematic Reviews and Meta-analysis Statement (PRISMA) were followed in our study (19, 20). We used the PubMed, Web of Science, and Medline to screen the eligible papers before 24 December 2021 using the following terms: ({osteosarcoma} OR {osteogenic sarcoma OR {Sarcoma, Osteogenic}) AND ({variation} OR {variant} OR {single nucleotide polymorphism} OR {polymorphism} OR {SNP}). Moreover, we also screened other relevant articles in the references of the included articles (**Figure 1**).

Criteria for Inclusion and Exclusion

The included criteria were as follows: (a) evaluation of the association between SNP and OS susceptibility in a case-control in humans; (b) pathologically confirmed OS; (c) providing sufficient information (such as genotype amount); (d) published articles with full text in English. The excluded criteria were as follows: (a) duplicate publications; (b) case reports, reviews, letters, conference abstracts, and meta-analysis; (c) the articles were about the survival/mortality rate of OS.

Data Extraction

The first author (DY) and co-first author (JT) independently extracted relevant information and then cross-checked each other. If any disagreement was found, discussion and reexamination were made with the third investigator (HD). The following details were extracted: first author, year of publication, variation in gene (rs numbers), gene name, genotype counts, ethnicity, and sample size. Apart from that, three ethnicities (Asian, Mixed and Caucasian) were mentioned in this research; "overall" indicated two or more. Regarding the same SNP with different modes of presentation, we adopted the most recent one on the website (https://www.ncbi.nlm.nih.gov/snp/). The quality of included studies was assessed based on the Newcastle Ottawa Scale (NOS; **Supplementary Table S1**).

Statistical Analysis

We conducted the study with Stata, version 12 (Stata, College Station, TX, USA), and $P < 0.05$ (two-sided) indicated the significance level in our study. We performed meta-analysis under three models (allelic, dominant, and recessive models) with at least two independent datasets; a subgroup analysis on the basis of ethnicity was also evaluated if necessary. We assessed the heterogeneity of the different studies using the Cochran's Q test and the I^2 statistic. Specifically, the I^2 values were assigned at three levels: ≥50%, 25%–50%, ≤25% (21, 22). We used the fixed effect model ($P_Q > 0.1$) and the random effect model ($P_Q < 0.1$). Furthermore, we conducted sensitivity analysis to evaluate whether significant ORs were lost by excluding the first published study or studies that deviated from the Hardy-Weinberg equilibrium (HWE) in controls. Potential publication bias and small study bias were assessed using Begg's test and Egger's test respectively [$P < 0.1$ is the significant level; (23, 24)].

Evaluation of Cumulative Evidence

The Venice Criteria and FPRP were respectively used to investigate the cumulative epidemiological credibility of significant relationships (17, 18, 25) (**Supporting Information for the Venice Criteria and FPRP**). Cumulative epidemiological evidence of statistical relationships was assigned as strong level (all A) or weak level (any C), or moderate level (a combination of A or B) based on Venice Criteria. Ultimately, evidence levels

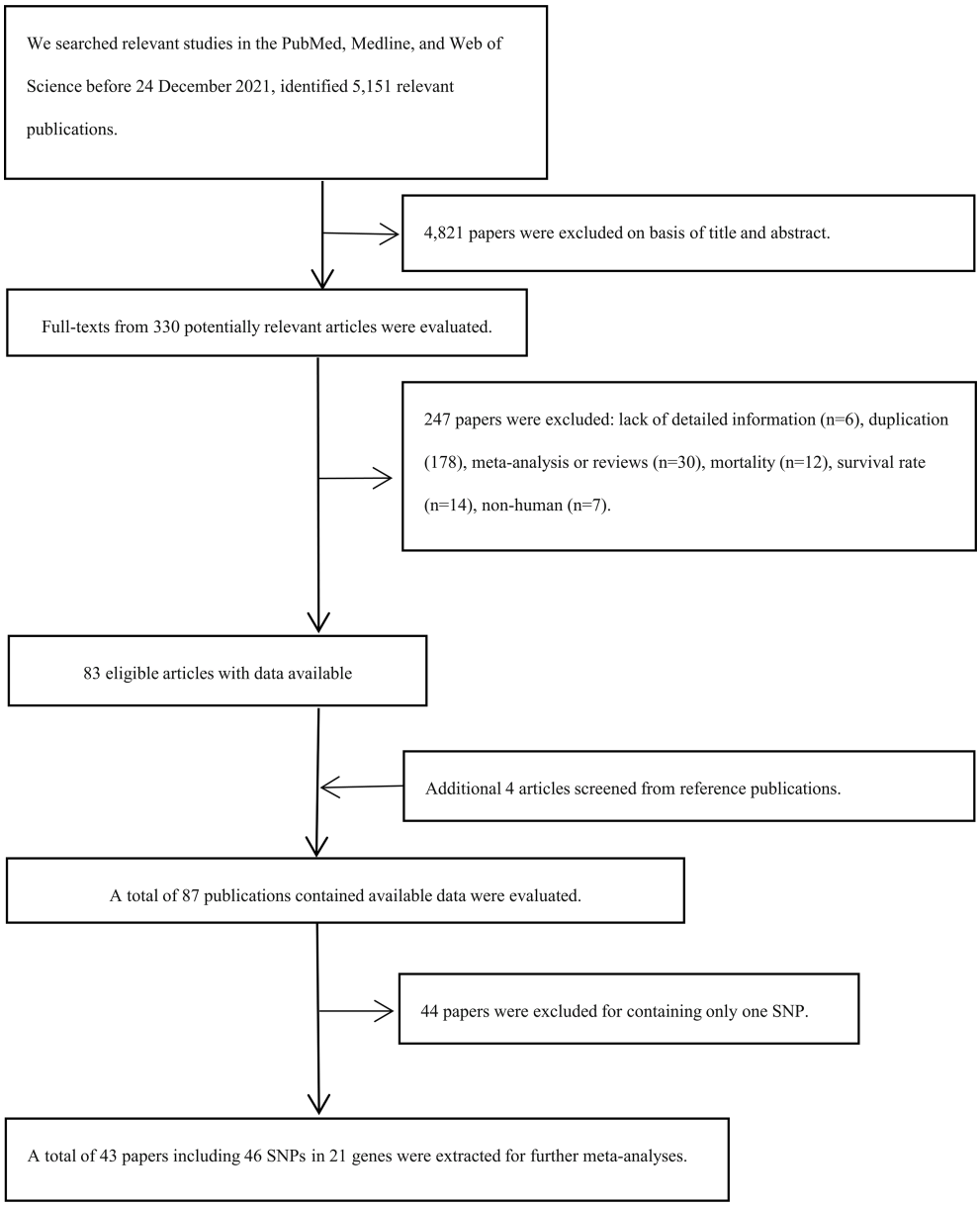

FIGURE 1 | Flow diagram of search strategy and study selection. *SNPs*, single nucleotide polymorphisms.

were adjusted according to the FPRP value (cumulative evidence could be downgraded or upgraded according to the FPRP value).

RESULTS

Characteristics of the Included Articles

Our research included 5,151 relevant publications, excluded 4,821 papers based on the title and abstract, and excluded 247 papers after a full text review. Apart from that, four papers were screened from reference publications (**Figure 1**). Finally, a total of 43 articles with 46 SNPs in 21 genes were extracted, including 10,255 cases and 13,733 controls (**Supplementary Table S2**). Furthermore, the mean study quality score for included papers was 6.88 ± 0.31 (ranged from 6 to 7) based on NOS (**Supplementary Table S1**).

Main Meta-Analyses

We conducted a meta-analysis between 46 SNPs in 21 genes and OS risk (a total of 153 associations); of these, 25 SNPs in 17 genes were statistically associated with susceptibility to OS (65 significant associations; **Supplementary Table S3**). Specifically,

APE1 rs1760944, *ERCC3* rs4150506, *HOTAIR* rs7958904, *IL8* rs4073, *MTAP* rs7023329, *MTAP* rs7027989, *PRKCG* rs454006, *RECQL5* rs820196, *VEGF* rs2010963, *VEGF* rs3025039, *VEGF* rs699947, *XRCC1* rs25487 and *XRCC3* rs861539 had significant association with the susceptibility to OS in Asians under three models (allelic model, dominant model, and recessive model). *BCAS1* rs3787547 was significantly associated with susceptibility to OS in Asians (allelic model and dominant model). *CTLA4* rs231775 had statistical relationship with OS susceptibility in overall population and in Asians under three models. For *CTLA4* rs5742909, it was statistically associated with OS susceptibility under recessive model in Asians. We found that *ERCC3* rs4150441 had significant association with OS risk under dominant model in Asians. We found that *HOTAIR* rs874945 was statistically associated with OS susceptibility in Asians under allelic model. *IL10* rs1800896 was significantly associated with risk of OS in overall population (allelic model and dominant model). We found that *IL6* rs1800795 had significant association with OS risk in Asians (allelic model and recessive model). *TNF-α* rs1800629 had statistical association with OS susceptibility in overall population under three models. Our results presented that *TP53* rs1042522 had statistical association with OS susceptibility in overall population (allelic model and dominant model), and in Caucasians (allelic model and dominant model). For *VEGF* rs1570360, our results showed that SNP rs1570360 had statistical association with OS susceptibility in Asians under allelic model. *VEGF* rs833061 had statistical relationship with OS susceptibility in Asians under allelic model. *VMP1* rs1295925 had statistical relationship with OS susceptibility in overall population (allelic model and dominant model; **Table 1**).

Cumulative Evidence of Association

We used the Venice Criteria to assess cumulative epidemiological credibility of significant associations (**Supplementary Table S3**). There were 25 grades A in the amount of evidence, 56 grades A in the replication of association, and 61 grades A in the protection from bias, respectively; there were 37, 7, and 0 grades B in these three criteria, respectively; and there were 3, 2, and 4 grades C were in these three criteria, respectively. The FPRP values were then used to evaluate the significant associations between the 25 SNPs and OS risk (65 associations). 29 associations between 16 SNPs in 14 genes and OS risk obtained a FPRP value < 0.05, as follows: *APE1* rs1760944 (two associations); *BCAS1* rs3787547 (two associations); *CTLA4* rs231775 (four associations); *ERCC3* rs4150506 (one association); *HOTAIR* rs7958904 (one association); *IL6* rs1800795 (one association); *IL8* rs4073 (one association); *MTAP* rs7023329 (two associations); *PRKCG* rs454006 (two associations); *RECQL5* rs820196 (one association); *TP53* rs1042522 (two associations); rs3025039, rs699947, and rs2010963 in *VEGF* (eight associations); *VMP1* rs1295925 (one association); *XRCC3* rs861539 (one association). 16 associations between 15 SNPs in 13 genes and OS risk obtained FPRP 0.05 to 0.2. 20 associations between 16 SNPs in 13 gens and OS risk obtained FPRP value >0.2. Finally, 31 associations with strong evidence were found between 17 SNPs in 14 genes and OS risk (**Table 1**), as follows: *APE1* rs1760944 (two associations); *BCAS1* rs3787547 (two associations); *CTLA4* rs231775 (four associations); *ERCC3* rs4150506 (one association); *HOTAIR* rs7958904 (one association); *IL6* rs1800795 (one association); *IL8* rs4073 (one association); rs7023329, rs7027989 in *MTAP* (three associations); *PRKCG* rs454006 (two associations); *RECQL5* rs820196 (one association); *TP53* rs1042522 (two associations); rs3025039, rs699947, and rs2010963 in *VEGF* (eight associations); *VMP1* rs1295925 (two associations); *XRCC3* rs861539 (one association). 17 associations with moderate evidence were found between 14 SNPs in 12 genes and OS risk; 17 associations with weak evidence were found between 14 SNPs in 11 genes and OS risk.

Heterogeneity, Bias, and Sensitivity Analysis

56 associations (86.15%) between 23 SNPs in 17 genes and OS risk obtained mild heterogeneity; 7 associations (10.77%) between six SNPs in five genes and OS risk obtained moderate heterogeneity; two associations (3.08%) between two SNPs in two genes and OS risk obtained high heterogeneity (**Table 1**). Publication bias ($p < 0.10$ in Begg's test) was found only in one association (*VEGF* rs3025039 under the dominant model in Asians). After deleting the first published study, the relationships between three SNPs in two genes and OS susceptibility were no longer significant (*CTLA4* rs231775 in the overall population under recessive association; *CTLA4* rs5742909 under recessive association in Asians; *VEGF* rs1570360 under allelic association in Asians; **Supplementary Table S3**).

DISCUSSION

Our study conducted a comprehensive and updated meta-analysis of the relationships between genetic variants and OS susceptibility. We conducted meta-analysis from 43 papers with 46 SNPs in 21 genes, and found that 25 SNPs in 17 genes were significantly associated with susceptibility to OS (65 significant associations). We further assessed the levels of epidemiological evidence for significant associations combining Venice Criteria as well as FPRP test. Finally, 31 associations with strong epidemiological credibility were found between 17 SNPs in 14 genes and OS risk, as follows: *APE1* rs1760944 (two associations); *BCAS1* rs3787547 (two associations); *CTLA4* rs231775 (four associations); *ERCC3* rs4150506 (one association); *HOTAIR* rs7958904 (one association); *IL6* rs1800795 (one association); *IL8* rs4073 (one association); rs7023329, rs7027989 in *MTAP* (three associations); *PRKCG* rs454006 (two associations); *RECQL5* rs820196 (one association); *TP53* rs1042522 (two associations); rs3025039, rs699947, and rs2010963 in *VEGF* (eight associations); *VMP1* rs1295925 (two associations); *XRCC3* rs861539 (one association).

The apurinic/apyrimidinic endodeoxyribonuclease 1 (*APE1*) gene may be involved in the specific activation of DNA repair and numerous malignancies (26, 27). This study presented strong evidence of the association between a polymorphism (rs1760944) and lower OS risk. SNP rs1760944 (T>G) may impair the binding affinity of octamer-binding transcription factor-1 (*Oct-1*), thus reducing *APE1* mRNA expression levels

TABLE 1 | Genetic variants showing significant associations with OS risk in main meta-analyses.

Gene	Variant	Allelic[a]	Ethnicity	Number evaluation		Genetic models	MAF	Effect model	Risk of meta-analysis				Venice Criteria[c]	FPRP values[d]	Credibility of evidence
				Studies	Cases/controls				OR[b] (95%CI)	I^2	P_Q	P_{value}			
APE1	rs1760944	T>G	Asian	2	378/616	Allelic	0.442	Fixed	0.692 (0.574–0.834)	0	0.701	<0.001	BAA	0.003	Strong
						Dominant		Fixed	0.610 (0.468–0.796)	0	0.748	<0.001	BAA	0.020	Strong
						Recessive		Fixed	0.642 (0.451–0.914)	0	0.867	0.014	BAA	0.388	Weak
BCAS1	rs3787547	G>A	Asian	2	1300/1300	Allelic	0.300	Fixed	1.222 (1.088–1.373)	0	0.703	0.001	AAA	0.014	Strong
						Dominant		Fixed	1.295 (1.110–1.511)	0	0.694	0.001	AAA	0.020	Strong
CTLA-4	rs231775	A>G	Overall	4	660/754	Allelic	0.623	Fixed	0.725 (0.620–0.846)	0	0.96	<0.001	AAA	0.001	Strong
						Dominant		Fixed	0.491 (0.360–0.668)	0	0.981	<0.001	AAA	0.004	Strong
						Recessive		Fixed	0.748 (0.596–0.938)	29.9%	0.233	0.012	BBC	0.212	Weak
			Asian	3	594/629	Allelic	0.672	Fixed	0.723 (0.613–0.853)	0	0.862	<0.001	AAA	0.003	Strong
						Dominant		Fixed	0.506 (0.354–0.722)	0	0.97	<0.001	AAA	0.049	Strong
						Recessive		Fixed	0.717 (0.569–0.903)	0	0.711	0.005	BAA	0.109	Moderate
	rs5742909	C>T	Asian	3	486/533	Recessive		Fixed	2.046 (1.028–4.073)	0.0%	0.591	0.042	CAC	0.807	Weak
ERCC3	rs4150441	T>C	Asian	2	522/1047	Dominant		Fixed	0.519 (0.357–0.755)	60.1%	0.113	0.001	ACA	0.108	Moderate
	rs4150506	G>A	Asian	2	522/1047	Allelic	0.230	Fixed	1.331 (1.123–1.576)	0	0.581	0.001	BAA	0.019	Strong
						Dominant		Fixed	1.348 (1.089–1.667)	0	0.775	0.006	BAA	0.117	Moderate
						Recessive		Fixed	1.622 (1.110–2.370)	0	0.498	0.012	BAA	0.408	Weak
HOTAIR	rs7958904	C>G	Asian	2	900/900	Allelic	0.710	Fixed	1.294 (1.115–1.501)	0	0.736	0.001	AAA	0.013	Strong
						Dominant		Fixed	1.636 (1.154–2.321)	0	0.961	0.006	AAA	0.260	Moderate
						Recessive		Fixed	1.298 (1.078–1.564)	0	0.768	0.006	BAA	0.110	Moderate
	rs874945	C>T	Asian	2	900/900	Allelic	0.189	Fixed	1.183 (1.006–1.393)	17.4%	0.271	0.042	BAA	0.455	Weak
IL-10	rs1800896	T>C	Overall	2	340/420	Allelic	0.391	Fixed	1.326 (1.060–1.657)	0	0.557	0.013	BAA	0.224	Weak
						Dominant		Fixed	1.398 (1.009–1.936)	33.9%	0.219	0.044	BBA	0.556	Weak
IL-6	rs1800795	C>G	Asian	2	322/322	Allelic	0.750	Random	0.563 (0.445–0.712)	0	0.805	<0.001	BAA	0.000	Strong
						Recessive		Random	0.420 (0.268–0.659)	47.6%	0.167	<0.001	BBA	0.121	Moderate
IL-8	rs4073	A>T	Asian	2	299/299	Allelic	0.776	Fixed	0.625 (0.483–0.809)	0	0.793	<0.001	BAA	0.021	Strong
						Dominant		Fixed	0.598 (0.366–0.975)	0	0.949	0.039	BAA	0.692	Weak
						Recessive		Fixed	0.590 (0.424–0.819)	0	0.823	0.002	BAA	0.116	Moderate
MTAP	rs7023329	A>G	Asian	2	392/1578	Allelic	0.512	Fixed	0.712 (0.615–0.844)	0	0.540	<0.001	AAA	0.002	Strong
						Dominant		Fixed	0.650 (0.510–0.828)	0	0.439	<0.001	AAA	0.022	Strong
						Recessive		Fixed	0.641 (0.484–0.848)	0	0.855	0.002	BAA	0.082	Moderate
	rs7027989	A>G	Asian	2	392/1578	Allelic	0.824	Fixed	0.761 (0.627–0.923)	0	0.905	0.006	AAA	0.104	Strong
						Recessive		Fixed	0.757 (0.601–0.954)	0	0.760	0.018	AAA	0.288	Moderate
						Dominant		Fixed	0.557 (0.328–0.945)	0	0.751	0.030	AAA	0.693	Moderate
PRKCG	rs454006	T>C	Asian	2	998/998	Allelic	0.293	Fixed	1.347 (1.178–1.539)	0	0.826	<0.001	AAA	0.000	Strong
						Dominant		Fixed	1.204 (1.010–1.437)	15.4%	0.277	0.039	AAA	0.432	Moderate
						Recessive		Fixed	1.989 (1.536–2.575)	0	0.596	<0.001	BAA	0.000	Strong
RECQL5	rs820196	T>C	Asian	2	397/441	Allelic	0.340	Fixed	1.445 (1.186–1.762)	0	0.742	<0.001	BAA	0.008	Strong
						Dominant		Fixed	1.487 (1.118–1.976)	0	0.844	0.006	BAA	0.184	Moderate
						Recessive		Fixed	2.153 (1.409–3.289)	0	0.700	<0.001	BAA	0.135	Moderate
TNF-α	rs1800629	G>A	Overall	2	160/259	Allelic	0.183	Fixed	1.743 (1.245–2.440)	0	0.582	0.001	BAA	0.107	Moderate
						Dominant		Fixed	1.640 (1.065–2.524)	0	0.427	0.025	BAA	0.576	Weak
						Recessive		Fixed	3.306 (1.541–7.093)	0	0.588	0.002	CAA	0.657	Weak
TP53	rs1042522	G>C	Overall	3	515/744	Allelic	0.499	Fixed	0.738 (0.618–0.881)	0.0%	0.754	0.001	AAA	0.017	Strong
						Dominant		Fixed	0.591 (0.445–0.784)	14.5%	0.310	<0.001	BAA	0.024	Strong
		G>C	Caucasian	2	305/324	Allelic	0.342	Fixed	0.764 (0.584–0.999)	0.0%	0.503	0.049	BAA	0.526	Weak
						Dominant		Fixed	0.534 (0.364–0.783)	47.4%	0.168	0.001	BBA	0.163	Moderate

(Continued)

TABLE 1 | Continued

Gene	Variant	Allelic[a]	Ethnicity	Number evaluation		Genetic models	MAF	Effect model	Risk of meta-analysis				Venice Criteria[c]	FPRP values[d]	Credibility of evidence
				Studies	Cases/controls				OR[b] (95%CI)	I^2	P_Q	P_{value}			
VEGF	rs1570360	A>G	Asian	3	527/692	Allelic	0.254	Fixed	1.229 (1.025–1.475)	0	0.774	0.026	BAC	0.341	Weak
	rs2010963	C>G	Asian	7	1489/1867	Allelic	0.338	Random	1.249 (1.089–1.432)	46.4%	0.083	0.001	ABA	0.027	Strong
						Dominant		Fixed	1.393 (1.190–1.630)	0	0.504	<0.001	AAA	0.001	Strong
						Recessive		Fixed	1.294 (1.098–1.524)	34.8%	0.163	0.002	BBA	0.038	Strong
	rs3025039	C>T	Asian	8	1671/2049	Allelic	0.230	Fixed	1.248 (1.120–1.391)	0	0.941	<0.001	AAA	0.001	Strong
						Dominant		Fixed	1.222 (1.066–1.399)	0	0.997	0.004	AAC	0.065	Weak
						Recessive		Fixed	1.596 (1.253–2.032)	0	0.702	<0.001	BAA	0.009	Strong
	rs699947	A>C	Asian	4	709/874	Allelic	0.679	Fixed	0.713 (0.615–0.827)	0	0.593	<0.001	AAA	0.000	Strong
						Dominant		Fixed	0.611 (0.462–0.810)	0	0.776	0.001	AAA	0.041	Strong
						Recessive		Fixed	0.685 (0.559–0.840)	0	0.687	<0.001	BAA	0.009	Strong
	rs833061	C>T	Asian	2	358/358	Allelic	0.624	Fixed	0.788 (0.638–0.974)	34.6%	0.216	0.027	BBA	0.358	Weak
VMP1	rs1295925	T>C	Asian	2	1300/1300	Allelic	0.450	Fixed	0.847 (0.759–0.945)	0	0.597	0.003	AAA	0.053	Strong
						Dominant		Fixed	0.767 (0.651–0.902)	0	0.646	0.001	AAA	0.026	Strong
XRCC1	rs25487	T>C	Asian	2	318/523	Allelic	0.481	Fixed	1.405 (1.132–1.745)	0	0.433	0.002	BAA	0.052	Moderate
						Dominant		Fixed	1.488 (1.055–2.099)	0	0.902	0.024	BAA	0.463	Weak
						Recessive		Fixed	1.564 (1.114–2.195)	50.1%	0.157	0.010	BCA	0.313	Weak
XRCC3	rs861539	G>A	Asian	2	288/440	Allelic	0.272	Fixed	1.572 (1.252–1.975)	0	0.882	<0.001	BAA	0.006	Strong
						Dominant		Fixed	1.573 (1.161–2.133)	0	0.902	0.003	BAA	0.151	Moderate
						Recessive		Fixed	2.230 (1.395–3.566)	0	0.896	0.001	CAA	0.240	Weak

APE1, apurinic/apyrimidinic endodeoxyribonuclease 1; BCAS1, brain enriched myelin associated protein 1; CTLA4, cytotoxic T-lymphocyte associated protein 4; ERCC3, excision repair cross-complementation 3; HOTAIR, HOX transcript antisense RNA; IL-10, interleukin-10; IL-6, interleukin-6; IL-8, interleukin-8; MTAP, methylthioadenosine phosphorylase; PRKCG, protein kinase C gamma; RECQL5, RecQ like helicase 5; TNF-α, tumor necrosis factor α; TP53, tumor protein p53; VEGF, vascular endothelial growth factor A; VMP1, vacuole membrane protein 1; XRCC1, X-ray repair cross complementing 1; XRCC3, X-ray repair cross complementing 3; A, adenine; C, cytosine; G, guanine; T, thymine; OR, odds ratio; CI, confidence interval; MAF, minor allelic frequency in control; NA, not applicable; FPRP, false positive report probability.

[a]*Allelic: Minor allelic (bold) versus major allelic (reference).*

[b]*OR: OR < 1, decrease the susceptibility of OS (protective factor); OR > 1, increase the susceptibility of OS (susceptive factor).*

[c]*Venice Criteria grades are for the amount of evidence, replication of the association, and protection from bias.*

[d]*The prior probability of FPRP is 0.05 and the FPRP level of noteworthiness is 0.20.*

and then decreasing the risk of OS (28) which is the same as in our meta-analysis. For *APE1* rs1760944, OS patients with G allele had better survival and less susceptible to metastasis, and lower risk of low differentiation tumor (29). The brain enriched myelin associated protein 1 (*BCAS1*) gene resides in a region at 20q13.2 and *BCAS1* rs3787547 may be related to the development of OS by altering the binding power of p53, which is one of the most critical tumor suppressors (30). Our meta-analysis found that *BCAS1* rs3787547 increased the susceptibility of OS with strong evidence in Asians (allelic and dominant model). The excision repair cross-complementation 3 (*ERCC3*) gene encodes a DNA helicase that plays an important role in nucleotide excision repair. The polymorphisms of *ERCC3* have been reported to be associated with several cancers, such as colorectal cancer, pancreatic cancer, breast cancer, and OS (5, 31, 32). The potential mechanism of OS susceptibility was deemed to be its functions as rate-limiting enzymes in the NER pathway (33). Our meta-analysis found that *ERCC3* rs4150506 increased the risk of OS in Asians under the allelic model (strong evidence), dominant model (moderate evidence), and recessive model (weak evidence). The HOX transcript antisense RNA (*HOTAIR*) gene is highly expressed in a variety of cancers, and deletion of HOTAIR can inhibit the aggressiveness of cancers (34). The research by Zhou et al. supported the hypothesis that SNP rs7958904 increased OS risk by influencing lncRNA expression, which was localized to a regulatory boundary in the HOXC cluster (35). Our meta-analysis found that *HOTAIR* rs7958904 increased the risk of OS in Asians (strong evidence in the allelic model, moderate evidence in dominant model, moderate evidence in recessive model), and *HOTAIR* rs874945 increases the risk of OS in Asians under the allelic model with weak evidence.

The interleukin-6 (*IL6*) gene encodes an inflammation cytokine and may be involved in key steps of tumor proliferation, apoptosis, angiogenesis, and differentiation (36). For *IL6* rs1800795, OS patients carrying G allele had better survival and less susceptible to metastasis (10). The interleukin-8 (*IL8*) gene plays a critical role in both the pathogenesis and progression of many human tumors. *IL8* rs4073 is known to affect *IL8* expression that regulates cancer progression through mitogenic and angiogenic factors (37, 38). For IL8 rs4073, OS patients carrying T allele had better Enneking stages and less susceptible to metastasis (39). Our meta-analysis provided strong evidence that *IL6* rs1800795 with the G allele and *IL8* rs4073 with the T allele could decrease the risk of OS under the allelic model in Asians.

The methylthioadenosine phosphorylase (*MTAP*) gene encodes an enzyme that saves methionine and adenine in polyamine metabolism. Inhibition of *MTAP* expression may be responsible for the development of tumor and *MTAP* polymorphisms were associated with some cancer risk, including OS (40, 41). Our meta-analysis also presented strong evidence that *MTAP* rs7023329 (under allelic and dominant model) and rs7027989 (under the allelic model) were associated with a lower risk of OS in Asians. Although the exact mechanism of SNP rs7023329 affect OS risk remains unknown, Zhi et al. hypothesized that SNP rs7023329 might coexist in linkage disequilibrium with one certain variants and affect its regulation machinery to associate with OS risk (42). The protein kinase C gamma (*PRKCG*) gene is located on chromosome 19q13.42 and functions as the major receptor for tumor promoters. Missense variants in exon 4 (C114Y/G123R/G123E) of the *PRKCG* gene have a relationship with tumor development and migration (43). Lu et al. discovered that *PRKCG* rs454006 associated with higher OS risk under allele and dominant model (44). Zheng et al. predicted that SNP rs454006 could cause a new splice donor site, then lead to incorrect translation of the nuclear cancer proteins, which can regulate oncogene products at the transcription level and result in the development of OS (45). Our study found that *PRKCG* rs454006 increased the risk of OS in Asians with strong evidence in the allelic model, moderate evidence in dominant model, and strong evidence in recessive model.

The RecQ like helicase 5 (*RECQL5*) gene is mapped on 17q25.1 and encodes a helicase protein that is essential for genome stability. The *RECQ* family plays a critical role in DNA repair and transcription. Therefore, *RECQL5* variants are considered candidate genes for human cancers (46). As our study found, *RECQL5* rs820196 was associated with higher risk of OS among Asians under allele model (strong evidence). However, the mechanism of how SNP rs820196 affected OS risk has not been revealed. The vacuole membrane protein 1 (*VMP1*) gene encodes a transmembrane protein that plays a key regulatory role in the autophagy process and acts as a tumor suppressors (47, 48). Normal expression of the *VMP1* protein is essential to maintain normal tissue homeostasis and integrity. SNP rs1295925 might affect the binding of p53 and eventually lead to OS susceptibility by affecting the promote or inhibit cell autophagy, and our meta-analysis presented that *VMP1* rs1295925 decreased the risk of OS in Asians with strong evidence [allelic model and dominant model; (49)]. The X-ray repair cross complementing 3 (*XRCC3*) gene encodes a protein that repairs DNA damage and maintains chromosome stability. *XRCC3* polymorphisms influence human cancer susceptibility by altering DNA repair efficiency (50). Our meta-analysis presented that *XRCC3* rs861539 could increase OS susceptibility with strong evidence under the allelic model in Asians. Although the above level of evidence was strong, each SNP only contains 2 datasets with small sample size mainly in Asians, which might reduce the credibility of the results. Therefore, more studies containing a large sample of different ethnicities are needed to evaluated the relationship between OS risk and SNPs above.

The cytotoxic T-lymphocyte associated protein 4 (*CTLA4*) gene encodes a protein that transmits an inhibitory signal to T cells, and plays an important role in increasing cancer susceptibility (51). *CTLA4* rs231775, a variant in which A is changed to G, causes an amino acid exchange and may increase the risk of OS through upregulating the *CTLA4* production and downregulating T cell activation (52, 53). A meta-analysis by Wang et al. revealed that the G allele of SNP rs231775 might function as a protective factor for OS risk (54) which is the same as in our meta-analysis. Our study provided strong evidence that *CTLA4* rs231775 was associated with lower risk of OS (G allele was protective factor) among all populations and Asians both under allelic model and dominant model. However, no

significant association was found among Caucasians, which suggesting that more studies were required to evaluate the relationships among Caucasians. The tumor protein p53 (*TP53*) gene is located on chromosome 17p13.1 and acts as a tumor suppressor (55). Savage et al. reported that *TP53* rs1042522 (G > C) increased OS risk under recessive model in a small number of Caucasians (98 cases and 67 controls) (56). However, the association was not significant in our study; instead, our meta-analysis found that *TP53* rs1042522 decreased OS risk in the allelic model (strong evidence) and the dominant model (strong evidence) among overall population, as well as in the allelic model (moderate evidence) and the dominant model (weak evidence) among Caucasians. The decrease in OS risk of SNP rs1042522 may be due to the encoding of a protein isomorph that induces transcription and apoptosis of the target gene (57). The vascular endothelial growth factor A (*VEGF*) gene encodes an angiogenesis cytokine, which induces proliferation and migration of vascular endothelial cells, and the genetic variants of *VEGF* are correlated with tumor risk (58). As our meta-analysis found, *VEGF* rs2010963 increased OS risk with strong evidence under three models among Asians; *VEGF* rs3025039 was associated with higher risk of OS under allelic model and recessive model among Asians (strong evidence); *VEGF* rs699947 decreased OS risk with strong evidence under three models in Asians. Although each SNP mentioned above contains more than two datasets, the sample size is still small, and the population involved is mainly Asian, suggesting that we need to do more research on large populations and different ethnicities in the future. Interestingly, a study performed by Wang et al. (16) revealed that *CTLA4* rs231775, *TP53* rs1042522, *VEGF* rs699947 increased the OS susceptibility in the allelic model, and *VEGF* rs2010963 decreased the OS susceptibility in the allelic model. These results contradicted our meta-analysis because they confused the major allele and the minor allele.

Our research also found that there were 17 relationships between 14 SNPs in 12 genes and OS susceptibility with moderate evidence, and 17 relationships between 14 SNPs in 11 genes and OS susceptibility with weak evidence. Furthermore, large prospective studies should be performed to elucidate the relationships with OS risk for these SNPs with moderate or weak evidence. Additionally, our study that analyzed the same SNP from different groups reported controversial conclusions due to the genetic models, race, and sample size.

Some unavoidable limitations should be noted: (i) although the extensive literature was searched, some papers may have been overlooked; (ii) There could be publication bias because only English articles are examined; (iii) subgroup analysis was conducted based on race (main in Asians and Caucasians) and genetic models, which decrease the credibility of some results; future study in much larger sample size and more races may be needed; and (iv) the errors or confusion of the major allele and the minor allele in the original articles could not be avoided. Therefore, large prospective studies are recommended to evaluate the relationship between OS susceptibility and these SNPs, and all results of our meta-analysis should be interpreted with caution until the molecular properties have been clarified.

Collectively, our research evaluated the cumulative evidence of significant associations of genetic variants with OS risk combining the Venice Criteria and the FPRP test to increase the persuasion and precision of the results. 17 variants in 14 genes with 31 associations were rated as strong evidence of OS susceptibility, 14 SNPs in 12 genes with 17 associations were moderate, and 14 SNPs in 11 genes with 17 associations were weak. Our findings provided useful information to identify the appropriate candidate SNPs and design future studies to evaluate the factors of SNPs for OS risk.

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.

AUTHOR CONTRIBUTIONS

WZ and HD contributed to the conception and design of the study. DY and JT performed the literature search, extracted the data, conducted the meta-analysis, and wrote the first draft of the manuscript. YX, NB, and FK contributed to manuscript editing. XF contributed to the manuscript review. WZ and HD contributed to supervise study, manuscript revision. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.912208/full#supplementary-material

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REVIEW
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Looking Beyond the Glioblastoma Mask: Is Genomics the Right Path?

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Glioblastomas are the most frequent and malignant brain tumor hallmarked by an invariably poor prognosis. They have been classically differentiated into primary isocitrate dehydrogenase 1 or 2 (*IDH1 -2*) wild-type (wt) glioblastoma (GBM) and secondary IDH mutant GBM, with *IDH* wt GBMs being commonly associated with older age and poor prognosis. Recently, genetic analyses have been integrated with epigenetic investigations, strongly implementing typing and subtyping of brain tumors, including GBMs, and leading to the new WHO 2021 classification. GBM genomic and epigenomic profile influences evolution, resistance, and therapeutic responses. However, differently from other tumors, there is a wide gap between the refined GBM profiling and the limited therapeutic opportunities. In addition, the different oncogenes and tumor suppressor genes involved in glial cell transformation, the heterogeneous nature of cancer, and the restricted access of drugs due to the blood–brain barrier have limited clinical advancements. This review will summarize the more relevant genetic alterations found in GBMs and highlight their potential role as potential therapeutic targets.

Keywords: glioblastoma, targeted therapy, EGFR, B-Raf, Met, NF-1

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INTRODUCTION

The most common malignant primitive tumor of the central nervous system, glioblastoma (GBM), shows some distinctive features: WHO grade IV—it is uniquely classified as "metastatic" even if it remains limited within the brain. As it is different from most kinds of cancers, oncological research faces an uphill struggle to find therapeutic significant advancements which are scarce since the 2005 STUPP pivotal trial (1, 2). The prognosis remains poor: 12–18 months median overall survival and 5% alive at 5 years (3). As shown in **Figure 1**, the timeline of glioblastoma treatments emphasized the lack of significant medical progress: a wait of 14 years after STUPP to find an improvement in survival in relapsed glioblastoma with regorafenib (4) and a wide array of novel treatments under investigation.

Pathological classification appears to be substantially surpassed by molecular classification since 2016 and increasingly in the new WHO 2021 edition (5). Alteration of specific GBM markers,

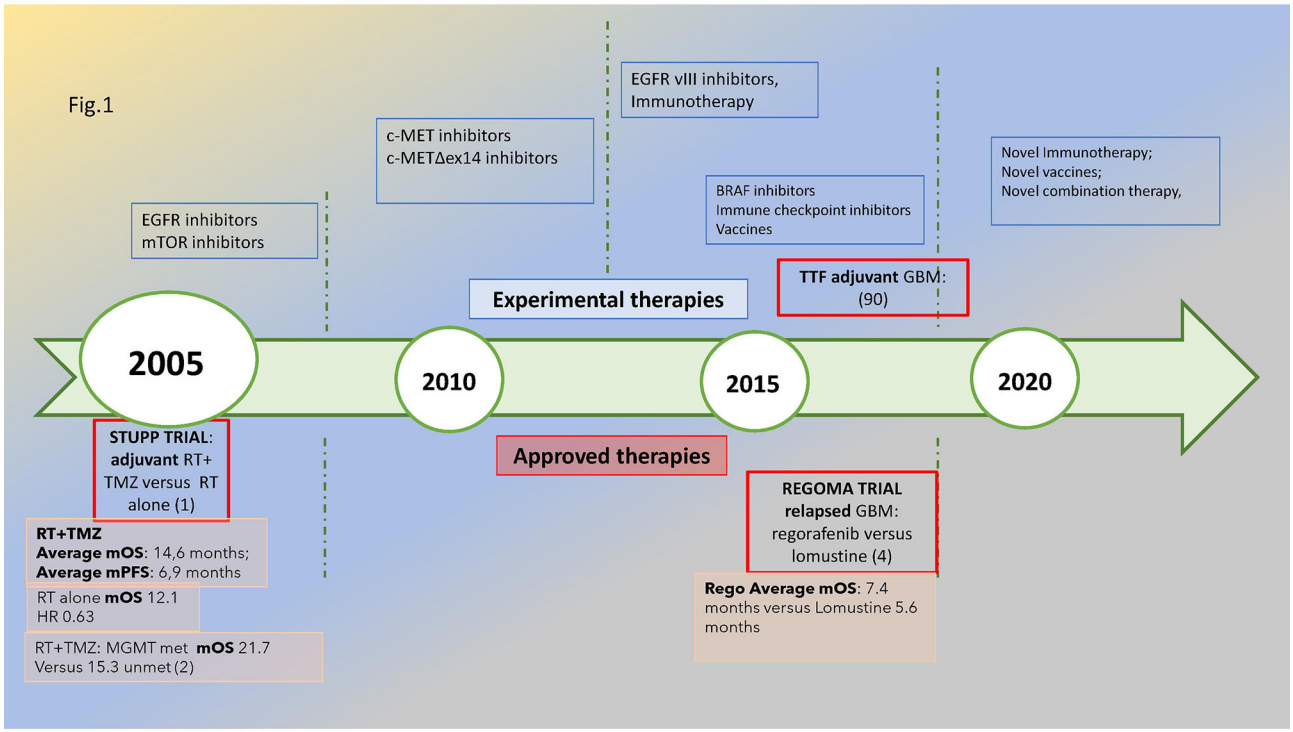

FIGURE 1 | Glioblastoma's treatment timeline: in the upper part of the figure, the novel treatments under investigation are reported, while in the lower part are the approved treatments in the adjuvant and relapsed phases with a reported significant improvement in survival, *i.e.*, STUPP and REGOMA trial, respectively, dated 2005 and 2019. The median overall survival for the experimental and control arms is also reported. The methylation of MGMT promoter is associated with improved survival compared with unmethylated subtypes. Met, methylated; unmet, unmethylated.

including the O(6)-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation, epidermal growth factor receptor (*EGFR*) overexpression, co-deletion of 1p and 19q, mutation in isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*) as well as telomerase reverse transcriptase gene (*TERT*) promoter, along with epigenome analysis not only underline the novel nomenclature but have a prognostic value and may guide treatment decisions. However, these molecular signatures do not automatically merge into precision medicine applications of immediate practical value, thus determining a certain discouragement towards analyses that requires high time and costs, with limited practical relevance.

In this review, we examine the most relevant molecular drivers of GBM which are comprehensively depicted in **Figure 2**, both from a molecular and a clinical point of view, being aware that we are far from really-practice-changing interventions but still in the world of "one, no one, and one hundred thousand". Like this drama, glioblastoma represents a complex conundrum. Following the track of other Pirandello's plays, we gave a title to each paragraph that calls to mind uncertainty, investigation (a player in search of an author, either of one or of no one), high expectations (the lord of the ship), what is unexpected but in some cases may be a turning point (the turn), the relationship with other signaling (the rules of the game), and an undefined identity (each on its own way). Through this walk into the challenging glioblastoma land, we will provide some insights into the complex genomics looking to the progress with desirable clinical relevance.

TARGETING *TERT*: A PLAYER IN SEARCH OF AN AUTHOR

At each cell division, telomeres become shorter; however, a specialized enzyme called telomerase provides the chromosome tips of additional DNA. Telomerase is a reverse transcriptase ribonucleoprotein enzyme coded by the *TERT* (telomerase reverse transcriptase) gene that copies the template RNA named telomerase RNA component (TERC) (**Figure 3A**). Telomerase critically ensures chromosome length and genomic stability during cell replication, with telomerase defects being, accordingly, associated with senescence and cellular death (6). Conversely, some mutations in the *TERT* promoter are oncogenic, resulting in cell immortalization and transformation. These mutations, firstly discovered in melanoma, include frequent cytidine-to-thymidine conversion and have been found at two genetic regions upstream of the transcriptional start site, specifically c.-124C>T and c.-146C>T (7) (**Figure 3B**). A low rate of self-renewal in GBM histological samples has been correlated to high *TERT* expression in various

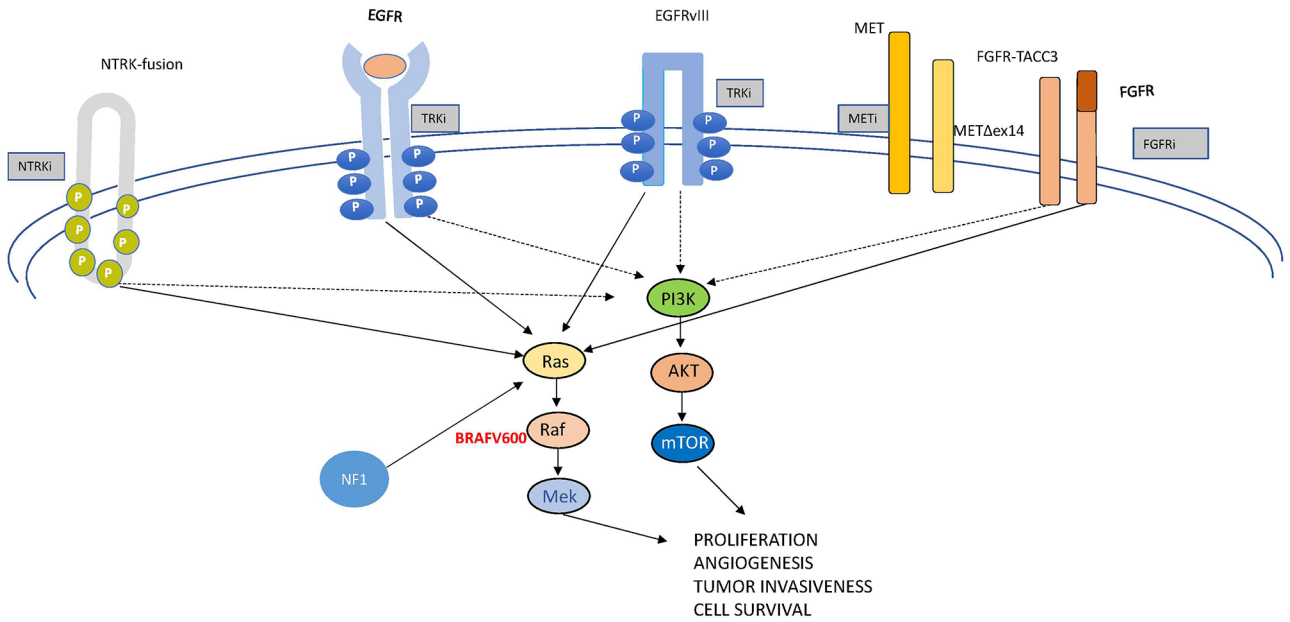

FIGURE 2 | A comprehensive representation of the relevant pathways in glioblastoma.

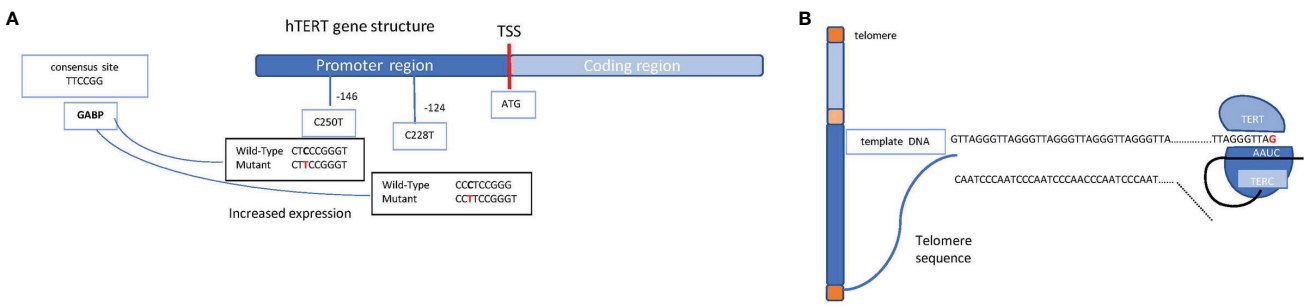

FIGURE 3 | Schematic representation of the hTERT gene structure and the telomerase complex. **(A)** Schematic mechanism of a chromosome (telomeres in orange, short arm in light blue, long arm in blue, and centromere in yellow) and the molecular mechanism through which TERT enzyme, supported by TERC, ensure the telomere length. **(B)** hTERT gene promoter region (in blue) and coding region (in light blue) are shown. The transcription start site (TSS) is indicated as a red bar; on the promoter region, the most common mutations which lead to an increased expression of the gene are shown (the indicated positions refer to the TSS). Shown on the left, in the light blue box, is the consensus sequence which takes place because of the single mutation, allowing the binding of the transcription factor GABP on the promoter.

cancer types, including melanomas, primary GBMs, liposarcomas, and hepatocellular carcinomas among others (8).

Mutations in the *TERT* promoter result in the generation of a novel binding site for the transcription factor GABP that, in turn, triggers TERT overexpression. Intriguingly, *TERT* mutations have been identified in about 80% of IDH wild-type GBMs and in 30% of IDH mutant GBMs, correlating with poor prognosis (9). These mutations may confer an increased benefit to temozolomide in *MGMT*-methylated GBMs (10, 11).

The role of *TERT* mutations in cell transformation and tumor aggressiveness has been documented in several preclinical studies. However, the number of available antitelomerase drugs is currently low, and only imetelstat (GRN163L) has entered in clinical practice. Imetelstat is a competitive inhibitor of *TERT* that acts by hindering the binding of telomerase to DNA (12). Interestingly, in GBM, imetelstat has been shown to reduce cell proliferation both *in vitro* and *in vivo*. Importantly, the drug was observed to cross the blood–brain barrier (BBB) and reduce tumor growth in tumor-engrafted mice (13). In addition, the association of imetelstat with classical radiotherapy and temozolomide drastically reduced GBM tumor growth *in vitro* and in pre-clinical studies (12). However, despite the promising results obtained, clinical trials have failed to prove imetelstat as effective on human solid tumors, probably because of the poor permeation

of the drug into tumor tissues and for critical effects, such as several intracranial hemorrhages in phase II trial NCT01836549 (14). To date, imetelstat remains under investigation only in a phase III study for myelofibrosis cure (14). Although pharmacological research is currently oriented to improve the pharmacological characteristics of imetelstat, new strategies targeting the enzymatic activity of TERT are being developed. The small molecule -6-thio-2′- deoxyguanosine, whose metabolite is preferentially incorporated into telomeres, changes DNA structure and inhibits transcription factor binding. This compound is actively tested in preclinical studies (15) and is under investigation in a phase II study involving patients with non-small cell lung cancer at late disease stages. Eribulin has also been shown to effectively inhibit TERT activity in GBM cells (16, 17); however, its development has been stopped early.

Other approaches to target telomerase include antisense oligonucleotides, small-molecule inhibitors targeting TERT or TERC, such as BIBR1532 (18), and vaccines including UCPVax and INO-5401. UCPVax has been investigated in a phase I/II clinical trial (NCT04280848) (14). It is a universal vaccine designed by employing small portions of telomerase peptides to induce strong TH1 CD4 T cell responses in oncological patients (NCT02818426) (14). Differently, INO-5401 uses a combination of three separated DNA plasmids to co-target the Wilms tumor gene-1 (WT1) antigen, prostate-specific membrane antigen, and human telomerase reverse transcriptase (*hTERT*) genes. It is currently in phase I/II clinical trials for newly diagnosed GBM patients together with INO-9012, which employs a DNA vector to overexpress human interleukin-12 (IL-12), and cemiplimab (NCT03491683) (14). This study is in an active—but not recruiting—phase, with June 2022 as the estimated date of completion.

To summarize, many clinical trials targeting TERT have not been concluded yet. Thus, its role in GBM treatment plan is still undecided. TERT is still "a character in search of an author".

TARGETING RECEPTOR TYROSINE KINASES AND THEIR DOWNSTREAM PATHWAYS

Targeting receptor tyrosine kinases (RTKs) are transmembrane-spanning receptors that, following ligand binding, undergo homo- or heterodimerization, leading to intracellular kinase domain activation and induction of a variety of downstream signaling pathways, including phosphatidylinositol 3 kinase (PI3K)/AKT/mTOR and RAS/MAPK. RTK activation enhances tumor progression and survival as well as metastatic potential and angiogenesis.

The Lord of the Ship: *EGFR*

Among all oncogenic pathways, epidermal growth factor signaling has the right credentials to be considered the driver of GBM tumorigenesis (19).

EGFR is part of the transmembrane HER receptor family which also includes HER2/neu, HER3, and HER4 and is located on chromosome band 7p12. More than 40 EGFR high- and low-affinity ligands are recognized (20). Frequently, classical and mesenchymal GBMs are characterized by chromosome 7 gains with amplification of *EGFR* (21). The amplification can be graded into low/moderate and high ratio between *EGFR* and chromosome 7 with a significant correlation with survival, which was worse in the highly amplified group (22).

Specifically, *EGFR* gene amplification, resulting in high levels of protein expression, is detected at a high frequency rate (more than 50%) in GBM (23) and is associated with poor prognosis. In **Figure 4A**, the alterations found in GBM along with that found in lung cancer are reported.

Of note is the fact that, in the majority of *EGFR*-amplified GBMs, an intragenic deletion in exons 2 to 7 leads to the distinctive production of the variant EGFRvIII, corresponding to a truncated constitutively active receptor (23). Besides gene amplification, the spectrum of the described EGFR alterations in GBM is quite heterogeneous—for example, EGFR overexpression can also result from increased gene transcription, without any DNA alterations, even if overexpression mostly correlates with gene amplification (24, 25). Additionally, in GBM, *EGFR* has been found to be constitutively active because of point mutations in the extracellular domain, especially A289V, R108K, and T263P (**Figure 4A**) (26). Regardless of the molecular mechanism causing constitutive activation, EGFR strongly induces GBM tumor growth and participates in other cell processes, such as autophagy, aerobic glycolysis, and biosynthesis of fatty acids and pyrimidines (**Figure 4B**) (27).

These observations altogether encouraged clinical trial studies of drugs targeting EGFR in GBM patients. However, until now, the results of the clinical trials involving tyrosine kinase inhibitors (TKIs) are quite disappointing since they have shown limited activity. Even type II TKIs, which, by binding to the inactive kinase, had the potential to be more active in GBM (28), have failed in clinical trials—for example, one such drug, lapatinib, failed to show a significant activity in GBM patients (29).

Currently, among the more potent tested TKIs (30), TAS2940, a small molecule inhibitor of ERBB family proteins HER2 and EGFR, has entered phase I trial (14) (NCT04982926). Failure reasons of drugs targeting EGFR in GBM, compared to therapeutic efficacy observed in other tumors, may depend on several reasons, including GBM tissue heterogeneity and the restricted access of TKIs due to the BBB (31). Considering these limitations, two ongoing clinical trials are evaluating the efficacy of two novel targeted agents able to cross the BBB: epitinib (HMPL-813), a potent and highly selective oral EGFR inhibitor, and WSD0922-FU, which prevents EGFR/EGFRvIII-mediated signaling (14, 32) (NCT04197934 and NCT03231501).

Another critical point underlying TKIs' failure is the frequent mutation in the EGFR extracellular domain in GBM. However, these mutations might make GBM particularly susceptible to targeted extracellular interventions (33). Accordingly, the anti-EGFR antibody GC1118 is currently tested in a phase II trial (14) (NCT03618667), following promising preclinical results (34). Depatuxizumab mafodotin (Depatux-M), a selective antibody-conjugated drug comprising an EGFR-targeting antibody (ABT-

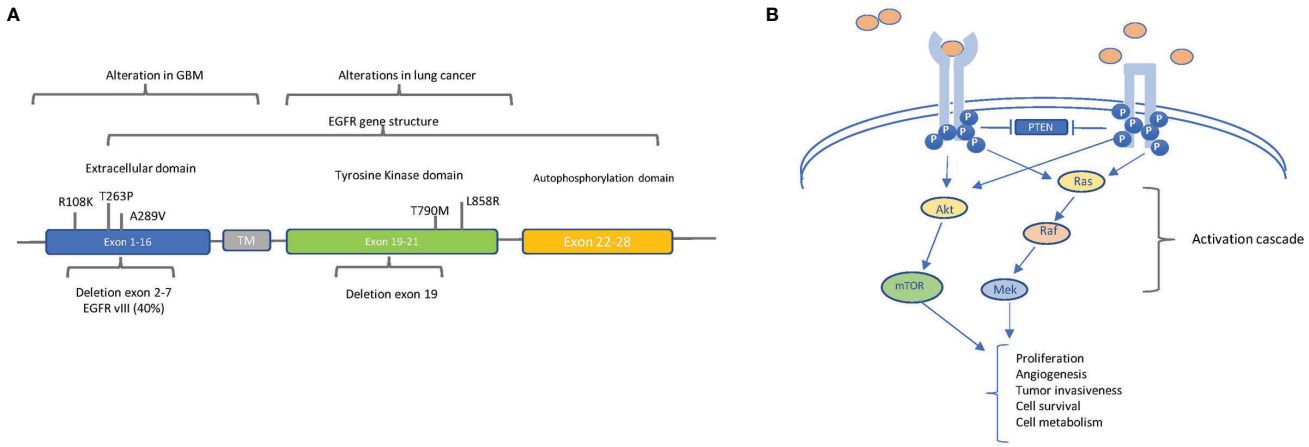

FIGURE 4 | The main oncogenic alterations of EGFR: **(A)** Localization of relevant alterations within the epidermal growth factor receptor (EGFR) gene in glioblastoma (GBM) and lung cancer. The structural organization of EGFR exons and respective domains is shown. The principal point mutations and deletions in GBM (in exons 1–16, extracellular domain) and in lung cancer (in exons 19–20, tyrosine kinase domain) are indicated. The frequency of intragenic deletion in exons 2 to 7 (leading to variant EGFRvIII) is indicated. **(B)** EGFR (left) and EGFRvIII (right) signaling pathways. EGFR and EGFRvIII trigger the AKT and MAPK pathways, but ligands (pink circles) can bind and activate only EGFR, whereas EGFRvIII is constitutively active in a ligand-independent manner. Block arrows indicate inhibition. Point arrows indicate activation. The downstream processes of the activation cascade are described.

414/806) together with the toxin monomethylauristatin-F, has instead shown no survival advantage in the phase III INTELLANCE-1 study, leading to the recommendation of trial stop by an independent data monitoring committee and the discontinuation of all ongoing related studies (35) (NCT02573324).

Additionally, the vaccine rindopepimut, targeting the GBM-peculiar EGFRvIII mutant, has been investigated in the series of ACT trials (36, 37). The phase II trial (ACTIVATE/ACT II) showed good tolerance with EGFRvIII-specific immunity, displaying encouraging results in increasing patients' survival as confirmed in the phase II trial (ACT III) (38). However, these promising therapeutic effects failed in the phase III trial ACT IV, in which rindopepimut alone was compared, in newly diagnosed GBM, to the standard regimen of temozolomide and radiation therapy after maximal surgical resection (39). Rindopepimut has also been investigated in the Re-ACT trial, a double-blind randomized phase II trial evaluating GBM patients injected with vaccine plus bevacizumab and a control injection of keyhole limpet hemocyanin concurrent with bevacizumab (40). Alarmingly, in the Re-ACT trial, the experimental arm was built on two tethering columns: rindopepimut coming from a negative phase III trial and bevacizumab, which has not demonstrated a survival-related improvement being FDA-approved for treating relapsing GBM only based on progression-free survival benefit.

The Turn: Ras-Raf Signaling

The pathway controlled by RAS and the downstream cascade of kinases (mitogen-activated protein kinase—MAPK—and extracellular-regulated kinase—ERK) (**Figure 2**) is critically involved in most tumors. It is often activated in GBM, even in the absence of RAS mutations, due to its overstimulation by RTKs, such as EGFR. BRAF, a key mediator of the MAPK pathway, has been found mutated in about 7% of tumors arising in the central nervous system (41). The most frequently described (~90%) oncogenic driver mutation in BRAF is represented by the substitution of valine by glutamic acid at amino acid 600 (V600E). The mutated protein boosts about 500× the MAPK/ERK activation, resulting in uncontrolled cell proliferation and survival (42). BRAFV600E was reported in 69% of epithelioid GBM in a recent systematic review performed on more than 13,000 patients (43).

BRAF class I inhibitors (BRAFi) selectively bind to the mutated V600E BRAF protein, thus inhibiting MAPK/ERK signaling and the related effects on tumor growth. This class encompasses three FDA compounds approved for the treatment of BRAFV600-mutated metastatic malignant melanomas: vemurafenib, dabrafenib, and encorafenib. Their use in melanoma has revealed that patients often acquire resistance to BRAFi through several molecular mechanisms, including the overactivation of RTKs such as EGFR (44). To overcome BRAFi resistance, a next-generation BRAF inhibitor, PLX8394, has been synthesized and reached phase I and II clinical trials (14) (NCT02428712), which include glioma patients. PLX8394 belongs to the novel dimer breakers that selectively target BRAF fusion proteins, splice variants as well as BRAF V600 monomers, leading to the inhibition of the overriding ERK signaling in tumors with sparing of BRAF function in normal cells in which signaling is driven by BRAF homodimers (44, 45). It should overcome resistance to the classical class I BRAF inhibitors by inducing a paradoxical, negative cooperativity effect, which means the activation of one BRAF monomer when the other is linked to a BRAF inhibitor (46).

Importantly, the combination of BRAF inhibitors with a drug inhibiting the downstream MEK protein reinforces the inhibition of MAPK/ERK signaling, delays the occurrence of acquired resistance, and reduces the adverse events related to

BRAF inhibitors used as single agents (47). Three MEK inhibitors—cobimetinib, trametinib, and binimetinib—reached clinical approval in the USA and Europe. Nevertheless, they have a low BBB crossing rate that is limited by P-glycoprotein (P-gp) and Bcrp as reported by *in vitro* studies (48).

In the recent Rare Oncology Agnostic Research basket trial, the rate of responses to the combination of BRAF/MEK inhibition obtained in high-grade as well as in low-grade glioma cohorts has been encouraging (49), thus advocating BRAF testing in clinical practice (50, 51). In detail, at a median follow-up of 12.7 months (IQR, 5.4–32.3) among the 45 patients with high-grade tumors, three complete responses and 12 partial responses were reported (ORR, 33%; 95% CI, 20–49). At a median follow-up of 32.2 months (IQR, 25.1–47.8), in the low-grade cohort of 13 patients, one complete, six partial, and two minor responses were achieved (ORR, 69%; 95% CI, 39–91). A pediatric rollover phase IV study is ongoing (NCT03975829) (14). A phase II clinical study with the BRAF/MEK inhibitor combo encorafenib plus binimetinib is ongoing, with a foreseen primary estimated completion in July 2025 (14) (NCT03973918). Binimetinib is in the preliminary clinical phases also in combination with a new, potent, selective, highly brain-penetrant, small-molecule inhibitor of BRAF V600, PF-07284890 (14) (NCT04543188).

Besides BRAF point mutations, particularly in pilocytic astrocytomas, KIAA1549–BRAF gene fusions have been found (52). In these tumors, a phase I clinical trial (NCT03429803) and a phase II FIREFLY study (NCT04775485) (14) are investigating the efficacy of the pan-RAF inhibitor DAY 101 (tovorafenib, formerly TAK-580, MLN2480). The FIRELIGHT trial (phase Ib/II NCT04985604), a multi-center, open-label umbrella master study, is also investigating DAY101 as monotherapy in phase II and, in association with the novel oral MEK inhibitor pimasertib, in a phase I study. DAY 101 and other pan-BRAF inhibitors, by inhibiting also the wild-type protein, have, on one hand, the potential to inhibit MAPK/ERK pathway regardless of the activating BRAF mutation and the ability to overcome some resistance mechanisms; on the other hand, the therapeutic index is expected to be low (53).

NF-1

Apart from BRAF mutations, in glioma, RAS/MAPK signaling (**Figure 2**) can be activated by neurofibromatosis 1 (*NF1*) gene inactivating mutations or deletions. The *NF1*-derived protein is named neurofibromin, which is a tumor suppressor RAS-GAP. The shutdown of RAS signaling, through the conversion of the GTP-bound active RAS form into the inactive GDP-bound form and the increasing levels of cAMP induced by neurofibromin, finally inhibits cell proliferation and survival (54). According to the vast evaluation performed by the Tumor Cancer Genome Atlas, a discrete percentage of GBMs (13 to 14%) are *NF-1*-mutated, and these tumors are characterized by a poor prognosis. *NF-1*-mutated GBMs are often associated with the mesenchymal subtype, with a bidirectional correspondence (55). Despite the fact that the loss of *NF-1* function is related to resistance to targeted therapies, MEK inhibitors may be effective against *NF-1*-mutated brain tumors (56). Among those, pediatric inoperable plexiform neurofibromas may be eligible for treatment with selumetinib which was acknowledged as orphan drug by the FDA (57). An ongoing phase III study (NCT03871257) is evaluating selumetinib in comparison with chemotherapy in low-grade *NF-1*-associated gliomas (14).

Interestingly, the tumors with *NF1* mutations, as compared with those with RAS or BRAF mutations, are characterized by a higher mutational burden and, thus, may be responsive to immunotherapy-based treatment strategy (58).

The Rules of the Game: Mesenchymal–Epithelial Transition Factor

Mesenchymal–epithelial transition (MET) is a receptor tyrosine kinase involved in several cell processes related not only to proliferation and cell survival but also to invasiveness and angiogenesis (**Figure 2**). In this capacity, it functions as a team player given the intricate crosstalk between MET and other signaling pathways. As an example, VEGFR and c-Met signaling cooperate in the control of angiogenesis and tumor growth (59, 60).

Overexpression is the most frequently found MET alteration, detected in 20–30% of high-grade gliomas, followed by amplification, found in 4% of primary GBM. About 3% of GBMs consist of a constitutively active ligand-independent MET protein, derived from exons 7 and 8 deletions in the *MET* gene (*MET*Δ7-8) (61). Additionally, the *MET* exon 14 skipping mutation (METΔex14) produces an abnormal receptor lacking the juxtamembrane domain which activates MET downstream effectors in a ligand-independent manner.

Crizotinib is one of the first MET inhibitors tested in clinical studies together with other small-molecule inhibitors and anti-MET antibodies. However, a relative paucity of them have been rescued and moved forward in advanced late-stage clinical trials (62, 63).

Capmatinib, a highly selective MET inhibitor (INC280), has shown an overall response of 41% in non-small cell lung cancer patients harboring a *MET*Δex14 mutation as compared with 29% in patients with *MET* amplification (64). The promising anticancer potential of this drug prompted the conduct of a phase I/II study (NCT01870726) using capmatinib alone and in combination with the pan-class I PI3K inhibitor buparlisib (65). Unfortunately, the published results were not particularly encouraging in terms of activity.

The MET inhibitor tepotinib has shown good tolerability and clinical activity in MET-dysregulated tumors. A phase II basket trial (NCT04647838) is ongoing to evaluate tepotinib in solid cancers with MET amplification or exon 14 mutation.

APL-101 is a novel, selective small-molecule MET inhibitor currently investigated in the SPARTA phase I/II trial (NCT03175224), including advanced solid tumors with METΔex14 and MET dysregulation (14).

Given the crosstalk between MET-induced and other signaling pathways, further research is looking towards combinatorial treatments to synergize and prevent resistance, such as VEGFR/c-Met dual-target inhibitors (59). One of them, dovitinib, reached phase II study but has not shown a clinically

meaningful activity (66), and the same fate has befallen tivozanib (67) and cabozantinib (68).

Each on Its Own Way: Fibroblast Growth Factor Receptor Oncogenic Mutations

Fibroblast growth factor receptor (FGFR) comprises a family of RTKs consisting of four members (FGFR1–4) which are involved in several tumor-cell-related processes, such as proliferation, survival, invasion, and vessel growth (**Figure 2**). Twenty-two ligands and cell adhesion molecules, including the neural cell adhesion molecule, are known to bind these receptors and activate downstream signaling, including the PI3K-AKT and Ras-BRAF-MEK-ERK pathways (69). Comprehensively, amplifications, mutations, and translocations of FGFR genes are described in different tumors (69) with a quite composite arrangement: gene amplification, abnormal activation, or single-nucleotide polymorphisms mostly pertain to *FGFR1* and *FGFR2*, while genetic fusions that involve *FGFR1* and *FGFR3* tyrosine kinase domains and the transforming acidic coiled-coil proteins generate oncoproteins. Similar to MET, an autocrine loop contributes to overstimulation of FGFR signaling.

FGFR inhibitors are in the earlier phase of clinical studies. Following on from the promising clinical results achieved by one of these compounds, infigratinib (BGJ398) in metastatic cholangiocarcinoma with *FGFR2* gene fusions or rearrangements (70), a phase I study (NCT04424966) is ongoing in recurrent high-grade glioma with definite mutations of *FGFR1* or *FGFR3* or translocations involving *FGFR3* (14).

AZD4547 is an oral TKI selective for FGFR1, 2, and 3 which showed only a modest activity in patients with advanced cancer who harbor *FGFR1*, *2*, or *3* alterations and enrolled in the arm of the National Cancer Institute—Molecular Analysis for Therapy Choice (NCT02465060) (71).

Either of One or of No One: Neurotrophic Tyrosine Receptor Kinase Fusions

The neurotrophic tyrosine receptor kinase (NTRK) family comprises three genes—*NTRK1* (1q21–q22), *NTRK2* (9q22.1), and *NTRK3* (15q25)—each encoding one receptor protein (TRKA, TRKB, TRKC or NTRK1, NTRK2, and NTRK3) (**Figure 5**) with the same characteristics of the other transmembrane receptors with tyrosine kinase activity (72). The recognized ligands nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NTF-3) exhibit a preferential binding with TRKA, TRKB, and TRKC, respectively (73–76). Upon ligand binding, receptor dimerization induces signals that promote cell survival and proliferation. The most common oncogenic NTRK aberrations produce fusion proteins able to activate signaling independently from ligand binding (76) (**Figure 5**). The constitutive activation of NTRK signaling induced by NTRK fusions has been recognized as oncogenic not only in different rare and aggressive tumors, such as salivary gland and infantile fibrosarcoma tumors (77), but also more commonly melanoma and thyroid carcinoma as well as lung, breast, and colon cancer (78, 79). NTRK fusions are less reported in glioma (0.55 to 2%) while exceeding 5% in pediatric high-grade gliomas (80). In some cases, the NTRK fusion correlates to the switch from low-grade to high-grade glioma (81).

Larotrectinib is the first FDA-approved powerful and selective TRK inhibitor. Both *in vitro* and *in vivo*, larotrectinib inhibits kinase activity by blocking ATP binding sites and, *in vivo*, potently suppresses the growth of tumor cancer with TRKA and TRKB fusion proteins (82). Following several positive preclinical investigations (83, 84), three trials (NCT02122913; NCT02637687, SCOUT; and NCT02576431, NAVIGATE) led to FDA approval, but it should be emphasized that only one was a phase II basket trial while the others were phase I studies. The combined analysis of the two of these trials documented that the responses induced by larotrectinib were significant in terms of number, duration, and speed of onset (85). In December 2020, an early phase I clinical trial (NCT04655404) was started to evaluate the disease control rate in high-grade pediatric glioma with NTRK fusion (14).

Entrectinib is another orally available inhibitor with activity on TRKA/B/C, ROS1, and ALK (86, 87) developed to reach a high concentration in the central nervous system that correlates to high intracranial activity as shown in preclinical models (88). Two phase I dose-escalation studies and a phase II basket trial STARTRK-2 (NCT02568267) supported the activity of entrectinib. In 2020, an integrated analysis of these three clinical trials (89) confirmed that entrectinib is an effective treatment for patients with NTRK fusion-positive solid tumors. The results of the ongoing STARTRK-2 and STARTRK-NG trials are awaited to confirm the activity of entrectinib in NTRK fusion-positive tumors (90).

Selitrectinib and repotrectinib are next-generation TRK inhibitors developed to be used at the presentation of resistance. Clinical trials are ongoing (NCT03215511 and NCT03093116) (14).

DISCUSSION

The therapeutic algorithm of GBM is based on some main indications with few evolutions over time. As proof, the Central Nervous System National Comprehensive Cancer Network Guidelines have not required any update for more than a year (91). Surgery with radical intent, at diagnosis and relapse, is a bearing pillar, whereas medical treatments consist of the dated STUPP protocol following resection and limited therapeutic options while on a progressive disease. A significant advancement over standard treatment has been obtained with the intensification of adjuvant temozolomide with tumor-treating fields, which interferes with cell growth. This treatment achieved a reduction of about 40% in the risk of progression and death in a large, randomized trial (92).

However, GBM is not only an aggressive and ominous disease but also distinctively affects the entire body functions through the tumor itself and related edema, with invalidating symptoms such as headache, speech disturbances, loss of motor abilities, amnesia, sleep disorders, seizures, fatigue, and psychiatric disorders, with the need for a specialized team to counteract each of them. In front of this parade of symptoms, supportive care also turns around steroids, antiseizure drugs, and a few other beneficial medications. This perspective is rather

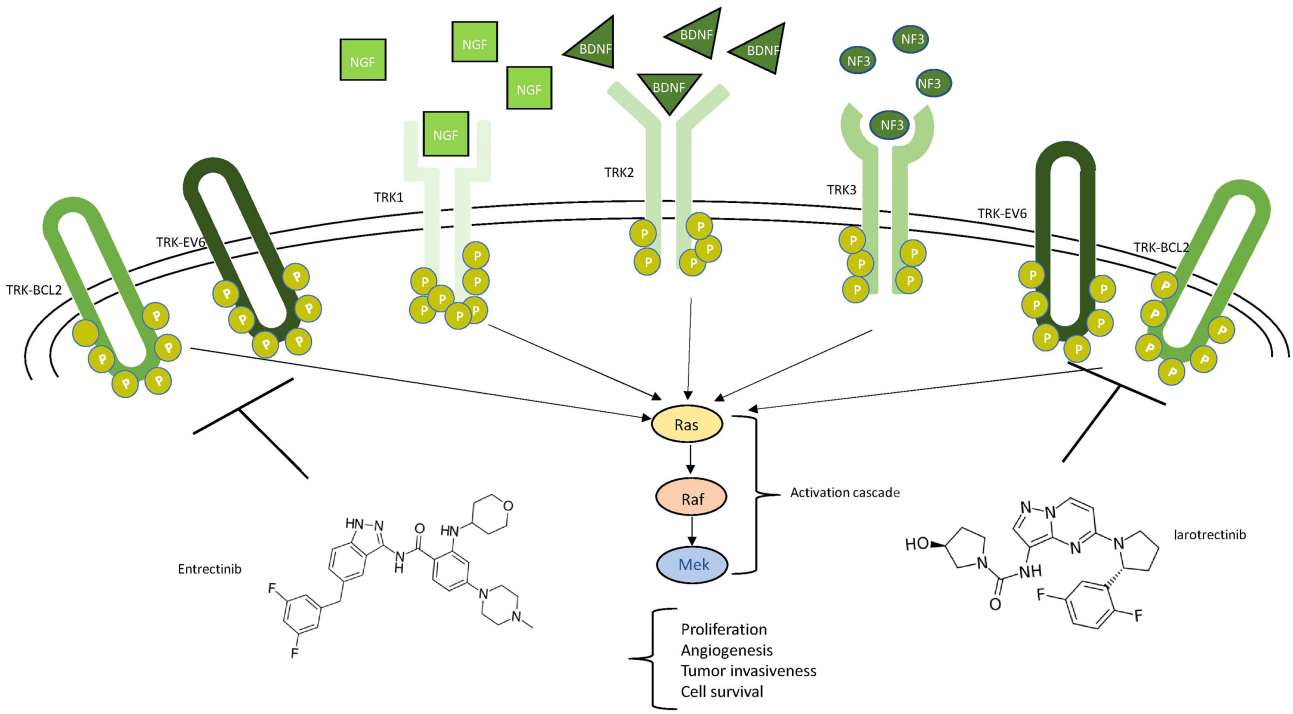

FIGURE 5 | Schematic view of NTRK signaling. Ligands (NGF in squares, BDNF in triangles, and NF3 in circles) and their respective receptors (TRK1/2/3) are represented. The main neurotrophic tyrosine receptor kinase (NTRK) fusion products (TRK-BCL1 and TRK-EV6) are represented as ellipticals as they do not need any ligand to be active. All the receptors trigger the MAPK pathway, leading to the indicated consequences. Two drugs, entrectinib and larotrectinib, can inhibit the NTRK aberrant forms as shown.

frustrating because of the instinctive comparison between the therapeutic advancements in several types of cancer with the insufficient medical progress and invariably poor prognosis of GBM patients.

Genomics has radically changed the outcomes of many tumors with identifiable actionable and druggable mutations. Otherwise, the identification of gene alterations and presumptive key pathways has not translated into practice-changing results in GBM. There are different reasons underlying this paradoxical discrepancy.

First, there is the selection of molecules for clinical studies. Many times, drugs active in cell and animal models fail to confirm any activity in clinical trials. Of note is that the pre-clinical evaluation of most RTK-targeting molecules has been conducted in models harboring a unique genetic alteration that is far from the heterogeneous nature of GBM. Moreover, predetermined selection criteria based on molecular tumor signatures may address the rational use of RTK-targeting compounds.

The BBB, tumor edema, and necrosis limit the rate of the drug ultimately reaching the target tumor so that a pharmacodynamically effective concentration is not attained. As intuitively recognized, even the most powerful drug should exert a limited effect if does not reach an active concentration in brain tumors. One way to overcome the limited drug transition through the BBB is local administration at surgery time when access to the tumor area is easier—for example, with gliadel wafers which, however, reported controversial results (93). The next-generation approaches, including biomaterials, alternative formulations, and targeted delivery, bear the promise to improve the glioblastoma therapy outcomes. Targeted delivery includes the selection of biochemical compounds interacting with a ligand highly expressed in brain tumor and studies of pharmacokinetics improving drug distribution and reducing elimination. The most promising approaches concern nanoparticles and exosomes loading the active cargo and efficiently carrying it at the tumor site.

Most studies are investigating the complex nature of glioblastoma which even increases if we look immediately outside the restricted field of tumor cells: the composite network of immune cells, blood vessels, and the microglia compartments which reciprocally interact. These cells are presumed to be more stable and perhaps targetable (94). However, it is hard to identify a unique hypothetical Achilles' heel.

Intensive medical research concern immunotherapy which, however, require being adaptively inclined to glioblastoma specificity. This tumor is fundamentally immune resistant as documented by some intrinsic features, such as low tumor mutational burden, a highly immunosuppressive microenvironment, and tumor heterogeneity, without counting systemic immunosuppression which is often associated with glioblastoma because of steroid concomitant use. Moreover, primitive and relapsed tumors are different in their gene signatures, thus exhibiting a different response to a defined treatment,

as recent studies suggested (95). This is the shape-shifting nature of glioblastoma—changing constantly its appearance to prevail over the host. The selection by different parameters, such as high towards low tumor mutational burden, may help to individualize treatment strategies. Moreover, the combination of procedures, such as radiotherapy, which itself increases antigen presentation with enhanced immunotherapy by the use of immune adjuvants or dendritic cells, bears the promise that the desert landscape of glioblastoma will change.

Looking at the role of gene pathways that preliminarily raise important expectations, such as *EGFR*, two main mechanisms have been suggested: target independence, namely, alterations in the target that becomes insensitive to inhibition, and target compensation; in other words, the activation of alternative pathways (96). GBM cells are probably dependent on several growth pathways and are particularly skilled to escape a one-modality attempt. The dynamics of GBM cells with their adaptive nature to change under therapeutic and metabolic pressure (97) and the role of microenvironment with other peculiar metabolic and molecular signatures (98) even complicate the enigmatic nature of this tumor. Since GBMs are characterized by multiple genetic as well as epigenetic mutations within the same tumor, it is fundamental to perform extensive research using single-cell technology to comprehensively define GBM heterogeneity. These results will not only elucidate the unclear GMB-related biological mechanisms but will also identify genomic signatures and address treatment strategies, including combinatorial therapy. On top of that, it remains also crucial to recognize new druggable targets driving GBM onset, maintenance, and progression that will contribute to changing the present treatment algorithms.

Concerning NTRK and BRAF, they are found only in a minority of adult cases. A relatively low percentage of a definite alteration is hard to represent in a paradigm shift for the whole. Moreover, the low rates of these alterations allow only for phase II and basket/umbrella trials, with phase III studies being unfeasible. Consequently, these studies are not candidates for evaluation through a standardized approach such as the European Society for Medical Oncology Magnitude of Clinical Benefit Scale aimed at defining the unbiased magnitude of the clinical benefit given by a new anticancer therapy (99).

To date, the expectations placed in precision medicine and, particularly, in genomics determine the heterogeneous use of cancer gene platforms worldwide, which does not always correspond to the principles of evidence-based medicine and available guidelines. In the future, it will be urgent to unravel the molecular pathways involved in GBM drug resistance mechanisms as well as improve drug delivery approaches to bypass BBB. Next-generation sequencing methods should be part of national and international studies, including data banking and platform trials integrated with artificial intelligence and machine-learning-based approaches, which can disclose the composite and mutable nature of glioblastoma.

AUTHOR CONTRIBUTIONS

LM, RDM, LA, NG, GB, and LC: conceptualization and methodology. LM, NG, and LA: writing, original draft preparation, and data curation. LM, MC, MB, DC, RV, GF, RDM, LA, NG, GB, LA, GF, and LC: visualization and supervision. LM, RDM, MB, DC, and RV: figures. LM, LA, and NG: writing—reviewing and editing. All authors contributed to the article and approved the submitted version.

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REVIEW
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The Emerging Role of RNA N6-Methyladenosine Modification in Pancreatic Cancer

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Pancreatic cancer (PC) is one of the most common malignant cancers, ranking the seventh highest causes of cancer-related deaths globally. Recently, RNA N6-methyladenosine (m6A) is emerging as one of the most abundant RNA modifications in eukaryote cells, involved in multiple RNA processes including RNA translocation, alternative splicing, maturation, stability, and degradation. As reported, m6A was dynamically and reversibly regulated by its "writers", "erasers", and "readers", Increasing evidence has revealed the vital role of m6A modification in the development of multiple types of cancers including PC. Currently, aberrant m6A modification level has been found in both PC tissues and cell lines. Moreover, abnormal expressions of m6A regulators and m6A-modified genes have been reported to contribute to the malignant development of PC. Here in this review, we will focus on the function and molecular mechanism of m6A-modulated RNAs including coding RNAs as well as non-coding RNAs. Then the m6A regulators will be summarized to reveal their potential applications in the clinical diagnosis, prognosis, and therapeutics of PC.

Keywords: pancreatic cancer, non-coding RNAs, coding RNAs, RNA methylation, N6-methyladenosine (m6A)

INTRODUCTION

Pancreatic cancer (PC) is the seventh highest leading cause of cancer mortality worldwide accompanied by poor prognosis as well as a 5-year survival rate of about 10% (1, 2). With the development of clinical diagnosis and treatment for PC in the past two decades, the survival rate of PC patients has increased yearly, while the mortality rate of PC patients remains high. According to the statistics, the death cases (466,000) of PC are close to its new cases (496,000) globally (1). Lacking typical clinical symptoms and early diagnosis biomarkers, numerous PC patients are

diagnosed at an advanced stage and miss the chance to get a surgical resection, resulting in the worse clinical outcome of PC patients. Thus, there is great urgency to clarify the initiation and progression of PC. Currently, aberrant genetic mutations (KRAS, p53, CDKN2A, SMAD4), dysregulation of the key signaling pathway (TGF-β, Wnt/β-catenin, Notch, Hippo, YAP), and epigenetic alterations (DNA methylation, RNA methylation, posttranslational modifications) have been reported to participate in PC development (3, 4). However, the molecular mechanism of PC progression remains largely unknown. Therefore, a comprehensive understanding of the pathogenesis and molecular regulatory mechanism of PC will greatly contribute to the early diagnosis, prognosis, and targeted therapeutics development of PC.

In recent years, RNA modifications, such as N6-methyladenosine (m6A), 5-methylcytosine (m5C), N1-methylguanosine(m1A), have been extensively reported in many cancers including PC (5). As one of the most abundant RNA modifications in eukaryotes, m6A has attracted more and more attention, which existed in RNAs including protein-coding RNAs as well as non-coding RNAs (ncRNAs). As reported, m6A modification is catalyzed by the methyltransferase (also called "m6A writers") and meanwhile can also be removed by the demethylases (also called "m6A eraser") (5, 6). Additionally, m6A-binding proteins (also called "m6A readers") recognize and bind to the m6A-modified RNAs to regulate RNA fate (5). In PC, a significantly increased m6A level has been observed in both PC tissues and cell lines, and an elevated m6A level was associated with poor prognosis of PC patients (7–11). So far, dysregulation of m6A-associated modulators and m6A-modified RNAs has been associated with PC cell growth, iron metabolism, glycolysis metabolism, stemness-like property, and metastasis.

In this review, we will systemically summarize the molecular mechanisms and biological functions of m6A modifications in both mRNA and ncRNAs as well as the m6A regulators in the initiation and progression of PC and then discuss the potential applications of m6A modifications in the clinical diagnosis, prognosis, and targeted therapy of PC.

M6A MODIFICATION

The RNA N6-methyladenosine (m6A) modification was defined to methylate the N6 position of adenosine, which was firstly reported in eukaryotic cells by Desrosiers et al. in 1974 (12). Up to now, m6A modification was considered to be the most abundant modification in RNAs (13, 14) and prefers to occur at the consensus motif RRACH (R=A or G, H=A, C, or U) of 3′-untranslated regions (3′UTRs), long internal exons (CDS), and near stop codons rather than randomly happens (15, 16). Nowadays, with the development of m6A detection-associated technologies, m6A modifications have been revealed in various types of RNAs including protein-coding RNA and ncRNAs, such as long non-coding RNAs (LncRNAs), microRNAs (miRNAs), circular RNAs (circRNAs), ribosomal RNA (rRNA), and transfer RNA (tRNA) (5, 17, 18). As shown in **Figure 1**, m6A modifications have been shown to be involved in RNA processes including nuclear export, miRNA maturation, alternative splicing, stability, translation, and degradation, thus participating in the initiation and progression of various diseases (19). As reported, m6A modification was dynamically and reversibly regulated by m6A methyltransferase ("writers"), m6A demethylases ("erasers"), and m6A-binding proteins ("readers") (**Figure 1**) (20).

Writers

m6A modification was installed by the methyltransferase complex (MTC), in which Wilms' tumor-1-associated protein

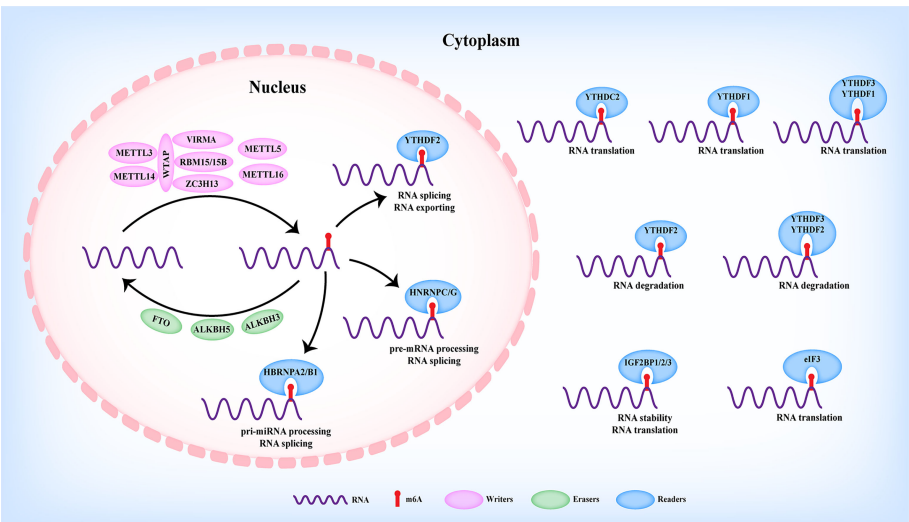

FIGURE 1 | The dynamic regulation of m6A modification in RNAs.

(WTAP), methyltransferase-like 3 (METTL3), and methyltransferase-like 14 (METTL14) formed the core component (**Figure 1**). METTL3 was the firstly identified m6A writer with catalytic activity to trigger m6A modification *via* the S-adenosylmethionine (SAM)-binding motif. Serving as a supporting structure without catalytic activity, METTL14 formed a METTL3/METTL14 complex with METTL3 to recognize the conserved RRACH motif (21, 22). WTAP was further revealed to interact with the METTL3/METTL14 complex to mediate their nuclear speckle localization, thus modulating target RNA m6A modification (23). Other m6A readers including Vir-like m6A RNA methyltransferase-associated protein (VIRMA/KIAA1429), RNA-binding motif protein 15/15B (RBM15/15B), and zinc finger CCCH domain-containing protein 13 (ZC3H13) have also been identified to participate in the m6A modification of MTC (**Figure 1**). For example, VIRMA recruited the catalytic METTL3/METTL14/WTAP complex to mediate m6A modification in the 3′UTR and near the stop codon (24). RBM15/15B could interact with and recruit the MTC to a specific site to enhance the m6A modification of the LncRNA X-inactive specific transcript (XIST), thereby facilitating XIST-mediated gene silencing (25). Moreover, ZC3H13 enhanced the nuclear translocation of the ZC3H13–WTAP–Virilizer–Hakai complex to facilitate m6A modification (26). Apart from the above writers, methyltransferase-like protein 5 (METTL5) and methyltransferase-like protein 16 (METTL16) were identified as m6A writers (**Figure 1**) (27–30).

Erasers

Contrary to the function of writers, m6A erasers exerted the demethylation of m6A modification of RNAs. Currently, erasers mainly contain fat mass and obesity-associated protein (FTO), alkB homolog 5 (ALKBH5) (**Figure 1**) (31, 32), both of which are primarily located in the nucleus and belong to the alkB family (32). As reported, FTO was the first identified eraser, participating in the m6A modification of nuclear RNAs (31). To date, FTO and ALKBH5 have been widely reported in modulating RNA m6A modification in various human cancers (19). Recently, alkB homolog 3 (ALKBH3) was revealed as a novel m6A eraser in mediating tRNA demethylation and protein translation (**Figure 1**) (33).

Readers

Unlike m6A writers or m6A erasers to add or remove the m6A modification of RNAs, readers could recognize and interact with m6A-motified RNAs, thereby modulating various RNA processes, such as alternative splicing, nuclear export, miRNA maturation, stability, degradation, and translation (**Figure 1**) (34). Currently, insulin-like growth factor 2-binding proteins (IGF2BPs), YTH family proteins (YTHDC1/2 and YTHDF1/2/3), heterogeneous nuclear ribonucleoprotein family (HNRNPC, HNRNPG, HNRNPA2B1), and eIF3 have been identified as m6A readers (**Figure 1**). Based on their cellular localization, m6A readers exerted different functions. On the one hand, m6A readers with nuclear localization including YTHDC1 (35–38), HNRNPA2B1 (39, 40), and HNRNPC/G (41, 42) were reported to be involved in pri-miRNA processing, splicing, and nuclear exporting of m6A-modified RNAs (**Figure 1**). On the other hand, m6A readers with cytoplasmic localization, such as YTHDF1 (43), YTHDF2 (44, 45), YTHDF3 (46, 47), YTHDC2 (48, 49), IGF2BPs (50), and eIF3 (51), were demonstrated to participate in the stability, translation, and degradation of m6A-modified RNAs (**Figure 1**). In addition, CCHC-type zinc finger nucleic-acid binding protein (CNBP) (52) and NF-κB associated protein (NKAP) (53) were recently uncovered as novel m6A readers involved in modulating stability and pri-miRNA processing.

ROLE OF RNA M6A MODIFICATION IN PC

m6A Modification of Coding RNAs in PC

WIF-1

Wnt inhibitory factor 1 (WIF-1) was firstly identified by Hsieh et al. as a secreted Wnt-binding protein to suppress Wnt signaling activity (54). Currently, the tumor-suppressive role of WIF-1 has been clarified in various cancers including PC (55–58).

As shown in **Table 1**, the WIF-1 protein level was downregulated in PC tissues, which was correlated with poor overall survival (OS) of PC patients (59). WIF-1 was further identified as a downstream target of ALKBH5 *via* m6A-seq and RNA-seq. The demethylase-ALKBH5 increases WIF-1 expression through reducing the m6A level of WIF-1 mRNA, which inhibits the Wnt signaling pathway *via* the WIF-1/Wnt axis. Functionally, knockdown of WIF-1 alleviated the ALKBH5-induced suppression of cell growth, migration, and invasion, while overexpression of WIF-1 attenuated the ALKBH5 deficiency-induced promotion of cell growth, migration, and invasion (56) (**Table 1**; **Figure 2**). All the above findings suggests the antitumor role of WIF-1 in the malignant progression of PC.

PER1

PERIOD1 (PER1) is a clock gene involved in circadian rhythm regulation, DNA damage, cell cycle, cell proliferation, and metastasis. The abnormal expression of PER1 has been shown in various types of cancers, and both oncogenic and tumor-suppressive roles of PER1 have been revealed (11, 60, 61, 67–76).

As reported, the expression of PER1 was significantly decreased in PC tissues as compared to the benign and adjacent normal tissues (11, 77) (**Table 1**). Moreover, the reduced expression of PER1 was associated with shorter OS of PC patients (11, 77). Analysis of RNA-seq and m6A-seq further revealed that PER1 was a downstream target of ALKBH5 (11) (**Table 1**). The expression of PER1 was increased in the presence of ALKBH5, whereas the deficiency of ALKBH5 led to a reduced PER1 expression, which was confirmed in PC through immunohistochemistry (IHC) and TCGA dataset analysis (11). The MeRIP-qPCR analysis revealed that the m6A level of PER1 was negatively regulated by the demethylase ALKBH5 (11) (**Table 1**; **Figure 2**). Additionally, YTHDF2 served as an m6A reader to trigger PER1 mRNA degradation. Furthermore, a

TABLE 1 | m6A modification of mRNAs in pancreatic cancer.

Name	Role	Expression	Function	Clinical significance	m6A regulator	Mechanisms	Refs
WIF-1	Suppressor	Down	WIF1 inhibited cell proliferation, migration, and invasion	OS↑	ALKBH5	1.ALKBH5 decreased the m6A level of WIF-1 and increasedWIF-1 expression, thus suppressing the Wnt signaling pathway *via* the AKLBH5/WIF-1/Wnt axis.	(56, 59)
PER1	Suppressor	Down	PER1 Inhibited cell proliferation and invasion	OS↑	ALKBH5 YTHDF2	1.ALKBH5 decreased the m6A level of PER1 mRNA and increased the PER1 expression. 2.YTHDF2 mediated PER1 mRNA degradation in an m6A-dependent way.	(11)
	Oncogene	Up	PER1 KD inhibited PC cell growth	–	–	1.TNF-α decreased PER1 expression	(60, 61)
PERP	Suppressor	Down	PERP Inhibited cell proliferation, migration, and invasion	–	METTL14 YTHDF2	1.METTL14 KD decreased the m6A level of PERP 3′UTR and increased the PERP expression 2.YTHDF2 recognized the m6A of PERP 3′UTR to promote PERP mRNA degradation.	(7, 62)
PIK3CB	Oncogene	Up	PIK3CB promoted the cell proliferation, migration, invasion, metastasis, and tumorigenesis of PTEN-deficient PC cells.	OS↓ DFS↓	METTL3 METTL14 WTAP YTHDF2	1.Knockdown of METTL3/METTL14/WTAP separately reduced the m6A level of PIK3CB while it increased PIK3CB expression. 2. YTHDF2 recognized and bound to m6A-modified PIK3CB to induce PIK3CB mRNA decay. 3.PIK3CB activated the Akt signaling pathway.	(63, 64)
PJA2	Suppressor	Down	PJA2 KD promoted PC cell growth, migration, and invasion *in vitro*	–	FTO YTHDF2	1.FTO reduced the m6A level of PJA2 and increased the PJA2 expression *via* YTHDF2 mediated mRNA decay. 2.PJA2 suppressed the Wnt signaling pathway.	(8)
NUCB1	Suppressor	Down	1.NUCB1 decreased PC cell proliferation and GEM-induced autophagy and UPR. 2.NUCB1 increased PC cell apoptosis	OS↑	METTL3 YTHDF2	1.METTL3 KD decreased the m6A enrichment of NUCB1 5′UTR. 2.YTHDF2 recognized and bound to m6A-modified NUCB1 5′UTR and decreased NUCB1 mRNA stability and expression. 3.NUCB1 suppressed the antitumor role of GEM *via* inactivating ATF6.	(9)
FBXL5	Suppressor	Down	FBXL5 decreased PC cell migration, invasion, and iron level.	OS↑	ALKBH5	1.ALKBH5 reduced the m6A level of FBXL5 and its RNA stability. 2.ALKBH5 increased FBXL5 expression. 3.FBXL5 promoted the ubiquitination of IRP2 and SNAI1 proteins, forming a ALKBH5-FBXL5-IRP2/SNAI1 axis.	(65)
SLC25A37	Oncogene	Down	SLC25A37 increased THE mitochondrial iron level and dysregulation of immunometabolism.	OS↓	ALKBH5	1.ALKBH5 reduced the m6A level of SLC25A37 and regulated the alternative splicing of SLC25A37. 2.ALKBH5 increased SLC25A37 expression.	(65, 66)
SLC25A28	Oncogene	Down	SLC25A28 increased THE mitochondrial iron level and dysregulation of immunometabolism.	–	ALKBH5	1.ALKBH5 reduced the m6A level of SLC25A28 and its RNA stability. 2.ALKBH5 increased SLC25A28 expression.	(65, 66)

PC, pancreatic cancer; KD, knockdown; up, upregulation in PC, down, downregulation in PC; OS: overall survival; DFS: disease-free survival.

positive feedback loop between ALKBH5 and PER1 was revealed, since PER1 attenuated the enhanced cell proliferation and invasion induced by ALKBH5 deficiency (11), while PER1 could in turn enhance p53 activation to elevate the ALKBH5 expression through the PER1–P53–ALKBH5 axis (11) (**Table 1**; **Figure 2**). Taken together, there is a novel positive feedback loop between ALKBH5 and PER1, and ALKBH5 triggers PER1 expression *via* in an m6A-YTHDF2-dependent manner to suppress PC progression. On the contrary, studies have also shown that PER1 was upregulated in PC tissues as compared to normal tissues (60). Furthermore, TNF-α treatment suppressed PER1 expression, and loss of PER1 suppressed cell proliferation and increased apoptosis of PC cells acting as an oncogene (60, 61). Therefore, PER1 might be a potential biomarker for PC prognosis and also a promising therapeutics target for PC treatment.

PERP

The P53 effector related to pmp-22 (PERP) was firstly reported as a transcriptional target of p53 involved in cell apoptosis (78). At present, a tumor-suppressive role of PERP has been confirmed in various cancers (62, 79–82).

Zhao et al. revealed that PERP was highly expressed in PC (83) (**Table 1**). PERP was further identified as a target of METTL14 through RNA sequencing and m6A sequencing. Acting as an m6A writer, METTL14 knockdown significantly decreased the m6A level of PERP-3′UTR, which stabilized the mRNA of PERP and further increased the expression of PERP.

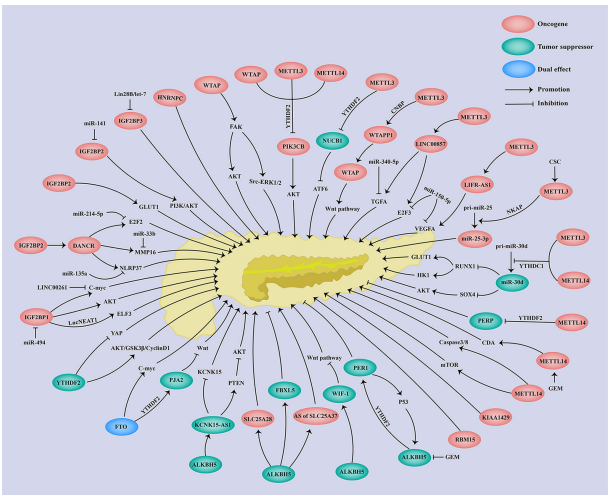

FIGURE 2 | Regulation network of m6A regulators and associated genes in pancreatic cancer.

In contrast, METTL14 could reduce the stability and expression of PERP mRNA. Mechanistically, METTL14 deficiency promoted PERP expression in an m6A-YTHDF2-dependented manner, in which YTHDF2 mediated PERP mRNA degradation (**Table 1**; **Figure 2**). Moreover, PERP inhibition could rescue the decreased cell proliferation, migration, and invasion induced by METTL14 knockdown (7) (**Table 1**). The above findings suggest a tumor-suppressive role of PERP in the malignant progression of PC.

PIK3CB

p110β/PIK3CB is a p110 catalytic subunit of phosphoinositide 3-kinases (PI3Ks), which together with p110α/PIK3CA, p110δ/PIK3CB, and the p85 regulatory subunit formed class IA PI3Ks (84). Aberrant expression of PIK3CB has been found in multiple cancers and is involved in tumor cell growth, metabolism, angiogenesis, metastasis, and multidrug resistance (63, 64, 85–93).

As reported, PIK3CB expression was remarkably elevated in PC tissues and a high expression of PIK3CB predicted poor prognosis of PC patients (63, 64) (**Table 1**). PIK3CB promoted cell proliferation, migration, invasion, and tumorigenesis in PTEN-deficient PC cells both *in vitro* and *in vivo via* activating the Akt signaling pathway (64) (**Table 1**; **Figure 2**). Furthermore, m6A writers including METTL3, METTL14, and WTAP could positively regulate the m6A level of PIK3CB and then reduce its expression in both mRNA and protein levels (**Table 1**, **Figure 2**). On the contrary, FTO, as an m6A eraser, significantly reduced the m6A level of PIK3CB and subsequently enhanced the PIK3CB expression (**Table 1**; **Figure 2**). Moreover, YTHDF2 could interact with m6A-modified PIK3CB to decrease PIK3CB mRNA stability as well as its protein expression (64) (**Table 1**; **Figure 2**). In summary, m6A regulators (METTL3, METTL14, WTAP, FTO) inhibited PIK3CB expression *via* an m6A-YTHDF2-dependent way. The oncogenic effect of PIK3CB in PTEN-deficient PC, indicating that PIK3CB is an emerging therapeutic target for PC.

PJA2

Praja ring finger ubiquitin ligase 2 (PJA2) is a RING-H2-type E3 ubiquitin ligase, which was firstly identified as an axotomy-suppressed gene in nerve cells (94) and played key roles in regulating the cAMP-dependent activation of PKA (95). Emerging evidence has shown that PJA2 was aberrantly expressed across cancers and acts as an oncogene or a tumor suppressor in thyroid cancer (96), glioblastoma (97), and gastric cancer (98, 99).

As shown, PJA2 was significantly upregulated in PC cells (8). Furthermore, PJA2 underwent m6A regulation of FTO, since FTO increased while loss of FTO decreased the expression and m6A level of PJA2 (8) (**Table 1**). Subsequently, Zeng et al. demonstrated that YTHDF2 but not YTHDF1 acted as an m6A reader to mediate PJA2 mRNA degradation and its downregulation (**Table 1**; **Figure 2**). Moreover, PJA2 deficiency could rescue the FTO-induced suppression of cell growth and metastasis *via* inhibiting the Wnt signaling pathway (8) (**Table 1**). Taken together, PJA2 acted as a tumor suppressor to regulate PC malignant behaviors *via* the FTO–PJA2–Wnt axis in an m6A-YTHDF2-dependent way, indicating that PJA2 is a new promising molecular target for PC therapeutic treatment.

NUCB1

Nucleobindins including NUCB1 and NUCB2 are DNA- and calcium-binding proteins (100, 101), involved in the development of various cancers (9, 102–107). Currently, an oncogenic role of NUCB1 has been found in colon cancer (105) and breast cancer (106).

As regards PC, NUCB1 was downregulated in both mRNA and protein levels of PC tissues, and an obviously poor prognosis was observed in PC patients with a lower expression of NUCB1 (9) (**Table 1**). In contrast to the effect of NUCB1 in colon cancer and breast cancer, NUCB1 suppressed the cell growth and promoted the apoptosis of PC cells both *in vitro* and *in vivo*, while loss of NUCB1 in turn promoted PC cell growth and inhibited cell apoptosis (9) (**Table 1**). Hua et al. further clarified that METTL3 induced the m6A modification of NUCB1 5′UTR, in which YTHDF2 mediated m6A-modified NUCB1 mRNA degradation (9) (**Table 1**; **Figure 2**). Functionally, NUCB1 inhibited the cell proliferation and promoted the antitumor effect of gemcitabine (GEM) on PC cells *in vitro* and *in vivo* (9). Moreover, NUCB1 also decreased autophagy and unfolded protein response (UPR)-induced by GEM *via* suppressing ATF6 activity (9) (**Table 1**; **Figure 2**). All the above findings demonstrate the m6A modulation of NUCB1 in an m6A–METTL3–YTHDF2-dependent way and a tumor-suppressive role of NUCB1 in PC progression.

FBXL5

F-box and leucine-rich repeat protein 5 (FBXL5) was firstly reported as a subunit of E3 ubiquitin ligase to promote the ubiquitination of $p150^{Glued}$ (108). IRP2 and Snail1 have been

identified as substrates of FBXL5 (109, 110). FBXL5 has been reported to contain a hemerythrin-like domain that binds to iron and oxygen, thereby being involved in iron homeostasis (109, 111). Likewise, iron and oxygen conditions could in turn regulate FBXL5 (109). Currently, both the oncogenic and tumor-suppressive roles of FBXL5 have been found across cancers including colon cancer and HCC (112–114).

Downregulation of FBXL5 has been detected in PC tissues, which was associated with poor prognosis of PC patients (65) (**Table 1**). FBXL5 was subsequently identified as a target of ALKBH5, which reduced the FBXL5 m6A level and enhanced mRNA stability to increase the FBXL5 expression (**Table 1**). Moreover, FBXL5 depletion successfully rescued the ALKBH5-mediated inhibition of intracellular iron accumulation, cell migration, and invasion through downregulating IRP2 and Snail (65). In a summary, FBXL5 served as a tumor suppressor in PC carcinogenesis through the ALKBH5–FBXL5–IRP2/SNAI1 axis in an m6A-dependent manner (**Figure 2**), indicating a potential role of FBXL5 in the diagnosis, prognosis, and target therapy of PC.

SLC25A28 and SLC25A37

Mitoferrin (MFRN) belongs to the mitochondrial solute carrier family (SLC25), located in the inner membrane (115). Mitoferrin consists of two isoforms: mitoferrin-1 (SLC25A37) and mitoferrin-2 (SLC25A28), which transport iron to the mitochondria (115). So far, both SLC25A37 and SLC25A28 have been involved in dysregulation of mitochondrial iron (116, 117). The tumor-suppressor roles of SLC25A37 and SLC25A28 have been confirmed since they were involved in tumor cell growth, ROS production, mitochondrial iron uptake, and ferroptosis (117–121).

SLC25A37 and SLC25A28 are lowly expressed in PC tissues (65). Li et al. found that a high expression of SLC25A37 but not SLC25A28 indicated shorter OS of PC patients (66) (**Table 1**). Huang et al. demonstrated that either SLC25A37 or SLC25A28 underwent m6A modification modulated by ALKBH5. Detailly, ALKBH5 demethylated SLC25A37 mRNA to modulate its alternative splicing (65) (**Table 1**). As for SLC25A28, ALKBH5 removed the m6A modification of SLC25A28 and thus promoted its mRNA stability and expression (65) (**Table 1**; **Figure 2**). More importantly, SLC25A37 or SLC25A28 was elevated by depletion of PINK1 or PARK2, enhanced mitochondrial iron level, and dysregulation of immunometabolism *via* the PINK1/PARK2–SLC25A37/SLC25A28–HIF1A–AIM2–HNGB1–CD274 axis and thereby triggered PC carcinogenesis (66). Therefore, SLC25A37 and SLC25A28 were regulated through both m6A-dependent and m6A-independent regulations in the modulation of PC progression.

m6A Modification of Non-Coding RNAs in PC

WTAPP1

LncRNA WTAPP1, short for Wilms tumor 1-associated protein pseudogene 1, has been shown to play key roles in tumor cell proliferation, migration, invasion, and angiogenesis (122–124).

Here in PC, WTAPP1 was markedly overexpressed in tumor tissues, which was correlated with poor prognosis of PC patients (52) (**Table 2**). An oncogenic role of WTAPP1 was found in PC, showing that WTAPP1 enhanced PC cell proliferation and invasion both *in vitro* and *in vivo* (**Table 2**). Deng et al. further revealed that METTL3 mediated the m6A modification of WTAPP1, in which CCHC-type zinc finger nucleic-acid binding protein (CNBP) served as an m6A reader and stabilized the RNA of WTAPP1 (52) (**Table 2**). Moreover, the pseudogene WTAPP1 could enhance the translation of WTAP and activate the Wnt signaling, contributing to the malignant progression of PDAC (52) (**Table 2**, **Figure 2**). In summary, WTAPP1 may be a potential diagnostic and prognostic biomarker as well as a promising therapeutic target for PC.

LINC00857

Long non-coding RNA LINC00857 was firstly revealed to be highly expressed in lung cancer and indicated poor survival of lung cancer patients (138). Up to now, the upregulation of LINC00857 has been found in multiple types of cancers and is involved in tumor cell growth, migration, invasion, glycolysis, autophagy, and radiosensitivity (139–143).

As reported, LINC00857 was overexpressed in both PC cells and tissues, and its upregulation was associated with shorter OS and disease-free survival of PC patients (125–127) (**Table 2**). Studies have shown that LINC00857 could increase PC cell proliferation, migration, and invasion and decrease cell apoptosis (125–127) (**Table 2**). In regard to the molecular regulation of LINC00857, Meng et al. demonstrated that m6A writer METTL3 elevated both the m6A level and RNA stability of LINC00857 to promote LINC00857 expression, and then LINC00857 functioned as a ceRNA to regulate E2F3 expression through sponging miR-150-5p, contributing to the malignant development of PC cells (125) (**Table 2**; **Figure 2**). Moreover, Li et al. further demonstrated that LINC00857 could also sponge miR-340-5p to enhance the TGFA expression in PC cells and then accelerate PC cell migration and invasion (126) (**Table 2**, **Figure 2**). Additionally, LINC00857 could also upregulate MET, STAT3, and CREB expression to enhance PC cell proliferation (127). Above all, LINC00857 exerts an oncogenic role in PC and may provide a possible therapeutic target for PC treatment.

DANCR

Long non-coding RNA differentiation antagonizing non-protein-coding RNA (DANCR) was firstly identified as a progenitor differentiation suppressor in 2012 (144). Later, both the oncogenic and tumor-suppressive roles of DANCR have been identified across cancers (128, 145), participating in modulating tumor cell growth, stemness-like properties, EMT, and chemoresistance (145).

Consistent with previous reports in various cancers, DANCR was also highly expressed in both PC cells and tissues (129, 130) (**Table 2**). Moreover, a high expression of DANCR predicted poor clinical outcomes in PC (130) (**Table 2**). Luo et al. demonstrated that DANCR deficiency significantly decreased the cell proliferation, migration, and invasion of PC through the DANCR/miR-33b/MMP16 axis, in which DANCR served as a miR-33b sponge (129) (**Table 2**; **Figure 2**). Consistent with Yao et al.'s reports, Tang et al. also revealed that DANCR enhanced cell proliferation and invasion *via* the DANCR/miR-214-5p/E2F2 axis or DANCR/miR-135a/NLRP37 (130, 131)

TABLE 2 | m6A modification of ncRNAs in pancreatic cancer.

Name	Role	Expression	Function	Clinical significance	m6A regulator	Mechanisms	Refs
WTAPP1	Oncogene	Up	WTAPP1 increased the PC cell growth and migration.	OS↓	METTL3 CNBP	1.METTL3 increased the m6A level and RNA expression of WTAPP1. 2.CNBP enhanced WTAPP1 RNA stability and subsequently accelerated WTAP translation and Wnt pathway activation.	(52)
LINC00857	Oncogene	Up	1.LINC00857 increased the PC cell proliferation, migration, and invasion. 2.LINC00857 reduced the PC cell apoptosis.	OS↓ DFS↓	METTL3	1.METTL3 increased the m6A modification, RNA stability, and expression of LINC00857 2.ceRNA: LINC00857/miR-150-5p/E2F3 axis. 3.ceRNA: LINC00857/miR-340-5p/TGFA axis 4.LINC00857 enhanced MET and then promoted STAT3 and CREB expression.	(125–127)
DANCR	Oncogene	Up	1.DANCR increased the PC cell proliferation, migration, invasion, stemness-like properties, and tumorigenesis. 2.DANCR inhibited PC cell apoptosis.	OS↓ PFS↓	IGF2BP2	1.IGF2BP2 recognized the m6A modification of DANCR and increased the DANCR RNA stability and expression 2.DANCR/miR-33b/MMP16 axis. DANCR sponge miR-33b to promote mmp16 expression 3.DANCR/miR-214-5p/E2F2 axis DANCR sponging miR-214-5p to promote E2F2 expression 4.DANCR/miR-135a/NLRP37 axis	(128–132)
KCNK15-AS1	Suppressor	Down	KCNK15-AS1 inhibited the PC cell proliferation, migration, and invasion.	DFS↑	ALKBH5	1.ALKBH5 decreased the m6a level of KCNK15-AS1 and enhanced the KCNK15-AS1 RNA stability and expression. 2.KCNK15-AS1inhibited KCNK15 translation. 3.KCNK15-AS1 enhanced PTEN to inhibit Akt signaling pathway.	(133, 134)
LIFR-AS1	Oncogene	Up	LIFR-AS1 KD suppressed the PC cell proliferation, migration, and invasion.	–	METTL3	1.METTL3 KD decreased the m6A level and RNA stability of LIFR-AS1 to reduce its expression. 2.LIFR-AS1 sponged miR-150-5p to promote VEGFA expression.	(135)
miR-25-3p	Oncogene	Up	miR-25-3p promoted the cell proliferation, migration, and invasion.	OS↓	METTL3 NKAP	1.CSC induced METTL3 expression *via* hypomethylation of METTL3. 2.METTL3 promoted miR-25-3p maturation *via* the NKAP-mediated m6A modification of pri-miR-25.	(53)
miR-30d	Suppressor	Down	miR-30d decreased the cell proliferation, migration, invasion, angiogenesis, and Warburg effect.	OS ↑ RFS↑ DFS↑	METTL3 METTL14 YTHDC1	1.METTL3/14 KD reduced the m6A enrichment of pri-miR-30d. 2.YTHDC1 induced the RNA decay of pri-miR-30d and increased the miR-30d expression. 3. miR-30d/RUNX1/GLUT1/HK1 axis. 4. miR-30d/SOX4/PI3K-Akt axis	(136, 137)

PC, pancreatic cancer; KD, knockdown; ncRNA, non-coding RNA; up, upregulation in PC, down, downregulation in PC; OS, overall survival; DFS, disease-free survival; PFS, progression-free survival; RFS, relapse-free survival.

(**Table 2**; **Figure 2**). In addition to ceRNA regulation, DANCR also underwent m6A regulation. In detail, IGF2BP2 acted as an m6A reader and could recognize and bind to DANCR to enhance its RNA stability and expression, thereby facilitating PC cell proliferation and stemness-like properties (128) (**Table 2**; **Figure 2**). The above findings indicate the oncogenic role of DANCR and its potential clinical application in PC prognosis and treatment.

KCNK15-AS1

LncRNA KCNK15 and WISP2 antisense RNA 1 (KCNK15-AS1 or RP11-445H22.4) were firstly found to be upregulated in osteoarthritis (146). Subsequently, an abnormal expression of KCNK15-AS1 was also observed among cancers.

As reported, there was a remarkable downregulation of KCNK15-AS1 in both PC tissues and cell line, and patients with a low expression of KCNK-AS1 have shown shorter OS (133, 134) (**Table 2**). RNA m6A demethylase ALKBH5 could bind to KCNK15-AS1 and thus enhance its RNA stability and expression through eliminating m6A modification (133, 134) (**Table 2**; **Figure 2**). More importantly, KCNK15-AS1 suppressed KCNK15 translation through interacting with the 5′UTR of KCNK15, while KCNK15-AS1 also promoted PTEN expression and thus inhibited the Akt pathway, thereby suppressing PC cell proliferation, migration, and invasion (133, 134) (**Table 2**; **Figure 2**). In summary, KCNK15-AS1 regulated by ALKBH5-mediated m6A demethylation acted as a tumor suppressor to suppress the malignant progression of PC cells

through the KCNK15–AS1/KCNK15 axis and KCNK15–AS1/PTEN/Akt axis, suggesting a promising therapeutic target for PC clinical treatment.

LncRNA LIFR-AS1

Leukemia inhibitory factor receptor antisense RNA 1 (LIFR-AS1) is a long non-coding RNA, which is transcribed from the LIFR gene. An abnormal expression of LIFR-AS1 has been found in various cancers and is involved in cancer development (147). The function of LIFR-AS1 is complex, since an antitumor role of LIFR-AS1 has been shown in glioma, breast cancer, and lung cancer, while an oncogenic role has been found in thyroid cancer, colorectal cancer, and osteosarcoma (147). Furthermore, both the oncogenic role and tumor suppressive role of LIFR-AS1 were revealed in gastric cancer (148, 149).

An obvious upregulation of LIFR-AS1 was observed in PC tissue and cell lines, which was correlated with poor clinical outcomes of PC patients (135) (**Table 2**). Knockdown of METTL3 reduced the m6A level of LIFR-AS1 and thus suppressed its RNA stability and expression (135) (**Table 2**, **Figure 2**). Moreover, a significant decrease in cell proliferation, migration, and invasion was observed following LIFR-AS1 inhibition (135) (**Table 2**). Additionally, a ceRNA regulation was also revealed showing that LIFR-AS1 could sponge miR-150-5p, thus activating downstream target VEGFA and promoting PC progression (135) (**Table 2**, **Figure 2**). These results revealed that LIFR-AS1 is an oncogenic gene in PC *via* the METTL3/LIFR-AS1/miR-150-5p/VEGFA axis in an m6A-dependent manner.

miR-25-3p

pri-miR-25 is the primary miRNA of miR-25-3p. miR-25 has been widely reported as an oncogenic miRNA. An abnormal expression of miR-25 has been found in multiple cancers (150, 151).

Here in PC, miR-25-3p was found to be highly expressed, which predicted the poor prognosis of PC patients (53) (**Table 2**). Zhang et al. further demonstrated that METTL3 induced by cigarette smoke condensate (CSC) could increase the m6A modification of pri-miR-25 *via* in a NKAP-dependent manner, in which NF-κB-associated protein (NKAP) functioned as an m6A reader of pri-miR-25 (53), thereby enhancing miR-25-3p maturation. Additionally, upregulated miR-25-3p could then activate the Akt signaling pathway through inhibiting the expression of its target-PHLPP2, thus promoting the cell migration and invasion of PC (53) (**Table 2**; **Figure 2**). In summary, the METTL3/miR-25-3p/PHLPP20/Akt axis exerts an oncogenic role in the carcinogenesis of PC patients who smoke.

miR-30d

miR-30d belongs to the miR-30 family, which is abnormally expressed across cancers. So far, both the oncogenic and antitumor roles of miR-30d have been revealed. miR-30d has been reported to remarkably suppress cell growth and metastasis of breast cancer (152), whereas upregulation of miR-30d enhanced tumor growth and angiogenesis of prostate cancer (153).

In PC, the expression of miR-30d was significantly decreased in both PC tissues and cell lines, which predicted a shorter OS, RFS, and DFS of PC patients (136, 137) (**Table 2**). More importantly, miR-30d overexpression inhibited PC cell growth, metastasis, and angiogenesis both *in vitro* and *in vivo* (136, 137) (**Table 2**). miR-30d was further shown to be involved in glycolysis regulation since miR-30d decreased the lactic acid level, glucose uptake, and ATP level while miR-30d inhibition increased the lactic acid level, glucose uptake, and ATP level of PC (**Table 2**). miR-30d has m6A modification and is regulated by METTL3, METTL14, and YTHDC1. In detail, YTHDC1 significantly promotes the RNA degradation of pri-miR-30d and increases the expression of miR-30d, and knockdown of METTL3/14 significantly reduces the m6A enrichment of pri-miR-30d (136) (**Table 2**; **Figure 2**). Moreover, RUNX1 and SOX4 were identified as a downstream target of miR-30d. Hou et al. demonstrated that RUNX1 deficiency could attenuate miR-30d inhibition-induced PC cell growth, metastasis, angiogenesis, and glycolysis *via* the miR-30d/RUNX1/GLUT1/HK1 axis (136) (**Table 2**; **Figure 2**). Xu et al. further revealed that SOX4 overexpression successfully rescued the antitumor effect of miR-30d *via* the miR-30d/SOX4/PI3K-Akt axis (137) (**Table 2**; **Figure 2**). Taking all the above into consideration, miR-30d is shown to be modulated by YTHDC1-mediated m6A modification and there is a tumor-suppressive role of the miR-30d/RUNX1/GLUT1/HK1 axis and miR-30d/SOX4/PI3K-Akt axis in PC progression, providing a possible application of miR-30d as a diagnosis and prognosis biomarker and also a therapeutic target for PC treatment.

FUNCTIONS OF M6A REGULATORS IN PANCREATIC CANCERS

Writers

WTAP

WTAP has been found to be highly expressed in PC tissue, which was correlated with shorter survival of PC patients (7, 52, 65, 154) (**Table 3**). As reported, WTAP triggered the cell proliferation, migration, and invasion and GEM resistance of PC cells, while knockdown of WTAP suppressed cell proliferation migration, and invasion and GEM resistance (52, 155) (**Table 3**).

As an m6A methylase, WTAP increased the m6A level of its target PIK3CB and enhanced the PIK3CB expression *via* an m6A-YTHDF2-mediated RNA decay of PIK3CB (64) (**Table 3**; **Figure 2**). Apart from m6A regulation, WTAP could also stabilize FAK mRNA and increase its expression through an m6A-indenpendent way, thereby activating the FAK-PI3K-AKT and FAK-SRC-GRB2-ERK1/2 signaling pathways (155) (**Table 3**; **Figure 2**). Moreover, GSK2256098, a specific FAK inhibitor, could attenuate WTAP-induced cell migration and invasion and GEM resistance of PC cells (155). As a key modulator to affect its downstream target genes, WTAP could also be regulated by its upstream genes. Deng et al. have shown that LncRNA WTAPP1 could recruit EIF3B to enhance WTAP

TABLE 3 | Functions of m6A writers in pancreatic cancer.

Name	Expression	Mechanism	Functions	Targets	Clinical significance	Refs
WTAP	Up	1.WTAP KD reduced the m6A level of PIK3CB and increased the PIK3CB expression *via* m6A-YTHDF2 mediated RNA decay of PIK3CB. 2.WTAP bound to and enhanced FAK mRNA stability to activate the FAK-Pl3K-AKT and FAK-SRC-GRB2-ERK1/2 signaling pathway. 3.LncRNA WTAPP1 enhanced WTAP translation and then the WTAP-activated Wnt signaling pathway.	WTAP increased PC cell proliferation, migration, and invasion and GEM resistance.	**m6A-dependent:** PIK3CB. **m6A-indenpendent:** FAK.	OS ↓	(7, 52, 64, 65, 154, 155)
METTL3	Up	1.METTL3 KD reduced the m6A level of PIK3CB and increased PIK3CB expression *via* the m6A-YTHDF2-mediated RNA degradation of PIK3CB. 2.METTL3 KD reduced the m6A enrichment of NUCB1 5′ UTR and increased the NUCB1 expression *via* YTHDF2-mediated RNA decay of NUCB1. 3.METTL3 KD reduced the m6A level of WTAPP1 and decreased its RNA stability and expression in an m6A-CNBP-dependent manner. 4.METTL3 KD reduced the m6A level, RNA stability, and expression of LIFR-AS1. 5.CSC induced the hypomethylation of the METTL3 promoter to enhance METTL3 expression, which increased the m6A level and expression of pri-miR-25 as well as miR-25-3p maturation in an NKAP-m6A-dependent way.	METTL3 promoted the cell proliferation, migration, invasion, stemness, and radio- and chemoresistance of PC cells.	**m6A-dependent**: PIK3CB, NUCB1, WTAPP1, LINC00857, LIFR-AS1, pri-miR-25, pri-miR-30d.	OS↓	(7, 9, 10, 52, 53, 64, 135, 156)
METTL14	Up	1.METTL14 KD reduced the m6A level of PIK3CB and PERP to increase their expression *via* m6A-YTHDF2-mediated RNA degradation. 2.METTL14 activated caspase3/8, mTOR pathway, and CDA expression. 3.SRFR5 regulated the AS of METTL14.	1.METTL14 increased PC cell proliferation, migration, invasion, metastasis, and chemoresistance. 2.METTL14 inhibited PC cell apoptosis and autophagy.	**m6A-dependent**: PIK3CB, PERP, pri-miR-30d.	OS↓	(7, 64, 157–159)
KIAA1429	Up	–	KIAA1429 KD inhibited PC cell proliferation.	–	OS↓	(160–162)
RBM15	Up	–	RBM15 KD inhibited PC cell proliferation,	–	OS↓, DFI↓ PFI↓, DSS↓	(163)

KD, knockdown; up, upregulation in PC; AS, alternative splicing; OS: overall survival; PFS: progression-free survival; DFI, disease-free interval; PFI, progression-free interval; DSS, disease-specific survival; -, no associated research.

translation, and then WTAP activated the Wnt signaling pathway, forming a functional WTAPP1/WTAP/Wnt axis[75] (**Table 3**; **Figure 2**). Additionally, knockdown of WTAP attenuated WTAPP1-induced PC cell proliferation, migration, and invasion (52) (**Table 3**). All the above findings indicate the oncogenic role of WTAP in PC in an m6A-dependent and m6A-independent way as well as the potential application of WTAP in PC prognosis and targeted therapy.

METTL3

Similar to WTAP, METTL3 was also shown to be upregulated in PC, which was correlated with shorter OS of PC patients (7, 10) (**Table 3**). According to the studies, METTL3 deficiency decreased PC cell proliferation, migration, invasion, stemness, and radio- and chemoresistance (10, 156) (**Table 3**). Acting as an m6A methylase, METTL3 knockdown obviously reduced the total RNA m6A level of PC cells (10). Several m6A-regulated targets of METTL3 have been identified, such as PIK3CB, NUCB1, WTAPP1, LINC00857, LIFR-AS1, pir-miR-25, and pri-miR-30d (**Table 3**).

Firstly, METTL3 inhibition reduced the m6A level of PIK3CB and increased the PIK3CB expression *via* YTHDF2-mediated mRNA decay (64). Later, Hua et al. found that the m6A enrichment of the NUCB1 5′UTR was notably decreased upon knockdown of METTL3 *via* in an m6A-YTHDF2-dependent way (9) (**Table 3**; **Figure 2**). In addition to coding RNAs, METTL3 also mediated the m6A regulation of non-coding RNAs. For instance, loss of METTL3 significantly reduced the m6A level of WTAPP1 as well as its expression, in which m6A reader CNBP enhanced the mRNA stability of WTAPP1 (52) (**Table 3**; **Figure 2**). Furthermore, METTL3 deficiency also decreased the m6A level and RNA stability of LINC00857 and LIFR-AS1, resulting in their downregulation (135) (**Table 3**; **Figure 2**). Smoke was a high-risk factor of PC, and smokers were reported to have a two-fold higher risk of PC than non-smokers (164). Interestingly, there was a significant upregulation of METTL3 in smokers as compared with non-smokers (53). Zhang et al. have further shown that cigarette smoke condensate (CSC) promoted METTL3 expression through hypomethylating the promoter of METTL3 (53), which enhanced the m6A level and expression of pri-miR-25 and miR-25-3p maturation *via* in an m6A-NKAP-dependent manner, in which NF-κB-associated protein (NKAP) functioned as an m6A reader of pri-miR-25 (53)

(**Table 3**; **Figure 2**). Additionally, METTL3 was also shown to affect the m6A enrichment of pri-miR-30d (136). In summary, METTL3 served as an oncogene in PC progression and provides a possible prognosis biomarker and therapeutic target for PC.

METTL14

An obvious upregulation of METTL14 has been found in both RNA and protein levels in PC tissues (7, 157) (**Table 3**). Patients with a higher expression of METTL14 have shown shorter OS (7) (**Table 2**). As mentioned before, METTL14 was involved in various cellular processes of PC cells, since METTL14 remarkably promoted PC cell proliferation, migration, and invasion; metastasis; cisplatin resistance; and GEM resistance while it inhibited the apoptosis and autophagy of PC cells (7, 157–159) (**Table 3**).

It was shown that loss of METTL14 increased cisplatin-induced cell apoptosis and autophagy by activating caspase3/8 and mTOR pathway, thereby enhancing the antitumor effect of cisplatin (157) (**Table 3**; **Figure 2**). Interestingly, GEM treatment specifically upregulated the expression of METTL14, without changes in other m6A regulators (158). However, GEM-induced METTL14 could in turn increase the GEM resistance *via* promoting CDA expression both *in vitro* and *in vivo (*158) (**Table 3**; **Figure 2**). Additionally, SRFR5 was shown to regulate the alternative splicing of METTL14, which formed a SRSF5–METTL14 axis to enhance PC cell growth and metastasis *in vitro* and *in vivo (*159) (**Table 3**). For m6A regulation, Tian et al. revealed that loss of METTL14 suppressed the m6A level and promoted the expression of PIK3CB *via* m6A-YTHDF2-mediated RNA decay of PIK3CB (64) (**Table 3**; **Figure 2**). Moreover, Wang et al. demonstrated that METTL14 deficiency also decreased the m6A level and thus increased the expression of PERP through m6A-YTHDF2-mediated degradation of PERP mRNA (7), resulting in cell growth and metastasis of PC (**Table 3**; **Figure 2**). In addition, METTL14 knockdown also deceased the m6A enrichment of pri-miR-30d (136). The above results suggest that both the m6A-dependent and m6A-independent regulation of METTL14 are involved in the carcinogenesis of PC, and METTL14 is a promising diagnosis and prognosis biomarker and chemotherapy resistance target for PC treatment.

KIAA1429

Vir-like m6A methyltransferase-associated (VIRMA, also named KIAA1429) was significantly upregulated in PC tissues as compared to normal tissues (160) (**Table 3**). Moreover, KIAA1429 was revealed as an independent risk factor for PC prognosis (165), and high expression of KIAA1429 was associated with shorter OS of PC patients (161, 162) (**Table 3**). Depletion of KIAA1429 remarkably reduced the cell proliferation of PC cells (162), indicating an oncogenic role of KIAA1429 in PC (**Table 3**; **Figure 2**).

RBM15

RNA-binding motif protein 15 (RBM15) has been identified as a methylase during m6A modification. According to TCGA and GTEx databases, RBM15 was highly upregulated in PC tissues and loss of RBM15 suppressed the cell proliferation of PC cells (163) (**Table 3**). Moreover, PC patients with a high expression of RBM15 have shown decreased OS, DFI, PFI, and DSS (163) (**Table 3**). Additionally, a highly correlated relationship between RBM15 expression and immune checkpoint markers was also revealed. The above findings suggest a favorable application of RBM15 in the prognosis and immunotherapy of PC.

Readers

IGF2BP1

It has been shown that an upregulation of IGF2BP1 was observed in PC tissues (166), which was associated with shorter OS of PC patients (166) (**Table 4**). Knockdown of IGF2BP1 dramatically reduced cell proliferation and induced G1 cell-cycle arrest and apoptosis (166, 167) (**Table 4**). IGF2BP1, an RNA-binding protein, attenuated the Linc00261-induced suppression of c-myc RNA stability through binding to Linc00261 (168) (**Table 4**; **Figure 2**). Moreover, IGF2BP1 cooperated with LncNEAT1 to increase the RNA stability of ELF3 and enhanced PC cell proliferation, migration, and invasion (167) (**Table 4**; **Figure 2**). In addition, miR-494 could target IGF2BP1 to suppress IGF2BP1 expression and then IGF2BP1 promoted PC progression *via* activating the Akt pathway (166) (**Table 4**; **Figure 2**). Therefore, IGF2BP1 might serve as a potential therapeutic target and prognostic biomarker for PC.

IGF2BP2

Consistent with IGF2BP1, IGF2BP2 was also significantly upregulated in PC tissues and cell lines (128, 169–172) (**Table 4**). A high expression of IGF2BP2 predicted a shorter OS of PC patients (128, 160–162, 169–173) (**Table 4**). Knockdown of IGF2BP2 significantly reduced PC cell growth and invasion (128, 162, 169, 171, 172) (**Table 4**). It has been reported that IGF2BP2 promotes the aerobic glycolysis of PC cells by binding to and stabilizing GLUT1 mRNA (169) (**Table 4**; **Figure 2**). IGF2BP2 was subsequently revealed as a potential target of miR-141 and to be involved in miR-141-induced PC cell growth *via* activating the PI3K-Akt signaling pathway (171) (**Table 4**; **Figure 2**). Acting as an m6A reader, IGF2BP2 could bind to DANCR to increase RNA stability *via* in an m6A-dependent manner and enhance the cell proliferation and stemness-like properties of PC cells (128) (**Table 4**; **Figure 2**). Thus, IGF2BP2 plays an oncogenic role in PC.

IGF2BP3

A high expression of IGF2BP3 in both PC tissues and cell lines was observed (172, 174, 175) (**Table 4**) and was correlated with shorter OS as well as PFS of PC patients (162, 172, 174–177) (**Table 4**). Knockdown of IGF2BP3 reduced the cell proliferation, migration, and invasion of PC cells (172, 178, 179). Mechanism-wise, IGF2BP3, located in cytoplasmic stress granules along with its downstream targets ARF6 and ARHGE4, promoted cell protrusion formation and enhanced PC cell invasion and metastasis (179, 180). A genome-wide analysis upon IGF2BP3 knockdown has further shown that IGF2BP3 was strongly

TABLE 4 | Functions of m6A readers and erasers in pancreatic cancer.

Name	Expression	Mechanism	Functions	Targets	Clinical significance	Refs
IGF2BP1	Up	1.IGF2BP1 increased the RNA stability of c-myc and ELF3. 2.miR-194 targeted IGF2BP1 to inhibit IGF2BP1 expression.	IGF2BP1 KD inhibited PC cell proliferation and induced G1 cell cycle arrest and apoptosis.	**m6A-independent**: c-myc, ELF3	OS↓	(166–168)
IGF2BP2	Up	1.IGF2BP2 promoted GLUT1 expression *via* stabilizing GLUT1 mRNA. 2. miR-141 downregulated IGF2BP2 to activate the PI3K-Akt pathway. 3. IGF2BP2 enhanced DANCR expression *via* binding to and stabilizing m6A-modified DANCR	IGF2BP2 promoted PC cell growth, invasion, aerobic glycolysis, and stemness-like properties.	**m6A-dependent**: DANCR. **m6A-independent**: GLUT1.	OS↓	(128, 160–162, 169–173)
IGF2BP3	Up	1.Lin28B/Let7 targets IGF2BP3 to downregulate IGF2BP3.	IGF2BP3 enhanced the PC cell proliferation, migration, invasion, metastasis, and stemness-like properties.	–	OS↓ PFS↓	(162, 172, 174–180)
YTHDF1	Up	–	–	–	–	(181, 182)
YTHDF2	Up	1.YTHDF2 acted as an m6A reader and induced the RNA degradation of m6A-modified PER1, PERP, PIK3CB, PJA2, and NUCB1. 2.YTHDF2 KD inhibited the Akt/GSK3β/CyclinD1 signaling pathway and activated the YAP signaling pathway.	YTHDF2 KD inhibited cell growth and promoted cell migration, invasion, and EMT.	**m6A-dependent**: PER1, PERP, PIK3CB, PJA2, NUCB1	OS↓ Advanced stage	(7–9, 11, 64, 65, 181–183)
YTHDF3	Up	–	–	–	–	(65)
YTHDC1	Down	YTHDC1 promoted miR-30d maturation through enhancing the m6A-dependent RNA degradation of pri-miR-30d.	YTHDC1 inhibited PC cell growth.	**m6A-dependent**: pri-miR-30d	OS↑ DFS↑	(136)
YTHDC2	Down	–	–	–	–	(136)
HNRNPC	–	–	HNRNPC KD inhibited PC cell proliferation.	–	OS↓	(162)
CNBP	–	CNBP enhanced RNA stability of WTAPP1.	–	–	–	(52)
ALKBH5	Down	1.ALKBH5 reduced the m6A level of PER1 to enhance the PER1 expression *via* in an m6A-YTHDF2-dependent way, forming a positive feedback loop of the ALKBH5/PER1/p53/ALKBH5 axis. 2.ALKBH5 reduced the m6A level while increasing the expression of WIF-1 and KCNK-AS1. 3.ALKBH5 promoted FBXL5 and SLC25A28 expression and also modulated the alternative splicing of SLC25A37 *via* m6A modification.	1.ALKBH5 decreased cell proliferation, migration, invasion, and GEM-resistance. 2.ALKBH5 modulated PC cell iron metabolism.	**m6A-dependent:** WIF-1, PER1, FBXL5, SLC25A28, SLC23A37, KCNK15-AS1.	OS↑ OS↓	(11, 56, 65, 133, 134, 184)
FTO	Down	1.FTO reduced the global m6A level of PC cells. 2.FTO reduced the m6A level of PJA2 and promoted the PJA2 expression *via* m6A-YTHDF2-mediated RNA degradation, suppressing the Wnt pathway.	FTO inhibited cell proliferation, migration, and invasion.	**m6A-dependent**: PJA2	OS↑ Lymph node metastasis↓	(8, 65)
	Up	FTO promoted c-myc expression *via* enhancing c-myc mRNA stability.	FTO KD reduced PC cell proliferation and enhanced apoptosis.	**m6A-independent**: c-myc	–	(185)

KD, knockdown; up, upregulation in PC, down, downregulation in PC; AS, alternative splicing; OS, overall survival; DFS, disease-free survival; PFS, progression-free survival; -, no associated research.

correlated with genes regulating cell migration, proliferation, and adhesion (178). Moreover, IGF2BP3 was identified as a target of Lin28B/Let7 and enhanced the cell growth and stemness-like properties of PC cells (177) (**Table 4**; **Figure 2**). Overall, IGF2BP3 might be a promising diagnostic and prognostic biomarker as well as therapeutic target for PC.

YTHDFs

YTHDF1, YTHDF2, and YTHDF3 were upregulated in PC tissues (65, 181–183) (**Table 4**). Among these YTHDF family genes, YTHDF2 has been extensively studied in PC, while few studies have reported the roles of YTHDF1 and YTHDF3 in PC. As previously reported, PC patients with a higher expression of YTHDF2 have shown a shorter OS (181) and advanced stage (183) (**Table 4**). Chen et al. found that knockdown of YTHDF2 inhibited cell growth through inhibiting the Akt/GSK3β/CyclinD1 signaling pathway (183) (**Table 4**; **Figure 2**). However, an enhancement of cell migration, invasion, and EMT was also observed upon YTHDF2 deficiency (183). Furthermore, loss of YTHDF2-mediated YAP signaling activation may participate in PC cell EMT (183) (**Table 4**; **Figure 2**). Referring to m6A regulation, YTHDF2 served as an m6A reader which could recognize and bind to m6A-modified PIK3CB, PERP, PER1, PJA2, and NUCB1 RNA, thereby mediating their RNA degradation (7–9, 11, 64) (**Table 4**;

Figure 2). The above findings indicate the critical roles of YTHDF2 in PC malignant progression.

YTHDCs

YTHDC1 and YTHDC2 were downregulated in PC tissues when compared to normal tissues (136) (**Table 4**). The upregulation of YTHDC1 predicted the longer OS and relapse-free survival (RFS) of PC patients (136) (**Table 4**). As an m6A reader, YTHDC1 triggered the degradation of pri-miR-30d and enhanced the maturation of miR-30d in an m6A-dependent manner (136) (**Table 4**; **Figure 2**). Finally, YTHDC1 was further found to suppress cell growth induced by miR-30d inhibition (136). Therefore, YTHDC1 might be a possible biomarker for PC prognosis and targeted therapy due to its antitumor effect.

HNRNPC

Few studies have reported the function of HNRNPC in PC. Hou et al. have shown that knockdown of HNRNPC significantly reduced the cell proliferation of PC cells (162) (**Table 4**). A high expression of HNRNPC was associated with a shorter OS of PC patients (162) (**Table 4**).

Erasers

FTO

A contradictory expression of FTO has been reported in PC. On the one hand, Tang et al. found that FTO was highly expressed in both PC tissues and cell lines (185) (**Table 4**). Loss of FTO inhibited cell proliferation and also enhanced the apoptosis of PC cells (185). Meanwhile, a significantly elevated m6A level of PC cells was detected after FTO knockdown through m6A dot-blot (185). Furthermore, FTO could interact with c-myc and enhance the expression and mRNA stability of c-myc, forming a functional FTO/c-myc axis (185) (**Table 4**; **Figure 2**). On the other hand, downregulation of FTO in PC tissues was also observed (8, 65) (**Table 3**). Furthermore, a low expression of FTO predicted a shorter OS of PC patients (8). FTO suppressed PC cell proliferation, migration, and invasion (8). Acting as an m6A demethylase, FTO reduced while FTO deficiency enhanced the total RNA m6A level of PC cells (8). Moreover, FTO reduced the m6A level of PJA2 and increased PJA2 expression *via* YTHDF2-mdeidated RNA degradation of PJA2, thereby suppressing the Wnt pathway and forming a functional FTO/YTHDF2/PJA2/Wnt axis to inhibit PC malignant progression (8) (**Table 4**; **Figure 2**). Taken together, FTO exerted both oncogenic and antitumor roles in the carcinogenesis of PC.

ALKBH5

Unlike the m6A writer expression in PC, ALKBH5 expression is significantly reduced in PC tissues, and PC patients with a low expression of ALKBH5 have shown a shorter OS (11, 56, 65). In contrast, Cho et al. revealed that a high expression of ALKBH5 was associated with a shorter OS of PC patients (184) (**Table 4**). So far, ALKBH5 has been reported to negatively regulate PC cell proliferation, migration, and invasion; iron metabolism; and GEM resistance (11, 56, 65, 133, 134) (**Table 4**). Currently, several m6A targets of ALKBH5 have been identified, such as WIF-1, PER1, FBXL5, SLC25A28, SLC25A37, and KCNK15-AS1. As reported, ALKBH5 reduced the m6A level of PER1 and then increased PER1 expression *via* in an m6A-YTHDF2-dependent way (11) (**Table 4**; **Figure 2**). Moreover, PER1 could in turn increase the ALKBH5 expression through activating p53 (11), suggesting a positive feedback loop between ALKBH5 and PER1 in promoting tumor growth and metastasis of PC (**Table 4**; **Figure 2**). Tang et al. found that ALKBH5, downregulated by GEM treatment, could also decrease the m6A level of WIF-1 and promote its expression to suppress the Wnt pathway, leading to PC cell growth, metastasis, and GEM resistance (56) (**Table 4**; **Figure 2**). In addition, ALKBH5 modulated the RNA stability of FBXL5 and SLC25A28, as well as the alternative splicing of SLC25A37 in an m6A-dependent manner (65), and reduced the cell migration and invasion and the intracellular iron level, thus preventing PC progression (65) (**Table 4**; **Figure 2**). He et al. further demonstrated that ALKBH5 remarkably enhanced the KCNK15-AS1 expression through decreasing the m6A level and stabilizing the KCNK15-AS1 mRNA, thereby suppressing cancer development (133, 134) (**Table 4**; **Figure 2**). Therefore, the above results revealed a tumor-suppressive role of ALKBH5 in PC, indicating a possible application of ALKBH5 for PC prognosis and chemoresistance prediction.

CONCLUSIONS AND PERSPECTIVES

In recent years, the molecular mechanisms of genetic and epigenetic regulation have been extensively studied in the occurrence and progression of PC. Notably, increasing attention has been paid to the m6A modifications in PC development. Here in this review, we focused on the function and molecular mechanism of m6A regulators and m6A-regulated genes. We summarized that m6A modifications exerted their functions mainly in two ways. Firstly, m6A modifications modulate mRNA methylation to affect their RNA stability as well as protein expression (**Table 1**; **Figure 2**). Secondly, m6A modifications regulate the methylation of ncRNAs including long non-coding RNAs (LncRNAs) and miRNAs and alter the ncRNA expression to participate in PC carcinogenesis (**Table 2**; **Figure 2**). In spite of the above findings, the molecular mechanism of m6A regulation in PC remains largely unknown. Increasing comprehensive and in-depth studies are required to elucidate the critical roles of m6A modification in the malignant progression of PC and to further identify novel promising diagnostic and prognostic biomarkers as well as therapeutic targets for PC, and finally explore their possible clinical applications. Moreover, further research is also required to be done to illustrate the m6A modulation in higher-risk factors of PC, such as smoking, obesity, diabetes, and chronic pancreatitis. Therefore, systematic and comprehensive studies are urgently needed to clarify the interplay between m6A regulation and PC malignant progression, paving the way for exploring more approaches for PC treatment.

AUTHOR CONTRIBUTIONS

XH, WSu, QX, and KT contributed to the idea and the manuscript. XL, WF, WSun, and QL collected the literature. JG contributed to the figure preparation. All authors contributed to the article and approved the submitted version.

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REVIEW
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Chromatin Dynamics in Digestive System Cancer: Commander and Regulator

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Digestive system tumors have a poor prognosis due to complex anatomy, insidious onset, challenges in early diagnosis, and chemoresistance. Epidemiological statistics has verified that digestive system tumors rank first in tumor-related death. Although a great number of studies are devoted to the molecular biological mechanism, early diagnostic markers, and application of new targeted drugs in digestive system tumors, the therapeutic effect is still not satisfactory. Epigenomic alterations including histone modification and chromatin remodeling are present in human cancers and are now known to cooperate with genetic changes to drive the cancer phenotype. Chromatin is the carrier of genetic information and consists of DNA, histones, non-histone proteins, and a small amount of RNA. Chromatin and nucleosomes control the stability of the eukaryotic genome and regulate DNA processes such as transcription, replication, and repair. The dynamic structure of chromatin plays a key role in this regulatory function. Structural fluctuations expose internal DNA and thus provide access to the nuclear machinery. The dynamic changes are affected by various complexes and epigenetic modifications. Variation of chromatin dynamics produces early and superior regulation of the expression of related genes and downstream pathways, thereby controlling tumor development. Intervention at the chromatin level can change the process of cancer earlier and is a feasible option for future tumor diagnosis and treatment. In this review, we introduced chromatin dynamics including chromatin remodeling, histone modifications, and chromatin accessibility, and current research on chromatin regulation in digestive system tumors was also summarized.

Keywords: digestive system tumor, epigenetics, chromatin dynamics, targeted therapy, clinical trials

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INTRODUCTION

Following a 19-year effort to sequence the full human genome, the landscape of human cancers began to be revealed. One of the most valuable results of this genome sequencing effort was that epigenetic and chromatin remodeling-centered processes were closely linked to cancer development (1). Cancer occurrence and progression are consequences of disruption of the mechanisms that regulate critical progress, such as cell proliferation, metabolism, apoptosis, and invasion, as well as other hallmark biological behaviors in cancer (1, 2). These disruptions are known as commonly

caused by early alterations at the chromatin and DNA levels. Chromatin is a multidimensional complex structure of genetic material that existed in the nucleus of interphase cells consisting of DNA, histones, non-histones, and a small amount of RNA. Genetic material evolves from DNA to densely packed chromosomes through four main stages, namely, the primary structure (nucleosomes), the secondary structure (solenoids), the tertiary structure (supersolenoid), and the quaternary chromatin (chromosome) (2). Chromatin remodeling and chromatin accessibility are important concepts of epigenetics. Chromatin remodeling is a molecular mechanism by which the packaging state of chromatin, the histones in nucleosomes, and the corresponding DNA molecules are altered during processes such as replication and recombination of gene expression (3). Chromatin accessibility is one of the categories of chromatin remodeling and refers to the extent to which eukaryotic DNA can bind to other regulatory factors after binding to components such as nucleosomes or transcription factors (TFs). These properties of chromatin reflect relatively early alterations in chromatin dynamics in the face of various endogenous mutations and environmental stresses and play an important role in physiological and pathological processes (4, 5).

Digestive system cancers rank first in tumor-associated death and rank second in the new case chart after reproductive system cancers (6). Since epigenetics was introduced in the 4th edition of the WHO classification of digestive system tumors in 2010, we have gained a deeper understanding of the etiology and pathogenesis of digestive system tumors (7). However, not all tumors and phenotypes have been studied at the level of chromatin dynamics, and available studies do not investigate chromatin regulation at the genome-wide level.

Here, we provide a brief overview of chromatin structure, chromatin remodeling, and chromatin accessibility, the landmark studies pertaining to their roles in digestive system tumors, and we also summarize relevant clinical trials and posit new directions for future research and therapeutic approaches.

CHROMATIN DISTURBANCES AND REGULATORY MODIFICATIONS IN DIGESTIVE SYSTEM CANCER

The concept "chromatin" was first coined by W. Flemming in 1880 (8). Chromatin is a moniliform complex composed of DNA, histones (H1, H2A, H2B, H3, and H4), non-histone proteins (enzymes that participate in DNA transcription and duplication), and a small amount of RNA forming in the nucleus during the interphase of the cell cycle (9). The structural monomers of chromatin, also termed the primary structure of chromatin, are nucleosomes. A nucleosome consists of an octamer of the four core histones encircled by 145~147 bp of DNA (10). Nucleosomes then coil, six per turn, and form the "solenoids" with an outer diameter of 30 nm, an inner diameter of 10 nm, and a pitch of 11 nm, which is called the secondary structure of chromatin (11). Subsequently, a cylindrical structure with a diameter of 0.4 μm will be formed by spiralization of the solenoids, which is named "supersolenoid," the tertiary structure of chromatin (12). Finally, the supersolenoids fold and form the quaternary chromatin, namely, chromosome. Topologically associating domains (TADs) emerge as a fundamental structural unit in the spatial organization of the genome that is thought to guide regulatory elements to cognate promoters. Disruption of TADs by chromatin rearrangements, such as chromatin remodeling, and histone modifications can result in gene misexpression and pathogenesis (13).

Chromatin remodeling and histone modifications may induce altered chromatin accessibility, and these three make major contributions to genome rearrangements. Chromatin accessibility was once termed as "a window into the genome," which refers to other factors' degree to physically rebind eukaryotic chromatinized DNA after histones and chromatin-binding factors bind to it (14). Dynamic change of chromatin accessibility constantly regulates DNA-based transactions including transcription, DNA replication, and repair. Factors such as nucleosome position and occupancy rate in the genome, chromatin remodeling complexes, histone modification, and DNA methylation are vital in determining and regulating the degree of chromatin accessibility. Histone modifiers, chromatin remodelers, and DNA modifiers dynamically regulate chromatin accessibility in different ways, such as ejecting nucleosomes and mutual charge repulsion. In this section, we summarized the effects of histone modifications, DNA modifications, and chromatin remodelers on chromatin accessibility (**Figure 1**) and their roles in the development of gastrointestinal tumors (**Figure 2**) (**Table 1**).

Histone Modification and Chromatin Accessibility in Digestive System Cancers

Histone tail modifications and the proteins that control them represent important components of chromatin regulation. Various types of chemical modification of histones such as histone acetylation, methylation, and ubiquitination give dynamic changes to nucleosome occupation and chromatin stages (39). Histone modifications exert their effect on the chromatin stage mainly through two mechanisms. First, the modifications directly influence the whole structure of chromatin, either short or long distances. Second, the modifications control the access of effector molecules. Since the discovery of highly transcriptional regions accompanied by hyperacetylated histones, over 150 different histone modification types have been identified, and their dysregulation can lead to inappropriate activation of oncogenes or, conversely, inactivation of tumor suppressors (40, 41).

Histone Acetylation

Histone acetylation, the first unveiled and most-studied histone modification type, was introduced in 1961, and the first histone acetyltransferase (HAT) and first histone deacetylase (HDAC) were discovered in 1996 (42). HATs (including P300/CBP, MYST family, and GNAT family) act on specific histone lysine residues in all four kinds of histones, thereby neutralizing the positive charge of lysine residues, weakening the charge-

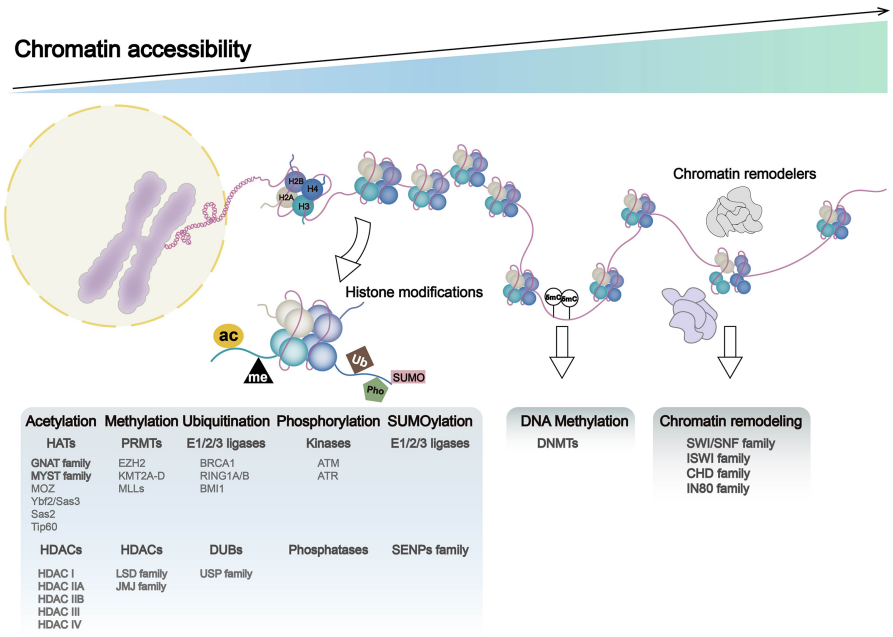

FIGURE 1 | Chromatin dynamics in genome: chromatin accessibility, histone modification, DNA methylation, and chromatin remodeling. DNA entangles histones (H2A, H2B, H3, and H4) to form nucleosomes, the basic functional unit of chromatin. Nucleosome occupancy in the genome, histone modifications, DNA methylation, and chromatin remodelers leads to alternations in chromatin accessibility, which regulates processes such as gene transcription and translation. Histone modifications include histone methylation, acetylation, ubiquitination, phosphorylation, and SUMOylation, with histone-modifying enzymes and associated gene expression abnormalities playing a major role in these processes. Chromatin remodeling complexes include SWI/SNF, ISWI, CHD, and IN80.

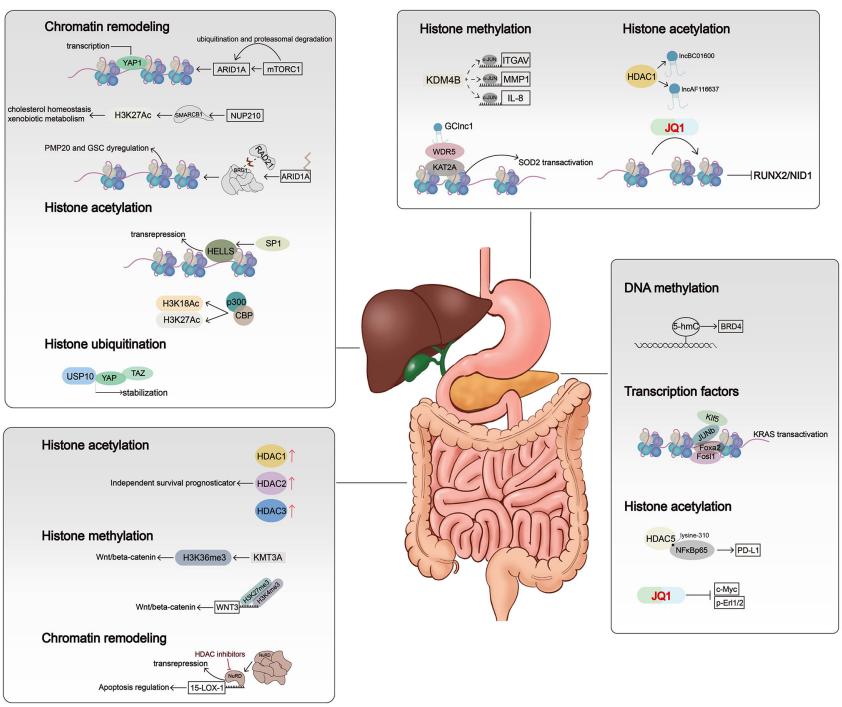

FIGURE 2 | Mechanisms of chromatin alterations regulating digestive system tumors. Histone modifications, chromatin remodelers, and DNA methylation affect critical signal pathways not only by altering gene expression levels but also by regulating chromatin accessibility.

TABLE 1 | Chromatin regulation and relative pathways in digestive system tumors.

Epigenomic modification	Cancer type	Element	Relative gene	Downstream effect	Reference
Histone acetylation	Hepatocellular carcinoma	P300/CBP	H3K27 H3K18	Chromatin accessibility	(15)
	Gastric cancer	HDAC1	lncBC01600 lncAF116637	/	(16)
	Gastric cancer	JQ1	RUNX2/NID1	Chromatin accessibility	(17)
	Pancreatic cancer	HDAC2	PDGFRa PDGFRb EGFR	TGF-β	(18)
	Pancreatic cancer	HDAC5	NF-κB p65	PD-L1	(19)
Histone methylation	Gastric cancer	GClnc1	SOD2 WDR5 KAT2A	Chromatin accessibility	(20)
	Hepatocellular carcinoma	NASP	P53 c-Myc	Chromatin accessibility	(21)
	Gastric cancer	KDM4B	IL-8 MMP1 ITGAV	/	(22)
	Colorectal cancer	KMT3A	WNT3	Wnt/β-catenin	(23, 24)
	Colorectal cancer	DPY30	ABHD5/YAP/c-Met	Chromatin accessibility	(25)
Histone ubiquitination	Cholangiocarcinoma	BAP1	/	Cell invasion and adhesion Cytoskeleton assembly-proteins	(26)
	Hepatocellular carcinoma	USP10	YAP/TAZ	/	(27)
SUMOylation	Pancreatic cancer	SAE2/UBA2, SAE1/UBE2I	MYC	MYC	(28)
DNA methylation	Colorectal cancer	DNMT	/	Wnt/β-catenin	(29)
5-Hydroxymethylcytosines	Pancreatic cancer	BRD4	/	/	(30)
SWI/SNF	Hepatocellular carcinoma	HELLS	CDH1	EMT	(31)
	Hepatocellular carcinoma	ARID1A	BRG1–RAD21	Chromatin accessibility	(32)
	Hepatocellular carcinoma	SMARCB1	NUP210	Chromatin accessibility	(33)
	Hepatocellular carcinoma	ARID1A	mTORC1	Chromatin accessibility	(34)
	Pancreatic cancer	ARID1A	ALDH1A1	KRAS	(35)
Regulation of transcription factors	Pancreatic cancer	KRAS mutation	Junb Fosl1 Klf5 Foxa2	Chromatin accessibility	(36)
	Pancreatic cancer	KRAS mutation	BRD4 IL33	Chromatin accessibility	(37)
	Intrahepatic cholangiocarcinoma	MFAP5	Notch1	Chromatin accessibility	(38)

dependent association between histones and DNA or adjacent histones, and thus facilitating various factors' contact to the loose region and making chromatin more accessible (43). Thus, histone acetylation is considered an active histone mark (44). The cyclic AMP response element-binding protein (CBP) often acts in conjugation with HATs P300 to form a CBP/P300 complex, which can further recruit other HATs like PCAF (P300/CBP-associated factor). Bi-allelic mutations of CBP and P300 have been observed in several cancers including colon cancer, breast cancer, and gastric cancer (45). The acetylation process can be reversed by HDACs, which tightly bind to negatively charged DNA and recover chromatin compaction. In humans, there are 18 HDACs belonging to four classes: the class I Rpd3-like proteins (HDAC1–3 and HDAC8), the class II Hda1-like proteins (HDAC4–7, HDAC9, and HDAC10), the class III Sir2-like proteins (SIRT1–7), and the class IV protein (HDAC11) (46). The class I, II, and IV HDACs are zinc-dependent, and the class III HDACs use NAD+ to generate nicotinamide and metabolite 20-*O*-acetyl-ADP-ribose during the process of deacetylation (47, 48). Dysregulation of class I and IV HDACs has been observed in gastric cancer, liver cancer, and colorectal cancer. For example, HDAC1 is involved in the promotion of gastric cancer cell proliferation, possibly by upregulating the expression of lncRNAs BC01600 and AF116637 in the tissues of patients with gastric cancer (16). In colorectal cancer, overexpression of HDAC1, HDAC2, and HDAC3 has been found, and HDAC2 expression was identified as an independent survival prognosticator (49). HDAC2 controlled the expression of pro-survival receptor tyrosine kinases connected to mesenchymal pancreatic ductal adenocarcinoma (PDAC), including PDGFRa, PDGFRb, and EGFR. The HDAC2-maintained program disrupted the tumor-suppressive arm of the TGF-β pathway, explaining impaired metastasis formation of HDAC2-deficient PDAC (18). HDAC5, a class IIa HDAC member, is downregulated in pancreatic cancer. HDAC5 regulates PD-L1 expression by directly interacting with NF-κB/p65 and reduces acetylation of p65 at lysine-310. Inhibition of HDAC5 sensitizes PDAC to immune checkpoint blockade (ICB) therapy (19). Several HDAC inhibitors (HDACis) have been approved by the US Food and Drug Administration (FDA) for cancer treatment or are currently being evaluated in clinical trials. Given that HDACi

monotherapy has been largely ineffective in solid tumors such as pancreatic cancer and liver cancer, the evaluation of combination regimens is currently ongoing (50).

Triggering changes in chromatin accessibility is another important mechanism by which histone acetylation affects (positively or negatively) tumor progression. Histone acetylation reduces the positive charge of histones and disrupts electrostatic interactions between histones and DNA. This leads to a more accessible chromatin structure, thereby facilitating DNA access by molecules such as TFs or protein elements. Evidence for this can be found in loci within a hyper-acetylated and transcriptionally competent chromatin environment that shows higher DNase sensitivity and therefore are generally accessible (51). Gastric cancer-associated lncRNA1 (GClnc1) upregulates superoxide dismutase 2 mitochondrial (*SOD2*) transcription by acting as a scaffold to recruit the WDR5 and KAT2A complexes to the *SOD2* promoter, increasing levels of H3K4 trimethylation and H3K9 acetylation in the *SOD2* promoter region and leading to increased chromatin accessibility (20). P300/CBP mediates increased acetylation of H3K18 and H3K27 leading to hepatocellular carcinoma progression, and a novel P300 inhibitor, B029-2, exerts an antitumor effect by reducing amino acid metabolism and nucleotide synthase gene (including *PSPH*, *PSAT1*, *ALDH18A1*, *TALDO1*, *ATIC*, and *DTYMK*) promoter regions of H3K18Ac and H3K27Ac levels, leading to decreased chromatin accessibility and antitumor effects (15). The bromodomain and extraterminal domain (BET) family contains proteins, such as BRD3 and BRD4, that alter chromatin accessibility by recognizing acetylated histone lysine residues and accumulate on hyperacetylated chromatin regions that act as active promoters or enhancers and recruit TFs and multiprotein complexes to facilitate transcription of target genes. The small-molecule BET inhibitor JQ1 masks the bromodomain acetyl-lysine binding pockets and is highly specific for BET family proteins, particularly bromodomain 4 (BRD4)-containing proteins. In gastric cancer, JQ1 downregulates chromatin accessibility and inhibits the RUNX2/NID1 signaling pathway, thereby preventing gastric cancer progression (17). JQ1 is also widely used in other gastrointestinal tumors. In pancreatic cancer, JQ1 inhibits pancreatic cancer cell proliferation by reducing c-Myc and p-Erl1/2 protein levels (52). It has also been shown that gemcitabine and JQ1 act synergistically in pancreatic cancer through the LXR/RXR activation pathway (53).

Histone Methylation

Histone methylation is a covalent modification that occurs at the lysine (K) residues of histone H3 and H4 by adding methyl groups, which is one of the most important post-transcriptional modifications. The methylation is catalyzed by the histone methyltransferase (HMT), which uses *S*-adenosyl-L-methionine (SAM) as the substrate to transfer methyl groups onto the lysine residues of histones. The amine group of lysine residues may bind one (mono-), two (di-), or three (tri-) methyl moieties (54, 55). The known methylation loci on histones were H3K4, H3K9, H3K27, H3K36, H3K79, and H4K20. Among these methylation loci, H3K4, H3K36, and H3K79 were found in highly active transcription regions, whereas H3K9 and H4K20 are hallmarks of silent transcription or heterochromatin (54). Histone arginine methylation is found to mostly happen on H3R2, H3R8, H3R17, H3R26, and H4R3 (56). Methyltransferases have quite a specificity in recognizing residues and modification states due to the sharing catalytic core, the SET domain. The protein arginine *N*-methyltransferase (PRMT) family leads the process of histone arginine methylation, which is considered less specific than the lysine methyltransferases (57). By increasing the affinity of protein structural domains for histone tails, the stability of nucleosomes is increased, and heterochromatin formation is promoted (55). Histone demethylase (KDM) includes the LSD family and JMJ family. LSD1 with FAD as cofactor forms complexes with Co.REST, BHC80, and HDAC1/2, and other proteins play a biological role (58). JMJ family has a JmjC domain, with Fe(ii) and Ot monoketoglutarate as cofactors, which can demethylate multiple sites such as H3K4, H3K9, and H3K36 (59). Regarding gastrointestinal carcinogenesis, histone lysine demethylase 4B (KDM4B) physically interacts with c-Jun at the promoter loci of *IL-8*, *MMP1*, and *ITGAV* through its demethylation activity, and infection with *Helicobacter pylori* results in a significant increase in the occupancy of KDM4B and c-Jun, leading to a significant attenuation of H3K9me3 signaling (22). In addition, another study identified three H3K27me modifier genes (*EZH2*, *KDM6A*, and *KDM6B*) that are individually associated with GC susceptibility through a synergistic triad of actions (60). As reported, all inter-single-nucleotide polymorphism (inter-SNP) interactions among these three genes together form a synergistic triad epistasis network of ring-type topology. The *EZH2–KDM6B* interaction is significant, but *EZH2–KDM6A* and *KDM6B–KDM6A* interactions are merely marginal. In colorectal carcinogenesis, mutations in Wnt/β-catenin signaling mediators may be among the earliest events that initiate and drive tumor progression. In the absence of *KMT3A*, the activity of the Wnt/β-catenin signaling pathway is enhanced due to a marked reduction in H3K36me3, which drives colorectal carcinogenesis (23). Furthermore, another study showed the decrease in H3K27me3 and the increase in H3K4me3 in the *WNT3* promoter region, suggesting that histone methylation directly activates the Wnt/β-catenin signaling pathway and promotes CRC initiation (24).

There is no precise conclusion as to how histone methylation affects chromatin accessibility. Some histone methylation patterns (H3K4 and H3K79 methylation) seem to be necessary for the binding of TFs. Several studies have shown that histone methylation can affect the higher-order chromatin structure by recruiting chromatin remodeling complexes. For instance, BPTF, the component of the chromatin remodeler NURF, contains a PHD finger that recognizes H3K4me3 (61). DPF3, the component of the BAF complex, contains a double PHD finger that interacts with methylated histones (62). Nuclear autoantigenic sperm proteins (NASPs) are molecular chaperones of histones, and deletion of NASP leads to cell

cycle accumulation at the S phase and failed replication (63). NASP deficiency induces histone pool disruption, mainly decreasing soluble H3, reducing H3K9me1 modification, and consequently causing chromatin to be more accessible, which helps prevent the development of hepatocellular carcinoma. With reduced cell proliferation due to NASP deletion, the expression levels of the pro-oncogenes *p53* and *c-Myc* were also decreased (21). In colorectal cancer, cancer stemness represents a major source of development and progression of CRC cells. The lipolytic factor *ABHD5* has been identified as an important tumor suppressor gene in CRC. Loss of *ABHD5* promotes c-Met activation to sustain CRC stemness in a non-canonical manner. Mechanistically, *ABHD5* interacts with the core subunit of the SET1A methyltransferase complex, DPY30. In the absence of *ABHD5*, DPY30 will translocate to the nucleus and support SET1A-mediated methylation of YAP and histone H3, which sequesters YAP in the nucleus and increases chromatin accessibility to promote *YAP*-induced transcription of c-Met (25).

Histone Phosphorylation

Histone phosphorylation, one of the most common post-translational modifications (PTMs), occurs at serine and tyrosine residues of histone proteins. Histone phosphorylation plays a similar role to histone acetylation in modulating nucleosome dynamics. Modified residues are imparted with a negative charge by phosphorylation, creating charge repulsion between histone and negatively charged DNA backbone, so that the association between DNA and histones can loosen and is less able to inhibit DNase I digestion (64, 65). In the human genome, histone H2A variant histone H2A.X is transformed into γH2A.X after phosphorylation at serine 139; this transformation is an essential part of the cellular response to DNA double-strand breaks (66). When phosphorylated by ATM or ATR kinases, γH2A.X recruits DNA repair-associated components to the double-strand break. It was also hypothesized that γH2A.X increases the level of chromatin accessibility to repair factors through charge repulsion (67). Histone phosphorylation can also alter the affinity of chromatin-binding proteins for their target molecules. For example, HP1 has a high affinity for H3K9me3, and when H3 serine 10 is phosphorylated, the binding of the HP1 chromosome group with H3K9me3 is inhibited (68–70).

Histone Ubiquitination

Histone ubiquitination includes monoubiquitination and poly-ubiquitination and results in a much larger covalent modification. The process of ubiquitination relies on three ubiquitin-activating enzymes. Ubiquitin-activating enzyme 1 (E1) first activates ubiquitin in an ATP-dependent manner and then binds to a cysteine residue of the ubiquitin-conjugating enzyme (E2) *via* a thioester bond. Finally, ubiquitin is transferred from the E2 enzyme to target lysine residues of specific substrate proteins by ubiquitin-protein isopeptide ligase (E3) (71). The process of ubiquitination can be reversed by deubiquitinating enzymes (DUBs). DUBs hydrolyze ester bonds, peptide bonds, or isopeptide bonds at the carboxyl terminus of ubiquitin, specifically separating ubiquitin from protein substrates and regulating the deubiquitination process. DUBs belong to the superfamily of proteases, including the ubiquitin deliberately modified enzyme family (USP). More than 90 DUBs have been identified, such as USP3, USP7, USP10, USP12, USP22, USP44, USP46, and USP49 (72). Immunohistochemical analyses revealed that aberrant histone ubiquitination patterns exist in many cancer types. Furthermore, DNA- and RNA-sequencing data show that genes encoding histone E3 ubiquitin ligases and DUBs are also frequently altered in cancers. RNF20 is the major H2B specific E3 ubiquitin ligase in mammalian cells. RNF20 represses gene expression by disrupting the interaction between TFIIS and the PAF1 elongation complex and inhibiting transcriptional elongation. Those effects are also dependent on the E3 ligase activity of RNF20 (73). In addition, RNF20-depleted cells show decreased expression of the *p53* and increased cell migration and tumorigenesis. USP22 is a ubiquitin hydrolase and catalyzes the removal of ubiquitin from monoubiquitinated histones H2A and H2B. In several studies, USP22 was found highly expressed in malignant tumor samples and associated with poor prognosis (74–76). Notably, USP22 has recently been found to function as a tumor suppressor in some tumors. For example, depletion of USP22 induced upregulation of secreted protein acidic and rich in cysteine (*SPARC*) by affecting H3K27ac and H2Bub1 occupancy on the *SPARC* gene in inflammation-associated colorectal cancer (77). In hepatocellular carcinoma, USP10 directly interacts with and stabilizes YAP/TAZ by reversing its proteolytic ubiquitination. This finding provides a rationale for potential therapeutic interventions in the treatment of patients with hepatocellular carcinoma harboring high levels of YAP/TAZ (27). BMI1 (also known as PCGF4), a member of PRC1 complex, can form homodimers and heterodimers with RING1 or PHC subunits9 that are critical for chromatin compaction. PTC596, a potent orally available BMI1 inhibitor, which can downregulate the anti-apoptosis factor MCL1, has progressed through phase I clinical trials for patients with advanced solid tumors (NCT02404480).

The effect of histone ubiquitination on chromatin accessibility is unclear, but some studies have shown that genes encoding ubiquitinases influence tumor progression by regulating chromatin accessibility. *BAP1* gene encodes a DUB and is identified as a tumor suppressor in many types of cancers including cholangiocarcinoma (78). *BAP1* targets multiple molecules and is involved in chromatin remodelers common with *PBRM1*, *IDH1*, *ARID1a*, and so on (78–80). With *BAP1* mutation, the ATAC-seq peaks were preferentially observed at TSS regions and the more accessible regions clustered in specific "hotspots" among the genome and a number of critical cell junction components; factors promoting cell invasion and adhesion and cytoskeleton assembly-proteins were noted to downregulate upon *BAP1* mutation in the global transcriptome. However, in *BAP1* mutation organoids, both decreases and increases in chromatin accessibility were observed in different genomic loci, which suggested that the function of *BAP1* might be divergent among various cell types (26). PRC1 complex contains a RING1 E3 ubiquitin ligase

(RING1A/B), which catalyzes the monoubiquitylation of histone H2A (that is, H2AK119Ub) and PcG RING finger proteins (PCGF1–6).

SUMOylation

SUMOylation is an important PTM that fine-tunes virtually all cell functions and pathological processes. SUMOylation occurs through a cascade of enzymes similar to ubiquitination, but SUMOylation utilizes only a single conjugating enzyme, UBC9, and a certain number of ligases compared to complex ubiquitination. Humans express five SUMO paralogs, SUMO-1, -2, -3, -4, and -5 (81). SUMO molecules regulate the structure and function of substrate proteins by covalently binding to lysine residues of those with the participation of the E1-activating enzyme, E2-binding enzyme, and E3 ligase (81). SUMO-specific proteases (SENPs) regulate the SUMOylation state of substrate proteins together with SUMO molecules, by specifically deSUMOylating modification of substrate target proteins (82). SUMOylation is widely involved in DNA damage response (DDR) and regulates DNA damage sensing and repair protein, which is mainly found in chromatin and nuclear bodies (83). SUMOylation can block the binding sites of substrate proteins and interaction domains and can affect the function of proteins by blocking protein-interaction domains. SUMOylation can also produce new docking sites to facilitate the interaction with other proteins. MYC protein activates SUMO-activating enzyme subunit1 (SAE1) transcription by binding to canonical E-Box sequences located close to the SAE1 transcription start site. In pancreatic cancer, members of the SUMO pathway including *SAE2/UBA2*, *SAE1*, or *UBE2I*, have been found to synthesize lethal MYC interaction (28). TRIM family proteins have both SUMO E3 ligase and ubiquitin E3 ligase activities and are involved in multiple cellular processes including carcinogenesis. Overexpression of TRIM29 enhances cell proliferation and transforming activity and promotes tumor growth by reducing the acetylation of *p53* (84). Nuclear factor-κB (*NF-κB*) is an important TF for carcinogenesis in chronic inflammatory diseases and plays a key role in promoting inflammation-associated carcinoma in the gastrointestinal tract (85). TRIM40 promotes the neddylation of inhibitor of NF-κB kinase subunit γ and consequently causes the inhibition of *NF-κB* activity (86).

DNA Methylation

DNA methylation and hydroxymethylation are important types of DNA modification in genome replication and transcription. DNA methylation plays a critical role in cell biology, including regulating gene expression, retro-element silencing, centromere stability and chromosome segregation in mitosis, X-chromosome inactivation, and monoallelic silencing of imprinted genes (87). In mammalian cells, DNA methylation is characterized by the addition of a methyl group at the carbon-5 position of cytosine base (5-methylcytosine (5-mC)) through the action of DNA methyltransferases (DNMTs) (87). 5-Hydroxymethylcytosine (5-hmC) is a further modified form of 5-mC, which is catalyzed by the Ten-Eleven Translocation (TET) protein family (88). DNA methylation mainly happens on "CpG islands" (clusters of CpG sites). CpG sites located within CpG islands are usually unmethylated in normal cells. They are activated in a transcriptionally permissive chromatin state that is characterized by combinations of post-translational histone modifications and special nucleosome organization (89). Unmethylated CpG sites within promoter CpG islands provide a binding platform for TFs to regulate gene expression (89), for example, specificity protein 1 (SP1), whose interactions with DNA are modulated by the presence or absence of DNA methylation at CpG islands (90). DNA methylation located in promoters is one of the most efficient patterns of gene transcription repression, which attributes to the function of remodeling chromatin. Until now, DNA methylation has been found to repress transcription in two ways. First, DNMTs can block the binding of transcriptional activators or coactivators with target sequences, thus directly inhibiting transcription initiation (91). Second, methyl-CpG-binding proteins (MeCPs) associated with chromatin remodelers can recognize DNA methylation sites and silence gene expression by recruiting co-repressors (92, 93). Distal regulatory regions such as tissue-specific enhancers are identified as CpG-poor and belong to lowly methylated regions (LMRs). It has been demonstrated that DNA methylation levels of enhancers are associated with gene activity at promoter–enhancer pairs, with a low level of 5-mC related to gene overexpression (94).

It is well known that global DNA methylation patterns are altered frequently in cancer development. Hypermethylation of CpG islands is common and mostly associated with the silencing of tumor suppressors, genes controlling cell growth, and downstream pathways. Numerous studies about locus-specific and genome-wide DNA methylation profiling have revealed multiple promoter-associated CpG islands that consistently undergo abnormal DNA hypermethylation in tumor cells (95). In addition, not only are single loci hypermethylated in cancer, but contiguous regions can become coordinately silenced and aberrantly hypermethylated. In colon cancer, CpG island Methylator Phenotypes (CIMPs) have been reported, enabling stratification of subtypes by a 5-mC signature (96). The expression of DNMT enzymes is also frequently disrupted in the tumor, which provides a feedback loop that drives alterations in DNA methylation patterns across the genome and has the potential to cause mutations in genomic sequence. Recently, DNMTs have been suggested as a potential epigenetic mechanism for maintaining cancer stem cells (CSCs). 5-Aza-2′-deoxycytidine (5-AzaDC), a novel DNMT inhibitor, was observed to significantly reduce the abundance of colorectal cancer CSCs and inhibit the growth of liver metastatic tumors by inhibiting the expression of active β-catenin and downregulating the Wnt signaling pathway (29).

During tumorigenesis, CpG-poor regions tend to undergo hypomethylation, resulting in the global decrease in DNA methylation characteristic of tumors. This phenomenon was first reported in colon adenocarcinoma and small cell lung cancer (97). DNA hypomethylation in cancer contributes to genomic instability and increased aneuploidy, both common

features of cancer genomes. It is widely accepted that the global loss of DNA methylation in cancer cells is accompanied by widespread genomic instability. However, a causal relationship remains to be clearly shown. Extensive global hypomethylation regions are associated with global changes in chromatin organization and structural changes.

Alongside the global alternation of 5-mC, regulation in 5-hmC has been also observed in many cancer types. High-throughput sequencing of 5-hmC in the genome of mouse embryonic stem cells showed that 5-hmC was mainly enriched in the exons of totipotency genes and near the transcription start point, and this site was often accompanied by lysine trimethylation modification at position 4 of histone H3 (H3K4me3). 5-hmC content was positively correlated with chromatin state, and the phenomenon of decreased 5-mC content but increased 5-hmC occurred at multiple gene active transcription sites (98–100). Researchers conducted a comprehensive genome-wide analysis of 5-hmC in pancreatic cancer and found that 5-hmC could be detected in both PDAC and control non-neoplastic pancreatic epithelial cells, though its level was lower than that of 5-mC (101). Moreover, they also observed that variability of 5-hmC was mostly increased and ubiquitous in PDAC cell lines compared to healthy cells. According to the data acquired from ATAC-seq, 5-hmC regions (DHMRs) showed high chromatin accessibility, as expected. *BRD4* was found to acquire 5-hmC modification at regions overlapped with H3K4me1 peaks. Overexpression of *BRD4* is found to be tightly related to 5-hmC modification at the enhancer of the *BRD4* sequence. Bromodomain inhibitors including JQ1 can competitively bind to the acetyl-lysine recognition sites of BET family bromodomain, thereby displacing *BRD4* from nuclear chromatin and inhibiting cancer initiation. These kinds of molecular targeting inhibitors are already tested in early-phase clinical trials and are expected to become effective targeting drugs for cancer (30).

Chromatin Remodelers

To achieve dynamic access to packaged DNA, cells have evolved a series of tailored regulation factors, named chromatin remodeling complex. The contribution of chromatin remodelers in regulating replication and transcription is obvious: i) specific remodelers can space nucleosomes correctly after replication to guarantee rational nucleosome position and properly arrange the whole genome. ii) Critical cis DNA elements are hidden among the densely packed nucleosomes, which lose the opportunity to interact with DNA-binding factors. Remodelers are able to slip the nucleosomes away and transiently expose the elements on the binding side. iii) The activities of DNA polymerases and RNA polymerases can be barriers to nucleosomes. Remodelers may help eject the nucleosomes or chaperone the histone octamers around the running polymerases (102). Because of this, chromatin remodeling complexes can be considered as important as other epigenetic mechanisms for oncogenesis. There are four different chromatin remodeler families that share a similar ATPase domain that has been identified: SWI/SNF family, ISWI family, CHD family, and INO80 family. The common properties of the four families are also described, including an affinity for the nucleosome, reorganization for covalent histone modifications, similar DNA-dependent ATPase domain, ATPase regulation domain, and chromatin or TF interaction domain. Apart from the common grounds, these four complexes are also special for their unique domains residing in catalytic ATPase and particular binding sites (103).

SWI/SNF Remodeler

SWI/SNF (switching defective/sucrose non-fermenting) family remodelers consist of 8 to 14 subunits. These family remodelers generally altered more than 20% of human malignancies (104). BAF and PBAF complex, whose specific ATPases were hBRM and BRG1, of the SWI/SNF family, are mainly included in human genome activity, and both contain a bromodomain (105). SWI/SNF plays a key role in chromatin remodeling and accessibility at promoters and enhancers by sliding and ejecting nucleosomes at multiple loci (106). Alternations in subunits of SWI/SNF complex and related genes play an important role in the development of digestive system tumors. For example, SNF2 is the most-studied example and interacts with various proteins including products of proto-oncogenes such as *p53*, *Rb*, and beta-catenin. HELicase, lymphoid-Specific (HELLS), also known as LSH, SMARCA6, or PASG, is a chromatin remodeling enzyme of the SNF2 family (107). Abnormal activity of TF SP1 in hepatocellular carcinoma leads to high expression of HELLS (31). At the epigenetic level, high HELLS expression increases nucleosome occupancy, decreases chromatin accessibility to enhancer regions, and inhibits the formation of nucleosome-free regions (NFRs) at TSSs. HELLS binds to the NFR of *CDH1*, which encodes E-cadherin and silences *CDH1* at the epigenetic level in hepatocellular carcinoma, thus contributing to EMT and cancer metastasis (31). SMARCB1, a subunit of the SWI/SNF complex, is significantly upregulated in hepatocellular carcinoma. SMARCB1 contributes to the stability of the BAF complex and its chromatin affinity. The putative tumor supporter, Nucleoporin210 (NUP210), is a critical coregulator of SMARCB1 chromatin remodeling activity, binds its enhancer, and alters H3K27Ac enrichment and downstream pathways, especially cholesterol homeostasis and xenobiotic metabolism (33).

The effect of the SWI/SNF family on chromatin accessibility has been most studied, including BRG1 (also known as SMARCA4), SNF5, BAF57, and BAF155 (108). *ARID1A* encodes a subunit of SWI/SNF, and its deletion in hepatocellular carcinoma induces conversion of the A/B region, remodeling of TADs, and a reduction in chromatin loops. RAD21 is a structural subunit of the chromatin structural element cohesin, and the ATPase BRG1 of the SWI/SNF complex can physically interact with RAD21. Lack of *ARID1A* markedly reduces BRG1–RAD21 coupling, leading to increased chromatin accessibility and promoting hepatocellular carcinoma metastasis (32). mTORC1 interacts with ARID1A protein in HCC and regulates ubiquitination and proteasomal degradation of ARID1A protein. The mTORC1–ARID1A axis promotes oncogenic chromatin remodeling, accessibility, and YAP-dependent transcription, thereby enhancing hepatocellular

carcinoma cell growth *in vitro* and tumor development *in vivo* (34). Remarkably, *ARID1A* shows a high expression level in primary tumors but shows a decreasing trend in metastatic lesions, indicating that *ARID1A* may be an initiating factor in HCC and be lost in the later lesions (109). In pancreatic cancer, *ARID1A* deletion promotes pancreatic tumorigenesis by increasing chromatin accessibility to the enhancer region of aldehyde dehydrogenase 1 family member A1 (*ALDH1A1*), upregulating *ALDH1A1* expression, and attenuating *KRAS*-induced senescence (35).

ISWI Remodeler

ISWI (imitation switch) family remodelers include 2 to 4 subunits and are conserved from budding yeast to humans (110). This family is special for its attendant proteins and a characteristic set of domains located at the C-terminal of ISWI family ATPases. Until now, two primary ATPases, SNF2L (SNF2-"like") and SNF2H (SNF2-"homolog") complexes, were identified to be composed of three ISWI family complexes in mammalian cells, namely, NURF, CHRAC, and ACF complexes (111). Instead of leading to the disruption of nucleosomes, the ISWI family remodelers rebuild the gap between nucleosomes, thereby promoting chromatin assembly and lower chromatin accessibility and inhibiting transcriptional process (112, 113). SMARCA5, an ATPase of the ISWI class of chromatin remodelers, is dysfunctional in leukemia and breast, lung, and gastric cancers. Following conditional haplo- or duplex SMARCA5 deletion, cells undergo accelerated growth arrest, enter senescence, and show a progressive increase in susceptibility to genotoxic damage. These phenotypic features were interpreted as a specific remodeling of the chromatin structure and transcriptome of primary cells prior to the onset of immortalization (114).

CHD Remodeler

CHD (chromodomain, helicase, DNA binding) family includes two chromodomains tandemly arranged at the N-terminal of catalytic subunits in addition to ATPase (115). CHD family has been unveiled as a "double-edged sword" in transcription, some of which eject or slide nucleosomes away to promote transcription, while others show suppressive effects. This property of the CHD family may partly rely on chromodomain diversity (116). The suppressive role of the CHD family is partly contributed by the Mi-2/NuRD (nucleosome remodeling and deacetylase) complex, a member of the CHD family in high eukaryotes, and forms large protein complexes including HDAC subunits (117). 15-Lipoxygenase-1 (15-LOX-1) is transcriptionally silenced in colon cancer cells, and its reactivation restores apoptosis to cancer cells. NuRD contributes to 15-LOX-1 transcription suppression *via* recruitment to the promoter, while HDACis can dissociate NuRD from the promoter to activate 15-LOX-1 transcription (118).

INO80 Remodeler

INO80 (inositol requiring 80) family contains more than 10 subunits and was originally discovered as a protein necessary for transcriptional activation of the gene *ino1* (119). "Split" ATPase domain distinguishes the INO80 family from other chromatin remodeler complexes, with a long insertion present in the ATPase domain and binds with helicase-related Rvb1/2 proteins or another ARP protein. Thus, INO80 has unique significance in representing a new class of ATPases (120). INO80 family complex remodels nucleosome structure by exchanging classical and variant histones (121). However, the specific mechanisms of how INO80 affects epigenetic inheritance still need to be further explored.

Transcription Factors

The fragments of accessible chromatin among the whole genome can be engaged with multiple binding factors, and the network between chromatin and TFs cooperatively controls the gene expression, playing an essential role in cancer development (4). With pancreatic cancer, normal pancreatic follicular cells are converted to duct-like cells in a process known as acinar-to-ductal metaplasia (ADM) (122–124). Meanwhile, a large number of pancreatic cancer precursor cells named pancreatic intraepithelial neoplasia (PanIN) are gradually generated in the pancreas of mice carrying *KRAS* mutation (125). Klf5 TF is highly expressed in human pancreatic cancer and is also expressed in normal pancreatic ductal cells and alveolar-to-ductal metaplasia (ADM). The KLF5-expressing ADM cells, called PDLP cells, have been shown to be a population of pancreatic cancer precursor cells that highly express a pro-oncogenic transcriptional regulatory network and have a strong differentiation capacity. Compared with normal pancreatic ductal cells, there are a large number of highly activated genes in PDLP, and the chromatin near these genes also becomes more accessible. The chromatin-accessible regions in PDLP cells are similar to those in PDAC cells. AP1, Ets, Fox, and Klf TF families are enriched in chromatin-accessible regions of PDLP, and the degree of chromatin accessibility is greatly downregulated after knockdown of Junb, Fosl1, and Klf5 (36). Pancreatitis associated with pancreatic tissue injury combined with *KRAS* mutation can also significantly accelerate the occurrence of early pancreatic cancer. Chromatin change associated with cancer initiation occurs within 48 h of pancreatic injury, indicating that chromatin remodeling changes occur at the initiation of pancreatic cancer (37). The cytokine interleukin-33 (IL-33) is rapidly activated in pancreatic tissue after injury (126). The presence of many IL-33-associated loci in the loose chromatin regions described above correlates with elevated *BRD4*-dependent IL-33 expression. Early in carcinogenesis, IL-33 links tissue damage with *KRAS* gene mutation-dependent epithelial plasticity to carcinogenesis (37). Microfibrillar-associated protein 5 (MFAP5) is an extracellular matrix (ECM) glycoprotein and a component of ECM microfibrils (38). Intrahepatic cholangiocarcinoma (ICC) patients with a higher level of MFAP5 are more likely with malignant progression and low survival rates. High expression of MFAP5 results in a more accessible chromatin landscape in specific regions, thereby promoting transcription of genes related to Notch1 pathways, subsequently accelerating the transition from G0/G1 phase to the S phase, and finally facilitating the aggressiveness of ICC.

Mutations of Epigenetic-Related Genes in Digestive System Cancers

As epigenetic regulators, histone modification, DNA methylation, and chromatin remodelers are an important layer of transcriptional regulation with the particularity to affect gene expression. Over the years, due to a large number of recurrent mutations, hundreds of novel driver genes have been characterized in cancers. However, it seems not well-documented to consider cancer only as the end product of accumulated somatic mutations. There exist few cancers with a limited number of somatic mutations such as thyroid cancer and marker cell carcinoma. Despite epigenetic-related genes being far less in numbers than the genes directly linked to cancer, the global impact on the genome cannot be ignored. Herein, we summarized several critical gene mutations associated with DNA methylation, histone modification, and chromatin remodeler SWI/SNF complexes in digestive system cancers (**Figure 3**).

DNMT enzymes, mainly DNMT1, DNMT3A, and DNMT3B, catalyze/regulate DNA methylation. DNMT1 maintains the methylation status of newly replicated DNA strands, while DNMT3A and DNMT3B are responsible for *de novo* DNA methylation. A meta-analysis in gastric cancer suggested that rs16999593 in *DNMT1* and rs1550117 in *DNMT3A* could contribute to GC risk and that rs1569686 in *DNMT3B* might be a protective factor (127). DNA methylation is not limited to the effect of DNMTs. The TET family (including TET1, TET2, and TET3) catalyzes the transformation from 5-mC to 5-hmC. Missense and truncating mutations in *TET* genes have been observed in almost all tumor types with relatively low frequency (0.1%–10% of cases). In colorectal cancer, up to 20% of patients were found to carry mutations in one or more of the *TET* genes (http://www.cBioPortal.org). However, it seems like mutation types of *TET* genes in solid tumors are often missense mutations with no significance.

As a complicated and far-reaching epigenetic entity, the impacts of mutations in histone-modifying enzyme-associated genes on tumors remain in the research focus. There are numerous reports showing the involvement of mutations in genes encoding HATs (*EP300*, *P300*, *CBP*, *MOZ*, etc.) in many cancers. The EP300 protein is a HAT that regulates transcription and chromatin dynamics. Six mutations of *EP300* gene were analyzed in 193 epithelial cancers (128). Of the six mutations, two were in primary tumors (a colorectal cancer and a breast cancer) and four were found in cancer cell lines (colorectal, breast, and pancreatic). In addition, missense alterations were found in primary colorectal cancer and two cancer cell lines (breast and pancreatic). These data show that *EP300* is mutated in epithelial cancers and behaves as a tumor-suppressor gene. *UTX* (also known as *KDM6A*) as a highly mutated gene encoding histone H3K27 demethylase has been reported in several cancer cell lines including colorectal adenocarcinoma and pancreatic adenocarcinoma (129).

Several members/subunits from chromatin remodeling families, such as *hSNF5/INI1*, *ARID1A*, and *MTA1*, are known to be mutated in human cancers. In the cancer spectrum, SWI/SNF complex has gained particular attention, as they are mutated in nearly 20% of human cancers (130). The frequency of *ARID1A* mutation is 17% in gastric cancer patients and 12%–13% in colorectal cancer patients. The mutations of *AIRD1A* are significantly associated with microsatellite instability (MSI) and Epstein–Barr virus (EBV) infection and also poorly differentiated grade and advanced tumor depth (131).

DEVELOPING TECHNOLOGIES FOR MEASURING CHROMATIN DYNAMICS

As mentioned earlier, eukaryotic genomes are hierarchically packaged into chromatin, and various forms of packaging play different roles in gene expression and regulation. The shortcut to comprehending the epigenetic information encoded in the chromatin mainly comes from high-throughput, genome-wide methods, which focus on chromatin accessibility, nucleosome position, and TF occupancy. In this section, we summarized four existing assays for measuring the chromatin stage and their principles (**Table 2**).

Chromatin immunoprecipitation (ChIP) is the first technique to be applied to large-scale epigenetic mapping, followed closely by ChIP-chip to enable genome-wide detection of DNA–protein interactions (132). ChIP-chip is based on microarray hybridization. However, this method is not widely used due to its low resolution, ambiguous surface introduced by probe design, and signal bias. With higher resolution, less noise, and greater coverage, ChIP sequencing (ChIP-seq) is gradually becoming one

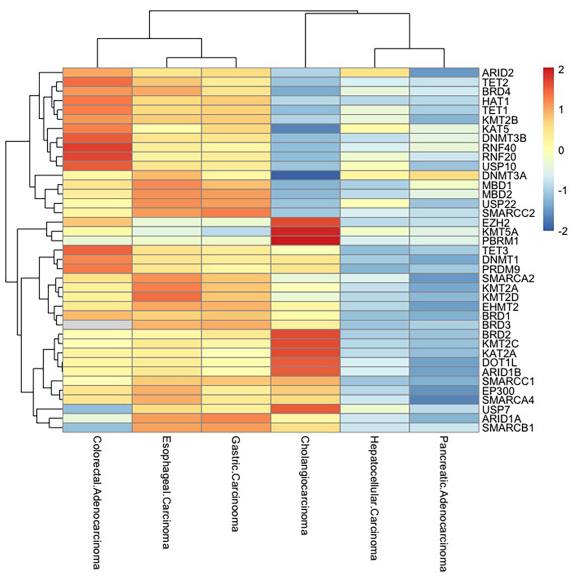

FIGURE 3 | Epigenetic-related gene mutations in digestive system cancers. Frequency of mutations in epigenetically critical genes in digestive system tumors (esophageal carcinoma, gastric carcinoma, hepatocellular carcinoma, pancreatic adenocarcinoma, colorectal adenocarcinoma, and cholangiocarcinoma) is shown as a heatmap. The mutation rates of these genes are high in esophageal carcinoma, gastric carcinoma, and colorectal adenocarcinoma, while pancreatic adenocarcinoma and hepatocellular carcinoma have low mutation rates.

TABLE 2 | High-throughput methods for chromatin detection.

Methods	Core elements	Target region	Critical experimental steps	Reference
ChIP-seq	Immunoprecipitation	Whole genome Specified region	10^5~10^7 cells Crosslinking Sonication Immunoprecipitation DNA purification Library and sequencing	(132–134)
MNase-seq	Micrococcal nuclease	Nucleosome occupancy	Crosslink with formaldehyde MNase extracts nucleosomes High-throughput sequence	(135–142)
DNase-seq	Endonuclease DNase1	Chromatin opening region Nucleosome occupancy TF occupancy	Crawford Stamatoyannopoulos	(143–150)
FAIRE-seq	Formaldehyde	Chromatin opening region	Crosslink with formaldehyde Shearing chromatins with sonication phenol Chloroform extraction DNA detection PCR NGS	(14, 151–155)
ATAC-seq	Tn5 transposase	Chromatin opening region Nucleosome occupancy DNA binding protein	500–50,000 cells Tn5 as adaptors High-throughput DNA sequencing PCR NGS	(156–159)

ChIP-seq, chromatin immunoprecipitation sequencing; MNase-seq, micrococcal nuclease sequencing; DNase-seq, deoxyribonuclease sequencing; FAIRE-seq, Formaldehyde-Assisted Isolation of Regulatory Elements sequencing; ATAC-seq, assay for transposase-accessible chromatin using sequencing; NGS, next-generation sequencing.

of the indispensable tools for epigenetics as second-generation sequencing becomes popular (133). Based on ChIP-seq, the development of single-cell ChIP-seq (scDrop-ChIP, sc-itChIP-seq, etc.) has helped to study the genetic diversity of heterogeneous cell populations and understand the evolution of tumor populations, allowing the clustering of cell populations based on the diversity of chromatin landscapes and the identification of chromatin features specific to each cell population. The disadvantage of single-cell ChIP-seq is that thousands of cells are required to obtain good clustering results (134). ChIP-seq and scChIP-seq are now widely applied in research related to tumors. The molecular dependencies of pancreatic cancer were mapped through ChIP-seq, RNA-seq, and genome-wide CRISPR analysis and revealed an unexpected utilization of immunoregulatory signals by pancreatic cancer epithelial cells (160). In a 2021 study, ChIP-seq was used to profile active enhancers at the genome-wide level in colorectal cancer patient tissues. As a result, 5,590 gain and 1,100 lost variant enhancer loci, and 334 gain and 121 lost variant super enhancer loci were identified (161). RNA-seq, MBD-seq, and H3K27ac ChIP-seq on gastric tissues and cell lines were performed, and 257,651 significant differentially methylated regions were identified in gastric cancer, which provide insight for understanding methylation changes at distal regulatory regions and reveal novel epigenetic targets in gastric cancer (162).

As our understanding of the structure and dynamics of chromatin has improved, techniques for detecting chromatin accessibility have also made great strides. MNase (micrococcal nuclease), an endo- and exo-nuclease, could preferentially digest naked DNA between nucleosomes, releasing nucleosomes from chromatin and retaining the DNA fragments that are protected by nucleosomes (135). Early in 1970, MNase digestion has been applied to detect chromatin structure in low-throughput sequence and later applied in tiled microarrays (136–138). Nowadays, MNase mainly is used together with next-generation sequencing (NGS) to qualitatively and quantitatively assess nucleosome messages in the whole genome (139). MNase-seq combined with ChIP-seq can probe regulatory factors or histone-tail modification relative to nucleosomes (140). At the single-cell level, scMNase-seq reproducibly detects an average of ~3, 0.9, and 700,000 unique fragments per cell type. The location of genome-wide nucleosomes in single cells is precisely defined, and subnucleosome-sized DNA fragments provide information on chromatin accessibility (141). However, MNase-seq has a lethal weakness, namely, sequence bias. It is easier and faster for MNase to cleave upstream of A or T, nearly about 30 times faster than it does on 5′ of G or C. Due to this bias in digesting level, careful and repeated enzymatic titrations must be supplied to improve the accuracy and credibility of MNase-seq (142).

Highly active regions of genomes commonly have an altering chromatin structure, thereby generating DNase hypersensitive sites (DHSs), which are chromatin accessible and can be cut by DNase1 (143). In the earlier DNase digestion assay, identification of DHSs relies on Southern blotting, and the detection regions on the genome were limited to a narrow range (144). Further improvement attempts to combine low-throughput sequence, real-time PCR, and hybridization to tiled microarrays. However, the efficiency and accuracy still remain unsatisfactory (145–147). DNase-seq ultimately became popular until the advent of NGS, which allows identifying DHSs among the whole genome specifically and sensitively (148). DNase-seq not only is able to

unveil chromatin accessibility among distinctive cell lines but also has the ability to show the single nucleosome position (149, 150). Additionally, DNase-seq footprints can reveal that TFs occupy chromatin qualitatively and quantitatively (163). Single-cell DNase sequencing (scDNase-seq) detects genome-wide DHSs starting from <1,000 cells of single or primary cell origin, and about 50% of bulky DHS promoter sites can be detected (164). However, several studies have demonstrated that DNase1 introduced cleavage bias. Furthermore, TFs bind to DNA transiently in living cells and are not shown in DNase-seq footprints (165).

FAIRE was first reported by Nagy and Lieb in 2003 (151) and then formally named in 2007 (166). In FAIRE-seq, chromatins are crosslinked with formaldehyde first in order to catch *in vivo* protein–DNA binding and then shearing chromatins with sonication, followed by phenol–chloroform extraction and detection of DNA within the aqueous phase. The regions where nucleosomes are depleted will be released into the aqueous phase of the solution, and subsequently, the chromatin-accessible subgroups of fragments can be detected by real-time PCR, tiling DNA microarrays or paired-end/single-end NGS (151, 152). The advantage of FAIRE-seq is that it directly enriches areas of active chromatin while nucleosome-depleted regions are not degraded (153, 154). Furthermore, the sequence-specific bias in MNase and DNase is overcome in FAIRE-seq (155), although the limitations of FAIRE-seq cannot be ignored, including its lower signal-to-noise rate compared with other assays and difficulty in data computation due to this high background (14).

ATAC-seq was first thoroughly described as "fast and sensitive epigenomic profiling of opening chromatin" by Jason D. Buenrestro et al. in 2013. In ATAC-seq, information such as nucleosome package and position, and DNA binding sites can be read (156). Usage of Tn5 transposase is considered the core driver in creating this technique (157, 158). In ATAC-seq, the accessible regions of chromatin are more likely for Tn5 transposase to integrate its adaptor into and generate highly intensive peaks due to steric hindrance. In contrast, the regions of lower chromatin accessibility seem to set a barrier to such transposition (156). In 2015, single-cell ATAC-seq (scATAC-seq) was developed to detect transposase-accessible chromatin by using sequencing integrated into programmable microfluidic platforms (ATAC-seq), dissecting single-cell epigenomic heterogeneity, and linking cis and trans effectors to variability in the accessibility profile of individual epigenomes (159).

Currently, ATAC-seq is the most commonly used method to detect chromatin accessibility. For instance, ATAC-seq was used to investigate epigenetic elements responsible for the differential response to anti-PD-1 therapy by quantitatively assessing the genome-wide chromatin accessibility of circulating CD8+ T cells in patients' peripheral blood. In this study, unique accessible regions of chromatin were identified to distinguish anti-PD-1 therapy responders from non-responders (167). Notably, ATAC-seq has been shown to have the potential to predict tumor prognosis. By ATAC-seq analyses of EpCAM+ PDAC epithelial cells sorted from 54 freshly resected human tumors, researchers found 1,092 chromatin loci displaying differential accessibility between patients with disease-free survival (DFS) < 1 year and patients with DFS > 1 year (168).

THERAPY TARGETING

Over the past several decades, research on chromatin dynamics and its relationship with disease, particularly cancer, has provided us with strong evidence of its potential for cancer therapy. Dynamic change in genomic architecture caused by intricate cross-linking of elements of chromatin almost controls the function of every cell. As described before, the chromatin stage can be regulated on several levels such as DNA sequence and histone modification. The regulating patterns include chromatin remodeling complexes, methylation, and acetylation. Undergoing various types of modification on different levels, the accessibility of chromatin to regulatory elements such as TFs and modifying enzymes will be altered. Subsequently, the global genome landscape also is changed and affects the expression of the downstream gene. A series of actions cause positive or negative influences on the process of the cell cycle. Research concentrating on chromatin targeting therapy is ongoing and has gained rapid development in several hotspots such as HDACs, PRC2, and *EZH2* (169).

Drugs targeting chromatin remodeling complexes and histone modifications are actively being tested in clinical trials and approved by the US FDA (**Table 3**) (170, 171), such as histone deacetylation inhibitors, histone demethylation inhibitors, and drugs targeting the SWI/SNF chromatin remodeling complex. Regimens of the above drugs alone or combined with conventional chemotherapeutic agents have been addressed in several clinical trials.

HDACis have been indicated as potent inducers of differentiation, growth arrest, and apoptosis induction. Vorinostat is a broad-based inhibitor of HDAC activity, inhibiting class I HDACs (HDAC1, HDAC2, HDAC3, HDAC8) and class II HDACs (HDAC6 and HDAC10, and HDAC11). Several clinical trials have been conducted to validate the use of vorinostat in combination with other chemotherapeutic agents (e.g., capecitabine and 5-FU) in colorectal cancer, pancreatic cancer, and other gastrointestinal tumors. Patients with gastrointestinal tumors (NCT00455351) showed better tolerability and stability when treated with vorinostat alone with a reduced dose (vorinostat 300 mg bid for 3 consecutive days followed by 4 days of rest) or combined with radiotherapy (172, 173). Pancreatic cancer patients showed good tolerance (NCT00983268) to the combination of vorinostat and capecitabine with radiation (174). Combinations of vorinostat with capecitabine, cisplatin, 5-FU, leucovorin, sorafenib tosylate, and other drugs have also been actively tried in several clinical trials on gastric cancer, colorectal cancer, and liver cancer. Resminostat is a new oral pan-HDACi that specifically targets HDAC1, HDAC2, and HDAC3. The effectiveness of resminostat in combination with several drugs such as sorafenib, cisplatin, and doxorubicin has been demonstrated. Resminostat combined with S-1 or FOLFIRI

TABLE 3 | Clinical trials targeting epigenetic modifiers in digestive system cancers.

Target	Drug	Tumor type	Strategy	Phase	Status	NCT number
Histone methylation	Guadecitabine (inhibitor of DNA methyltransferase)	Hepatocellular Carcinoma	Guadecitabine	I/II	Completed	NCT01752933
		Colorectal cancer	Guadecitabine	I/II	Completed	NCT01896856
	Tazemetostat (Target EZH2)	Solid/advanced solid tumor	Tazemetostat	II	Recruiting	NCT05023655
			Itraconazole Rifampin Tazemetostat	I	Active, not recruiting	NCT04537715
			Tazemetostat	I	Recruiting	NCT04241835
			Tazemetostat	II	Active, not recruiting	NCT03213665
			Tazemetostat Durvalumab	II	Recruiting	NCT04705818
Histone acetylation	Vorinostat (inhibitor of HDAC1, HDAC2, HDAC3, HDAC6)	Pancreatic cancer	Vorinostat+capecitabine +radiotherapy	I	Completed	NCT00983268
		Gastrointestinal tumors	Vorinostat+pelvic radiation	I	Completed	NCT00455351
			Vorinostat+5-FU+irinotecan hydrochloride +leucovorin calcium	I	Completed	NCT00537121
		Gastric cancer	Vorinostat+capecitabine+cisplatin	I/II	Completed	NCT01045538
		Hepatocellular Carcinoma	Vorinostat+sorafenib tosylate	I	Completed	NCT01075113
	Domatinostat (inhibitor of class I HDACs)	Gastrointestinal tumors	Domatinostat	II	Unknown	NCT03812796
	Resminostat (Inhibitor of class I HDACs)	Cholangiocarcinoma	Reminostat+FOLFIRI	I/II	Completed	NCT01277406
		Gastrointestinal tumors	Reminostat+sorafenib	I/II	Completed	NCT00943449 NCT02400788
			Reminostat	II	Completed	NCT00098527
		Pancreatic cancer	Romidepin+azacitidine+nab-paclitaxel +gemcitabine Reminostat+nab-paclitaxel+gemcitabine	I/II	Recruiting	NCT04257448
			Reminostat+S-1	I	Completed	JapicCTI152,864
		Gastric cancer	Reminostat	II	Completed	NCT00077337
			Reminostat+FOLFIRI	I/II	Completed	NCT01277406
	Depsipeptide (inhibitor of class I HDACs)	Pancreatic cancer	Depsipeptide+gemcitabine	I/II	Completed	NCT00379639
Chromatin remodelers	Palbociclib (CDK4/6 inhibitor)	Pancreatic cancer	Palbociclib+ulixertinib	I	Active, not recruiting	NCT03454035
			Palbociclib	II	Completed	NCT02806648
			Palbociclib+ binimetinib	Early I	Recruiting	NCT04870034
		Gastrointestinal tumors	Palbociclib	II	Completed	NCT01907607
		Hepatocellular carcinoma	Palbociclib	II	Active, not recruiting	NCT01356628
		Colorectal cancer	Palbociclib+cetuximab	I	Active, not recruiting	NCT03454035
			Palbociclib+binimetinib	II	Active, not recruiting	NCT03981614
			Palbociclib+binimetinib	Early I	Recruiting	NCT04870034
			Palbociclib+ Cetuximab +Encorafenib+ERAS-007	I/II	Recruiting	NCT05039177
	Rucaparib (PARP inhibitor)	Pancreatic cancer	Rucaparib	II	Active, not recruiting	NCT03140670
	Dasatinib (targeting S100)	Pancreatic cancer	Dasatinib+placebo	II	Completed	NCT01395017
			Dasatinib+mFOLFOX6	II	Completed	NCT01652976
		Gastrointestinal tumors	Dasatinib	II	Completed	NCT00568750
	Bortezomib	Hepatocellular Carcinoma	Bortezomib+doxorubicin	II	Completed	NCT00083226
	Olaparib (PARP inhibitor)	Pancreatic cancer	Bortezomib+doxorubicin	II	Completed	NCT00083226

chemotherapy regimens has also been applied in patients with pancreatic cancer and colorectal carcinoma and demonstrated promising efficacy. However, according to the clinical trials mentioned above, the side effects of these drugs are not negligible, including diarrhea, anorexia, fatigue, and rash. Better regimens and dose assessments are yet to be proven.

Clinical trials targeting histone methylation modifiers have focused on the effects on hematological malignancies such as

stomatous lymphoma and non-Hodgkin's lymphoma. As for digestive system cancer, guadecitabine alone has been examined closely in phase I/II clinical trials of colorectal cancer and hepatocellular carcinoma (NCT01896856, NCT01752933). Guadecitabine was administered at two doses in patients with advanced hepatocellular carcinoma who had failed sorafenib treatment (NCT01752933). The median survival of included patients was 294 and 245 days, and the most serious adverse reaction was hematopoietic system dysfunction. Future research should pay more attention to these aspects in order to identify new treatment options for cancers of the digestive tract.

SWI/SNF has the broadest function of the four chromatin remodeling complexes, and drugs targeting this complex have been involved in several clinical trials, such as palbociclib, olaparib, rucaparib, bortezomib, and abemaciclib. Pancreatic neuroendocrine cancer patients with palbociclib alone had an overall survival of 33 months (NCT02806648). In patients with PDAC treated with abemaciclib+LY3023414+gemcitabine+capecitabine in different combinations, the overall survival was only about 6–10 months (NCT02981342).Conclusion and perspective

The regulation of chromatin dynamics by transcriptional elements and related complexes affects various pathways of digestive system tumor development, metastasis, and drug resistance and provides complex and precise control of various biological behaviors including cell cycle, metabolic program, and tumor microenvironment. The individual heterogeneity of tumors poses a very serious challenge for clinical treatment, and chromatin, which integrates genetic and epigenetic information, is a promising avenue to realize personalized treatment.

In the past decade or so, tremendous progress has been made in the field of chromatin regulation and cancer mechanisms, owing to in-depth investigations of chromatin regulatory factors, how these regulatory elements act on tumors, and attempts of targeting drugs in clinical therapy. In parallel, the invention of sequencing technologies such as ATAC-seq has further advanced our understanding of chromatin regulatory features, histone modifications, etc.

The current exploration of chromatin dynamics is primarily restricted to the regulation of extra-chromatin factors. It is worthwhile to consider whether chromatin already has potential accessible features in the early stage of formation. What is more, chromatin modulation-based tumor treatment strategies are rarely used in clinical training. First, there are still many limitations in regulating gene networks at the chromatin level, for example, the escape mechanisms and complexity of tumor signaling pathways under various stress stimuli, as well as diversities in the expression of a gene in a large patient population and the individual heterogeneity of downstream signaling pathways in each patient. Second, existing techniques for determining chromatin status still have many drawbacks and limitations, and there are no methods that can present the complete and dynamic genomic status of tumor patients and intervene. Application to individualized tumor treatment still requires much exploration and a long-term course of clinical trials. With the development of chromatin characterization and application, individualized tumor therapy is becoming unveiled.

AUTHOR CONTRIBUTIONS

ZL and BZ contributed equally to this review. WW, BZ, and ZL raised the concept. ZL conducted the literature review and wrote the first draft. BZ and CQ drew the figures. TL and YW organized the tables. All authors revised and approved the final manuscript.

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GLOSSARY

TADs	topologically associating domains
HAT	histone acetyltransferase
HDAC	histone deacetylase
HDACi	HDAC inhibitor
PDAC	pancreatic ductal adenocarcinoma
CBP	cyclic AMP response element-binding protein
ICB	immune checkpoint blockade
GClnc1	gastric cancer-associated lncRNA1
SOD2	superoxide dismutase 2 mitochondrial
BET	bromodomain and extraterminal domain
BRD4	bromodomain 4
HMT	histone methyltransferase
SAM	*S*-adenosy-{{sc}}l{{/sc}}-methionine
PRMT	protein arginine *N*-methyltransferase
KDM	histone demethylase
NASPs	nuclear autoantigenic sperm proteins
PTMs	post-translational modifications
DUBs	deubiquitinases
E1	ubiquitin-activating enzyme 1
E2	ubiquitin-conjugating enzyme
E3	ubiquitin-protein isopeptide ligase
USP	ubiquitin deliberately modified enzyme family
SENPs	SUMO-specific proteases
DDR	DNA damage response
NF-κB	nuclear factor-κB
5-mC	5-methylcytosine
DNMTs	DNA methyltransferases
MeCPs	methyl-CpG-binding proteins
5-hmC	5-hydroxymethylcytosine
TET	Ten-Eleven Translocation
SWI/SNF family	switching defective/sucrose non-fermenting family
ISWI family	imitation switch family
SNF2L	SNF2-"like
SNF2H	SNF2-"homologue"
CHD family	chromodomain, helicase, DNA binding family
NuRD	nucleosome remodeling and deacetylase
INO80 family	inositol requiring 80 family
HELLS	HELicase
NFRs	nucleosome-free regions
ADM	acinar-to-ductal metaplasia
PanIN	pancreatic intraepithelial neoplasia
ICC	intrahepatic cholangiocarcinoma
MFAP5	microfibrillar-associated protein 5
ECM	extracellular matrix
LTR	long terminal repeat
GC	gastric cancer
HCC	hepatocellular cancer
ChIP	chromatin immunoprecipitation
ChIP-seq	chromatin immunoprecipitation sequencing
ATAC-seq	assay for transposase-accessible chromatin using sequencing
NGS	next-generation sequencing
scATAC-seq	single-cell ATAC-seq
MNase	micrococcal nuclease
MNase-seq	micrococcal nuclease sequencing
DNase-seq	deoxyribonuclease sequencing
DHSs	DNase hypersensitive sites
scDNase-seq	single-cell DNase sequencing
FAIRE-seq	Formaldehyde-Assisted Isolation of Regulatory Elements sequencing

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TElomeric repeat-containing RNA (TERRA): Physiological functions and relevance in cancer

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Telomeres are complex protective structures located at the ends of linear eukaryotic chromosomes. Their purpose is to prevent genomic instability. Research progress in telomere biology during the past decades has identified a network of telomeric transcripts of which the best-studied is TElomeric Repeat-containing RNA (TERRA). TERRA was shown to be important not only for the preservation of telomere homeostasis and genomic stability but also for the expression of hundreds of genes across the human genome. These findings added a new level of complexity to telomere biology. Herein we provide insights on the telomere transcriptome, its relevance for proper telomere function, and its implications in human pathology. We also discuss possible clinical opportunities of exosomal telomere transcripts detection as a biomarker in cancer precision medicine.

KEYWORDS

TERRA, long non-coding RNA, telomere homeostasis, cancer, Alternative lengthening of telomeres

Introduction

Telomeres

Human chromosomes end with telomeres, the structures comprised of hundreds to thousands of hexameric DNA repeats (5′-TTAGGGn-3′ in vertebrates) and terminated by a single-stranded guanine-rich (G-rich) overhang (1). Telomeres are approximately 6 to 20 kilobases long in humans (2), with considerable length heterogeneity between

tissues of an individual and even between distinct chromosomes within a cell (3). Due to the abundance of guanine, telomeres facilitate the formation of the structures called "G-quadruplexes" where guanines alignments are stabilized by hydrogen bonds (4). Furthermore, telomeres form lariat-like structures called T- and D-loops by invading the 3′ single-stranded (ss)DNA overhang into the double-stranded telomeric site (5). These structures are indispensable for the proper function of telomeres, and their formation has to be strictly regulated during the cell cycle by proteins of the shelterin multimeric complex (6, 7).

Shelterin consists of six protein subunits, namely TRF1, TRF2, RAP1, TIN2, TPP1, and POT1 (8). TRF1 and TRF2 subunits are recruited to canonical double-stranded telomeric DNA (9). Both proteins, along with RAP1 are connected *via* the TIN2 protein bridge, which binds TPP1, an interacting partner of the POT1 shelterin subunit (10). POT1 has a high affinity to the 3′ ssDNA G-rich overhang. Overall, shelterin mediates the proper formation of telomeric chromatin following DNA replication (8). The key function of the shelterin complex is to assist the T-loop formation, repression of 5′ end hyper-resection, and avert inappropriate activation of DNA damage response (DDR) pathways at the ends of chromosomes (8). Emerging evidence indicates that the shelterin function and proper telomere homeostasis, in general, are regulated by telomere transcripts known as TERRA "TElomeric Repeat-containing RNA" (11).

Telomeres naturally become progressively shorter with each cell division due to the end-replication problem (7). Critically shortened telomeres elicit a DDR pathway which may trigger apoptosis or a replicative senescence state (12) also known as the M1 stage (13). Additionally, the accumulation of DNA damage at the ends of chromosomes was observed in non-dividing differentiated somatic cells (14) presumably due to the action of DNA damaging agents (15). Such DNA damage is accumulated in the form of Telomere-associated DDR foci (16). Bypass of the senescence, occurring for example *via* de-activation of tumor suppressors such as p53 (17), p21 (18), Rb (19), along with telomere uncapping potentially result in massive genomic instability, and ultimately in malignant transformation. To avoid apoptosis and acquire an immortal phenotype, premalignant cells have to stabilize telomeres *via* the reactivation of telomerase or by alternative lengthening of telomeres (ALT), the two pivotal telomere maintenance mechanisms (TMMs) (2, 20). Currently known TMMs have recently been shown to harbor distinct TERRA expression patterns (11). In the present article, we also discuss differences in TERRA expression between telomerase and ALT-positive tumors.

TElomeric repeat-containing RNA (TERRA) and its functions

Telomeres were historically viewed as generally heterochromatic and thus creating a transcriptionally repressive chromatin environment (21). In 1989 Rudenko and Van der Ploeg identified a heterogeneous population of RNA transcripts containing telomeric repeats in protozoa (*Trypanosoma brucei*) (22). The evidence of telomeric transcription in mammals was provided in 2007 when Azzalin et al. discovered TERRA molecules in a human cervical cell line (HeLa) (23). As shown by northern blot and RT-PCR, telomeric and subtelomeric regions are actively transcribed into TERRA molecules which are made of subtelomeric-derived RNA and UUAGGG repeats (23). In 2008 Schoeftner and Blasco characterized TERRA molecules as a novel structural component of telomeric chromatin having the capacity to regulate telomerase activity (24).

TERRA is a long non-coding (lnc) RNA, with transcription starting in the subtelomeric regions and terminating within the region of telomeric repeats. The telomeric C-rich strand is utilized as a template for TERRA transcription (25). TERRA is heterogeneous in its length ranging from 100 bases up to 10 kilobases (16, 17), while the majority of TERRA contains a (UUAGGG)n telomeric repeat tract with an average length of 200 bases. Therefore, the length heterogeneity of TERRA is probably due to the subtelomere-derived sequences (25)

Deciphering the role of TERRA was a major unresolved question of telomere biology in the past decade as TERRA loci were unknown, preventing further functional studies (26) and because of unsuccessful efforts to fully deplete TERRA molecules (27). However, in 2017, new insights in a complex landscape of TERRA functions were achieved by successful degradation of TERRA *in vivo* (27). The following chapter summarizes the key roles of TERRA in physiological and pathological processes (summarized in Figure 1).

TERRA as a regulator of chromatin structure

Though the specific functions of lncRNAs remain ill-defined, the molecules are linked with modulation of chromatin structure and recruitment of chromatin-modifying proteins to distinct genome regions. Based on the TERRA-protein interactome, an extensive network of TERRA-associated proteins has been identified in mouse embryonic stem cells (27). One of the crucial proteins identified in the study was ATRX, a chromatin remodeler frequently mutated in ALT-positive cancer cells. TERRA antagonizes ATRX localization at telomeric sites, having an impact on telomeric chromatin structure (27). Furthermore, TERRA and ATRX foci are not restricted to telomeres. TERRA and ATRX were shown to share genomic targets and modulate the expression of hundreds of genes across the genome (27), such as downregulation of genes involved in TOR signaling and upregulation of those with positive effects on telomere capping and organ morphogenesis (27). TERRA at loci co-occupied by ATRX promotes gene expression while the ATRX protein exerts

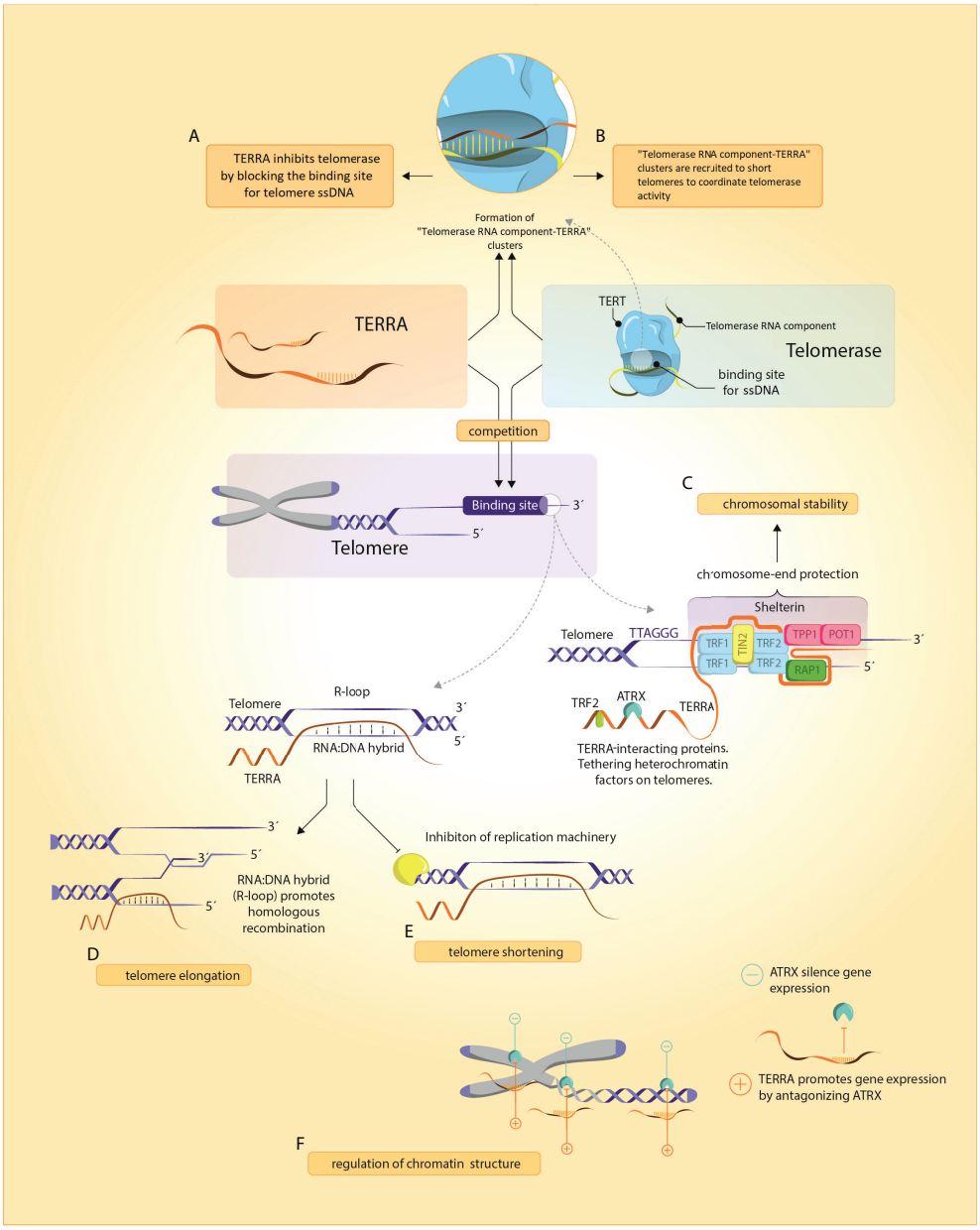

FIGURE 1
TERRA and its functions. **(A, B)** TERRA presumably binds to Telomerase RNA component through base-complementary pairing and blocks telomerase binding to telomeric ssDNA. In yeasts, Telomerase RNA component-TERRA clusters are localized at short telomeres where they coordinate telomerase activity, **(C)** TERRA and shelterin are implicated in chromosome-end protection by assembling secondary protective structures including R- and T-loops and G-quadruplexes **(D, E)** Regulation of telomeres by TERRA can induce either a shortening (by inhibition of telomerase activity and repressing *TERT* expression) or elongation (by homologous recombination promotion), **(F)** TERRA remodels chromatin structure through an antagonistic interaction with ATRX.

the opposite function (27). Overall, TERRA molecules along with ATRX and other chromatin remodeling proteins bind to chromatin on a global scale with the highest density at chromosome ends and regulate chromatin structure and expression of hundreds of genes. Thus, TERRA is both cis- and trans-acting at telomeric sites and on the global genome, respectively (27).

TERRA as a scaffold for shelterin subunits

TERRA also constitutes a hub for shelterin protein subunits. The nucleotide repeats of TERRA enable the formation of G-quadruplexes within TERRA itself similarly as in telomeric regions. TERRA G-quadruplexes are bound to telomeric DNA

via the TRF2 shelterin subunit and physically interact also with TRF1 (28, 29). It has been documented that inhibition of TERRA-TRF2 interaction results in an altered localization of TERRA and induction of DDR (11). Depletion of TRF2 was shown to induce massive DDR at telomeres and formation of telomere dysfunction-induced foci (30). These cellular events result in an increased level of TERRA which in turn associates with lysine demethylase LSD1 (30). In this study, TERRA was also shown to enhance binding affinity between LSD1 and the nuclease MRE11, both crucial for the processing of uncapped telomeres (30). Another study demonstrated changes in TERRA expression and the interaction between TERRA and SUV39H1 H3K9 histone methyltransferase at damaged telomeres following TRF2 depletion. The accumulation of H3K9me3 at damaged telomeres promotes chromosome end-to-end fusion (31). The results define the critical role of TERRA during pathological telomere dysfunction events and indicate that TERRA does not function only as a scaffold for shelterin but at the same time, shelterin can affect TERRA expression as well. Thus, TERRA is critical for telomere protection, preservation of proper telomeric chromatin architecture, and prevention of inappropriate DDR events at telomeric loci (32–34).

In addition, shelterin recognizes and regulates many genes adjacent to interstitial telomere sequences (ITS) spread across the human genome by telomere looping (35–38). For example, it has been suggested that long telomeres with enriched TRF2 silence the telomerase reverse transcriptase (TERT) locus *via* Telomere Position Effect-Over Long Distances (TPE-OLD) (39). If telomeres are too short, telomere length-dependent loops are not possible, which, in turn, may increase TERT expression (39).

TERRA and telomerase activity

TERRA was found to bind core telomerase components including the telomerase RNA template (TERC) through base pair interaction and TERT polypeptide, acting as a direct regulator of telomerase activity (40). hTERC forms several domains within its 451 nucleotides such as the scaRNA domain (binds Dyskerin), CR4/CR5 domain, and Pseudoknot/template domain which is associated with TERT polypeptide (41). The Template Region within TERC contains 3′-CAAUCCCAAUC-5′ nucleotides. Indeed, the 3′ end of TERRA is complementary to the telomerase RNA template region (25, 42), although it is currently unclear whether TERRA binds the TERC template region (40, 43). Redon et al. demonstrated that synthetic TERRA molecules containing 5′-UUAGGG-3′ repeats base pair with the TERC and also interact with telomerase catalytic subunit TERT [possibly binds to so-called anchor site in TERT (40)]. Redon et al. suggested a more complex effect of TERRA on telomerase than mere competition with telomeric DNA substrates (40).

An *in vivo* study on mouse embryonic stem cells demonstrated a 2-fold upregulation of telomerase activity following TERRA depletion (27). Furthermore, TERRA and TERC were shown to colocalize *in vivo* (27). Therefore, it has been surmised that TERRA negatively controls telomerase *in vivo* (27). *In vitro* study demonstrated that $(UUAGGG)_3$ RNAs mimicking TERRA molecules inhibit telomerase activity (42). TERRA, on the other hand, was reported to promote telomerase-mediated telomere elongation in yeast *Schizosaccharomyces pombe* (44). Also, TERRA was shown to form TERRA-TLC1, a yeast telomerase RNA similar to hTERC, clusters, which are in turn recruited to short telomeres where those RNA foci help coordinate nucleation and activity of telomerase (45). However, whether the interactions between TERRA and telomerase have positive or negative effects on the activity of the latter in humans needs to be further elucidated (46).

TERRA and its effect on telomere length

Another TERRA function is associated with direct regulation of telomere length (47, 48). As described in budding yeast *Saccharomyces cerevisiae*, TERRA transcription stimulates the 5′-3′ activity of Exonuclease 1 at chromosome ends, by which it regulates the telomere shortening rate (47).

In yeasts and telomerase-positive human cancer cells, TERRA fluctuates during the cell cycle, with the highest concentration of TERRA in the early G1 phase and clearance of TERRA from chromatin during the S/G2 phase (30). Dysregulation of the TERRA through the cell cycle was documented in ALT-positive cancer cells due to the loss of ATRX (49). Association between TERRA and telomeric chromatin *via* RAD51 DNA recombinase creates RNA : DNA hybrid structures called R-loops (50). R-loops have to be removed from chromatin upon replication of telomeric loci. Otherwise, their retention would lead to replicative stress, activation of DDR, and excessive telomere shortening (18, 31). In contrast, RNA : DNA hybrids at telomeres may induce telomere elongation *via* telomeric DNA recombination events which frequently occur in ALT-positive cancer cells. A recent study using the U2OS osteosarcoma cell line established that TERRA transcripts actively destabilize telomere integrity in ALT-positive cancer cells and that the inhibition of TERRA expression impairs the accumulation of DDR markers at telomeric sites and reduces ALT features (51). Therefore, TERRA transcripts seem to be a major trigger of ALT activity. The data suggest that TERRA transcription manipulation may be a potential therapeutic target in tumors utilizing the ALT mechanism for telomere elongation (51).

Also, based on a systematic analysis of telomere length carried out on more than 18 000 samples from many different cancer types, Barthel et al. demonstrated telomere shortening in 70% of cancer tissues compared with non-cancerous mucosa (52). The paradoxical question of telomere biology emerged from this and previous studies: Why the majority of cancer cells harbor short telomeres in spite of telomerase activation? One of the reasons is probably that telomerase activity in cancer cells enhances the level of TERRA (53) which was shown to

negatively correlate with the expression of interferon-stimulated gene (ISG) (54). Therefore, an increased TERRA signal represses ISG expression and tumor growth (54). Overall, the finding suggests that there might be a connection to cancer cells harboring short telomeres as a beneficial state for tumor progression (53, 55). However, further studies exploring the role of TERRA regarding telomere homeostasis are required.

TERRA expression

Subtelomeres

TERRA expression is regulated and initiated from subtelomeres (48), chromosomal regions adjacent to terminal telomeric repeats (56). Thus, TERRA contains subtelomeric sequences at its 5′ end followed by canonical tracts of UUAGGG-3′ repeats transcribed from telomeres (50). Subtelomeres differ greatly in size among organisms, ranging from 10 kilobases in budding yeast to 500 kilobases in humans (48).

Putative TERRA promoter regions at multiple human subtelomeres were first identified by Azzalin et al. and by several independent studies (23, 50). One of the major transcription loci is embedded at the 20q subtelomere (26, 57). Experiments based on the ablation of approximately 8.1kb long fragment from the 20q subtelomere using the CRISPR-Cas9 method resulted in an almost complete downregulation of TERRA expression in 20q TERRA-KO U2OS osteosarcoma ALT-positive cells, telomere shortening, and the induction of massive DDR. This study was also the first to demonstrate the crucial importance of TERRA molecules for telomere homeostasis maintenance (57). Silva et al., who further elaborated on the origin of TERRA in U2OS cells, showed multiple other chromosome ends physiologically relevant for TERRA transcription. The group engineered Transcription Activator-Like Effectors ([TALEs], a plasmid based system) targeting consensus sequences located within twenty putative human subtelomeres with the purpouse to suppress TERRA expression. The group established that TERRA transcription suppression weakens ALT activity and suggested that the low level of TERRA molecules previously documented in 20q-TERRA-KO cells may, besides the 20q deletion, also arise due to short telomeres or clonal variability (51).

It is required to note that the copy number variation of the 20q13.3 subtelomeric region was identified in association with gastric (58) and sporadic colorectal cancer (59). The 20q13.3 amplification target in the tumors is most likely ADRM1, an integral plasma membrane protein involved in cell adhesion. Upregulation of ADRM1 at RNA and protein levels was reported to increase growth, proliferation, and migration in cancer cells (58). It would be interesting to analyze if other human malignancies contain similar chromosomal rearrangements. Critical information in this topic may be obtained by further studies focusing on systematic inhibition of specific TERRA promoters.

Epigenetic modifications of subtelomeres

Subtelomeres are CpG-enriched and frequently contain heterochromatic methylation patterns of histone H3 and H4 (H3K9me3, H4K20me3). These patterns are recognized and bound by heterochromatin protein 1 (HP1) (60). A consensus on these histone marks and a subtelomere chromatin structure is missing (44). The highest concentration of CpG islands is located within two distal kilobases of subtelomeres and gradually decreases upstream towards the distal end of the chromosome. The disparity in subtelomeric methylation was revealed while examining different human cell types. Subtelomeres undergo extensive methylation during embryo development (61). Sperm cells have hypomethylated subtelomeres, while human peripheral blood leukocytes have a high level of methylation in subtelomeric regions (61). Interestingly, cancer cells, irrespectively of TMM, display variation in methylation of subtelomeric CpG islands and deregulated TERRA expression (42). Those CpG islands are, in general, heavily methylated in telomerase-positive cancer cells. This epigenetic state results in dampened TERRA expression. The maintenance of subtelomeric heterochromatin state and low TERRA levels may be therefore necessary for telomerase function in telomerase-positive tumors presumably due to the effect of TERRA on telomerase activity (62). On the other hand, ALT-positive tumor cells show, in comparison with telomerase-positive cells, heterogeneous methylation changes in subtelomeric loci and a high level of TERRA transcripts, which may be essential for the maintenance of telomeres in those cells (62).

During and after transcription, TERRA is subject to co-/post-transcriptional modifications. The RNA processing varies between individual TERRA transcripts creating biochemically different TERRA fractions with remarkably diverse biological functions (25). TERRA is transcribed by RNA Polymerase II and therefore has a canonical 7-methylguanosine cap structure at 5′ ends like most coding RNA species. Only a minor fraction of TERRA has been shown to contain poly-A tail (poly(A)+), affecting its stability and affinity to chromatin (11, 25). Poly (A)+ TERRA population is present mainly in the nucleoplasm and has a weak chromatin affinity, while poly(A)- TERRA, in addition to being located at the nucleoplasm, associates with DNA predominantly at telomeric and other chromatin sites (25).

Regulation of TERRA expression

Expression of TERRA was shown to be regulated by major tumor suppressors (63, 64). Tutton et al. documented induction of TERRA expression upon treatment of human colorectal cancer (CRC) cells with etoposide, a drug producing DNA double-strand breaks. Notably, TERRA expression under such stress conditions is

dependent on the p53 transcription factor, which recognizes the non-canonical p53 binding sites within subtelomeric regions. This binding confers transcription enhancer-like functions and results in increased TERRA transcription. Thus, p53 provides a direct safeguard for human telomeres (64).

Furthermore, tumor suppressor Rb1 modifies telomeric chromatin architecture by regulating TERRA expression. Rb1 was demonstrated to bind human subtelomeres. Haploinsufficiency of RB1 leads to reduced TERRA levels, telomere shortening, and increased genomic instability, a common phenotypic feature of Rb1 deficient cells (i.e., osteosarcoma) (63). Additionally, Rb1 deficiency is associated with a shift in the patterns of telomeric histone modifications which, in turn, results in relaxed and unprotected chromatin (63). Overall, the non-canonical activity of Rb1 is associated with telomere homeostasis *via* regulation of TERRA expression (63).

Vohhodina et al. observed an increased TERRA expression in BRCA1-deficient cells. At telomeres and subtelomeres, BRCA1 depletion led to an altered chromatin architecture which resulted in elevated RNA Polymerase II binding to these regions. Moreover, in the absence of BRCA1, elevated R-loop levels were detected at subtelomeric CpG-island-containing TERRA promoters. Increased frequency of R-loops was associated with reduced recruitment of DNA methyltransferase, hypomethylation of TERRA promoter regions, and increased TERRA expression. Based on these observations, it can be proposed that BRCA1 regulates TERRA expression *via* the suppression of R-loop formation at subtelomeres (65).

TERRA expression is also tightly connected to cellular stress and DDR. For example, in response to heat stress, TERRA is upregulated by the heat shock factor 1 (HSF1) which was documented to bind subtelomeric regions in HeLa cell lines. Moreover, Koskas et al. detected a significantly higher frequency of DDR at telomeres in HSF1-KO cells compared to wild-type cells when cultured in the same conditions (66, 67). Interestingly, TERRA induction appeared to be a dynamic response to oxidative stress. Upon exposure to oxidative stress, TERRA expression is increased. If the stressor is removed, TERRA expression reverts after (66). Therefore, it seems that chromatin changes in subtelomeric regions displayed some sort of transcriptional memory to secure rapid expression of genes when stress was repeated.

TERRA expression is also influenced by cytoskeleton reorganization. TERRA level decreases together with decreasing surface stiffness of the cell. Cytoskeleton alterations may be produced by treatment with paclitaxel or colcemid, ultimately resulting in increased TERRA levels (67). Also, telomeres are under physiological circumstances associated with the nuclear envelope. The most recent findings on fission and budding yeast demonstrated elevated TERRA expression following detachment of telomeres from the nuclear envelope. This observation remains to be established in human cells (68). However, we can speculate whether impaired telomere-nuclear envelope interactions in humans and thereby misregulation of TERRA expression are connected with telomere-associated diseases including progeria, telomeropathies, and also cancer.

Deregulation of TERRA expression in human pathology

Several studies have shown the association of deregulated TERRA expression with cancer. However, the role of TERRA in human solid cancers remains largely unexplored. Downregulation of TERRA along with TRF1 and upregulation of TRF2 was identified in tumor tissue of patients diagnosed with hepatocellular carcinoma (HCC) (69). Decreased level of TERRA was associated with poor prognosis of the patients and with accelerated cell growth and metastatic progression of HCC both *in vivo* and *in vitro*. Additionally, TERRA knockdown in HCC cell lines led to a significant increase in telomerase activity, telomere elongation, and increased formation of metastasis, suggesting that depleting TERRA favors the metastatic spread in HCC (69). Authors of another study found a significant reduction in TERRA expression, along with high telomerase activity and short telomeres, in endometrial cancers compared with noncancerous endometrial tissues (70). Other studies found downregulated TERRA expression in squamous cell carcinoma and astrocytoma predisposing the patients to poorer clinical outcome (71, 72). Also, in patients with astrocytoma, TERRA level correlated with the activity of telomerase, telomere length, and clinical stage (71). By contrast, upregulation of TERRA was observed in a mouse model of medulloblastoma and human cancer biopsies derived from lung, colon, ovary, breast, and stomach (73). The authors showed that TERRA concentrates in rapidly proliferating normal and cancer cells and forms foci in the nuclear regions (72). To our best knowledge, only one study has evaluated TERRA expression and the outcome of patients diagnosed with CRC. Patients with high TERRA expression and low preoperative carcinoembryonic antigen level had improved disease-free survival (74). Previous observations that telomere length may relate to cell radiosensitivity (75) were refuted by Smirnova et al. In their article, variability in TERRA levels and telomere length did not affect sensitivity to ionizing radiation in different human cell lines, including breast, gastric cancer and cervical carcinoma (76). Overall, dysregulation of TERRA was present in various human cancer tissues. TERRA was shown to accumulate and form foci in rapidly proliferating progenitor and tumor cells, supporting the presumption that TERRA expression is coupled with cell proliferation (73). The available data indicate that variation in TERRA expression across different malignancies may be tumor-type specific.

An epigenetic state of subtelomeric regions may play a critical role in TERRA expression and TMM decision too. Hypermethylated subtelomeric CpG islands in telomerase-positive cancer cell lines were detected by Nergadze et al., while demethylation of these sequences reflected in increased

TERRA expression (77). In ALT-dependent cancer cells, frequent occurrence of ATRX/DAXX mutations is accompanied by DNA hypomethylation in subtelomeric regions (78). ATRX/DAXX mutations are found notably in pancreatic neuroendocrine tumors (79), and CNS malignancies (80). Depletion of ATRX and/or DAXX in the presence of various genotoxic agents is sufficient to induce ALT phenotype (81). ATRX loss induces gradual decondensation of telomere heterochromatin leading to telomeric replication stress and DDR (82). Consecutively, a cell is forced to switch to ALT to secure telomere length maintenance (82). Overall, this observation raised the possibility of distinct subtelomeric epigenetic patterns between telomerase and ALT-positive cancer cells (42).

Cell-free TERRA as a potential diagnostic marker

Extracellular cell-free TERRA molecules (cfTERRA) have been identified in exosomes secreted into body fluids. cfTERRA is usually around 200 nucleotides in length due to post-transcriptional processing or aborted transcription from longer forms of intracellular TERRA. It has been suggested that cfTERRA levels are correlated with intracellular TERRA expression. cfTERRA is associated with histones and the binding together with high resistance to RNase contributes to cfTERRA stability and abundance in tissue and cells. Using RNA-seq analyses, cfTERRA was identified among the 20 most frequent extracellular transcripts derived from human blood plasma (83). However, the quantity of cfTERRA does not seem to be unique for malignancies, as no differences have been found between healthy subjects and various cancer patients, such as breast, colon, duct, kidney, lung, liver melanoma, ovarian, prostate, and stomach. In addition, increased cfTERRA was detected in extracellular exosomes following induced telomere dysfunction (83).

cfTERRA might belong to a family of molecules known as alarmins. These molecules (Danger Associated Molecular Pattern – DAMP; or Pathogen Associated Molecular Pattern – PAMP) are signaling cellular damage, or viral and bacterial infection (84). cfTERRA was shown to modulate the expression of the inflammatory cytokines TNFalpha and IL6 in recipient cells which represent communication between dysfunctional telomeres and inflammation through DAMP-like signaling (85). This observation may provide a mechanistic explanation of how disrupted telomere homeostasis contributes to the inflammatory cascade reaction and senescence towards neighboring cells, a bystander effect due to senescence-associated secretory phenotype (83, 86). As cfTERRA is also present at low levels in normal human plasma, it cannot stand as a single biomarker for diagnosis in itself. However, enrichment of cfTERRA along with other DAMPs might serve as a potential biomarker for the noninvasive detection of diseases associated with telomere dysfunction including cancer and telomeropathties such as familial pulmonary fibrosis, dyskeratosis congenita or aplastic anemia.

Conclusion

In this article, we have pointed out the relevance of TERRA, a novel and exciting field of telomere biology, in the context of human physiological and pathological processes. Understanding TERRA functions is of great interest in basic medicine as TERRA regulation is altered in human diseases including cancer. However, the expression level of TERRA varies in a tumor type-specific manner (80). Based on the current knowledge, TERRA is a potential therapeutic target in different malignancies. Further studies are needed to clarify whether cancer cells harboring different TMMs have diverse methylation of subtelomeres and different patterns of TERRA expression.

Increased cfTERRA have been detected in response to telomere dysfunction, suggesting its potential use as a biomarker for the detection of early stages of cancers and other telomere-driven diseases. It is also important to further elucidate the crosstalk between cfTERRA, inflammation, and tumor microenvironment.

Overall, a deeper insight into TERRA regulation could help us understand its role in telomere maintenance and genome stability.

Author contributions

Conceptualization: MKr; Funding acquisition: LV, MKr and KT; Visualization: PS; Writing and original draft: MKr, KT, and MKa; Review and editing: PV, RK, and TB. All authors contributed to the article and approved the submitted version.

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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.

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Polymorphisms in ERCC4 and ERCC5 and risk of cancers: Systematic research synopsis, meta-analysis, and epidemiological evidence

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The variants of DNA repair genes have been widely reported to be associated with cancer risk in the past decades. As were two crucial members of nucleotide excision repair pathway, *ERCC4* and *ERCC5* polymorphisms are linked with susceptibility to multiple cancers, but the conclusions were controversial. In this updated meta-analysis concerned with *ERCC4* and *ERCC5* single-nucleotide polymorphisms (SNPs), 160 eligible publications were identified, and we exerted the meta-analysis of correlations between 24 variants and 19 types of cancer. Venice criteria and the false-positive report probability were used to evaluate a cumulative evidence of significant associations. We conducted functional annotations for those strong associations using data from the Encyclopedia of DNA Elements (ENCODE) Project. We obtained 11 polymorphisms significantly related to changed susceptibility to 11 cancers ($p < 0.05$). Strong evidence was assigned to four variant-related cancer risks in Asians (*ERCC4* rs744154 with bladder cancer, *ERCC5* rs2296147 with esophageal cancer, *ERCC5* rs17655 with laryngeal cancer and uterine cancer, and *ERCC5* rs751402 with gastric cancer), moderate to six SNPs with a risk of eight cancers, and weak to nine SNPs with nine cancers. Data from ENCODE and other public databases showed that the loci of these SNPs with strong evidence might fall in putative functional regions. In conclusion, this paper summarizes comprehensive evidence that common variants of *ERCC4* and *ERCC5* genes are strongly associated with the risk of bladder cancer, esophageal cancer, laryngeal cancer, uterine cancer, and gastric cancer and elucidates the crucial role of the DNA repair genes in the genetic predisposition to human cancers.

KEYWORDS

genetic variant, cancer, susceptibility, epidemiology, ERCC gene

Introduction

Cancer has become one of the major and most formidable obstacles to extending human life; the number of newly diagnosed cancer patients and cancer deaths worldwide reached 18.1 million and 9.6 million in 2018 (1). Among the complex array of carcinogenic factors, genetic variants have been shown in many studies to play a crucial role in the pathogenesis of cancer in recent decades (1, 2). Increasing genetic studies are being made to reveal the potential association between genetic polymorphism implicated in signaling pathways and the discordance of cancer predisposition among individuals.

In the process of metabolism, many factors such as exposure to environmental carcinogens and toxic metabolites may lead to the occurrence of DNA damage (3, 4). Under normal circumstances, our body deals with DNA damage through a complex set of repair mechanisms so that the hereditary material is balanced and stable to keep the body healthy. Nucleotide excision repair (*NER*) is one of the well-studied DNA repair pathways in human body, which reverses the multiform damage of the double-helix DNA with four steps: the recognition of lesion, the demarcation and unwinding of the impaired DNA fragment, oligonucleotide excision, and the ligation of new strands (5–7). The mutations of *NER* genes alter the capacity of DNA damage repairment, further resulting in an individual discrepancy of the risk of malignancy in tissue cells. Previous studies have identified that ineffective *NER* may give rise to incidence of a rare disease called xeroderma pigmentosum (*XP*), which can significantly increase the risk of skin cancer (3, 8).

As known to date, the functional performance of the *NER* pathway involved the participation of at least eight pivotal genes (*XP A-G* and *ERCC1*). The *XPF* gene, also known as excision repair cross-complementation group 4 (*ERCC4*), is located on chromosome 16p13.2 and consists of 11 exons that span approximately 28.2 *kb* (9). The proteins encoded by the *ERCC4* gene and *ERCC1* gene play a synergistic role in the *NER* pathway when participating in the excision of the damaged fragment (10, 11). Located on chromosome 13q22-33, consisting of 15 exons and 14 introns, the *XPG* gene is also termed as *ERCC5*, and the special endonuclease is produced by which it is indispensably enrolled in the two incision steps of the *NER* process (12). A growing number of genetic evidence indicated that the single-nucleotide polymorphisms (SNPs) in the *ERCC4* and *ERCC5* genes may vary susceptibility to malignant tumor; previous studies have demonstrated that *ERCC4* rs1800067 was associated with the risks of lung cancer, breast cancer, and glioma (13–15). Interestingly, the SNP rs17655 could trigger the occurrence of bladder cancer, leukemia, and glioma (16–18). Moreover, this SNP could downregulate the risk of head and neck cancer (19, 20) Variants other than the above-mentioned two SNPs in *ERCC4* and *ERCC5* have also been tested for the underlying relationship with the susceptibility to cancers, with inconsistent conclusions appearing due to the limitations of the sample and population.

Meta-analyses aiming to explore the relationship between *ERCC4* and *ERCC5* variants and the kinds of human cancers were continuously published (21, 22). However, most of these studies involved a single SNP and/or a single cancer; the conclusions are not always consistent, and the functional mechanisms remain unclear. Although in previous published meta-analysis studies, a single SNP with the risk of individual cancer was investigated, the results were still inconsistent. Furthermore, a comprehensive research synopsis with systematic functional annotation has not been performed to evaluate the epidemiological evidence of genetic associations between *ERCC4* or *ERCC5* genes and the risk of cancers till now. The purpose of the current study was to elucidate the role of all studied SNPs in *ERCC4* and *ERCC5* in the tendency of all implicated types of cancer. We firstly did meta-analysis with data collected from all relevant studies so far; then, the statistical power of generated significant evidence was detected. Finally, a systematic functional annotation was conducted for seeking the molecular mechanisms of approved connection.

Methods and materials

We did this work with strict adherence to the guidelines of the Human Genome Epidemiology Network for systematic review of genetic association studies and Meta-analyses of Observational Studies in Epidemiology (MOOSE) and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement (PRISMA) guidelines (see Supplementary Table S1) (23–26)

Literature searching and identification

A systematical article-searching was performed in the three most popular electronic databases: PubMed, Medline, and Web of Science. Eligible published studies up to 30 August 2021 were collected by using the following terms: "excision repair cross complementing group or *ERCC* or xeroderma pigmentosum group or *XP*" and "cancer or carcinoma or malignant tumor or adenocarcinoma" and "mutation or variant or variation or polymorphism or SNP or genotype." Aside from articles originated from database, studies identified from meta-analyses and references were also added to the list.

Criteria for inclusion and exclusion

We included genetic studies that meet the criteria below: (1) aiming to test the relationships between the *ERCC4* and/or *ERCC5*

gene and the risk of cancer in case–control, cross-section, or cohort studies, (2) original articles published in a journal in English, (3) the concrete sample size of case and control groups and the quantity of genotype and/or allelic distributions were provided. Ineligible studies were excluded for these reasons: (1) studies researched the association between polymorphisms of other subgroup genes of *ERCC* and cancer risk; (2) meta-analyses, systematic reviews, pooled analyses, and duplicated publications; (3) adequate data (e.g., the amount of genotype) could not be acquired; and (4) studies focused only on the prognosis and survival of cancer patients, not cancer incidence.

Data extraction and management

The authentic and precise data were independently extracted by two participators from qualified studies; all the divergences that occurred through the process were resolved by discussing with the corresponding author. Detailed information presented in the form including the first author, the year of publishing, cancer site, cancer type, country/region, ethnicity, genotyping methods, gene name, allele genotype and genotype distribution for each polymorphism, and minor allelic frequency (MAF). Ethnicity was comprised of four categories [Asian (East Asian descent), Caucasian (European descent), African (African descent), or others (including people from other countries such as Indians, Native Hawaiians, Latinos, Hispanics, and the mixed)] based on the criterion that at least 80% of the study populations belonged to one of these groups; "overall populations" integrates two or more. If the same genetic variant was reported in more than one study, we selected the most recently published study with the greatest number and most integrated participants. The specific minor allelic of each SNP were obtained from the website (https://www.ncbi.nlm.nih.gov/snp/).

Statistical analysis

Meta-analyses were executed on the variants of more than one dataset, in which we employed three models: allelic, dominant, and recessive models for calculating the pooled ORs (Supplementary Table S2). We also carried out a subgroup analysis of ethnicity among the SNPs with sufficient data. The heterogeneity across involved studies was examined by the utilization of Cochran's *Q* statistic and the I^2 test (27, 28). Briefly, the I^2 value was categorized into $I^2 \leq 25\%$, $25\% < I^2 < 50\%$, and $I^2 \geq 50\%$, which represented no or little heterogeneity, moderate heterogeneity, and large heterogeneity, respectively. Different kinds of models were employed according to the *P*-value generated from the *Q* statistic; the random effect model was used when the *P*-value <0.1, and the fixed effect model was appropriate for other circumstances. Furthermore, sensitivity analysis was applied to test the stability of significant ORs, which means producing a new OR value by excluding a single study (dataset), and/or the first published study, and/or studies that disobeyed the Hardy–Weinberg equilibrium (HWE) in the controls; it is an unstable association if the statistical significance was lost. We checked bias in two aspects: Begg's test for potential publication bias and Egger's test for small-study bias (29, 30). In this study, the strategy of affirming findings to be statistically significant was *P*-value <0.05 in the meta-analysis and *P*-value <0.10 in tests of heterogeneity and biases. An association was considered to be non-statistically significant if the 95% CI included 1.0 or if the P-value was ≥0.05. Statistical analyses were conducted utilizing Stata, version 12 (Stata, College Station, TX, USA).

Assessment of cumulative evidence

The epidemiological credibility of statistically significant findings was primarily evaluated with Venice criteria (Supplementary Notes for Venice criteria) (23). Combined with the ratings of the three criteria (amount of evidence, replication, and protection from bias) and then got the assignment of grades as A, B, or C separately, the epidemiological evidence was ranked as strong, moderate, or weak. The amount of evidence was graded based on the result of sum of the tested alleles or genotype numbers in cases and controls, the sum more than 1,000, between 100 and 1,000, or less than 100 was graded as A, B, or C, respectively. To grade the replication, the consequences of heterogeneity estimation were employed as follows: A signified $I^2 \leq 25\%$, B signified $25\% < I^2 < 50\%$, and C signified $I^2 \geq 50\%$. The grade of protection from bias was generated from comprehensively analyzing the outcome of sensitivity analysis, statistic of publication bias and small study bias, and assessment of an excess of significant findings. Eventually, grade A was assigned if no apparent bias was observed, or bias was unable for illuminating the presence of association, grade B would be assigned if we got moderate bias, and grade C was assigned if there was evident bias or bias could explain the existence of association. Meanwhile, connection intention was a non-negligible factor of the evaluation of the protection from bias; grade C was assigned on this criterion when the pooled OR was less than 1.15 (or more than 0.87 in a protection effect). However, this rule would be invalid if this significant finding had been replicated extensively by large collaborative studies including GWAS or GWAS meta-analysis (31). We strictly adhere to the checklist when checking the sources of bias in different settings proposed by the Venice criteria (see Supplementary Information Notes). Subsequently, those significant findings with grade A for all three criteria were determined as strong-credibility epidemiological evidence, those with grades were composed of A and B were determined as moderate-credibility evidence, and those with C assigned to any of three categories were considered as weak-credibility evidence.

A prior probability of 0.05 and a false-positive report probability (FPRP) cut-off value of 0.2 in the FPRP assay should be performed to detect the potential false-positive

results among significant associations and assess whether these associations should be excluded, as Wacholder et al. recommended (32). If the calculated FPRP value was below the prespecified noteworthiness value of 0.2, we would consider the association noteworthy, indicating that the association might be true. The true evidence was graded by the FPRP value: <0.05, 0.05–0.2, or >0.2, indicating strong, moderate, or weak, respectively. With a strong magnitude of FPRP, the credibility of evidence would be upgraded from weak to moderate and from moderate to strong; if FPRP was assigned as weak, we would downgrade the credibility of association from strong to moderate and from moderate to weak. We utilized an Excel spreadsheet established by Wacholder et al. for calculating the FPRP values and corresponding statistical power.

Functional annotation

The underlying functional role of the variants of *ERCC4* and *ERCC5* genes was evaluated with information from the Encyclopedia of DNA Elements (ENCODE) tool HaploReg (v4.1) (32) as well as UCSC Genome browser (http://genome.ucsc.edu/). Furthermore, the current work explored genome-wide cis-eQTL data in multiple tissues from the Genotype-Tissue Expression Project (33) and the Multiple Tissue Human Expression Resource Project (33) databases in order to reveal whether these genes might explain the observed findings in these loci.

Results

Characteristics of included studies

Initially, 3,118 studies were retrieved from PubMed, Medline, and Web of Science (Figure 1). After reviewing the title and abstract, we filtered out 672 articles related to the *ERCC* gene and cancer risk; those were excluded because of duplication or no correlation. Then, 498 articles were excluded due to the lack of eligible data: not SNPs of *ERCC4* and *ERCC5*; no amount of genotype; and prognosis and survival related. Next, 38 papers of meta-analysis and review were excluded; additional 24 studies were added from related meta-analyses and references. Ultimately, 160 publications were eligible, including 192 datasets in 84 publications of *ERCC4* and 280 datasets in 123 articles of *ERCC5* (47 articles containing data about both *ERCC4* and *ERCC5*). The demographical characteristics of all available publications are summarized in Supplementary Table S3. In current study, 55,446 cases of 19 types of cancer and 61,855 controls were enrolled in these 192 datasets for the investigation of the implication of 40 *ERCC4* variants on cancer susceptibility, and as for *ERCC5*, we collected 38 SNPs distributed in 55,393 cases of 22 types of cancer and 66,872 controls. A total of 19 types of cancer and 24 SNPs of both *ERCC4* and *ERCC5* were

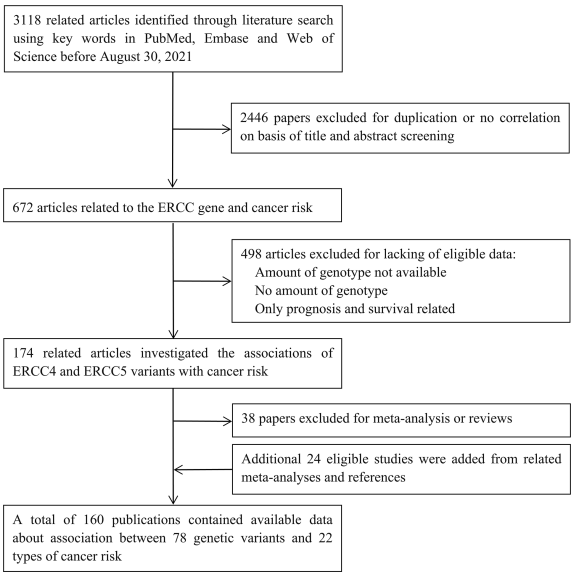

FIGURE 1
Flow diagram of literature search and study selection.

incorporated into meta-analysis because there were at least two serviceable datasets.

Main meta-analyses

ERCC4

We executed meta-analysis on the correlation between 12 variants of *ERCC4* and the risk of 13 types of cancer, and four SNPs (rs744154, rs1800067, rs2276466, and rs1799801) were figured out to be significantly associated with risk of three cancers (bladder cancer, gastric cancer, and glioma) (Table 1). To be specific, rs744154 (C vs. G) was confirmed to be a risk factor of bladder cancer in Asians in the allelic model (OR = 1.566, 95% CI = 1.233-1.989, $p < 0.001$) and recessive model (OR = 1.731, 95% CI = 1.296-2.313, $p < 0.001$). Two SNPs were significantly associated with the risk of glioma, an increased susceptibility was observed for rs2276466 (G vs. C) in Asians (allelic model: OR = 1.332, 95% CI = 1.101-1.612, $p = 0.003$; dominant model: OR = 1.336, 95% CI = 1.030-1.733, $p = 0.029$; recessive model: OR = 1.553, 95% CI = 1.094-2.206, $p = 0.014$); nevertheless, we demonstrated that rs1800067 (A vs. G) was a protective factor in the occurrence of glioma in the overall population (allelic model: OR = 0.634, 95% CI = 0.426-0.944, $p = 0.025$; recessive model: OR = 0.528, 95% CI = 0.350-0.796, $p = 0.002$). In addition, significant associations with the risk of gastric cancer were revealed for rs1799801 (C vs. T) in the overall population in the dominant model (OR = 0.755, 95% CI = 0.614-0.930, $p = 0.008$), and for rs744154 (C vs. G) in Asians (allelic model: OR = 0.790, 95% CI = 0.666-0.937,

p< 0.007; dominant model: OR = 0.681, 95% CI = 0.535-0.866, p = 0.020).

The results of subgroup analysis by ethnicity showed that rs1800067 (A vs. G) could decrease the risk of glioma in Asians (allelic model: OR = 0.543, 95% CI = 0.389-0.760, p < 0.001; dominant model: OR = 0.502, 95% CI = 0.312-0.808, p = 0.005; recessive model: OR = 0.535, 95% CI = 0.347-0.826, p = 0.005) but did not in Caucasians. In addition, there was no relationship between rs1799801 (C vs. T) and gastric cancer risk in Asians but in Caucasians (allelic model: OR = 0.698, 95% CI = 0.505-0.963, p < 0.029; dominant model: OR = 0.567, 95% CI = 0.378-0.849, p = 0.006).

ERCC5

A total of 12 SNPs of the *ERCC5* gene and 15 types of cancer were involved into meta-analyses (Table 1). Rs17655 (C vs. G); the most extensively researched variants were testified to be significantly associated with the risk of nine cancers in the overall population, including leukemia (allelic model: OR = 1.176, 95% CI = 1.017-1.360, p = 0.029; dominant model: OR = 1.169, 95% CI = 1.041-1.313, p = 0.009), colorectal cancer (allelic model: OR = 1.053, 95% CI = 1.006-1.102, p = 0.027; dominant model: OR = 1.132, 95% CI = 1.020-1.255, p = 0.019), head and neck cancer (recessive model: OR = 0.787, 95% CI = 0.627-0.989, p = 0.040), laryngeal cancer (recessive model: OR = 0.571, 95% CI = 0.533-0.753, p < 0.001), prostate cancer (dominant model: OR = 1.149, 95% CI = 1.005-1.312, p = 0.042), and uterine cancer (allelic model: OR = 1.239, 95% CI = 1.050-1.463, p = 0.011; dominant model: OR = 1.315, 95% CI = 1.039-1.664, p = 0.023; recessive model: OR = 1.355, 95% CI = 1.142-1.608, p = 0.001).

Through the subgroup analysis by ethnicity, we got these following associations between rs17655 (C vs. G) and cancers: increased risk of leukemia in Caucasians (allelic model: OR = 1.298, 95% CI = 1.007-1.671, p = 0.044; dominant model: OR = 1.285, 95% CI = 1.065-1.550, p = 0.009; recessive model: OR = 1.511, 95% CI = 1.093-2.090, p = 0.013), colorectal cancer in Caucasians (dominant model: OR = 1.118, 95% CI = 1.006-1.242, p = 0.038), oral cancer in Asians (allelic model: OR = 1.334, 95% CI = 1.105-1.611, p = 0.003; dominant model: OR = 1.414, 95% CI = 1.059-1.889, p = 0.019; recessive model: OR = 1.485, 95% CI = 1.082-2.038, p = 0.014), prostate cancer in Caucasians (allelic model: OR = 1.208, 95% CI = 1.003-1.454, p =< 0.046), gastric cancer in Caucasians (allelic model: OR = 1.282, 95% CI = 1.024-1.606, p = 0.030; recessive model: OR = 1.513, 95% CI = 1.126-2.034, p = 0.006), uterine cancer in Asians (allelic model: OR = 01.365, 95% CI = 1.190-1.565, p < 0.001; dominant model: OR = 1.618, 95% CI = 1.286-2.035, p < 0.001; recessive model: OR = 1.387, 95% CI = 1.121-1.715, p = 0.003); a decreased risk of head and neck cancer in Asians (dominant model: OR = 0.796, 95% CI = 0.649-0.976, p = 0.028), laryngeal cancer in Asians (allelic model: OR = 0.636, 95% CI = 0.520-0.779, p < 0.001; dominant model: OR = 0.613, 95% CI = 0.444-0.847, p = 0.003; recessive model: OR = 0.574, 95% CI = 0.427-0.772, p < 0.001), and thyroid cancer in Caucasians in the recessive model (OR = 0.501, 95% CI = 0.313-0.801, p = 0.004).

With the exception of rs17655 (C vs. G), six SNPs (rs1047768, rs2094258, rs2296147, rs2228959, rs751402, and rs873601) of *ERCC5* were also demonstrated to significantly alter the susceptibility of cancers. We found that rs1047768 (C vs. T) remarkably increased the risk of colorectal cancer in Asians in the recessive model (OR = 1.218, 95% CI = 1.006-1.474, p = 0.044), in contrast, it is a protective factor of colorectal cancer in Caucasians in the allelic model (OR = 0.876, 95% CI = 0.774-0.991, p = 0.036). Another significant association with the risk of colorectal cancer was observed for rs2094258 (T vs. C) in Asians (allelic model: OR = 1.128, 95% CI = 1.043-1.219, p = 0.002; dominant model: OR = 1.141, 95% CI = 1.025-1.270, p = 0.015; recessive model: OR = 1.232, 95% CI = 1.051-1.445, p = 0.010). It was uncovered that rs2296147 (T vs. C) polymorphism was relevant to the decreased risk of esophageal cancer in Asians (allelic model: OR = 0.825, 95% CI = 0.741-0.919, p < 0.001; dominant model: OR = 0.783, 95% CI = 0.687-0.892, p < 0.001), and the same association was shown between rs2228959 (A vs. C) and lung cancer in Asians (allelic model: OR = 0.370, 95% CI = 0.283-0.484, p < 0.001; dominant model: OR = 0.429, 95% CI = 0.188-0.979, p = 0.044). When researching the incidence of gastric cancer in Asians, a protective effect was observed in the implication of rs751402 (G vs. A) polymorphism on gastric cancer (allelic model: OR = 0.865, 95% CI = 0.784-0.954, p = 0.004; dominant model: OR = 0.802, 95% CI = 0.657-0.980, p = 0.031; recessive model: OR = 0.867, 95% CI = 0.794-0.946, p = 0.001); however, the opposite effect appeared when it comes to rs873601 (A vs. G) in the allelic model (OR = 1.069, 95% CI = 1.007-1.135, p = 0.029) and recessive model (OR = 1.133, 95% CI = 1.026-1.251, p = 0.014).

Non-significant association in meta-analyses

We additionally found that among those associations lack of statistical significance, five polymorphisms (two of *ERCC4* and three of *ERCC5*) had no evidence of relationship with four cancers risk in meta-analyses with at least 3,000 cases and 3,000 controls (Table 2).

Heterogeneity, sensitivity analyses, and publication bias

Among all the significant findings of the correlation between variants of *ERCC4* and *ERCC5* and cancer risk, little heterogeneity (I^2 ≤ 25%) was discovered in 29 (53.7%)

TABLE 1 Genetic variants showing significant summary odds ratios for different cancer risks in main meta-analyses in all three genetic models.

Gene	Variant	Alleles[a]	Cancer site	Ethnicity	MAF[b]	Genetic models	Effect model	Number Evaluation		Risk of Meta-Analysis		Heterogeneity		Venice criteria Grade[c]	FPRP values[d]	Credibility of evidence[e]
								Study	Sample size (case/controls)	OR (95% CI)	P value	I^2 (%)	P_Q			
ERCC4	rs744154	C vs. G	Bladder	Asian	0.7213	Allelic	Fixed	2	484 (234/250)	1.566 (1.233-1.989)	0.000	0.0	0.789	AAA	0.012	Strong
ERCC4	rs744154	C vs. G	Bladder	Asian	0.7213	Recessive	Fixed	2	484 (234/250)	1.731 (1.296-2.313)	0.000	0.0	0.862	BAA	0.023	Strong
ERCC4	rs1800067	A vs. G	Brain (Glioma)	Overall	0.7253	Allelic	Random	5	3502 (1481/2021)	0.634 (0.426-0.944)	0.025	82.9	0.000	ACC	0.540	Weak
ERCC4	rs1800067	A vs. G	Brain (Glioma)	Overall	0.7253	Recessive	Random	5	3502 (1481/2021)	0.528 (0.350-0.796)	0.002	69.3	0.011	ACA	0.247	Weak
ERCC4	rs1800067	A vs. G	Brain (Glioma)	Asian	0.9263	Allelic	Random	4	2645 (1119/1526)	0.543 (0.389-0.760)	0.000	69.5	0.020	ACA	0.057	Weak
ERCC4	rs1800067	A vs. G	Brain (Glioma)	Asian	0.9263	Dominant	Random	4	2645 (1119/1526)	0.502 (0.312-0.808)	0.005	0.0	0.825	AAC	0.416	Weak
ERCC4	rs1800067	A vs. G	Brain (Glioma)	Asian	0.9263	Recessive	Random	4	2645 (1119/1526)	0.535 (0.347-0.826)	0.005	76.7	0.005	ACC	0.361	Weak
ERCC4	rs2276466	G vs. C	Brain (Glioma)	Asian	0.3243	Allelic	Fixed	2	930 (432/498)	1.332 (1.101-1.612)	0.003	0.0	0.878	BAA	0.065	Moderate
ERCC4	rs2276466	G vs. C	Brain (Glioma)	Asian	0.3243	Dominant	Fixed	2	930 (432/498)	1.336 (1.030-1.733)	0.029	0.0	0.881	BAA	0.4240	Weak
ERCC4	rs2276466	G vs. C	Brain (Glioma)	Asian	0.3243	Recessive	Fixed	2	930 (432/498)	1.553 (1.094-2.206)	0.014	0.0	0.917	BAA	0.385	Weak
ERCC4	rs1799801	C vs. T	Stomach	Overall	0.4027	Dominant	Fixed	3	2796 (1327/1469)	0.755 (0.614-0.930)	0.008	42.2	0.177	ABA	0.151	Moderate
ERCC4	rs744154	C vs. G	Stomach	Asian	0.4854	Allelic	Fixed	2	1504 (681/823)	0.790 (0.666-0.937)	0.007	62.2	0.104	ACA	0.117	Weak
ERCC4	rs744154	C vs. G	Stomach	Asian	0.4854	Dominant	Fixed	2	1504 (681/823)	0.681 (0.535-0.866)	0.020	37.6	0.2060	BBA	0.055	Moderate
ERCC5	rs17655	C vs. G	Blood	Overall	0.2597	Allelic	Random	7	5100 (2592/2508)	1.176 (1.017-1.360)	0.029	50.2	0.061	ACA	0.354	Weak
ERCC5	rs17655	C vs. G	Blood	Overall	0.2597	Dominant	Fixed	7	5100 (2592/2508)	1.169 (1.041-1.313)	0.009	37.3	0.144	ABA	0.138	Moderate
ERCC5	rs17655	C vs. G	Blood	Caucasian	0.2517	Allelic	Random	4	2114 (1014/1100)	1.298 (1.007-1.671)	0.044	59.0	0.062	ACA	0.484	Weak
ERCC5	rs17655	C vs. G	Blood	Caucasian	0.2517	Dominant	Fixed	4	2114 (1014/1100)	1.285 (1.065-1.550)	0.009	61.0	0.051	BCA	0.149	Weak
ERCC5	rs17655	C vs. G	Blood	Caucasian	0.2517	Recessive	Fixed	4	2114 (1014/1100)	1.511 (1.093-2.090)	0.013	0.0	0.472	BAA	0.332	Weak
ERCC5	rs1047768	C vs. T	Colorectum	Asian	0.2815	Recessive	Random	2	55889 (2755/2834)	1.218 (1.006-1.474)	0.044	0.0	0.367	BAA	0.452	Weak
ERCC5	rs1047768	C vs. T	Colorectum	Caucasian	0.5512	Allelic	Fixed	2	3305 (1135/2170)	0.876 (0.774-0.991)	0.036	44.5	0.180	BBC	0.402	Weak
ERCC5	rs17655	C vs. G	Colorectum	Overall	0.3667	Allelic	Fixed	13	17943 (8068/9875)	1.053 (1.006-1.102)	0.027	18.3	0.259	AAC	0.331	Weak
ERCC5	rs17655	C vs. G	Colorectum	Overall	0.3667	Dominant	Random	13	17943 (8068/9875)	1.132 (1.020-1.255)	0.019	53.3	0.012	ACC	0.260	Weak
ERCC5	rs17655	C vs. G	Colorectum	Caucasian	0.2283	Dominant	Random	7	7206 (2858/4348)	1.118 (1.006-1.242)	0.038	0.0	0.431	AAC	0.417	Weak
ERCC5	rs2094258	T vs. C	Colorectum	Asian	0.3394	Allelic	Fixed	2	5589 (2755/2834)	1.128 (1.043-1.219)	0.002	9.0	0.295	AAC	0.043	Moderate
ERCC5	rs2094258	T vs. C	Colorectum	Asian	0.3394	Dominant	Fixed	2	5589 (2755/2834)	1.141 (1.025-1.270)	0.015	0.0	0.706	AAC	0.231	Weak
ERCC5	rs2094258	T vs. C	Colorectum	Asian	0.3394	Recessive	Fixed	2	5589 (2755/2834)	1.232 (1.051-1.445)	0.010	59.8	0.115	BCA	0.165	Weak
ERCC5	rs2296147	T vs. C	Esophagus	Asian	0.2550	Allelic	Fixed	2	4292 (1672/2620)	0.825 (0.741-0.919)	0.000	0.0	0.531	AAA	0.009	Strong
ERCC5	rs2296147	T vs. C	Esophagus	Asian	0.2550	Dominant	Fixed	2	4292 (1672/2620)	0.783 (0.687-0.892)	0.000	0.0	0.868	AAA	0.004	Strong
ERCC5	rs17655	C vs. G	Head and Neck	Overall	0.4481	Recessive	Random	6	6772 (2919/3853)	0.787 (0.627-0.989)	0.040	58.8	0.033	ACC	0.451	Weak
ERCC5	rs17655	C vs. G	Head and Neck	Asian	0.5252	Dominant	Fixed	3	2069 (783/1286)	0.796 (0.649-0.976)	0.028	54.0	0.114	ACC	0.360	Weak
ERCC5	rs17655	C vs. G	Laryngeal	Overall	0.3732	Recessive	Fixed	3	1667 (634/1033)	0.571 (0.533-0.753)	0.000	0.0	0.933	BAC	0.010	Moderate

(Continued)

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TABLE 1 Continued

Gene	Variant	Alleles[a]	Cancer site	Ethnicity	MAF[b]	Genetic models	Effect model	Number Evaluation		Risk of Meta-Analysis		Heterogeneity		Venice criteria Grade[c]	FPRP values[d]	Credibility of evidence[e]
								Study	Sample size (case/controls)	OR (95% CI)	P value	I^2 (%)	P_Q			
ERCC5	rs17655	C vs. G	Laryngeal	Asian	0.6049	Allelic	Random	2	772 (386/386)	0.636 (0.520-0.779)	0.000	0.0	0.683	BAA	0.001	Strong
ERCC5	rs17655	C vs. G	Laryngeal	Asian	0.6049	Dominant	Random	2	772 (386/386)	0.613 (0.444-0.847)	0.003	0.0	0.770	BAA	0.158	Moderate
ERCC5	rs17655	C vs. G	Laryngeal	Asian	0.6049	Recessive	Fixed	2	772 (386/386)	0.574 (0.427-0.772)	0.000	0.0	0.720	BAA	0.025	Strong
ERCC5	rs2228959	A vs. C	Lung	Asian	0.2186	Allelic	Fixed	2	984 (492/492)	0.370 (0.283-0.484)	0.000	61.6	0.107	BCA	0.000	Moderate
ERCC5	rs2228959	A vs. C	Lung	Asian	0.2186	Dominant	Random	2	984 (492/492)	0.429 (0.188-0.979)	0.044	66.9	0.082	BCA	0.852	Weak
ERCC5	rs17655	C vs. G	Oral	Asian	0.4433	Allelic	Fixed	2	965 (424/541)	1.334 (1.105-1.611)	0.003	0.0	0.365	BAA	0.054	Moderate
ERCC5	rs17655	C vs. G	Oral	Asian	0.4433	Dominant	Fixed	2	965 (424/541)	1.414 (1.059-1.889)	0.019	0.0	0.974	BAA	0.359	Weak
ERCC5	rs17655	C vs. G	Oral	Asian	0.4433	Recessive	Fixed	2	965 (424/541)	1.485 (1.082-2.038)	0.014	52.3	0.148	BCA	0.347	Weak
ERCC5	rs17655	C vs. G	Prostate	Overall	0.3844	Dominant	Fixed	4	4755 (2128/2627)	1.149 (1.005-1.312)	0.042	24.8	0.263	AAC	0.419	Weak
ERCC5	rs17655	C vs. G	Stomach	Caucasian	0.5808	Allelic	Fixed	2	1493 (643/850)	1.282 (1.024-1.606)	0.030	18.3	0.269	AAA	0.404	Moderate
ERCC5	rs17655	C vs. G	Stomach	Caucasian	0.5808	Recessive	Random	2	1493 (643/850)	1.513 (1.126-2.034)	0.006	0.0	0.686	BAA	0.187	Moderate
ERCC5	rs751402	G vs. A	Stomach	Asian	0.6558	Allelic	Random	10	8565 (3989/4576)	0.865 (0.784-0.954)	0.004	51.8	0.028	ACA	0.044	Moderate
ERCC5	rs751402	G vs. A	Stomach	Asian	0.6558	Dominant	Random	10	8565 (3989/4576)	0.802 (0.657-0.980)	0.031	50.4	0.034	ACC	0.379	Weak
ERCC5	rs751402	G vs. A	Stomach	Asian	0.6558	Recessive	Fixed	10	8565 (3989/4576)	0.867 (0.794-0.946)	0.001	6.7	0.380	AAA	0.010	Strong
ERCC5	rs873601	A vs. G	Stomach	Asian	0.4757	Allelic	Fixed	6	8717 (4177/4540)	1.069 (1.007-1.135)	0.029	29.2	0.216	ABC	0.444	Weak
ERCC5	rs873601	A vs. G	Stomach	Asian	0.4757	Recessive	Fixed	6	8717 (4177/4540)	1.133 (1.026-1.251)	0.014	22.5	0.265	AAC	0.195	Weak
ERCC5	rs17655	C vs. G	Thyroid	Caucasian	0.5551	Recessive	Fixed	2	647 (181/466)	0.501 (0.313-0.801)	0.004	0.0	0.491	BAA	0.388	Weak
ERCC5	rs17655	C vs. G	Uterus	Overall	0.3544	Allelic	Random	5	4431 (1936/2495)	1.239 (1.050-1.463)	0.011	63.5	0.027	ACA	0.181	Weak
ERCC5	rs17655	C vs. G	Uterus	Overall	0.3544	Dominant	Random	5	4431 (1936/2495)	1.315 (1.039-1.664)	0.023	63.5	0.027	ACC	0.332	Weak
ERCC5	rs17655	C vs. G	Uterus	Overall	0.3544	Recessive	Fixed	5	4431 (1936/2495)	1.355 (1.142-1.608)	0.001	0.0	0.482	AAA	0.011	Strong
ERCC5	rs17655	C vs. G	Cervix	Asian	0.4764	Allelic	Random	2	1800 (678/1122)	1.365 (1.190-1.565)	0.000	0.0	0.483	AAA	0.000	Strong
ERCC5	rs17655	C vs. G	Cervix	Asian	0.4764	Dominant	Random	2	1800 (678/1122)	1.618 (1.286-2.035)	0.000	0.0	0.820	AAA	0.003	Strong
ERCC5	rs17655	C vs. G	Cervix	Asian	0.4764	Recessive	Fixed	2	1800 (678/1122)	1.387 (1.121-1.715)	0.003	43.6	0.183	BBA	0.059	Moderate

OR, odds ratio; A, adenine; T, thymine; G, guanine; C, cytosine; ERCC: excision repair cross-complementation.
[a]Minor alleles vs. major alleles (reference).
[b]Frequency of minor allele in controls.
[c]Strength of epidemiological evidence based on the Venice criteria.
[d]FPRP values at prior probability of 0.05 at power OR of 1.5, and the FPRP level of noteworthiness is 0.20.
[e]Degree of epidemiological credibility based on the combination of results from Venice guidelines and FPRP tests.

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TABLE 2 Variants showing no relation to cancer risk in meta-analyses with at least 3,000 cases and 3,000 controls in additive model.

Gene	Variant	Alleles[a]	Cancer site	Ethnicity	MAF[b]	Effect model	Number Evaluation		Risk of Meta-Analysis		Heterogeneity		Power (%)	The value of Power (%) if the MAF is 0.2	The value of power (%) if the MAF is 0.1
							Studies	Sample size (case/controls)	OR (95% CI)	P value	I^2 (%)	P_Q			
ERCC4	rs1800067	A vs. G	Breast	Overall	0.1040	Random	12	17,885 (9,310/8,575)	1.011 (0902-1.133)	0.850	37.5	0.091	98.2	100.0	97.9
ERCC4	rs1800067	A vs. G	Breast	Caucasian	0.0696	Random	9	15,228 (7,936/7,292)	1.042 (0.924-1.176)	0.502	38.6	0.111	87.9	99.8	95.7
ERCC4	rs744154	C vs. G	Breast	Overall	0.3517	Fixed	4	81,066 (41,439/39,627)	1.000 (0.978-1.023)	0.997	16.5	0.309	100.0	100.0	100.0
ERCC4	rs744154	C vs. G	Breast	Caucasian	0.3598	Fixed	2	75,040 (3,8088/36,952)	0.996 (0.972-1.019)	0.714	40.3	0.195	100.0	100.0	100.0
ERCC5	rs17655	C vs. G	Breast	Overall	0.3003	Random	15	17,222 (8,341/8,881)	1.039 (0.959-1.125)	0.355	51.6	0.011	100.0	99.9	97.6
ERCC5	rs17655	C vs. G	Breast	Caucasian	0.2392	Random	8	12,053 (5,873/6,180)	1.071 (0.962-1.195)	0.109	58.8	0.017	99.6	99.2	91.0
ERCC5	rs17655	C vs. G	Lung	Overall	0.4252	Random	11	9,728 (4,284/5,444)	1.078 (0.961-1.209)	0.199	65.2	0.001	99.5	96.2	80.5
ERCC5	rs17655	C vs. G	Skin	Caucasian	0.3245	Fixed	10	11,284 (5,162/6,122)	0.951 (0.890-1.015)	0.133	0.0	0.670	99.7	98.4	87.2
ERCC5	rs2094258	T vs. C	Stomach	Asian	0.3885	Random	9	9,884 (4,648/5,236)	1.022 (0.929-1.124)	0.652	54.9	0.023	99.7	97.3	83.6
ERCC5	rs2296147	C vs. T	Stomach	Asian	0.2121	Fixed	5	7,589 (3,699/3,890)	0.989 (0.915-1.069)	0.778	33.7	0.197	94.3	93.4	74.6

OR, odds ratio; A, adenine; C, cytosine; G, guanine; T, thymine; ERCC: excision repair cross-complementation.
[a]Minor alleles vs. major alleles (reference).
[b]Frequency of minor allele in controls.

relationships; moderate (25% < I^2 < 50%) and large (I^2 ≥ 50%) heterogeneity were figured out in 6 (11.1%) and 19 (35.2%) associations.(Table 1) The results of sensitivity analysis are shown in Table 1. We identified that 12 associations were dusted on the account of the removal of a single study (dataset); the first published and/or studies deviated from HWE in controls, including rs1800067 with glioma in the overall population in the allelic model and in the Asians in the dominant model, rs17655 with colorectal cancer in overall population in the allelic model and in Caucasians in the dominant model, rs17655 with head and neck cancer in the overall population in recessive and in Asians in the dominant model, rs17655 with laryngeal cancer in the overall population in the recessive model, rs17655 with prostate cancer in the overall population in dominant model, rs751402 and gastric cancer in Asians in the dominant model, rs873601 and gastric cancer in the allelic and recessive model, and rs17655 with uterine cancer in the overall population in the dominant model. The evidence of significant publication bias (p < 0.1) was found in two connections (rs17655 with head and neck cancer in the overall population in recessive, rs1800067 with glioma in Asians in the recessive model). We could not test the excess of significant finding because of the absence of data of the genotype or allele in most of the studies (Supplementary Table S4).

Cumulative evidence of significant findings

We conducted epidemiological evidence evaluation on all of the 54 significant associations, 10 of which were rated as strong credibility, 13 results were rated as moderate credibility, and 31 associations were rated as weak credibility (Table 1). Firstly, by assessing the amount of evidence of the Venice criteria, we got 32 relationships that were assigned grade A, and 22 others were assigned grade B. In terms of replication, grade A was distributed in 29 findings, grade B in 6 findings, and grade C in 19 results. As for protection from bias, grades A, B, and C were assigned to 37, 0, and 17 associations. In summary, 17, 8, and 29 evidence were separately determined as strong, moderate, and weak credibility in the Venice criteria (Table 2). Subsequently, the FPRP values of all the significant findings were computed for the evaluation of the probability of true association. With the result of the FPRP value < 0.05, the rate of credibility was upgraded from moderate to strong in three findings (rs744154 and bladder cancer in Asians in the recessive model, rs17655 and laryngeal cancer in Asians in allelic and recessive models), and from weak to moderate in four associations (rs2094258 and colorectal cancer in Asians in the allelic model, rs17655 and laryngeal cancer in the overall population in the recessive model, rs2228959 and lung cancer in Asians in the allelic model, rs751402 and gastric cancer in Asians in the allelic model). On the contrary, owing to FPRP values >0.2, the credibility of evidence in one connection

(rs17655 and gastric cancer in Caucasians in the allelic model) were downgraded from strong to moderate, and six of the findings (rs2276466 and glioma in Asians in dominant and recessive models, rs17655 and leukemia in Caucasians in the recessive model, rs1047768 and colorectal cancer in Asians in the recessive model, rs17655 and oral cancer in Asians in the dominant model, rs17655 and thyroid cancer in Caucasians in the recessive model) were downgraded from moderate to weak. Ultimately, we got 10 strong-credibility evidence incorporating rs744154 and bladder cancer in Asians in allelic and recessive models, rs2296147 and esophageal cancer in Asians in allelic and dominant models, rs17655 and laryngeal cancer in Asians in allelic and recessive models, rs751402 and gastric cancer in Asians in the recessive model, and rs17655 and uterine cancer in overall population in the recessive model and in Asians in allelic and dominant models (Supplementary Table S5).

Functional annotation

Referring to the data gained from the Encyclopedia of DNA Elements tool HaploReg v4.1, we analyzed the functional roles of those four variants strongly associated with five cancers (Table 3). Results showed that rs744154 mapped to intronic regions, rs2296147 and rs751402 mapped to 5'UTR, and rs17655 was annotated as missense. All these four SNPs might be identified as expression quantitative trait loci (eQTLs) for many genes in various tissue types; two SNPs might be located within the histone modification regions of enhancers and three SNPs in promoters and sites exhibiting DNase I hypersensitivity. Furthermore, we also found that rs2296147 and rs751402 had the alteration in transcription factor binding and all these four variants may affect transcriptional regulatory element activity in this region. Subsequently, as the consequence of the function evaluation using the PolyPhen-2 web server (34), the unique non-synonymous variant rs17655 was qualitatively predicted to be "probably damaging" with a naïve Bayes posterior probability of more than 0.85 (Figure 2). In addition, the linkage disequilibrium (LD) plots explained that the regions represented by significant SNPs had distinct genetic structures among European, Asian, and African ancestries (Figure 3, Figure 4). In addition, the Genotype-Tissue Expression Project revealed that rs744154 is eQTLs for *ERCC4*, whereas rs2296147, rs17655, and rs751402 are eQTLs for *ERCC5*, respectively. Additionally, rs744154 is associated with a decrease in *ERCC4* gene expression in muscle tissues and in *MKL2* gene expression in colon tissues; rs2296147 is associated with a decrease in *BIVM* gene expression and an increase in *METTL21EP* gene expression in esophagus tissues; and rs751402 is associated with a decrease in *BIVM* gene expression in breast tissues and in *ERCC5* gene expression in esophagus tissues (Supplementary Table S6).

Discussion

The *NER* pathway plays a crucial role in maintaining genomic integrity and preventing carcinogenesis by continuously monitoring and repairing various forms of DNA damage (35). *ERCC4* and *ERCC5* were indispensable component members of the *NER* pathway; numerous studies were conducted to investigate the correlations between the SNPs of *ERCC4* or *ERCC5* and the risk of cancers. However, most previous meta-analyses focused unilaterally on a single SNP and/or an individual cancer type; furthermore, the conclusions of which were inconsonant, resulting from the related small sample size and diversity of population (22, 36–38). To the best of our knowledge, the present study was the first work to comprehensively elucidate whether the studied variants of both *ERCC4* and *ERCC5* were associated with cancer risk and then to evaluate the credibility of significantly epidemiological evidence using the Venice criteria and FPRP tests. We exacted data from a total of 472 datasets in 160 literatures; the relationship among 19 types of cancers and 25 polymorphisms was involved into meta-analyses for assessment. We had 54 associations to be demonstrated as statistically significant, as mentioned above, 10 of which were rated as strong-credibility

TABLE 3 Summary of functional annotations for four single-nucleotide polymorphisms in ERCC4 and ERCC5 with five cancer sites risk (strong epidemiological credibility).

Variant	Gene	Position[a]	Annotation	Promoter histone marks[b]	Enhancer histone marks[c]	DNAse[d]	Proteins bound[e]	Motifs changed[f]
rs744154	ERCC4	13921224	Intronic	17 tissues	17 tissues	6 tissues		Smad
rs2296147	ERCC5	102846025	5'-UTR	24 tissues		53 tissues	17 bound proteins	BDP1
rs17655	ERCC5	102875652	Missense		ESDR, BRN			DEC,GR,Nkx2
rs751402	ERCC5	102845848	5'-UTR	24 tissues		53 tissues	48 bound proteins	E2F,IRC900814,Pou3f2

[a]The chromosome position is based on NCBI Build 37;
[b]Histone modification of H3K4me1 and H3K27ac (tissue types: if >3, only the number is included);
[c]Histone modification of H3K4me3 (tissue types: if >3, only the number is included);
[d]Levels of DNase I hypersensitivity (tissue types: if >3, only the number is included);
[e]Alteration in transcription factor binding (disruptions: if >3, only the number is included);
[f]Alteration in regulatory motif (disruptions: if >3, only the number is included).

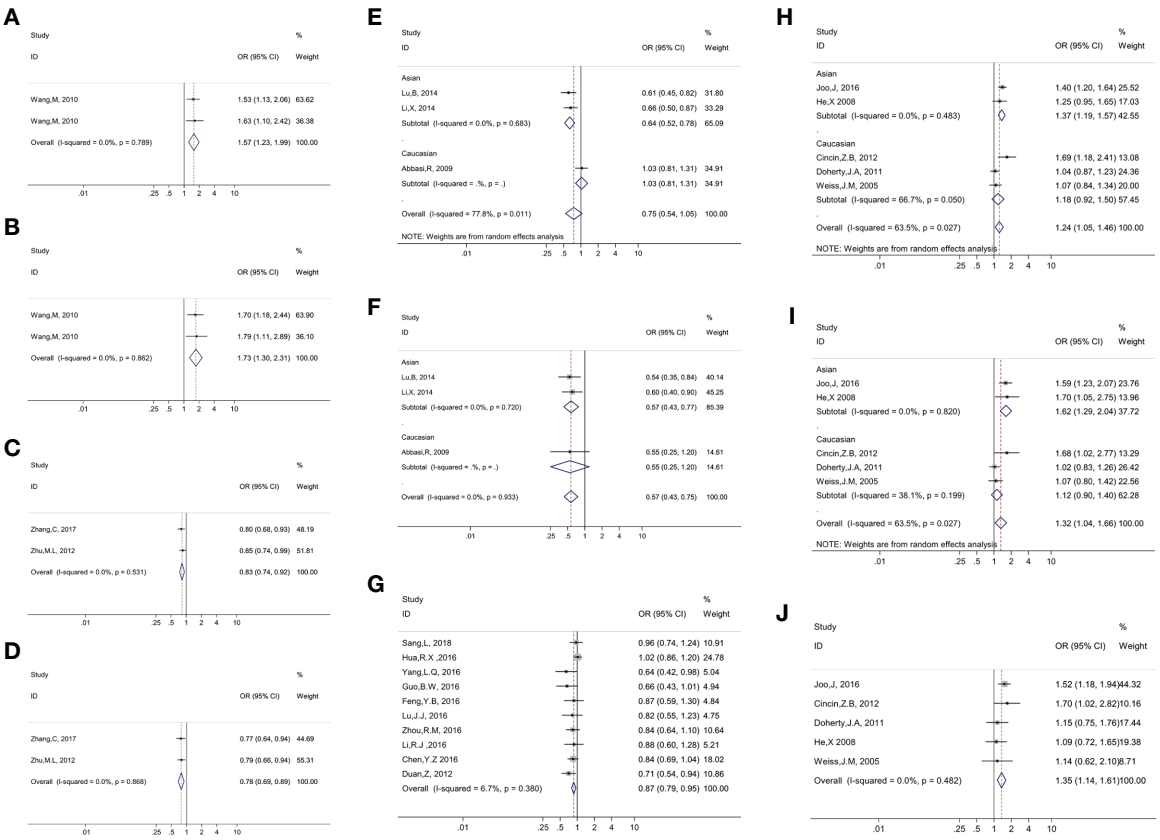

FIGURE 2
Presented forest plot of 10 strong-credibility evidence: **(A).** association between ERCC4 rs744154 and bladder cancer risk in the Asian population in the allelic model; **(B).** association between ERCC4 rs744154 and bladder cancer risk in the Asian population in the recessive model; **(C).** association between ERCC5 rs2296147 and esophageal cancer risk in the Asian population in the allelic model; **(D).** association between ERCC5 rs2296147 and esophageal cancer risk in the Asian population in the dominant model; **(E).** association between ERCC5 rs17655 and laryngeal cancer risk in the allelic model, stratified by ethnicity; **(F).** association between ERCC5 rs17655 and laryngeal cancer risk in the recessive model, stratified by ethnicity; **(G).** association between ERCC5 rs751402 and gastric cancer risk in Asian population in the recessive model; **(H).** association between ERCC5 rs17655 and Uterine cancer risk in the allelic model, stratified by ethnicity; **(I).** association between ERCC5 rs17655 and uterine cancer risk in the dominant model, stratified by ethnicity; **(J).** association between ERCC5 rs17655 and uterine cancer risk in overall population in the recessive model.

evidence; moderate and weak credibility were graded to 13 and 31 significant findings. Moreover, the result of functional annotation indicated that these four SNPs (rs744154 in *ERCC4*, rs2296147, and rs17655 and rs751402 in *ERCC5*) with a strong evidence of a significant association might fall in several putative functional regions of *ERCC4* and *ERCC5* genes (Table 3). Briefly, our research offers comprehensive epidemiological evidence that common variants of the *ERCC4* and *ERCC5* genes show association with the predisposition of bladder cancer, esophageal cancer, laryngeal cancer, uterine cancer, and gastric cancer.

An obligate heterodimer complex is formed by proteins encoded by *ERCC4* and *ERCC1* genes, which could operate a 5' incision to the DNA lesion in the irreversible dual-incision process of *NER* (39). The current evidence showed that four SNPs of the *ERCC4* gene (rs744154, rs1800067, rs2276466, and rs1799801) were significantly associated with risk of three cancers (bladder cancer, glioma, and gastric cancer). A former meta-analysis reported that no significant correlation was found between rs744154 and cancer risk (36), but with a larger sample size, we revealed a strong effect of increasing bladder cancer risk with rs744154 in Asian population under allelic and recessive models. In the same population, C allele and GC/CC genotypes were related to a protective effect on the gastric cancer risk compared with the G allele and GG genotype. Wang et al. and Chu et al. indicated that rs744154 was in LD with -357A > C polymorphism in the *ERCC4* promoter, then altered the expression of *ERCC4* mRNA and protein, and finally affected the susceptibility to bladder and gastric cancer (40, 41). Meanwhile, the TC/CC genotypes of rs1799801 polymorphism were proven to be protective factors of gastric cancer in comparison with the TT genotype in overpopulation; however,

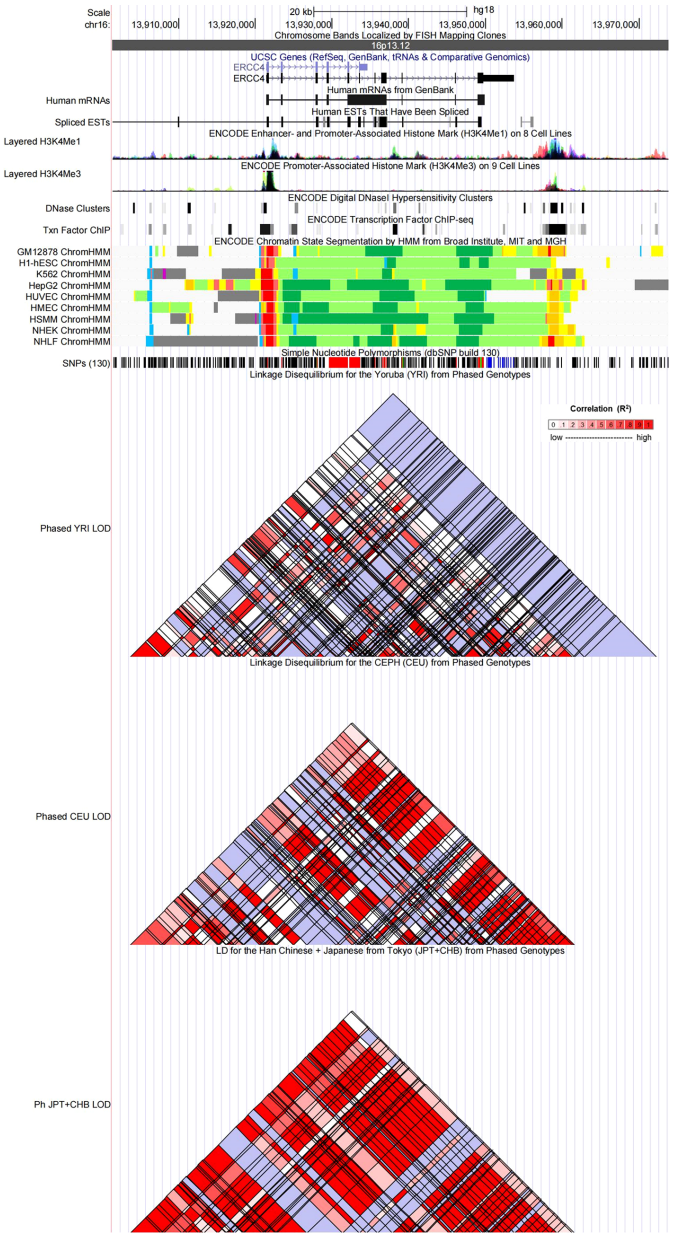

FIGURE 3
Evidence from the Encyclopedia of DNA Elements (ENCODE) data for the regulatory function of variants in 16p13.12 using the UCSC Genome Browser. The plot represents 16p13.12 within a 20-kb window centered on the ERCC4 gene region. Tracks (from top to bottom) in each of the plots are Genome Base Position, Chromosome Bands, UCSC Genes, Human messenger RNAs from GenBank, Human expressed sequence tag (ESTs) That Have Been Spliced, ENCODE Enhancer and Promoter-Associated Histone Mark (H3K4Me1) on 8 Cell Lines, ENCODE Promoter-Associated Histone Mark (H3K4Me3) on 9 Cell Lines, ENCODE Digital DNaseI Hypersensitivity Clusters, ENCODE Transcription Factor ChIP-seq, ENCODE Chromatin State Segmentation by Hidden Markov Model (HMM) from Broad Institute (bright red, active promoter; light red, weak promoter; purple, inactive/poised promoter; orange, strong enhancer; yellow, weak/poised enhancer; blue, insulator; dark green, transcriptional transition/elongation; light green, weak transcribed; gray, polycomb-repressed; light gray, heterochromatin/low signal/repetitive/copy number variation), Simple Nucleotide Polymorphisms (dbSNP build 130), Linkage Disequilibrium for the Yoruba (YRI) from Phased Genotypes, Linkage Disequilibrium for the CEPH (CEU) from Phased Genotypes and LD for the Han Chinese + Japanese from Tokyo (JPT+CHB) from Phased Genotypes.

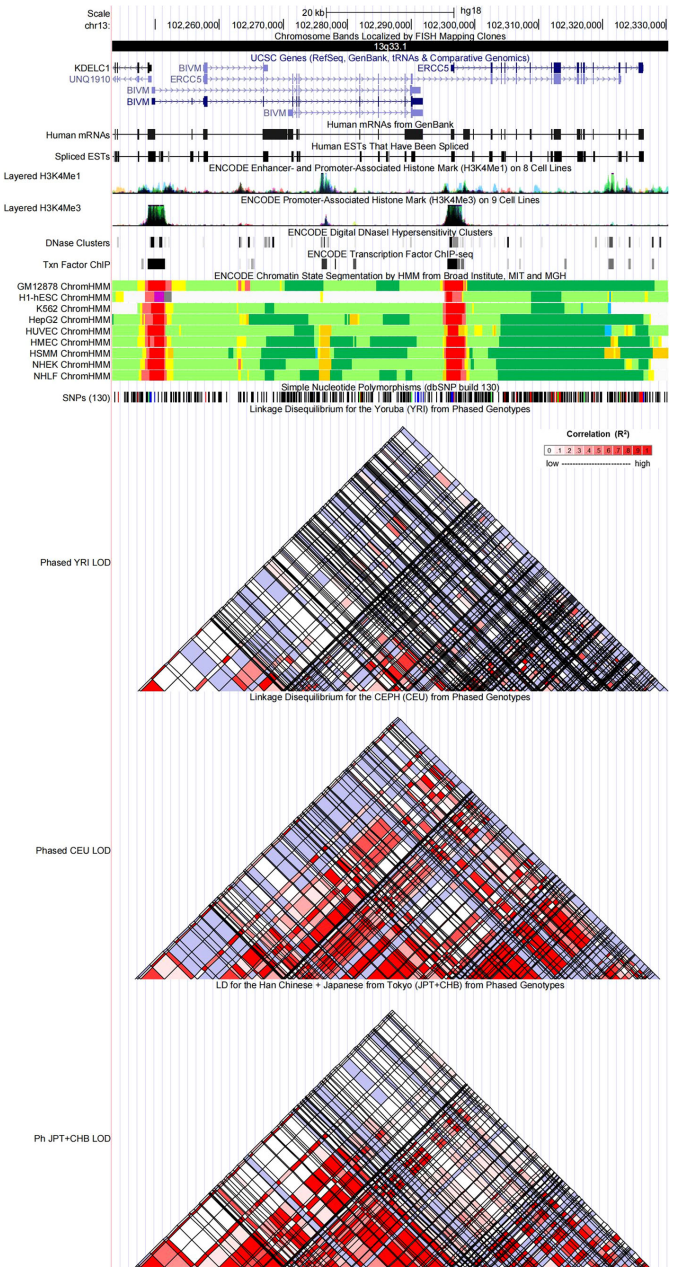

FIGURE 4
Evidence from the ENCODE data for the regulatory function of variants in 13q33.1 using the UCSC Genome Browser. The plot represents 13q33.1 within a 20-kb window centered on the ERCC5 gene region. Tracks (from top to bottom) in each of the plots are Genome Base Position, Chromosome Bands, UCSC Genes, Human messenger RNAs from GenBank, Human expressed sequence tag (ESTs) That Have Been Spliced, ENCODE Enhancer and Promoter-Associated Histone Mark (H3K4Me1) on 8 Cell Lines, ENCODE Promoter-Associated Histone Mark (H3K4Me3) on 9 Cell Lines, ENCODE Digital DNaseI Hypersensitivity Clusters, ENCODE Transcription Factor ChIP-seq, ENCODE Chromatin State Segmentation by Hidden Markov Model (HMM) from Broad Institute (bright red, active promoter; light red, weak promoter; purple, inactive/poised promoter; orange, strong enhancer; yellow, weak/poised enhancer; blue, insulator; dark green, transcriptional transition/elongation; light green, weak transcribed; gray, polycomb-repressed; light gray, heterochromatin/low signal/repetitive/copy number variation), Simple Nucleotide Polymorphisms (dbSNP build 130), Linkage Disequilibrium for the Yoruba (YRI) from Phased Genotypes, Linkage Disequilibrium for the CEPH (CEU) from Phased Genotypes and LD for the Han Chinese + Japanese from Tokyo (JPT+CHB) from Phased Genotypes.

the statistical significance only appeared in Caucasians when exerting subgroup analysis, not in Asians, considering there was a single dataset of Caucasians. Larger-group studies were needed to further confirm this association. The genetic variants of *ERCC4* were reported to be associated with glioma risk before (42), and in our study, we discovered that rs1800067 and rs2276466 could alter the susceptibility of glioma, but with the reason of the replication of studies, protection from bias and/or FPRP > 0.2, the strength of evidence was moderate or weak, following different genetic models. Further studies are recommended for improving the confidence level of evidence.

The product expressed by *ERCC5* (*XPG*) is an endonuclease, which is mainly in charge of recognizing and cutting DNA lesions on the 3' side, and the genetic alterations of *ERCC5* might impact the DNA repair capacity as a result of insufficient and loss-of-function proteins, thereby causing the initiation of carcinogenesis (5, 12, 43). As the result of our meta-analysis, seven SNPs were significantly linked to the risk of various types of cancer, including rs17655, rs1047768, rs2094258, rs2296147, rs2228959, rs751402, and rs873601, and we obtained four significant findings (rs17655 in laryngeal and uterine cancer, rs2296147 in esophageal cancer, and rs751402 in gastric cancer) with strong credibility for accumulating epidemiological evidence. The phase 3 of the 1000 Genomes Project (44) (Supplementary Table S7) suggested that rs2296147 is in weak LD with rs17655 in Asians (r^2 = 0.2366), Africans (r^2 = 0.1354), and Europeans (r^2 = 0.1354) and is in weak LD with rs751402 in Asians (r^2 = 0.1470), Africans (r^2 = 0.0883), and Europeans (r^2 = 0.2125); rs17655 is in weak LD with rs751402 in Asians (r^2 = 0.2450), Africans (r^2 = 0.1948), and Europeans (r^2 = 0.0679). According to the results, there might exist different causal variants and functional mechanisms in the relationships of variants in the *ERCC5* genes with esophageal cancer, gastric cancer, laryngeal cancer, and uterus and cervical cancer predisposition. Current evidence showed that rs17655 (C vs. G) polymorphism is the most widely studied SNP of *ERCC5*, triggering a replacement of a single amino acid from aspartate to histidine (4). Similar to the result of former research (20), we found that the C allele and CC+ GC genotype could strongly reduce the risk of laryngeal cancer among Asian individuals. Li et al. also pointed out that the variant-related risk may be adjusted by the smoking and alcohol drinking status (20). Apart from laryngeal cancer, the current evidence showed that variant rs17655 was strongly associated with uterine cancer, but conversely, the heterozygotes and homozygotes of this variant were linked to an increased risk of uterine cancer than wild controls. We further observed that this strong evidence of correlation was mainly derived from rs17655 with cervical cancer in the Asian population when stratified by ethnicity and the cancer type; null association was found in Caucasians with endometrial cancer.

In the past couple of years, the relationship between *ERCC5* variants and gastric cancer risk drew much attention of researchers, especially for Chinese investigators. It has been reported that rs873601 (A vs. G), rs2296147 (C vs. T), rs2094258 (T vs. C), rs751402 (G vs. A) were significantly associated with increased or decreased susceptibility of gastric cancer (45–47). What's interesting is that null significant finding appeared in a study which exploring correlation of all above mentioned SNPs of *ERCC5* and gastric cancer risk (48). With the largest sample size thus far in present study, we acquired that three variants of *ERCC5* were probably involved in carcinogenesis of gastric cancer, rs17655 in Caucasians and rs873601 in Asians was related to an increased risk. rs751402 was strongly associated with a reduced risk of gastric cancer in Asian population. Former study has reported that accumulation of these risk genotypes could reinforce the link between *ERCC5* and gastric cancer risk (48), and some studies figured out that the *Helicobacter pylori* infection may enhance the genetic effect on altered gastric cancer risk (48, 49).

Another strong evidence of association was that rs2296147 (T vs. C) mutant of *ERCC5* could significantly downregulate the susceptibility of esophageal cancer in Asians under allelic and dominant genetic models. A previous study indicated that the locus rs2296147 is located in the 5'UTR, possibly the transcription factor– binding site (TFBS), which hinted that the mutant rs2296147 might affect the transcription process and finally affect the development of malignant tumor (50, 51). However, the specific mechanisms of a potential carcinogenic effect is still unclear; further studies are necessary for finding out whether the rs2296147 locus is the pathogenic SNP.

In this present study, we evaluated the associations between 78 SNPs in *ERCC4* and *ERCC5* and 22 cancer risks based on each SNP extracted from one data source, and then calculated the FPRP values of significant findings; two associations (rs2276466 with gastric cancer risk in Asians, rs2094258 with neuroblastoma in Asians) were affirmed as strong evidence (Supplementary Table S8). This will provide reference directions for future research.

Our study demonstrated that two SNPs on *ERCC4* and three SNPs on *ERCC5* were observed in a sample of at least 3,000 cases, 3,000 controls, which offers over 89% power to detect an OR of 1.15 in an allelic model for a variant with an MAF of 20%. Further investigations evaluating the following relationships (rs1800067, rs744154 and rs17655 with breast cancer, rs17655 with lung cancer and skin cancer, and rs2094258 and rs2296147 with stomach cancer) will probably not yield meaningful results.

In spite of the largest-sample and comprehensive evaluation of variants related to the risk of cancers, there were several limitations in our study. Firstly, a few literatures might be neglected because we only enrolled studies published in English and the searching stratagem had some drawbacks. Secondly, we could not exact sufficient data for the assessment of the interaction among different variants and the adjustment effect of environment factors like smoking, *H. pylori* infection, and others. Thirdly, we did not carry out a detailed subgroup

analysis of cancer types ascribed to the heterogeneity of cancer typing among eligible studies. Fourthly, the excess of significant findings was not further evaluated due to insufficient data. Finally, some of the significant findings were identified with moderate or weak credibility; part of the reasons may be generated from related small samples in the subgroup of ethnicity under different genetic models, so studies with sufficient subgroups are warranted for the validation of our findings.

Conclusion

In this extensively updated meta-analysis, 11 SNPs were proven to be significantly associated with cancer risk, and four variant-related cancer risks (one for *ERCC4* and three for *ERCC5*) were graded as strong-credibility cumulative epidemiological evidence. These results of high statistical efficacy confirmed once again that SNPs in DNA repair genes play a crucial role in the development of cancer. Therefore, future studies on the pathogenesis of these genes will be conducive to improving the prevention and treatment of cancer.

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.

Author contributions

Specific contributions are as follows: study design: HC, CZ, TL. Data collection: CZ, XL, ZY. Data management and analysis: CZ, LY, CY. Manuscript drafting: XL, CZ. Manuscript review: all. All authors read and approved the final manuscript.

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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.

The reviewer XM declare a shared parent affiliation with the author CZ to the handling editor at the time of review.

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Supplementary material

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Current understanding on the role of CCT3 in cancer research

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Chaperonin containing TCP1 Subunit 3 (CCT3) is an important member of the chaperone protein family, providing a favorable environment for the correct folding of proteins in cell division, proliferation, and apoptosis pathways, which is involved in a variety of biological processes as well as the development and invasion of many malignant tumors. Many malignancies have been extensively examined with CCT3. It is presently used as a possible target for the treatment of many malignancies since it is not only a novel biomarker for the screening and diagnosis of different tumors, but it is also closely associated with tumor progression, prognosis, and survival. Recent studies have shown that the expression of CCT3 is up-regulated in some tumors, such as liver cancer, breast cancer, colon cancer, acute myeloid leukemia, etc. In this paper, we review the role of CCT3 in various tumors.

KEYWORDS

CCT3, TCP1, cancer, biomarker, relationship

Introduction

Chaperonin containing TCP1 (CCT) comprises a family of eukaryotic chaperones that have a cylindrical structure composed of two rings stacked opposite each other. Each loop consists of eight homologous but distinct subunits (CCT1-8). The eight subunits of CCT are structurally similar, consisting of a conserved equatorial domain and an actively variable apical domain (1). All these chaperones, whose equatorial domains are structurally and functionally identical, recognize different motifs in the substrate protein. Cell survival depends on CCT, which interacts with roughly 5-10% of the proteome (2). It has been proved that CCT plays an important role in the folding of many proteins involved in cancer, including Von Hippel-Lindau (3, 4) and p53 (5); proto-oncoprotein signal transduction proteins (6); and cell cycle regulatory proteins (7, 8). CCT chaperones

Abbreviations: CCT: chaperonin containing t-complex; TCP1: T-complex protein 1; HCC: hepatocellular carcinoma.

regulate the folding of signal transduction (STAT3). In many types of cancer, STAT3 acts as a mucoprotein by delivering hormone-peptide signals to the nucleus. STAT3 has been shown *in vitro* and *in vivo* to be a substrate for the chaperone protein CCT, which contributes to its biosynthesis and activity.

CCT3 is the major subunit of the chaperone CCT complex, whose gene is located on chromosome 1. CCT3 has a molecular weight of approximately 60 kDas, and specific functions during protein folding and refolding. The CCT3 chaperone protein shares significant sequence similarities with other members of the CCT family, as well as conserved domains with other distant chaperone proteins (9, 10). The chaperone proteins play a crucial role in protein homeostasis and proteomic stability. Molecular chaperones cooperate to correctly guide protein folding pathways, intracellular localization, and proteolytic translation (11). The major functional partners of a chaperone molecule depend on their ability to transiently bind a nascent hydrophobic region or a stress-denatured polypeptide and prevent misfolding. In addition to their key role in proper folding to avoid protein mismatches, chaperones also function as targeting proteins for degradation (12). For aggregated proteins that cannot be unfolded, chaperone-mediated autophagy or a helper selective autophagy pathway is necessary to remove the damaged proteins (13, 14). Protein homeostasis is critical to normal cellular function. Thus, disrupted protein homeostasis is the basis of various diseases, such as cancer. Inevitably, molecular chaperone pathways have been implicated in the development of cancer (15, 16). Studies have shown that CCT3 might regulate insulin-like growth factor-1 (IGF-1) signaling, actin cytoskeletal signaling, and phosphatase and tensin homolog (PTEN) signaling pathways. CCT3 might also play a role in regulating microtubule structure and function (by capturing centromeres), which would affect the sensitivity of cells to these microtubule targets. CCT3 also has an important role in the tumorigenesis of epithelial cells and the growth and survival of cancer cells (17).

CCT3 related research in tumors

Cell survival depends on the family of eukaryotic chaperones. Available information on the dysregulation of chaperone proteins reveals that CCT and its subunits are essential for the emergence of various cancers, including breast cancer (18), acute myeloid leukemia (19), hepatocellular carcinoma (20), cholangiocarcinoma (21), and colon cancer (22). Table 1 shows the brief history of cct3 in tumorigenesis and development.

Liver cancer

CCT3 plays an important role in the tumorigenesis and progression of HCC. The mRNA and protein expression of CCT3 in hepatocellular carcinoma (HCC) tissues are higher than those in non-HCC tissues (33). Wong et al. founded that the expression level of CCT3 in tumors was significantly higher than that of matched adjacent non-malignant liver tissues in 10 cases of HCC with amplicon 1q21-q22 ($P \leqq 0.04$) (23). The PPI of CCT3-related genes is enriched in KEGG signaling pathways, indicating that CCT3 may regulate HCC by targeting related sites and gene enrichment pathways. According to the Kyoto Encyclopedia of Genes and Genomes pathway analysis, CCT3 can influence HCC occurrence and development through the cell cycle and DNA replication pathways. Through the phosphorylation of transcription factors (Figure 1A) and STAT3, which enter the nucleus of liver cancer cells (Figure 1B), CCT3 has an impact on the development of liver cancer in HCC. CCT3 is a target affecting STAT3 activation and plays a critical role in the translocation of (p)STAT3 and STAT3 from the cytoplasm into the nucleus. HCC progression is partly affected by knockdown of CCT3, which may negatively regulate activation of the IL6/STAT3 signaling pathway and affect the progression of HCC partly by having an impact on the transport of (p)STAT3/STAT3 into the nuclei of HCC cells (42). By maintaining proper chromosome alignment and segregation (29), CCT3 can play a part in regulating microtubule structure and function (capturing the attachment site). By preventing the ubiquitination of YAP and TFCP2 brought on by poly (RC) binding protein 2 (PCBP2) in a beta-transducin repeat containing E3 ubiquitin protein ligase (trcp)-independent way, CCT3 extended their half-lives (28). YAP and TFCP2 are recognized as upstream triggers to increase the protein stability of YAP and TFCP2 in liver cancer cells. Bioinformatics prediction and luciferase reporter assays validated that circ-CCT3 could promote HCC progression through the mir-1287-5p/TEA domain transcription factor 1 (TEAD1) axis (43). Circ-CCT3 acted as a sponge for mir-1287-5p to enhance TEA domain transcription factor 1 (TEAD1) expression, which subsequently contributed to the activation of PTCH1 and LOX and consequently promotes tumorigenesis and progression. In addition, CCT3 can be associated with hepatocyte lipid metabolism in cells and *in vivo via* the long-stranded non-coding RNA LINC00326. Perturbation of the CCT3-LINC00326 regulatory network results in decreased intracellular lipid accumulation and increased lipid degradation, as well as diminished tumor growth *in vivo* (38).

Analysis of some recent cancer-driven genes identified CCT3 as a novel biomarker for liver cancer screening and diagnosis (44). In particular, for AFP-negative and small HCC patients, the CCT3 protein level showed good correlation with HCC etiology, tumor size, TNM stage, and Child-Pugh classification. The progression of malignancy is positively linked with the degree of CCT3 expression (29, 42). CCT3 overexpression is associated with poorer clinical outcomes and aggressive clinicopathological features, suggesting a poor

TABLE 1 A brief history of CCT3 development.

Time	Related Research and Achievements
1997	Research: Exploration of the CCT subunit. Achievement: CCT consists of 8 subunits and arrangement between subunits (1).
2001	Research: Colon cancer Achievement: As a useful tumor marker (22).
2003	Research: The non-malignant liver tissues. Achievement: CCT3 may represent targets in the progression of hepatocellular carcinoma (23).
2005	Research: Ovarian cancer Achievement: CCT3 is an effective marker for ovarian cancer (24).
2009	Research: Colon cancer Achievement: Discovery human advanced colon cancer (25).
2013	Research: Cholangiocarcinoma Achievement: CCT3 is a potential biomarker for cholangiocarcinoma (CCA) (26).
2015	Research: Breast cancer Achievement: CT3 is critical for the survival of breast cancer cells (18).
	Research: Osteosarcoma Achievement: CCT3 is candidate driver genes of importance in OS tumorigenesis (27).
	Research: Hepatocellular carcinoma Achievement: CCT3 supports proper mitotic progression and cell proliferation in hepatocellular carcinoma cells (28).
	Research: hepatocellular carcinoma Achievement: CCT3 predicts poor prognosis in hepatocellular carcinoma (29).
2016	Research: Acute myeloid leukemia Achievement: CCT modulates the activity of leukemogenic fusion oncoprotein (19).
	Research: Esophageal carcinoma Achievement: CCT3 plays an important role in the development of EC (30).
2017	Research: Gastric cancer Achievement: CCT3 is vital for gastric cancer growth (17).
2018	Research: Papillary thyroid carcinoma Achievement: CCT3 presents as a potential molecular marker of PTC (21).
	Research: Glioblastoma Achievement: CCT3 expression was significantly elevated in glioblastoma (31).
2019	Research: Tumor-repopulating cells (TRCs) Achievement: CCT3 inhibits TRCs-induced tumor formation (32).
	Research: Liver cancer Achievement: CCT3 might represent a promising biomarker for liver cancer (33).
	Research: Colorectal cancer Achievement: circ-CCT3 Enhance Colorectal Cancer Metastasis (34).
	Research: Multiple myeloma Achievement: high expression of CCT3 may serve as an indicator in diagnosis and prognosis of MM patients (35).
2020	Research: Breast cancer Achievement: Suppression of CCT3 inhibits the proliferation and migration in breast cancer cells (36).
	Research: Head and neck squamous cancer (HNSC) Achievement: CCT3 is a biomarker for improving HNSC survival and prognosis (37).
2021	Research: Hepatocellular carcinoma Achievement: CCT3-LINC00326 axis regulates hepatocarcinogenic lipid metabolism (38).
	Research: Breast and prostate cancers Achievement: CCT3 suppression prompts apoptotic machinery through oxidative stress and energy deprivation in breast and prostate cancers (39).
	Research: Lung adenocarcinoma (LUAD) Achievement: CCT3 promote cisplatin resistance of lung adenocarcinoma (LUAD) cells through the JAK2/STAT3 pathway (40).
	Research: Cervical cancer Achievement: Upregulation of CCT3 promotes cervical cancer progression through FN1 (41).

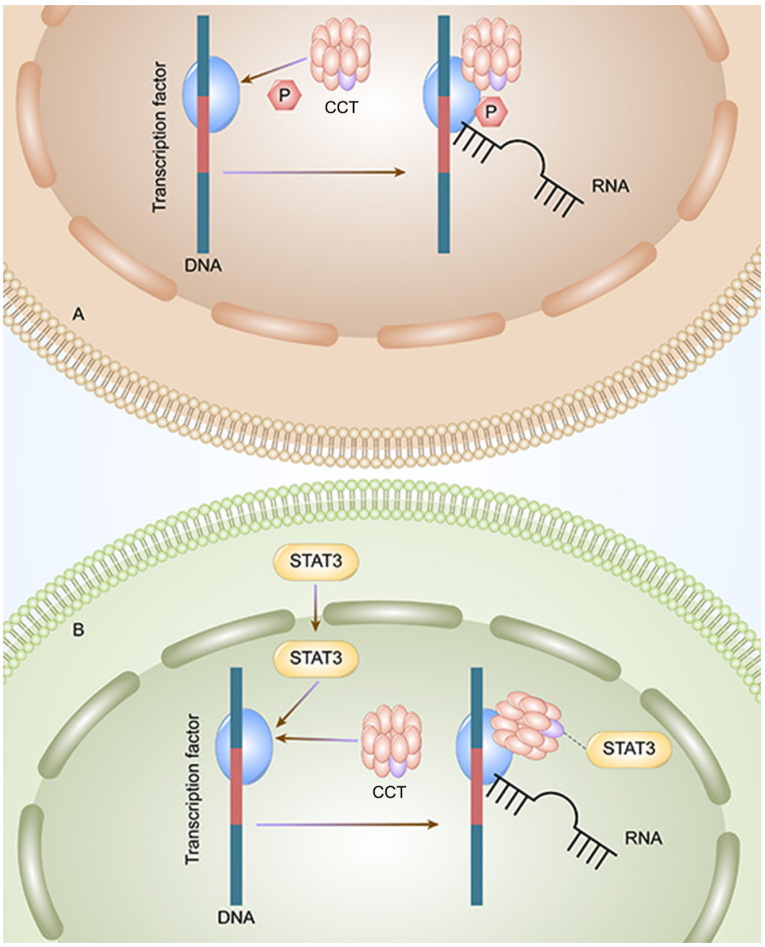

FIGURE 1
CCT3 affects the progression of liver cancer by two methods. **A**: CCT can initiate the transcription by phosphorylating the transcription factors, and the HCC tumor cells can proliferate. **B**: CCT activates STAT3 and initiates the transcription and proliferation of HCC tumor cells.

prognosis for patients with HCC (33). In addition, CCT3 contributed to the invasion capacity of cells. This showed that CCT3 expression may be associated with metastasis in HCC. In conclusion, CCT3 is involved in the carcinogenesis and progression of HCC and serves as a potential therapeutic target and biomarker in hepatocellular carcinoma (28, 33).

Breast cancer

CCT3 was significantly upregulated in a large proportion of human breast cancer tissues, and its overexpression was also significantly correlated with breast cancer clinical characteristics, including the clinical stage and the TNM classification. Both the mRNA and the protein levels of CCT3 are potential diagnostic biomarkers and therapeutic targets for breast cancer (45). Xu et al. found that CCT3 regulates breast cancer tumorigenesis by promoting cell proliferation and cell cycle progression. The knockdown of CCT3 inhibited proliferation, metastasis, and apoptosis of breast cancer cells, and the mechanism may be related to the regulation of the cell cycle, apoptosis, and multiple signal transduction pathways (36). They confirmed that the knockdown of CCT3 can induce apoptosis in breast cancer with the annexin method in this study. Perhaps the mechanism is related to CDC20 and p53. They are substrates of CCT. CDC20 is known to modulate key anti-apoptotic proteins Mcl-1 and Bim (46, 47), and p53 mediates cell apoptosis by activating the mitochondrial pathway and death receptor-induced apoptosis pathway (48). Secondly, down regulation of CCT3 significantly inhibited NF-κB activity and reduced the proliferation and metastatic capacity of breast cancer cells (30, 36). Qu et al. further explored the mechanism of action of CCT3 in breast cancer was performed through gain- and loss-of-function studies and found that CCT3 can directly bind mir-223 through the ceRNA network between mir-223 and β-catenin, thus affecting the activation of the Wnt/β-catenin

signaling pathway, attenuating the regulation of mir-223 in the Wnt/β-catenin pathway and promoting breast cancer cell proliferation and tumorigenicity (45). In this study, they found that CCT3 can promote β-catenin nuclear translocation, and there is a high possibility that CCT3 may recruit β-catenin into the nucleus through direct binding. Wnt/β-catenin signaling was activated when CCT3 was knocked down. The target genes downstream, such as cyclin D1 and c-myc, were then transcribed with the help of β-catenin. These Wnt/β-catenin target genes promote breast cancer cell G1/S transition and other oncogene transcriptions, maintaining the malignant proliferative ability (Table 2). In breast and prostate cancers, miRNA-mediated CCT3 inhibition can disrupt intracellular ROS homeostasis, leading to elevated ROS levels, altering intracellular free amino acid water and distribution for energy metabolism and promoting apoptotic mechanisms through oxidative stress and energy deprivation (39).

Lung cancer

Investigations according to the GEPIA (Gene Expression Profiling Interactive Analysis) web portal demonstrated that CCT3 expression levels were significantly upregulated in both lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) tissues. Furthermore, it was concluded from the Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases that a high expression level of CCT3 was associated with the poor prognosis of LUAD patients, albeit not in LUSC patients. Therefore, Xu et al. speculated that CCT3 may be closely involved in the tumorigenesis and progression of non-small cell lung cancer (NSCLC), and it might have a more prominent role in LUAD (40). Shi et al. found through further studies revealed that silencing of CCT3 resulted in the inhibition of Yes-associated protein 1 (YAP1) in NSCLC cells, decreasing the expression of YAP1 target genes and producing antitumor effects in NSCLC. In NSCLC cells, activation of the YAP1 by forced expression of constitutively active YAP1 mutants reversed the antitumor effects induced by CCT3 inhibition. This study unveils a possible role for the CCT3/YAP1 axis in NSCLC and suggests CCT3 as a candidate anticancer target (49). The increased expression of CCT3 in lung adenocarcinoma may lead to uncontrolled cell proliferation by promoting the expression of cyclin B1/CDK1 and thus accelerating cell cycle progression (40). Under cisplatin treatment, cell cycle protein B1 and CDK1 protein levels in lung adenocarcinoma cells were significantly reduced after CCT3 knockdown. The downregulation of CCT3 significantly inhibited the proliferation, invasion, and migration of lung adenocarcinoma cells, resulting in increased apoptosis and enhanced expression of the apoptosis marker cleaved caspase-3 (34) and leading to significant G2/M cell cycle arrest and apoptosis in lung adenocarcinoma cells, significantly reducing the tumorigenicity of cisplatin-treated lung adenocarcinoma

TABLE 2 CCT3 can affect tumor progression through STAT3, cdc20, p53, NF-κB, the wnt pathway, the VEGF pathway.

Signaling pathway or transcription factor	Progression	Impact on cancer
JAK/STAT3	STAT3 is an important downstream signaling molecule of numerous growth factors and cytokines, and participates in various biological processes, such as cell proliferation, differentiation, and survival. STAT3 can be activated by nonreceptor tyrosine kinases such as Janus kinases (JAKs) in a tyrosine phosphorylation dependent-manner. CCT3 down regulation could sensitize lung adenocarcinoma cells to cisplatin by inhibiting the Janus kinase 2/signal transducer and activator of the transcription 3 (JAK2/STAT3) pathway.	CCT3 overexpression might affect the progression of multi-ple myeloma through the JAK/STAT3 pathway. The JAK2/STAT3 pathway has been considered a promising target for chemotherapeutic interference ascribed to its persistent activation in human carcinomas. CCT3 may be a new molecular target to overcome cisplatin resistance of LUAD patients
Cdc20,P53	Cdc20 is known to modulate key anti-apoptic proteins Mcl-1 and Bim, and p53 mediates cell apoptosis by activating mitochondrial pathway and death receptor-induced apoptotic pathway. CDC20 was frequently upregulated in many types of malignancies and remarkably suppressed by ectopic introduction of p53.	P53 inhibits tumor cell growth through the indirect regulation of CDC20 and that CDC20 might be a good potential therapeutic target for a broad spectrum of human cancer.
NF-κB	Down regulation of CCT3 significantly inhibited NF-κB activity and reduced the proliferation and metastatic capacity of breast cancer cells	Overexpression of NFκB rescued the effect of CCT3 on the proliferation and migration of breast cancer cells.
The Wnt/ βpathway	Wnt/β-catenin is a key signaling pathway in cancer cell proliferation. Wnt signaling was highly activated in MDA-MB-231 and T47D cells when CCT-3 was upregulated. In contrast, knocking down of CCT-3 knocked down the Wnt signaling significantly. The protein level of p-GSK- 3β and β-catenin nuclear accumulation increased in CCT3-overexpressed MDA-MB-231 and T47D cells. CCT-3 also affected the expression levels of β-catenin downstream effectors such as cyclin D1 and c-myc.	CCT-3 may promote breast cancer tumorigenesis at least in part *via* activating the Wnt/β-catenin signaling pathway.
The VEGF pathway.	Circ-CCT3 depletion attenuates invasion and induces apoptosis of CRC cells through mir-613/WNT3 or VEGFA. Thus, circ-CCT3 can enhance colorectal 173 cancer metastasis by regulating VEGFA.	Circ-CCT3 plays an oncogenic role in CRC metastasis through mir-613/VEGFA and Wnt signaling.

cells. In addition, CCT3 downregulation could sensitize lung adenocarcinoma cells to cisplatin by inhibiting the Janus kinase 2/signal transducer and activator of the transcription 3 (JAK2/STAT3) pathway. It is suggested that CCT3 may be a new molecular target to overcome cisplatin resistance in LUAD patients (40) (Table 2).

Cervical cancer

Dou et al. examined the effect of CCT3 on the proliferation and migration of cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) *in vitro* through various experiments, including proliferation, Transwell, and flow cytometric assays. The results showed that CCT3 expression was significantly upregulated in CESC. *In vitro*, silencing CCT3 inhibited the proliferation, migration, and invasion of CESC cells. Downregulation of the CCT3 gene promoted apoptosis and cell cycle arrest in CESC cells and inhibited the expression of fibronectin 1 (FN1) protein. Furthermore, rescue assays demonstrated that CCT3 could promote the proliferation and migration process of CESC through FN1 (50). Saioa Mendaza et al. experimentally found that mir-877-3p promotes Squamous Cell Carcinoma of the Cervix (SCCC) cell migration and invasion by regulating cytoskeletal protein folding, mainly through the CCT complex, and that anti-mir-877-3p increased the expression of CCT3 in SCCC, leading to abnormal folding of actin and microtubulin, thereby impairing cell migration and invasion ability. Overall, CCT3 may be considered a new and promising biomarker that is closely related to the progression, prognosis, and survival of CESC and may become a therapeutic target for CESC (26).

Multiple myeloma

Qian et al. study analyzed 2220 multiple myeloma patients (2380 samples) using 10 independent GEO datasets with different bioinformatics analysis methods and found that CCT3 was significantly overexpressed in multiple myeloma patients and that CCT3 overexpression was a poor predictor of survival, and its functional intensity was positively correlated with disease progression. CCT3 may play a supporting role in myeloid diagnosis. KEGG pathway analysis indicated that the CCT3 targeted genes were involved in the JAK-STAT3 signaling pathway, the Hippo signaling pathway, the WNT signaling pathway, and two pathways centralizing in leukemia-related terms (namely acute myeloid leukemia and chronic myeloid leukemia) (41, 51, 52). GSEA of gene sets differentially regulated in the CCT3 high and CCT3 low groups revealed that leukocyte migration, regulation of leukocyte migration, IL6/JAK/STAT3 signaling, and regulation of STAT cascade gene sets were significantly upregulated in the CCT3 high group. These results suggest that high CCT3 expression is associated with leukemia and the JAK-STAT3 signaling pathway, and that CCT3 expression may promote multiple myeloma progression by regulating MYC mainly through the JAK-STAT3 signaling pathway. These findings suggest that high CCT3 expression may serve as an indicator for the diagnosis and prognosis of multiple myeloma patients and a potential target for the future treatment of multiple myeloma (53).

Papillary thyroid carcinoma

Susannah Hallal et al. used TCGA data to interrogate gene expression levels and DNA copy numbers *in silico* for all eight subunits. Glioblastoma tissue had significantly higher levels of CCT2, CCT3, CCT5, CCT6A, and CCT7 gene expression relative to healthy brain tissue (52, 54). Human papillary thyroid cancer (PTC) tissues had significantly greater levels of CCT3 expression than did healthy paraneoplastic tissues (21). CCT3 is essential for the survival and growth of PTC cells, and the lentivirus-mediated knockdown of the CCT3 gene significantly reduced the proliferation and cell cycle progression of K1 cells and induced apoptosis in K1 cells. In addition, enhanced G2/M cycle blockade could also lead to increased apoptosis after CCT3 knockdown. The current findings suggest that CCT3 is a significant oncogene in PTC, and it was found that an increase in CCT3 expression was associated with the tumor area of PTC. In conclusion, CCT3 may be a promising potential candidate gene for molecular diagnosis and treatment of PTC and may serve as a predictive prognostic indicator for PTC patients and a molecular target for PTC treatment (21).

Malignant melanoma

Protein is the actual functional molecule in cells and is responsible for almost all the biochemical activities of cells and is the basis of biological functions. CCT3 regulates the pathogenesis and metastasis of melanoma at the protein level. Roobol et al. reported that CCT3 is an important partner of the folded cytoskeletal component, and elevated CCT3 expression might impair the proper folding and assembly of complex proteins (35). CCT3 may regulate IGF-1 signaling, actin cytoskeletal signaling, and PTEN signaling pathways, which are known to function in epithelial tumorigenesis and cancer cell growth and play an important role in survival. CCT3 might play a role in regulating microtubule structure and function (capturing centromeres), which would affect the sensitivity of cells to these microtubule targets. In addition, cytoskeletal proteins (27), including actin and tubulin (31, 37), many cell cycle regulators (7), and tumor suppressors (37), are also substrates of CCT.

Numerous studies have shown that many proteins play a key role in the occurrence and metastasis of malignant melanoma

(MM). Baruthio et al. detected a total of more than 100 proteins in the detergent-resistant membrane of melanoma cell lines, and melanoma cells are particularly resistant to detergents. Membrane protein structure is related to the degree of malignancy, suggesting that some membrane proteins might play a role in the progression of melanoma (55). Sinha et al. used two-dimensional electrophoresis and proteomics methods to analyze the protein expression profiles of drug-resistant melanoma cells and found that voltage-dependent anion channel proteins were overexpressed. Molecular chaperones were also overexpressed in resistant melanoma cells, including heat shock proteins (32). BRaf proto-oncogene, serine/threonine kinase (BRAF) is the most frequently mutated serine-threonine protein kinase involved in the pathogenesis of melanoma and is mutated in most melanoma and melanocyte lesions. However, BRAF mutations mainly occur in the progression of melanoma and rarely occur in the advanced stages, suggesting that this pathway plays a role in the initiation and progression of melanoma (56). The dephosphorylated protein cuts the filaments near the serosa to cause actin to polymerize to create new spikes to promote melanoma cell invasion.

In other settings, increased levels of plasma membrane intrinsic protein 2 (PIP2) can be achieved by binding and replacing actin filament capping proteins, such as gelsolin, at the ends of actin filaments (high levels of gelsolin reduce melanoma cell metastasis), thereby promoting actin polymerization. The mechanism of actin polymerization is dependent on the availability of intracellular actin monomers, which are associated with melanoma metastasis (24) (Figure 2). The structure of actin is V-shaped, and the two arms are composed of two large and one small domain, and the gap between them is visible with nucleotide (ATP) adhesion. CCT binds to ATP and has weak ATPase activity. After actin enters the CCT side cavity, it binds tightly to the apical domain of CCT through the arm of the large domain. The driving force for cell body movement comes from the interaction of actin and myoglobin, which is mainly regulated by phosphorylation of non-muscle cell types. Many phosphorylation pathways that affect regulatory myosin subunits are up-regulated in metastatic melanoma cells. Rhoc increases actin-myosin contractility by binding to ROCK1 and ROCK2, and high expression of Rhoc is associated with melanoma metastasis. Inhibition of rhoc reduces the invasiveness of melanoma cells (57). Experiments have shown that the microtubule network acts on the adhesion point to separate it, which is beneficial to the protein transfer induced by dynein. Homomorphic tubulin is homomorphic. It enters the inner cavity of the CCT side loop to form a CCT-tubulin complex. When tubulin binds to the CCT side loop, it stops the information exchange of the loops, preventing the other loop from binding to other substrates. Some proteins are currently used for the diagnosis of MM. However, the diagnosis of melanoma by using a single protein index remains insufficient.

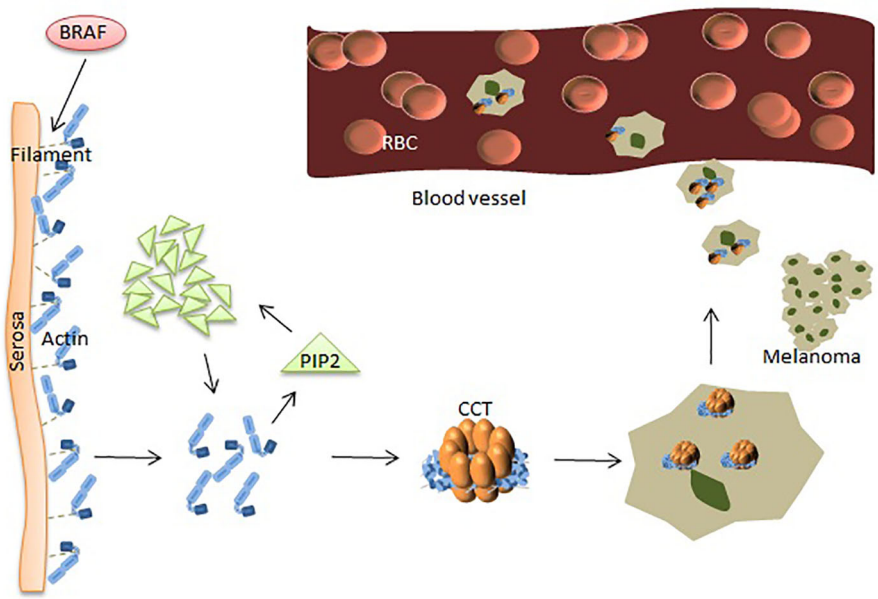

FIGURE 2
CCT plays a key role in the progression of melanoma. BRAF makes dephosphorylated protein cut the filaments near the serosa, causing polymerization of actin monomers. The increased amount of actin acts on PIP2, and the clustered PIP2s, in turn, increase actin monomers. Polymerized actin binds between the two rings of CCT and provides the driving force for movements leading to MM cells' metastasis.

Other tumors

At present, there are many researches on CCT3 in liver cancer, breast cancer, lung cancer, and so on. There has also been some research into other tumors.

Gastric cancer

CCT3 was found to be important in the growth and survival of gastric cancer. Li et al. used immunohistochemistry to show that CCT3 expression levels were found to be higher in surgical specimens from 26 gastric cancer patients than in non-cancerous epithelial tissue adjacent to cancer. RNA interference was used to knock down the expression of CCT3 in gastric cancer cell lines. Cellular knockdown of CCT3 inhibited proliferation and colony formation, reduced cell viability, and promoted apoptosis of gastric cancer cells *in vitro*. Gene expression analysis showed that CCT3 knockdown was associated with down-regulation of mitogen-activated protein kinase 7, cell division cycle 42, cyclin D3 and up-regulation of cyclin-dependent kinase 2 and 6 (17).

Colorectal cancer

Li et al. used reverse transcription-quantitative PCR and western blot to detect the mRNA and protein levels of each gene and found that circ-CCT3 was highly expressed in human clinical CRC tumors. Low levels of circ-CCT3 are strongly associated with higher survival and tumor metastasis in CRC patients. Circ-CCT3 plays an oncogenic role in CRC metastasis through mir-613/VEGFA and Wnt signaling. Mechanistically, circ-CCT3 directly interacts with mir-613, then regulates VEGFA and WNT3 gene expression. Phenotypically, circ-CCT3 depletion attenuates invasion and induces apoptosis of CRC cells through mir-613/WNT3 or VEGFA. Thus, circ-CCT3 can enhance colorectal cancer metastasis by regulating VEGFA and WNT3 signaling through sponge uptake of mir-613 (25, 34) (Table 2).

Esophageal cancer

Su et al. quantitative real-time polymerase chain reaction (qPCR) was used to verify the expression level of differentially expressed genes (DEGs) in esophageal carcinoma (EC) and identified significant differentially expressed genes (DEGs) of CCT3 that play a key role in the tumor development of esophageal cancer (EC). CCT3 can also be considered as a potential candidate biomarker for therapeutic targets of esophageal cancer (30). A proteomics-based study by Shi et al. showed that CCT3 is a potential biomarker for cholangiocarcinoma (CCA) and has the potential to be used as a new tumor marker for the early detection of CCA (58).

Osteosarcoma

Xiong et al. concluded from a comprehensive analysis of osteosarcoma (OS) gene expression and genomic aberration data that CCT3 is an important pivotal protein in the development of osteosarcoma and plays an important role in the progression of osteosarcoma. Furthermore, CCT3 is an important specific driver gene in osteosarcoma. It can be an excellent candidate biomarker for OS diagnosis and may be used to improve the diagnostic markers for neonatal osteosarcoma (59).

Head and neck squamous cell carcinoma

The GEO, Oncomine, and ALCAN databases were used to examine CCT3 expression in head and neck squamous cell carcinoma (HNSCC). The results showed that CCT3 expression was significantly up-regulated in HNSCC at both mRNA and protein levels. Up-regulated CCT3 expression was associated with various clinicopathological parameters. Gene set enrichment analysis (GSEA)analysis indicated that high expression of CCT3 was closely correlated with tumor-related signaling pathway mTOR pathway (MTORC1/PI3K AKT mTOR) and HNSCC cell survival. In addition, overexpression of CCT3 was associated with unfolded protein response, DNA repair, and the p53 pathway, which may contribute to the progress of HNSCC. Cellular knockdown of CCT3 significantly inhibited cell growth and invasion of HNSCC cell lines. Thus, CCT3 could be a prognostic marker and potential therapeutic target in HNSCC (60, 61).

MCKD1

Wolf et al. collected clinical data and blood samples from 257 individuals (including 75 affected individuals) from 26 different relatives. Mutation analysis was performed on 37 genes (374 exons) in 23 patients with MCKD1 in a defined critical region. In addition, for nine relatives, RT-PCR analysis of the sequenced genes was performed to screen for mutations that activate hidden splice sites. Mutational analysis of all 37 positional candidate genes revealed sequence variation in CCT3, which was isolated from each affected relative and was not found in 96 healthy individuals, suggesting that CCT3 is important to MCKD1 (62).

Ovarian cancer

Peters et al. experimentally identified several potential novel biomarkers for ovarian cancers, including CCT3. Taken together, these data identified no elevated expression of genes in primary ovarian cancer but confirmed a valid existing marker, which is CCT3 (63).

Study of tumor-repopulating cells

Tumor-repopulating cells (TRCs) are cancer stem cell (CSC)-like cells with highly tumorigenic and self-renewing abilities. Huang et al. identified CCT3 as a potential new stem cell-associated gene by integrating a network of membrane

proteins, the WNT pathway, and cancer-related genes. Not only is it a key molecule in the selection of tumor-repopulating cells (TRCs), but it is also important for mechanotransduction and influences the selection process of TRCs. The number of TRC colonies was dramatically decreased by silencing CCT3, and TRC growth and selection were also slowed. Additional research revealed that CCT3 increased stemness and cell proliferation *in vitro*, while its inhibition prevented the development of tumors brought on by TRCs (64).

Conclusion and perspectives

In this review, we first briefly introduce the structure and function of CCT, and then focus on the role of CCT3 in a variety of tumors. We discuss the multiple regulatory mechanisms involved in CCT3 in eukaryotes. For example, overexpression of CCT3 promotes the proliferation and differentiation of cancer cells and induces apoptosis, thereby inhibiting cell proliferation. CCT3 has been shown to mediate the folding of many proteins involved in tumorigenesis. Great progress has been made in elucidating the role of CCT3 in tumorigenesis through STAT3, Wnt, PI3K-AKT and other related signaling pathways and its expression mechanism in diseases. Although the experiments of tumor therapy and prognosis targeting CCT3 have been carried out widely and achieved some results, further work is needed to study the role of CCT3 as a tumor marker and drug-targeted therapy in tumors. It can be used to develop strategies to identify new cancer therapies. Therefore, it is necessary to develop new methods and further experimental studies to improve our understanding of the role of CCT3 in tumor development and progression.

Author contributions

GJ and WLL provided direction and guidance throughout the preparation of this manuscript. WLL drafted the manuscript. YL, XY and QSL designed the tables and imagines. YJS ,YZL, YCL and JQZ collected and prepared the related literature. GJ and WLL reviewed and made significant revisions to the manuscript. All authors contributed to the article and approved the submitted version article and approved the submitted version.

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.

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LncmiRHG-MIR100HG: A new budding star in cancer

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MIR100HG, also known as lncRNA mir-100-let-7a-2-mir-125b-1 cluster host gene, is a new and critical regulator in cancers in recent years. MIR100HG is dysregulated in various cancers and plays an oncogenic or tumor-suppressive role, which participates in many tumor cell biology processes and cancer-related pathways. The errant expression of MIR100HG has inspired people to investigate the function of MIR100HG and its diagnostic and therapeutic potential in cancers. Many studies have indicated that dysregulated expression of MIR100HG is markedly correlated with poor prognosis and clinicopathological features. In this review, we will highlight the characteristics and introduce the role of MIR100HG in different cancers, and summarize the molecular mechanism, pathways, chemoresistance, and current research progress of MIR100HG in cancers. Furthermore, some open questions in this rapidly advancing field are proposed. These updates clarify our understanding of MIR100HG in cancers, which may pave the way for the application of MIR100HG-targeting approaches in future cancer diagnosis, prognosis, and therapy.

KEYWORDS

lncRNA, lncmiRHG, MIR100HG, cancer, biomarker, therapeutic target

Introduction

Cancer is the primary barrier to increasing life expectancy in the countries of the world for its multistage development process and genetic and environmental factors (1, 2). According to the report from the international cancer research team in 2020, the number of new cancer cases and cancer-related death number increased by about 19.3 million and 10 million in the world, respectively (2). Although the understanding of molecular mechanisms in cancers has increased substantially in recent years (3, 4), the recurrence and death rates of cancer patients are still not satisfactory (2). Thus, there is an urgent need to find new effective biomarkers and treatment targets.

Long noncoding RNA (lncRNA) is over 200 nucleotides in length, with few exons, weak constraints on evolutionary processes, relatively low abundance, and lacking obvious open reading frames, resulting in protein-coding defects (5, 6). These transcripts are transcribed by RNA polymerase II and exhibit representative mRNA-like characteristics (7), and many lncRNAs are evolutionarily not conserved (8). At the molecular level, increasing evidence suggests that lncRNAs perform chromatin organization, epigenetic, transcriptional, and post-transcriptional RNA regulation through multiple mechanisms (9–13). Therefore, lncRNAs may function as tumor-suppressive and oncogenic genes, affecting the malignant behaviors of cancer cells. Moreover, the expression levels of lncRNA are dynamically modulated in a tissue, cell, or development-specific way. Consequently, lncRNA has been regarded as a promising biomarker for diagnosis and prognosis or treatment targets of tumors (14).

MiRNA-host gene lncRNAs (lnc-miRHGs) are a special type of lncRNA. Different from the various subclasses of lncRNAs that were previously categorized according to functions, genomic locations, or expression patterns (15), lncmiRHGs refer to lncRNAs containing miRNAs in exon or intron sequences (16). Typically, about 17.5% of miRNAs are derived from lncRNAs (17). At present, the biogenesis and function of lnc-miRHG-processed miRNAs are adequately studied. Some lnc-miRHGs are abnormally expressed in different disorders with promising diagnostic and prognostic biomarkers potentials. Nevertheless, it is hardly known whether an accurate and stable splice pool of lnc-miRHGs derived from the pri-miRHG during the miRNA process performs a required cellular function or no function as a by-product of miRNA processing. So far, limited research has revealed miRNA-independent functions of lnc-miRHGs, such as lncRNA H19, RMST, PVT1, and MIR31HG (18). Nevertheless, the roles of lnc-miRHGs in diverse cancers are still not studied.

LncRNA MIR100HG is one of the recently studied lncmiRHGs subclasses, encoding three miRNAs (mir-125b-1, 100, and let-7a-2) within introns. Emerging evidence showed that MIR100HG expression levels were increasing in multiple tumors in comparison to normal tissues (19–22). Besides, MIR100HG overexpression remarkably enhances tumor behaviors (23–26). However, controversial literature also reported that MIR100HG played an important role in reducing the proliferation and invasiveness of tumor cells (27, 28). However, there is still no substantial progress in the clinical application of MIR100HG.

In this review, we will highlight the characteristics, mechanisms, pathways, chemoresistance, and current research progress of MIR100HG in expression patterns, functions, and clinicopathological characteristics of cancers.

Identification and characterization of MIR100HG

MIR100HG, also known as lncRNA mir-100-let-7a-2-mir-125b-1 cluster host gene, lncRNA-N2, linc-NeD125, or AGD1, is a lnc-miRHG located on chromosome 11q24.1 with 17 exons. MIR100HG has an intronic coding region (BLID), which acts as a pro-apoptotic element through the caspase-dependent mitochondrial cell death pathway (29)(Figure 1).103 transcripts of MIR100HG were found from the LNCipedia database with the length ranging from 242 to 7061 bp (extracted from NCBI database). MIR100HG is mostly located in the nucleus but also little in the cytoplasm (30). Non-reference sequence annotated MIR100HG is a predominantly cytoplasmic, neuronal-inducible, long intergenic noncoding RNA that includes miR-125b- 1 in introns but does not contain let-7a-2 and miR-100 and therefore renamed neuronal differentiation lncRNA host miR-125 (linc-NeD125). Linc-NeD125 not only acts as a miRNA precursor, but other features suggest that it may also have intrinsic functions, such as its specific induction of neuronal differentiation, advanced evolutionary conservation in primates, and accumulation as a spliced stable molecule under differentiation stimuli (28). While lncRNA-N2 contains the miRNAs mir-125b and let7 within its introns but does not contain miR-100. LncRNAs-N2 were expressed in brain structures. It is reported that host miRNAs are critical to neurogenesis. LncRNA-N2 promotes neurogenesis by maintaining the levels of miR-125b and let7a in neural progenitor cells. The best secondary structure prediction of MIR100HG by the R fold Web server (from the Vienna software package) has a minimum free energy of -746.40

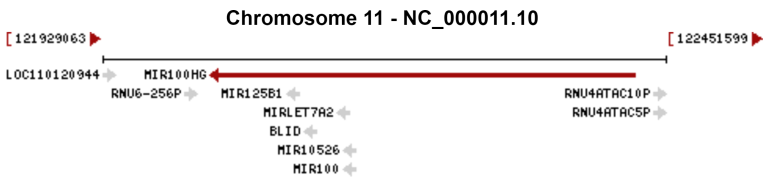

FIGURE 1
The genomic context of the MIR100HG. The genomic context of the MIR100HG was extracted from NCBI database (http://www.ncbi.nlm.nih.gov/gene/399959). Source: U.S. National Library of Medicine.

kcal/mol, and the dotted brackets are drawn with the results illustrated in Figure 2. MIR100HG was originally discovered in a human transcriptome analysis (31), identified as a key role in neural stem cell neuronal differentiation (32) and mesenchymal stem cell fate determination (33), and then discovered in diverse malignant tumors (19–22). Results from the normal tissues of the Human Protein Atlas (HPA) project RNA-seq demonstrated the broad expression of MIR100HGs in the ovary (RPKM 32.2) and gall bladder (RPKM 20.3) compared with other human tissues. To explore the MIR100HG expression in multiple tumors, MIR100HG expression levels were evaluated by the GEPIA2 tool according to the Cancer Genome Atlas (TCGA) database. The results demonstrated that MIR100HG was remarkably downregulated in multiple malignant tumors of the urogenital system, including bladder urothelial cancer, ovarian serous cystadenocarcinoma, breast invasive cancer, endocervical adenocarcinoma, uterine corpus endometrial cancer, and some cancers of the respiratory system, containing lung squamous cell cancer and lung adenocarcinoma (Figure 3). Furthermore, the prognostic value of MIR100HG expression levels in diverse carcinomas was analyzed *via* the GEPIA2 tool. Higher MIR100HG expression suggested shorter overall survival (OS) and disease-free survival (DFS) in brain glioma with lower grade and stomach adenocarcinoma and showed favorable OS and DFS in skin cutaneous melanoma (Figure 4). These results revealed that MIR100HG may function as diagnostic and prognostic predictors in diverse malignant tumors.

Expression regulation, pattern, functions, and clinicopathological characteristics of MIR100HG

The expression pattern, functions, and clinicopathological characteristics of MIR100HG in diverse cancers have been summarized (Tables 1, 2; Figure 5).

Brain tumors

Medulloblastoma (MB) is the most common malignant brain tumor in children (62). Among them, group 4 medulloblastoma (G4MB) and type 3 medulloblastoma (G3MB) account for about 60%, and the prognosis is relatively poor, especially since the targeted therapy of G3MB is still in its infancy, and the pathogenesis is unclear (63). Laneve et al. (28) found that the expression of linc-NeD125 was significantly higher in G4MB than in normal brain tissues, and its expression was also significantly higher than the other three types of MBs. While Kesherwan et al (64) found that MIR100HG was significantly down-regulated in G3MB. Downregulation of linc-NeD125 reduces G4 cell proliferation. In addition, G3MB has a worse prognosis than G4MB. Interestingly, overexpression of linc-NeD125 in the G3MB cell model acquired specific G4MB molecular signatures. In other words, overexpression of linc-NeD125 in the G3MB cell model increases the protein production of the G4MB driver gene with a significant reduction of the high proliferation, migration, and invasion of G3MB cells, which gives them capabilities that G4MB-like cells possess. Therefore, the action mode of linc-NeD125 in MB deserves a more in-depth study for possible therapeutic applications. Controversially, linc-NeD125 and its contained miR-125b-1 function as negative regulators in human neuroblastoma cell proliferation, apoptosis, and viability. Linc-NeD125 can reduce cell proliferation and activate the anti-apoptotic factor BCL-2, and mainly controls cell viability by preventing cell cycle progression independently of host miRNAs (27). Therefore, linc-NeD125 may play different regulatory roles in different brain tumors, and the related mechanisms need to be further studied.

Head and neck squamous cell carcinoma

Most squamous cell carcinomas of the head and neck are diagnosed as advanced, which is the sixth most common carcinoma in the world (65, 66). Wilkins et al. (34) performed a genome-scale assessment of the OS contribution of common RNA SNPs to HNSCC and site-specific disease and found that the MIR100HG variant rs1816158 is related to OS in oral cancer. Similarly, Zhou et al. (35) also discovered that MIR100HG was significantly associated with OS in patients with tongue squamous cell carcinoma. Meanwhile, expression of MIR100HG-derived miR-100 was significantly related to OS in head and neck cancers, while other MIR100HG-derived miRs, 125b-1 and let7a-2, were not related to OS. The C allele in rs1816158, which is related to an increased risk of death, increases miR-100 expression. Another study discovered that the MIR100HG was up-regulated in the tumor tissue of laryngeal squamous cell carcinoma (LSCC) patients, while miR-204-5p was down-regulated. And their significant negative correlation was not identified in adjacent healthy tissues, indicating that there is a pathological mediator between the two RNAs. MIR100HG may promote the proliferation, migration, and invasion of cancer cells in LSCC *via* downregulating miR-204-5p, while overexpression of miR-204-5p merely partially reduced the enhancement of MIR100HG overexpression on the malignant behaviors of cancer cells, indicating MIR100HG may also have multiple downstream effectors in LSCC. But the pathological mediators and downstream effectors haven't been revealed. Besides, the expression of MIR100HG was significantly increased with the increase of AJCC staging, while the expression of miR-204-5p was significantly decreased (36). Moreover, MIR100HG, miR-100, and miR125b were simultaneously upregulated in HNSCC cell lines which were acquired and resisted in new cetuximab, while inhibition of MIR100HG, miR-100, and miR125b can restore cetuximab responsiveness *in vitro* and vivo, which also provides novel ideas for the therapeutic

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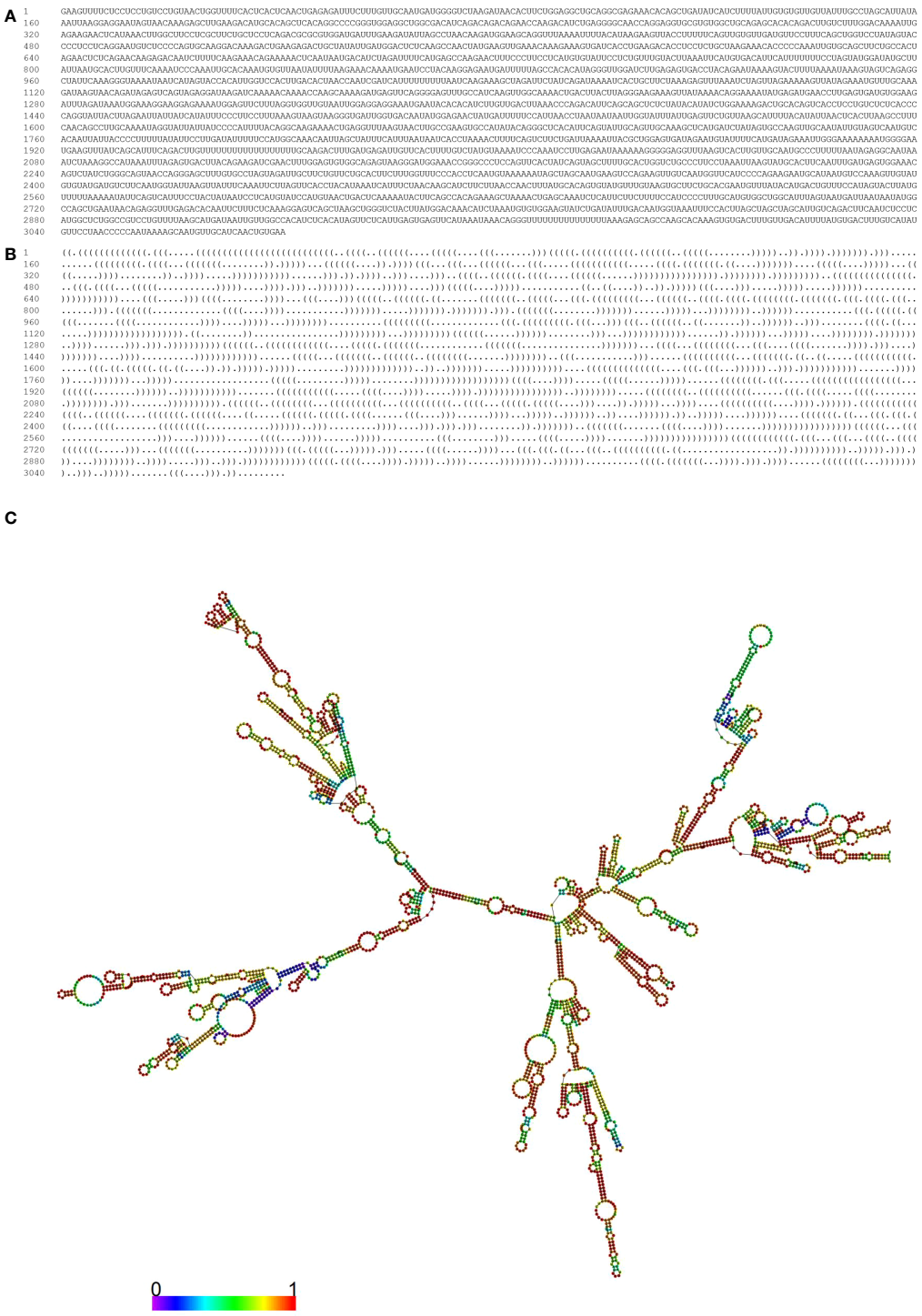

FIGURE 2
Optimal secondary structure of the MIR100HG. Prediction of the optimal secondary structure of the MIR100HG (Forena format) with-746.40 kcal/mol with its dot-bracket notation using the R-fold web server. **(A)** sequence; **(B)** dot–bracket notation; **(C)** the optimal secondary structure of the MIR100HG. In this structure, the color of the nucleotides is as follows: A, yellow; U, blue; C, green; G, red.

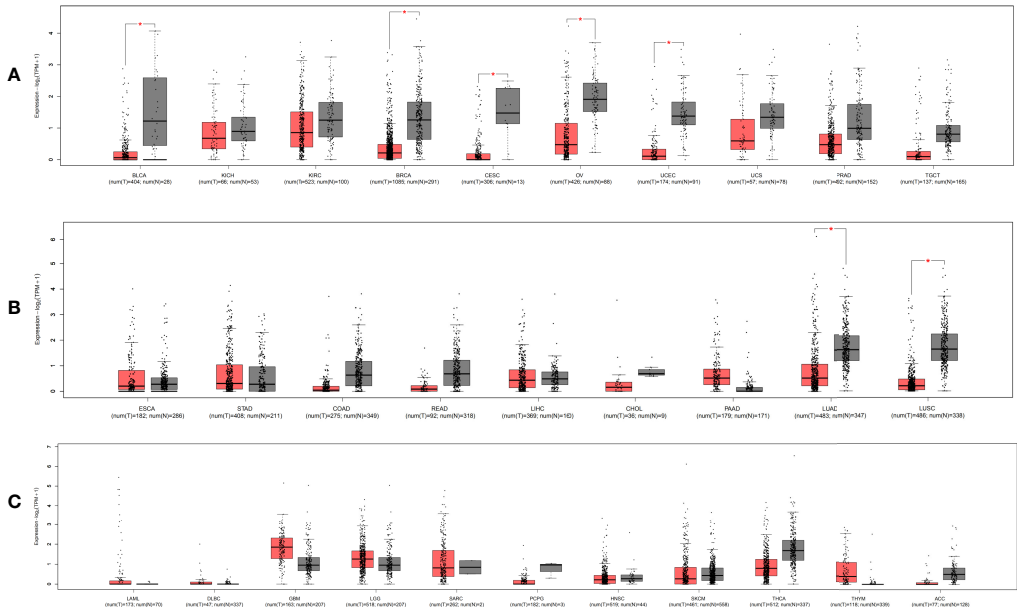

FIGURE 3
The relative expression level of MIR100HG in pan-cancer and adjacent normal tissues. The expression level of MIR100HG in different tissue of the digestive and respiratory systems. **(A)** urogenital system; **(B)** and other systems; **(C)** * |Log2FC|>1, $p < 0.01$. Data extracted from GEPIA2, all abbreviations in this figure refers to TCGA database (Supplementary Table 1).

methods of HNSCC cetuximab resistance (24). These results demonstrated that MIR100HG acts as an oncogenic lncRNA in head and neck cancers, which may represent a potential diagnostic biomarker or a novel therapeutic target for cancers.

Papillary thyroid cancer

Papillary thyroid cancer (PTC) is the most common subtype of thyroid cancer. Yang et al. (37)identified that MIR100HG was differentially expressed between cancerous and normal tissues and significantly downregulated in PTC tumors. Additionally, MIR100HG was co-expressed with CDHR3, which was targeted by hsa-miR-34a-5p. The epigenetic regulation among hsa-miR-34a-5p, CDHR3, and MIR100HG may participate in the pathological mechanism of PTC. MIR100HG was significantly but not independently associated with OS of PTC patients (61). These results indicated that MIR100HG may promote the progress of PTC by sponging the hsa-miR-34a-5p/CDHR3 axis, providing a potential marker in PTC patients. However, the expression and regulation mechanism between them has not been validated.

Breast cancer

Breast cancer is the most common malignant tumor in women and has become the main cause of cancer-

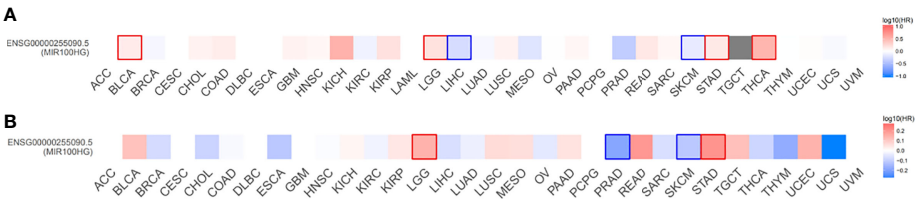

FIGURE 4
Overall survival **(A)** and disease-free survival **(B)** significance map of MIR100HG. The cut-off value was determined using the quartile of MIR100HG expression. Significance was defined as $p < 0.05$ and labeled with bold. Red represents high MIR100HG expression which suggests worse overall survival and disease-free survival; Blue represents high MIR100HG expression which suggests favorable overall survival and disease-free survival. Data extracted from GEPIA2, all abbreviations in this figure refers to TCGA database (Supplementary Table 2).

TABLE 1 Functional characterization of MIR100HG in cancers.

Cancer types	Expression	Model used	Functions	Genes/proteins/pathways affected	Role	References
Brain tumor	Upregulated/ Downregulated	Cell lines Human tissues	Cell apoptosis, cycle proliferation, migration, and invasion	BCL-2, miR-19a-3p, miR-19b-3p, mir-106a-5p, CDK6, MYCN, SNCAIP, and KDM6A	Oncogenic/ Suppressive	(27, 28)
Head and neck squamous cell carcinoma	Upregulated	TCGA database Cell lines Human tissues	Cell proliferation, migration, invasion, and chemoresistance	Variant rs1816158, miR-204-5p, miR-100, miR-125b, DKK1, ZNRF3, RNF43, DKK3, APC2, and Wnt signal pathway	Oncogenic	(24, 34–36)
Papillary thyroid cancer	Downregulated	TCGA database	–	Hsa-miR-34a-5p and CDHR3	Suppressive	(37)
Breast cancer	Upregulated	TCGA database Cell lines Human tissues Mice	Cell apoptosis, cycle proliferation, migration, invasion, and EMT	OTX1, p27, p21, cyclin D1, miR-5590-3p, mi100, SMARCA5, E-cadherin, MET, SMO, SEMA3C, CCND1, ERK/MAPK signal pathway, and cell cycle signal pathway	Oncogenic	(19, 20, 38)
Hepatocellular carcinoma	Upregulated	Cell lines Human tissues	Cell viability, migration, and invasion	MiR-146b-5p and CBX6	Oncogenic	(39)
Lung cancer	Downregulated	Array Express database Human tissues	–	–	Suppressive	(40)
Gastric cancer	Upregulated	GEO and TCGA database Cell lines Human tissues	Cell proliferation, migration, invasion, activation of T cells, immune escape, and chemoresistance	Dilated cardiomyopathy, the glutathione metabolism, peroxisome and glycosphingolipid biosynthesis, vascular smooth muscle contraction, focal adhesion, cGMP–PKG, calcium signaling, TGF beta signaling pathways, muscle organ development, cytoskeleton organization, muscle contraction biological process CXXC4, and CDK18-ERK1/2 axis.	Oncogenic	(25, 26, 41–43)
Pancreatic ductal adenocarcinoma	Upregulated	TCGA database Cell lines Human tissues Mice	Stemness, EMT, motility, metastasis, and tumorigenesis	TGF-β, LIN28B, miR-100, miR-125b-1, let-7a, SMAD2/3/4, TP53, apoptosis, and DNA damage crucial pathways	Oncogenic	(44, 45)
Colorectal cancer	Upregulated	TCGA database Cell lines Human tissues Mice	Cell migration, invasion, and chemoresistance, EMT, and cycle arrest	HNRNPA2B1, TCF7L2, miR-100, miR-125b, Wnt signal pathway, DKK1, ZNRF3, RNF43, DKK, APC2, β-catenin, HDAC6, and p57	Oncogenic	(23, 24, 46, 47)
Bladder cancer	Upregulated/ Downregulated	Cell lines Human tissues Mice	Cell proliferation, migration, invasion, and tumor formation	HNRNPA2B1, miR-142-5p, and CALD1	Oncogenic/ Suppressive	(48–52)
Cervical cancer	Downregulated	GEO and TCGA database Cell lines Human tissues	–	Gap junction and TGF-β signal pathway	Suppressive	(21, 22, 53, 54)
Osteosarcoma	Upregulated	GWA data Cell lines	Cell proliferation, cycle arrest, and apoptosis	ELK1, EZH2, LATS1/2, Hippo, PI3K and Rb signal pathway	Oncogenic	(55, 56)

(Continued)

TABLE 1 Continued

Cancer types	Expression	Model used	Functions	Genes/proteins/pathways affected	Role	References
		Human tissues Dogs				
Leukemia	Upregulated	Cell lines Human tissues	Cell proliferation, viability, apoptosis, and necrosis	MiR-100, miR-125b-1, and TGFβ	Oncogenic	(57–60)

related death. It can be divided into different subtypes. Triple-negative breast cancer (TNBC) is the most aggressive and high-risk occurrence subtype (67, 68). Chen et al. (38) showed that MIR100HG was significantly up-regulated in TNBC tissues and cells, and knockdown of MIR100HG inhibited the growth of TNBC cells and tumors *in vivo* and vitro. Consistently, Wang et al. (19) discovered that MIR100HG can promote the proliferation of TNBC cells. Overexpression of MIR100HG in TNBC MDA-MB-231 cells could increase the proportion of cells in the S phase, while knockdown of MIR100HG resulted in cell arrest in the G1 phase. Meanwhile, MIR100HG could promote DNA replication in TNBC cells. However, the coding potential calculator used to predict translational capacity showed that MIR100HG had no potential or ability to convert to protein. The expression of MIR100HG was significantly higher in TNBC than in other subtypes based on TCGA database. Wang et al. (20) further verified at the cellular level and found that MIR100HG could be detected in a variety of breast cancer cell lines, and its expression level in TNBC cell lines was indeed higher than in others. High expression of MIR100HG was related to a low survival rate. Additionally, Chen et al. (38) found that MIR100HG could also augment the expression of the OTX1 gene *via* sponging the miR-5590-3p, thereby promoting the malignant behaviors of TNBC cells, and inhibiting the apoptosis and cell cycle arrest in G0/G1 phase. Mir-100 embedded in the host gene MIR100HG also suppressed tumorigenicity, motility, and invasiveness of breast tumor cells besides miR-5590-3p, but miR-100 was generally downregulated in all subtypes. DNA hypermethylation of MIR100HG may lead to the downregulation or loss of the miR-100, while miR-100 expression is not only regulated by the aforementioned epigenetic silencing but also regulated by transcriptional activation. ZEB1 is usually observed in triple-negative and basal-like breast tumors and the activity of the mir-100 promoter is also significantly increased. Although it remains controversial whether EMT is related to increased tumorigenicity (38). Chen et al. (38) found that miR-100 is a new EMT inducer. EMT induction and tumor inhibition of mir-100 are conducted at different targets. Specifically, miR-100 inhibits EMT by targeting the CDH1 promoter methylation

TABLE 2 Clinical significance of MIR100HG in cancers.

Cancer types	Model used	Clinicopathologic features	References
Head and neck squamous cell carcinoma	TCGA database Human tissues	AJCC stage, resistance, and overall survival	(24, 34, 36)
Papillary thyroid cancer	GEO and TCGA database	Overall survival	(61)
Breast cancer	Mice	Larger tumor size and poor survival	(19, 20)
Hepatocellular carcinoma	**Human tissues**	TNM stage and Edmondson-Steiner grading	(39)
Gastric cancer	GEO and TCGA database Human tissues	Survival time, TNM stage, clinical stage, tumor invasion, lymph node metastasis, distant metastasis, overall survival, and disease-free survival	(25, 41, 42)
Pancreatic ductal adenocarcinoma	Human tissues	Overall survival and disease-free survival	(44)
Colorectal cancer	TCGA database Human tissues	T stage, lymph node metastasis, distant metastasis, AJCC stage, histological differentiation, poor survival, and unfavorable prognosis	(23, 46)
Bladder cancer	GEO and TCGA database Human tissues	Poor clinical outcome, poor prognosis, chemosensitivity, histological grade and clinical stage	(48, 50, 51)
Cervical cancer	GEO and TCGA database Human tissues	Lymph node metastasis and poor prognosis	(21, 53)
Osteosarcoma	Human tissues	Tumor size, advanced clinical stage, and poor prognosis	(55)

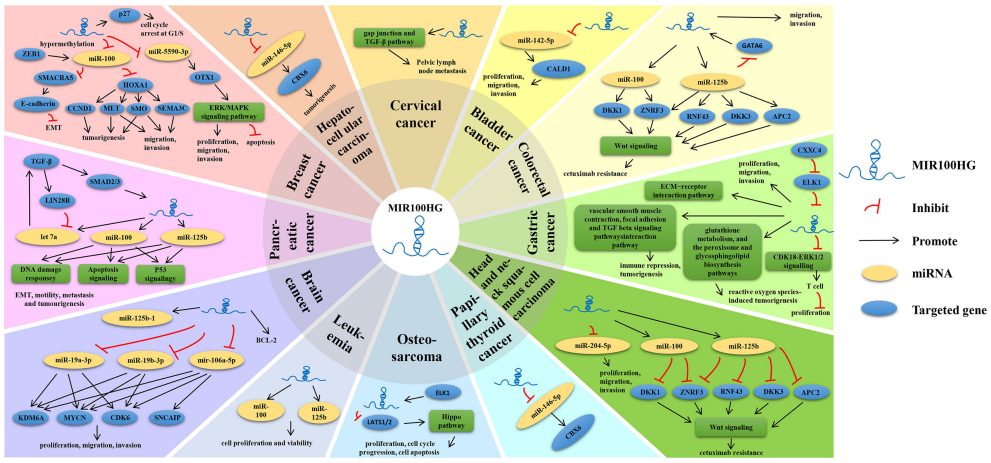

FIGURE 5
Multiple known regulatory mechanisms of MIR100HG in various human cancers.

regulator SMARCA5 to downregulate E-cadherin. Moreover, miR-100 can also inhibit its multiple downstream targets involved in tumorigenesis by directly targeting HOXA1, such as MET, SMO, and CCND1, and downstream targets SEMA3C, MET, and SMO involved in cell migration and invasion. Generally, it is believed that MIR100HG may be an oncogene in TNBC and may serve as a potential prognostic biomarker and therapeutic target for TNBC. Currently, there is no clear treatment guideline for TNBC. Therefore, it is necessary to further study the regulatory mechanism of MIR100HG in TNBC.

Lung cancer

Lung cancer is the second most common cancer in the world in 2020 and the main cause of cancer-related death (69). Non-small-cell lung cancer (NSCLC) accounts for approximately 80%-85% of all subtypes. Yu et al. (40) performed differential expression analysis on the GSE19188 microarray dataset obtained from the ArrayExpress database, including 91 tumor tissues and 65 adjacent non-cancer tissues, and found that the expression of MIR100HG was reduced in tumor tissue compared with non-cancer tissue samples, but there was no differential expression between histological categories. Moreover, the HG-U133Plus2.0 array used by the researchers can only represent some but not all of the potential lncRNAs. As mentioned above, MIR100HG may represent a potential therapeutic target for NSCLC patients. However, there is still limited information on the possible biological functions and mechanisms of MIR100HG. Thus, more comprehensive and in-depth analytical studies are urgently required to clarify the function of MIR100HG in lung cancer.

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is common primary liver cancer, which is accompanied by chronic viral hepatitis B or C and cirrhosis. Li et al. (39) discovered that MIR100HG expression was upregulated in HCC patient tissues and cells and related to the HCC progression. Increased MIR100HG expression was related to advanced TNM stage (III+IV) and Edmondson-Steiner grading (III+IV) in HCC patients and can promote the viability, migration, and invasion of HCC Cells. Bioinformatics analysis results demonstrated that miR-146b-5p was directly targeted by MIR100HG, while the binding site of miR-146b-5p was located on the 3′UTR of CBX6. The elimination of MiR-146b-5p or the overexpression of CBX6 can inhibit the MIR100HG suppression on tumorigenesis of HCC cells (39). However, the specific functional pathway has not been revealed. In summary, MIR100HG may represent a new biomarker for HCC.

Gastric cancer

Gastric cancer (GC) is the fourth most common tumor type and the second most common cancer-related death in the world (70). It is found that MIR100HG was highly expressed in gastric cancer by bioinformatics methods, and its expression was significantly related to the TNM stage, but not with lymph node metastasis (LNM) or age (25). Differently, another research group (41) further explored and declared that high expression of MIR100HG has a positive correlation with clinical stage, invasion, and lymph node and distant metastasis, but not with gender, age, histological grade, and HP infection. Furthermore, high expression of lncRNA MIR100HG predicts short OS in GC

patients, and high MIR100HG expression is also an independent adverse prognostic factor of OS in GC patients. Additionally, Wu et al. (42) identified lncRNA MIR100HG, LINC00205, TRHDE-AS1, OVAAL, and LINC00106 as independent prognostic factors of GC from TCGA and GEO databases by bioinformatics analysis. LINC00106 was considered to be a protective factor. The remaining four lncRNAs are risk factors. They established a risk score model including these five lncRNAs to predict OS and DFS in GC patients, especially in stage II-IV GC patients. Another group (43) also constructed a lncRNA signature including MIR100HG and other 10 lncRNAs, which was associated with the prognosis of GC and can be a more effective prognostic factor for GC patients.

Further experiments *in vitro* showed that low expression of MIR100HG inhibited proliferation, migration, and invasion of GC cells. Recently, a research group (26) analyzed the GC dataset through the GEO database and revealed that CXXC4 was a significantly down-regulated gene in GC. In contrast, ELK1 was significantly upregulated in GC. CXXC4 and ELK1 were identified to be co-expressed in GCs. ELK1 and MIR100HG were also co-expressed. Simultaneously, JASPAR prediction showed that ELK1 binds to the MIR100HG promoter region. Overexpression of CXXC4 can inhibit the phosphorylation of ELK1 and reduce its nuclear translocation, resulting in weakened binding of ELK1 to the MIR100HG promoter, which in turn inhibits CDK18-ERK1/2 signaling, promotes T cell activation, and activates CD3+ T cells. The increased secretion of IFN-γ inhibited the immune escape of GC cells and decreased the proliferative capacity of GC cells. Inhibition of the CXXC4/ELK1/MIR100HG pathway suppressed immune evasion of GC cells, which highlights a possible treatment target for GC. Overall, MIR100HG may serve as a novel target for the diagnosis and treatment of GC.

Pancreatic ductal adenocarcinoma

Pancreatic duct adenocarcinoma (PDAC) is a high mortality cancer with about a 6% survival rate of 5 years (71). Ottaviani et al. (44, 45) revealed that TGF-β can directly promote the transcription of MIR100HG by activating SMAD2/3/4 transcription factors (TFs). Although MIR100HG derived miR-100, let-7a, and miR125b, only miR-100 and miR-125b were up-regulated, and let-7a was unchanged, thereby promoting epithelial-to-mesenchymal transition (EMT) and tumorigenesis in PDAC cells. miR-125b is the most important effector of TGF-β-mediated tumorigenesis, as inhibition of miR-125b or miR-100 affects the ability of TGF-β to induce cell motility and promote spindle cells, but only miR-125b was able to reverse the ability of TGF-β to induce tumorigenesis *in vivo* and vitro. Phenotypically, miR-100 and miR-125b are involved in the EMT, motility, metastasis, stemness formation, and tumorigenesis of PDAC. Moreover, high levels of the two miRNAs were associated with decreased OS and DFS in PDAC patients. They can regulate key pathways in PDAC progressions, such as apoptosis, TP53, and DNA damage, and also regulate many genes involved in suppressing p53 and DNA damage response pathways important for this common metastatic disease progression. However, more efforts should be devoted to illustrating other regulatory mechanisms and clinical implications of MIR100HG in PADC. Of note, MIR100HG may become a promising candidate for PDAC targeted therapy.

Colorectal cancer

Colorectal cancer (CRC) has a high mortality rate, and tumor invasion and distant metastasis are the main causes of cancer-related death in CRC patients (72). MIR100HG, miR-100, and miR-125b may be potential predictive biomarkers for cetuximab (CTX) resistance. CRC cell lines were accompanied by overexpression of MIR100HG, mir-100, and mir-125b in the case of new and acquired CTX resistance. Similarly, this may also occur in patients with KRAS/NRAS/BRAF mutations and CTX resistance. Besides, analysis of the TCGA CRC database demonstrated a stage-dependent increase in the expression of MIR100HG (24). In further experiments, Li et al. (23) found that MIR100HG was significantly increased in CRC tissues compared with normal mucosal tissues. The expression of MIR100HG in advanced stage tissues was significantly higher than that in the early stage and may be related to the aggressive phenotype of CRC patients. Furthermore, MIR100HG overexpression may be an important factor in CRC metastasis, prognosis, and survival. The high expression of MIR100HG showed a positive correlation with TNM stage, lymph node and distant metastasis, AJCC stage, and tumor histological differentiation. However, there was no correlation between high MIR100HG expression and clinical parameters, such as age, sex, tumor size, tumor location, and vascular invasion status. The DFS and OS of CRC patients with high MIR100HG expression were shorter than those of patients with low MIR100HG expression. In addition, MIR100HG expression, AJCC stage, T classification, N classification, M classification, and tumor differentiation could be considered independent prognostic factors for DFS and OS. The expression of MIR100HG was related to EMT markers and was a positive regulator of EMT. Different experiments *in vitro* and vivo still confirmed that MIR100HG sustained cetuximab resistance and overexpression of MIR100HG promoted migration and invasion of CRC cells *in vitro*, as well as the liver metastasis ability *in vivo* (46). Overexpression of MIR100HG induced cell cycle G0/G1 arrest and repressed cell proliferation *via* p57 upregulation *in vitro* and *in vivo* (47). Above all, MIR100HG plays a key role in CRC progression and serves as a novel prognostic biomarker for CRC. The regulation of MIR100HG expression level may be a potential treatment

strategy for CRC patients, especially those with liver metastases. Although MIR100HG acts as a miRNA host gene, its overexpression can upregulate miR-100 and miR-125b, there are few studies on the effect of MIR100HG on the expression levels of miR-100 and miR-125b in CRC. Therefore, the effect of regulating MIR100HG expression on the expression levels of miR-100 and -125b, and how MIR100HG promotes the progression of CRC remains to be studied. Overall, it is believed that MIR100HG may serve as a potential prognostic biomarker and therapeutic target for CRC.

Bladder cancer

Bladder cancer (BC) is the second most common cancer in men worldwide. It has multiple genetic as well as phenotypic features. Even with systemic therapy, most patients eventually relapse or metastasize (73). Muscle-invasive bladder cancer (MIBC) is considered to be the main cause of BC-related death (74). Nevertheless, its mechanism remains largely unknown. Wang et al. (48) conducted a comprehensive analysis of mRNA, lncRNAs, and miRNAs between MIBC and non-tumor bladder samples based on the TCGA database and found that MIR100HG was significantly differentially expressed between MIBC and normal samples and was downregulated in MIBC. On the contrary, Zhang et al. (49) found that MIR100HG was significantly up-regulated in BC tissues in comparison to adjacent tissues, and its high expression was positively correlated with the histological grade and clinical stage of BC. Besides, the expression of MIR100HG was negatively related to the OS of patients (50). MIR100HG is an independent prognostic factor for BC. Additionally, the genomic instability-related lncRNA signature including MIR100HG and other four lncRNAs (CFAP58-DT, LINC02446, AC078880.3, and LINC01833) was also an independent prognostic factor of BC (51). In further experimental studies, Ying et al. (52) discovered that high expression of MIR100HG inhibits the proliferation and invasion of BC cells, and HNRNPA2B1 expression is down-regulated while MIR100HG is overexpressed. Additionally, overexpression of MIR100HG inhibits tumor formation in nude mice *in vivo*. However, Zhang et al. (49) found that high expression of MIR100HG significantly enhanced the proliferation, migration, and invasion abilities of BC cells. Taken together, these findings suggested that MIR100HG may play a vital role in BC and more studies should be replicated for the controversial results to validate the specificity and sensitivity of the MIR100HG product or individual MIR100HG as biomarkers.

Cervical cancer

Carcinoma of the uterine cervix (CACX) is the fourth leading cause of female cancer death (2). Papillomavirus infection is a major causative factor for CACX in high-risk populations, and the long latency period for tumor development after the infection has other genetic and epigenetic changes that lead to deregulation of cellular pathways, which in turn affect protein-coding genes and non-protein-coding genes (75). MIR100HG was found significantly differentially expressed and down-regulated in CACX (53) and CACX patients with pelvic lymph node metastasis (PLNM) (21), and pathway analysis showed a significant correlation with the "cell growth and proliferation" and "cancer" phenotypes (54). Zhang et al. (53) established a prognostic model of CACX with genomic instability-associated lncRNAs including MIR100HG, AC107464.2, and AP001527.2. Besides, the biological function of MIR100HG was correlated with promoter methylation of CACX. However, contrary to the expression result, CACX and CACX patients with PLNM with high expression of MIR100HG showed poor prognosis by Kaplan-Meier analysis based on the TCGA cohort (21, 54). PLNM is the most important prognostic parameter in CACX, especially in the early stage of CACX. Thus, effective PLNM detection is crucial for choosing the best treatment plan. GSEA showed that the RICKMAN_METASTASIS_UP gene set had a higher normal enrichment score and a positive correlation with the gene profile. Finally, this result confirms an important value of MIR100HG in the tumor LNM, which will guide further related *in vivo* and *in vitro* experimental studies and CACX metastasis treatment in the future.

Osteosarcoma

Osteosarcoma is a typical malignant bone tumor mostly discovered in adolescents (76), and its poor prognosis is mainly due to rapid growth and early metastasis (77). MIR100HG has been revealed overexpressed in osteosarcoma cell lines and patient samples. Overexpression of MIR100HG indicated larger tumors and advanced clinical stage. In addition, the expression of MIR100HG showed a negative correlation with the OS rate, and the overexpression of MIR100HG suggested a poor prognosis. Cell function experiments showed that knockdown of MIR100HG inhibited OS cell proliferation, promoted cell apoptosis, and induced cell cycle arrest in G0/G1 phase (55). Su et al. (55) studied the upstream mechanism of MIR100HG and found that ELK1 is a potential transcriptional activator of MIR100HG. Experimental verification indicated that the 1332bp to 1323bp sites in the MIR100HG promoter region were responsible for ELK1-induced transcriptional activation. ELK1 has a high expression in both osteosarcoma tissues and cell lines and was positively associated with the expression of MIR100HG. ELK1 induces MIR100HG epigenetic silencing of LATS1/2 upregulation by binding to the histone methylation regulator EZH2. The expressions of LATS1 and LATS2 were negatively correlated with MIR100HG, and LATS1/2 could partially reduce MIR100HG-regulated cell proliferation,

cell cycle progression, and apoptosis. In the same year, Zapata et al. (56) also found that MIR100HG serves as a new candidate gene that can function alone or in combination with other selected genes such as PHLPP1 and BRIP3 that are also in related PI3K and Rb pathways. Importantly, this model will shed light on cancer regulatory mechanisms and the resulting patterns of somatic mutation. Treatments validated in model organisms could enable small, gene-targeted clinical trials in pet dogs, which not only greatly improves the success rate of human trials, but also provides key information on the mechanisms of drug resistance. The above findings supported the fact that MIR100HG, as an oncogenic lncRNA, could be a novel biomarker in Osteosarcoma.

Acute myeloid leukemia

Acute myeloid leukemia (AML) is a highly aggressive hematological tumor that usually occurs in adults. Particularly, children with Down syndrome (DS) have a 400-fold increased risk of developing acute megakaryoblastic leukemia (AMKL) (57), but DS-AMKL patients have a favorable prognosis compared with non-DS-AMKL patients. Emmrich et al. (57) found that miR-125b-2 was highly expressed in DS-AMKL and non-DS-AMKL. Whereas the polycistronic homolog of miR-99a-125b-2 on hsa21 and miR-100-125b-1 in the MIR100HG intron on hsa11 have the same configuration. MIR00HG is more expressed in erythrocytes, hematopoietic stem/progenitor cells, and B cells in comparison to other blood cell lines. The MIR100HG expression in the AMKL cell line is higher than that in other leukemia cell lines. Regression analysis confirmed that MIR100HG was positively correlated with its miRNA polycistronic. Knockdown of MIR100HG impairs cell replication efficiency and viability in AMKL cells while altering the expression of lineage surface markers, increasing the percentage of CD36+ cells and decreasing the proportion of CD41+ cells. MIR100HG induces apoptosis of human megakaryocytic leukemia cells through up-regulating the expression of TGF-β, inhibiting the proliferation of human AMKL cell lines, and inducing their apoptosis and necrosis (58, 59). MIR100HG is mainly located in the nucleus, which makes it a role in regulating erythroid megakaryocyte development, while further studies are needed to determine the protein interaction target and to pinpoint the accurate subnuclear region and DNA target sequence of MIR100HG. The above results suggest that MIR100HG may be a new marker for improving AML treatment.

Studies on acute promyelocytic leukemia (APL) have reported that the up-regulation of MIR100HG is related to the proliferation of primary APL cells. Degradation of MIR100HG by antisense LNA GapmeRs can induce apoptosis, necrosis, and inhibit cell proliferation in APL cells. Therefore, inhibition of MIR100HG is a novel approach to control APL cell proliferation, which can be considered for the treatment of APL alone or in combination with existing treatments, or for patients who are resistant to conventional APL therapy (60). Corresponding clinical trials are still necessary to verify the effectiveness of MIR100HG for APL.

Mechanism of MIR100HG

The regulatory mechanisms of MIR100HG are diverse. According to current research, MIR100HG is mainly involved in pre-transcriptional regulation and post-transcriptional regulation. Pre-transcriptional regulation is mainly (1) as a promoter of RNA binding proteins (RNA binding proteins, RBPs) (2); as a structural component to form nucleic acid-protein complexes with proteins; post-transcriptional regulation is mainly (3) as ceRNA; (4) as miRNA precursor (Table 1 and Figure 5).

A promoter of RNA binding proteins

MIR100HG positively regulates the association between RBP HuR and multiple target mRNAs. The 3' end of MIR100HG is rich in U sequences. MIR100HG takes advantage of the U-rich motif to interact with HuR and HuR target mRNAs (18). Consistent with the aforementioned function of MIR100HG as an RNA-binding protein promoter, Panagiotis Papoutsoglou et al. (78) extended this model in the context of TGFβ tumor biology. The 3′ untranslated region of TGFB1 mRNA contains AU-rich sequences recognized by HuR, and MIR100HG promotes the formation of a complex between the RNA-binding protein HuR and TGFβ1 mRNA, thereby stabilizing the mRNA and enhancing autocrine TGFβ1 production and autoreactivity. Researchers suggested that HuR contains three RNA recognition motifs, one of which promotes HuR dimerization. Thus, there may be a mechanism whereby MIR100HG binds to one RNA recognition motif to promote TGFβ1 mRNA and the second RNA recognition motif in the dimerized HuR sequential combination. On the other hand, this lncRNA is in turn directly regulated by RBP. The RBP TDP-43 can directly regulate the expression and control stability of the mature form of linc-NeD125 merely under differentiation conditions, while this lncRNA is stabilized (27).

As a structural component to form nucleic acid-protein complexes with proteins

Chen et al. (38) discovered that the expression of MIR100HG was significantly up-regulated in TNBC tissues and cells and MIR100HG can affect the progression of TNBC

by regulating the expression of p27. Through bioinformatics prediction, three ncRNA binding motif triplex-forming oligonucleotides (TFOs) were found in MIR100HG, which specifically bind to p27 to form an RNA-DNA triple structure. They are located at 275-352nt (TFO1), 462-557nt (TFO2), and 2635-2688nt (TFO3) in the MIR100HG sequence, respectively. But the three TFOs do not act synergistically, and TFO1 is a functional binding site on the p27 site. Generally, lncRNAs in tumors interact with the EZH2 gene by trimethylation of lysine 27 of histone 3 on the p27 promoter, inhibit p27, or directly bind and activate the p27 promoter (79, 80). MIR100HG binds to the p27 gene locus through the TFO1 sequence to form an RNA-DNA triple structure, recruits epigenetic modification proteins to directly bind and activate the p27 promoter to regulate p27 expression, and is involved in the regulation of cell proliferation in TNBC. Additionally, both p21 and p27 are cyclin-dependent kinases (CDK) inhibitors, while cyclin D1 is necessary to G1 cell cycle progression. After MIR100HG overexpression, the expression of both p21 and p27 is reduced, and cyclin D1 expression is increased. p21, p27, and cyclin D1 are G1/S checkpoint cell cycle regulator proteins. p21 and p27 function on the CDK4/6-cyclin D1 complex, thereby preventing cell cycle progression in the G1 phase, while cell cycle D1 facilitates the G1/S transition. However, the locus-specific formation of RNA-DNA triplet structures by MIR100HG with p21 or cyclin D1 has not been discovered in bioinformatics predictions, but it cannot be ruled out that MIR100HG may also directly regulate p21 and cyclin D1 through other mechanisms. But it can't be ruled out that MIR100HG is possible to regulate p21 and cyclin D1 through other mechanisms (20).

As ceRNA

LncRNAs are often reported as molecular sponges that bind to miRNAs to inhibit the binding of miRNAs to target mRNAs and are involved in regulating cancer progression. However, MIR100HG does not play a role in TNBC cells through its host miRNAs but plays a critical role in the occurrence and progression of TNBC through other miRNAs in the ceRNA regulation mode. Chen et al. (38) found that MIR100HG can up-regulate the expression of the OTX1 gene by targeting miR-5590-3p, thereby promoting the malignant behaviors of TNBC cells, and inhibiting their apoptosis and cell cycle arrest in G0/G1 phase.Linc-NeD125 as a ceRNA can recruit the miRNA-induced silencing complex miRISC and directly bind the miRNAs miR-19a-3p, miR-19b-3p, and miR-106a-5p, sequestering the three miRNAs, resulting in the dysregulation of four target genes CDK6, MYCN, SNCAIP, and KDM6A, which are major drivers of G4MB (28). MIR100HG may promote the proliferation, migration, and invasion of cancer cells in LSCC by downregulating miR-204-5p, and MIR100HG may also have multiple downstream effectors in LSCC (36). MIR100HG deficiency inhibited the tumorigenesis in HCC cells by targeting the miR-146b-5p/CBX6 axis (39). MIR100HG can regulate CALD1 expression by sponging miR-142-5p to inhibit the proliferation, migration, and invasion of bladder cancer cells (49).

As miRNA precursor

Bevilacqua et al. (27) suggested that linc-NeD125 and its miR-125b-1 function as negative regulators in human neuroblastoma cell proliferation. These two-overlapping noncoding RNAs are coordinated *in vitro* during neuronal differentiation, and their expression is regulated by distinct mechanisms. While the production of miR-125b-1 is dependent on transcriptional regulation, linc-NeD125 keeps it under control at the post-transcriptional level by regulating its stability. On the other hand, linc-NeD125 acts independently of host miRNAs by attenuating cell proliferation and activating the anti-apoptotic factor BCL-2.

MIR100HG participates in multiple signaling pathways

The MIR100HG participates in multiple signaling pathways in diverse cancers (Table 1).

Wnt/β-catenin signaling pathway

The Wnt/β-catenin signaling pathway appears repeatedly in the occurrence and progression of different cancers and is very important for regulating cell proliferation, survival, migration, and other processes. There are many inhibitors of this pathway, including the DKK family, APC, ZNRF3, RNF43, etc. (24) Lu et al. (24) discovered that MIR100HG, miR-100, and miR125b were simultaneously up-regulated under acquired and new cetuximab resistance in HNSCC and CRC cell lines. Five negative regulators (DKK1, DKK3, ZNRF3, RNF43, and APC2) of the typical Wnt signaling pathway are synergistically downregulated by miR-100 and miR-125b. DKK1 and ZNRF3 are targeted by miR-100, and ZNRF3, RNF43, DKK3, and APC2 are targeted by miR-125b to increase the Wnt/β-catenin signaling pathway, resulting in resistance to cetuximab. Inhibition of Wnt signaling restored cetuximab responsiveness *in vitro* and *in vivo*. However, effects of the full-length 3-kb MIR100HG transcript on Wnt signaling cannot be ruled out. In addition, according to the analysis of the TCGA database, the research group also found that the expression of GATA6 was decreased in stage IV CRC, while the expression of MIR100HG was increased, and the increase in the expression of miR-125b

could enhance the inhibitory effect of GATA6, which in turn promoted the increased expression of MIR100HG. In this case, GATA6 can indirectly prevent cetuximab resistance caused by enhanced Wnt signaling to exert a tumor suppressor effect, which is different from previous studies in that GATA6 promotes tumorigenesis by activating Wnt signaling (81). Additionally, the study group further demonstrated that MIR100HG interacted with hnRNPA2B1 to augment Wnt signaling by stabilizing TCF7L2 mRNA, which is a core component that binds to nuclear β-catenin to promote downstream Wnt target gene transcription. hnRNPA2B1 recognized the N6-methyladenosine (m6A) site of TCF7L2 mRNA in the presence of MIR100HG. TCF7L2 activated MIR100HG transcription. Above all, MIR100HG functions in concert with their encoded miR-100/125b to enhance Wnt signaling activity in the setting of advanced CRC, but at different levels of Wnt signaling *via* complementary mechanisms (46). β-catenin/TCF4 can bind to the MIR100HG promoter. Additionally, MIR100HG was negatively correlated with HDAC6 and β-catenin in CRC specimens. β-catenin forced expression reduced primary and mature lnc-MIR100HG levels and the enrichment of H3K27Ac. HDAC6 was recruited to the MIR100HG promoter and downregulated H3K27Ac enrichment in a β-catenin-dependent manner (47). Above all, it provides new ideas for further therapeutic approaches.

ERK/MAPK signaling pathway

The ERK/MAPK signaling pathway is the most studied MAPK signaling pathway (38). Chen et al. (38) found that knockdown of MIR100HG *in vitro* and *in vivo* decreased the expression of OTX1 by upregulating miR-5590-3p and successfully inhibits the activation of the ERK/MAPK signaling pathway in TNBC, thereby inhibiting tumorigenesis.

TGF-β signaling pathway

TGFβ signaling pathway is signaling through membrane receptors. Membrane receptor activates effector transcription factors SMADs and MAPKs, which regulate target genes that control cell cycle, migration, extracellular matrix remodeling, and EMT. TGFβ can promote the stemness, invasiveness, and metastasis of advanced tumors (78). Shang et al. (21) found that MIR100HG has a key value in CACX LNM by regulating the TGF-β pathway. Moreover, Noordhuis et al. (22) confirmed that disrupting the TGF-β pathway may promote the malignant progression of cervical dysplasia in human CACX, further suggesting that MIR100HG is possible to participate in the regulation of early CACX LNM through the TGF-β pathway. Ottaviani et al. (44, 45) found that TGFβ induced MIR100HG transcription in pancreatic cancer cells. Although MIR100HG derived miR-100, let-7a, and 125b, it only induced the expression of miR125b and miR-100, but not miR let-7a -2 expression. The lack of change in let-7a may be due to the post-transcriptional downregulation of let-7a levels induced by TGF-β in LIN28B. Such a system of primary polycistronic miRNA transcript upregulation and post-transcriptional repression of specific miRNAs is critical to TGF-β responses in PDAC. In addition, various MIR100HG miRNAs mediated by TGFβ in keratinocytes and different cancer cells may reflect different physiological outcomes. TGFβ induces antitumor let-7a-2-3p to promote its antiproliferative capacity. Conversely, TGFβ often loses its antiproliferative capacity and instead promotes EMT and stem cells, including increased expression of EMT-promoting miR-100 and miR-125b in pancreatic cancer. As previously stated, MIR100HG may be an oncogenic or tumor suppressor, while the dual role of MIR100HG in cancer may be related to the dual role of TGFβ. Existing studies suggest that TGFβ has antitumor properties in some cancers and tumorigenic effects in others, which deserves to be explored in future studies.

YAP-hippo signaling pathway

The Hippo signaling pathway is evolutionarily conservative, and YAP is its main effector molecule. Inactivation of the Hippo signaling pathway induces down-regulation of MST1/LATS1 and up-regulation of YAP1, which are the core factors of the pathway. Dysregulation of Hippo signaling has been found in many human tumors and is closely associated with the acquisition of malignant features (82). Su et al. (55) studied the downstream mechanism of MIR100HG and showed that MIR100HG plays an oncogenic role in OS by inactivating the Hippo signaling pathway, as silenced MIR100HG leads to increased MST1, LATS1, LATS2 protein levels and decreased YAP1 protein levels.

Other signaling pathways

Zapata et al. (56) found that MIR100HG can be considered as a new candidate gene in a risk model of dog osteosarcoma genome scanning, which can function individually or in combination with other genes MYCN, Akt2, MTMR7/9, FGF9, PHLPP1, and BRIP3 also selected in the related PI3K and Rb pathways. Shang et al. (21) further suggested that MIR100HG may be involved in the regulation of LNM in early cervical cancer in various ways. MIR100HG not only participates in the TGF-β pathway, but also participates in gap junction, and most of the mRNAs co-expressed with MIR100HG participate in the gap junction pathway. In multiple PDAC patients, high expression of miR-100 or 125b was correlated with decreased OS and DFS. The two miR-100 or 125b of the MIR100HG host

regulate key pathways of PDAC progression, such as TP53, apoptosis, and DNA damage, and also regulate many genes involved in repressing p53 and DNA damage response pathways. These genes are critical for the progression of this common metastatic disease. In the study of gastric cancer, Li et al. (25) showed that MIR100HG is related to muscle tissue and cytoskeletal organization and participates in the interaction of ECM receptors, and these functions have been confirmed to be related to tumor metastasis. MIR100HG is positively associated with vascular smooth muscle contraction, local adhesion, and TGF-β signaling pathways, which is associated with immunosuppression and tumorigenesis, while inversely associated with glutathione metabolism, peroxisome, and glycosphingolipid biosynthesis pathways, mainly involved in reactive oxygen species-induced tumorigenesis.

MIR100HG and chemoresistance

Cetuximab

CTX is a monoclonal antibody against EGFR, which binds to the extracellular domain of EGFR and enhances the internalization and degradation of receptors. It is effective for metastatic CRC. When CTX is used as a monotherapy, EGFR monoclonal antibody produces durable responses in 12-17% of patients. When the mAb is combined with chemotherapy, there is a response rate of up to 72%. However, drug resistance frequently emerges with little known about resistance mechanisms (24). Lu et al. (24) established CTX-resistant cell lines of CRC and HNSCC in a three-dimensional medium and found that MIR100HG and its miR-100 and 125b were upregulated in CTX cell lines, and miR-100 and 125b synergistically drive cancer cell resistance to CTX. MiR-100 and 125b can suppress several Wnt inhibitory regulators and upregulate the level of the Wnt signaling pathway, resulting in cell resistance to CTX. This study has important clinical application value and can be used to treat CTX-resistant progressive CRC and HNSCC.

Sorafenib

Sorafenib (SFB) is a biaryl urea oral multikinase inhibitor, which is a commonly used drug in clinical molecular targeted therapy for liver cancer. Reducing the drug resistance of liver cancer cells has a positive significance for improving the therapeutic efficacy (83). He et al. (83) found that MIR100HG was upregulated and miR-142-5p was downregulated in sorafenib-resistant Huh7/SFB cells compared with Huh7 cells. MIR100HG targets and negatively regulates miR-142-5p expression. Inhibition of MIR100HG expression can improve the inhibition rate of sorafenib on Huh7/SFB cells, promote sorafenib-induced apoptosis and p21 and Bax protein expression in Huh7/SFB cells and inhibit CyclinD1 and Bcl-2 protein expression. Inhibition of miR-142-5p expression could reverse the effects of inhibiting MIR100HG expression on sorafenib-induced Huh7/SFB cell viability, apoptosis, and expression of related proteins p21, Bax, CyclinD1, and Bcl-2. This provides a new idea for improving the drug resistance of liver cancer to SFB and improving the therapeutic efficacy of liver cancer.

Gemcitabine

Gemcitabine (GEM) is a pyrimidine nucleotide analog, belonging to the antimetabolite class of anticancer drugs. It mainly acts on the DNA S phase, preventing DNA synthesis and leading to cell apoptosis. MiRNAs of the Let-7 family induced EMT reversal in GEM-resistant PDAC cells. MiR-100 and 125b are upregulated in GEM-resistant cells compared with Let-7 family members and promote EMT in PDAC. Targeting non-coding RNAs has been used for anticancer therapy. Inhibiting MIR100HG, miR-100, and 125b can improve PDAC resistance to GEM, which can be regarded as a novel approach to treating PDAC and used as biomarkers for stratified PDAC (44, 45).

Docetaxel, cisplatin, and S-1

The combination of docetaxel, cisplatin, and S-1 (DCS) is a common chemotherapy regimen for GC. Zhang et al (43) constructed a lncRNA signature including MIR100HG and other 10 lncRNAs, which was associated with the prognosis of GC and can be a more effective prognostic factor for GC patients. Furthermore, this model can well predict chemotherapy drug response and immune infiltration of GC patients. Therefore, the above results have uncovered that MIR100HG combined with other 10 lncRNAs as a new DCS therapy-related lncRNA signature could accurately predict outcomes for gastric cancer patients.

Summary and prospect

MIR100HG has been proven to be key regulator of human gene expression. Accumulating researchers have reported that MIR100HG is upregulated in many malignant tumors, containing leukemia, head and neck carcinoma, breast carcinoma, pancreatic ductal adenocarcinoma, osteosarcoma, gastric cancer, colorectal cancer, hepatocellular carcinoma and downregulated in a few tumors, including lung cancer, papillary thyroid cancer, and cervical cancer. While MIR100HG is upregulated or downregulated in brain tumor and bladder cancer (Figure 5). The regulatory mechanisms of MIR100HG are very complex and involve many steps, including as a

promoter of RBPs, as a structural component to form nucleic acid-protein complexes with proteins, as ceRNA, and as a miRNA precursor. MIR100HG mainly regulates the occurrence and development of diseases through the Wnt/β-catenin, ERK/MAPK, TGF-β, YAP-Hippo, and other signaling pathways. MIR100HG played an oncogenic or suppressive role that is involved in various tumor cell biology processes including proliferation, cell cycle, apoptosis, migration and invasion, metastasis, drug resistance, and EMT (Table 1). Meanwhile, dysregulated expression of MIR100HG is markedly correlated with poor prognosis and clinicopathological features including tumor size, AJCC stage, TNM stage, LNM, distant metastasis, prognosis, DFS, OS, and chemoresistance (Table 2). Therefore, MIR100HG is expected to serve as a promising disease diagnostic and prognostic biomarker or novel treatment target.

While the regulatory mechanisms of MIR100HG in multiple cancers have been investigated, studies on MIR100HG remain in the primary stage, and many key issues need to be further addressed. First and foremost, how to distinguish between effects mediated by MIR100HG and its residing miRNAs. Determining the independent roles that exist for host MIR100HG in different cancers is an open area of investigation. Besides, the reason why MIR100HG can play the opposite role as an inhibitor or promoter in different tumors should be investigated. What's more, an experimental in-depth study to precisely find the upstream and downstream molecular mechanisms of MIR100HG in different cancers is needed. For example, regulators and targets including mRNA or other miRNAs involved in the aberrant expression of MIR100HG in tumors remain rarely known, and the molecular mechanism of MIR100HG-mediated RNA-binding protein regulation still needs to be clarified in further studies. In addition, there is still a lack of large independent cohort studies for further verification. The physiological role of MIR100HG, the interaction between MIR100HG and the cancer microenvironment, and the function of MIR100HG in immune response, cell metabolism, starvation/autophagy, and neo-vascularization need to be investigated. What's more, a set of cellular senescence-associated miRNAs (SA-miRNAs) derived from the oncogenic MIR17HG and tumor-inhibiting MIR100HG clusters are effective controllers of complex and coordinated interactions among multiple cellular sub-processes related to cellular senescence. Importantly, it proved the functional significance of these SA-miRNAs to establish an aging phenotype in adult adipose stem cells (84). This raises the crucial issue of whether MIR100HG is involved in the pathogenesis of other diseases related to aging or high-fat metabolism in addition to tumor diseases. Although MIR100HG participates to regulate the progression of the tumor, the biological function of MIR100HG remains largely unknown. The diversification of structure enables RNAs to conduct various functions (85, 86). However, so far, no researchers have focused on the structures of MIR100HG. Thus, it is greatly important to investigate the biological function of MIR100HG in diseases by exploring the structure of MIR100HG.

In summary, we are optimistic that this review will contribute to a better understanding of MIR100HG and its relationship with a variety of cancers and the relevant knowledge can lay a solid foundation for the practical application of MIR100HG in the future.

Author contributions

YW contributed to the conceptualization, design, formal analysis, and final approval of the submitted version. YW, ZW, and SY collected and analyzed literature. YW, LS, and DL contributed to the manuscript writing. LS contributed to supervision and funding acquisition. All the authors conceived and approved the final manuscript. All authors contributed to the article and approved the submitted version.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.997532/full#supplementary-material

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The oncogenic roles of JC polyomavirus in cancer

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JC polyomavirus (JCPyV) belongs to the human polyomavirus family. Based on alternative splicing, the early region encodes the large and small T antigens, while the late region encodes the capsid structural proteins (VP1, VP2, and VP3) and the agnoprotein. The regulatory transcription factors for JCPyV include Sp1, TCF-4, DDX1, YB-1, LCP-1, Purα, GF-1, and NF-1. JCPyV enters tonsillar tissue through the intake of raw sewage, inhalation of air droplets, or parent-to-child transmission. It persists quiescently in lymphoid and renal tissues during latency. Both TGF-β1 and TNF-α stimulates JCPyV multiplication, while interferon-γ suppresses the process. The distinct distribution of caspid receptors (α-2, 6-linked sialic acid, non-sialylated glycosaminoglycans, and serotonin) determines the infection capabilities of JCPyV virions, and JCPyV entry is mediated by clathrin-mediated endocytosis. In permissive cells, JCPyV undergoes lytic proliferation and causes progressive multifocal leukoencephalopathy, while its DNA is inserted into genomic DNA and leads to carcinogenesis in non-permissive cells. T antigen targets p53, β-catenin, IRS, Rb, TGF-β1, PI3K/Akt and AMPK signal pathways in cancer cells. Intracranial injection of T antigen into animals results in neural tumors, and transgenic mice develop neural tumors, lens tumor, breast cancer, gastric, Vater's, colorectal and pancreatic cancers, insulinoma, and hepatocellular carcinoma. Additionally, JCPyV DNA and its encoded products can be detected in the brain tissues of PML patients and brain, oral, esophageal, gastric, colorectal, breast, cervical, pancreatic, and hepatocellular cancer tissues. Therefore, JCPyV might represent an etiological risk factor for carcinogenesis and should be evaluated for early prevention, diagnosis, and treatment of cancers.

KEYWORDS

JC polyomavirus, cancer, oncogenesis, signal pathway, virus replication, virus infection

Introduction

JC polyomavirus (JCPyV) belongs to the human non-enveloped polyomavirus family in combination of SV40 and BK viruses. The genomic DNA homology between JCPyV and SV40 or BK viruses is 69% or 75%, respectively, showing their close evolutionary relationships (1). A serological study has indicated asymptomatic JCPyV infection in 46.1% of 1-month-old infants, 80.7% of 1- year-old infants, 85.9% of 2-year-old children, 85.5% of 3-year-old children, and about 90% of the adult population (2). As shown in Figure 1, JCPyV consists of a small, circular, double-stranded DNA genome of 5,130 base pairs and icosahedral capsids. The transcription of early and late coding regions occurs to produce small t and large T antigens by an interposed transcription control region. The late region encodes the capsid structural proteins (VP1, VP2, and VP3) by alternative splicing and a small regulatory protein (agnoprotein). T and t antigens are responsible for DNA replication, and the VP proteins for assemble with viral DNA to form virions (1). JCPyV may be activated for cell lysis under immunosuppressive conditions (e.g., HIV infection or the transplantation of bone marrow, liver, lung or kidney), and therefore is an established etiologic factor of demyelinating progressive multifocal leukoencephalopathy (PML) (3–7). Moreover, JCPyV could infect the enteric glia and cause chronic idiopathic intestinal pseudo-obstruction (8), or result in male lower urinary tract symptoms (9). The autoimmune diseases of JCPyV-associated brain syndromes include multiple sclerosis (MS), Crohn's disease, and psoriasis, which were not previously considered as predisposing factors for PML (10). In non-permissive cells (i.e., cells that do not allow viral replication), JCPyV infection causes either abortive infection or malignant transformation (1) (Figure 2).

The infection and replication of JCPyV

As shown in Figure 3, JCPyV enters the human body through the intake of raw sewage or the inhalation of air droplets, and persists quiescently in tonsillar lymphoid and renal tissues during latency (11). Parent-to-child transmission is also common for its propagation (12). After asymptomatic primary infection in childhood, the virus spreads through the bloodstream from the primary sites of infection to secondary sites (kidney and lymphoid tissues, peripheral blood leukocytes, and brain tissue) (13). JCPyV DNA replication occurs primarily in lymphoid and glial cells that contain transcription factors specific for JCPyV (14, 15).

JCPyV infects human cells by the interaction of capsid VP proteins with receptors on JCPyV-sensitive cells, followed by endocytosis and nuclear transport of JCPyV virions. In the nucleus, the viral DNA is uncoated, initiating the transcription of the early region (16). The tissue-specific distribution of the VP receptors (α 2, 6-linked sialic acid, non-sialylated glycosaminoglycans, and serotonin) determines the different infection capabilities of JCPyV (17–21). JCPyV infection is dependent on the interactions between VP capsid proteins and asparagine N-linked sialic acids or the serotonin 5-hydroxytryptamine 2A receptor (5-HT2AR) on the cell

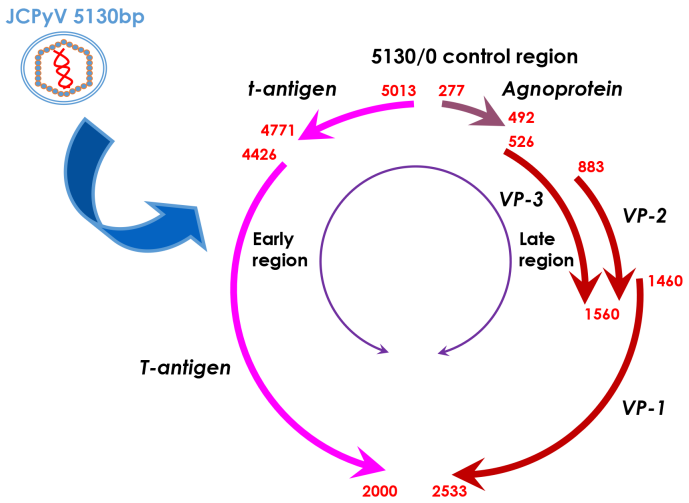

FIGURE 1
The genomic DNA structure of JCPyV. JCPyV has icosahedral capsids and small, circular, double-stranded genomic DNA of 5130 base pairs. It is composed of early and late coding regions, which are transcribed in opposite directions initiated by a transcriptional control region. The early region encodes both small and large T antigens by alternative splicing. The late region encodes the capsid structural proteins (VP1, VP2 and VP3) by alternative splicing and a small regulatory protein, agnoprotein.

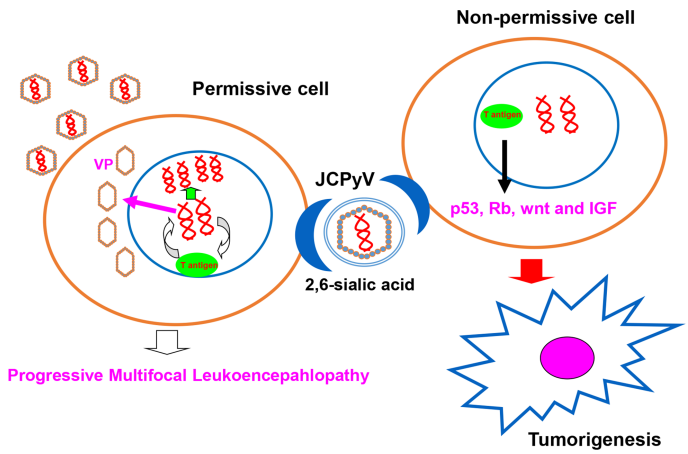

FIGURE 2
JCPyV infection outcome. JCPyV infection is initiated by its binding to JCPyV-sensitive cell surfaces. JCPyV capsids undergo endocytosis *via* capsid receptors (e.g., α 2, 6-linked sialic acid, non-sialylated glycosaminoglycans, and serotonin). In permissive cells, JCPyV may be activated for cell lysis and cause progressive multifocal leukoencephalopathy under immunosuppression (e.g., HIV infection, immunosuppressive drugs for organ transplantation, and cancer chemotherapy). In non-permissive cells, T antigen DNA from JCPyV can be inserted into genomic DNA, and T antigen can induce the malignant transformation of normal cells by targeting the p53, Rb, wnt, and IGF signal pathways.

surface. Treatment with an inhibitor of N-linked glycosylation (tunicamycin), 5HT2AR antagonists (ketanserin and ritanserin), or anti-5HT2aR antibody reduces JCPyV infection, while treatment with PNGase F to remove N-linked oligosaccharides does not influence JCPyV infection (18, 19, 22). VP1-composed virion-like particles (VLPs) can bind to sialoglycoproteins (α1 acid-glycoprotein, transferrin receptor, and fetuin) and glycolipids (gangliosides and lactosylceramide) (23). Exposure to either anti-VP1 antibody or sialidase to hydrolyze sialic acid residues can suppress viral entry into host cells. After interaction between capsid proteins and their receptors, JCPyV binds to caveolin-1 and undergoes eps15 and Rab5-GTPase-mediated internalization and clathrin-dependent endocytosis (24), which is facilitated by the interaction of β-arrestin with 5-hydroxytryptamine receptors (25).

After the entry of JCPyV into cells, TCF-4-T-antigen complex binds to the JCPyV promoter in U87-MG cells, increasing the ability of the T antigen to replicate viral DNA (26). LCP-1 also interacts with the lytic control element and differentially regulates T antigen expression (27). Glial factor 1 (GF-1) has homology with the central region of Sμbp-2 and can bind to the promoter B-regulatory domain of JCPyV (28). Purα interacts with T antigen to modulate T-antigen-mediated transcriptional activation, while the Purα-BAG-1 complex suppresses JCPyV DNA replication in glial cells (29, 30). The terminal core kinase of the MAPK cascade (MAPK-ERK) facilitates the transcription of the JCPyV by up-regulating the transcription factors downstream of the MAPK cascade (i.e., c-myc and SMAD4) and shuttling them to the nucleus (31), while SF2/ASF (splicing factor 2/alternative splicing factor) weakens the transcription and alternative splicing of JCPyV genes *via* direct interaction with the viral promoter (32), and retinoic acid-inducible gene I (RIG-I) and cGMP-AMP synthase negatively controls JCPyV replication in human astrocytes (33).

Moreover, HIV-1 induces cytokines that reactivate JCPyV to cause PML in the brain, suggesting a direct correlation between inflammatory cytokines and the susceptibility to JCPyV infection (34, 35). The treatment of glial cells with IFNα and IFNβ increases the endogenous levels of C/EBPβ-LIP, which inhibits basal and NF-κB-stimulated JCPyV transcription *via* the NF-κB-C/EBPβ-LIP -JCPyV DNA complex (36). Both TGF-β1 and TNF-α can stimulate JCPyV multiplication and increase the overall number of infected cells *via* the Smad and NF-κB pathways, respectively (37). Tat and Fast1 can cooperate with Smad2, 3, and 4 at the JCPyV DNA control region, stimulating its gene transcription in oligodendroglial cells (38). JCPyV infection significantly increases nuclear HIF-1α levels in glial cells, which binds to and activates the JCPyV early promoter *via* Smad3 and Smad4 (39). IL-1β dramatically increases JCPyV transcription in glial cells *via* NF-1 binding to the JCPyV enhancer region *via* the PKC pathways (40). However, interferon-γ inhibits JCPyV replication by down-regulating T antigen expression *via* Jak1 signaling (41).

According to recent literatures, topoisomerase I inhibitors (β-lapachone and topotecan) are found to inhibit JCPyV infection in neuroblastoma cells (42). Irisolidone, an isoflavone metabolite, negatively modulates JCPyV gene expression by suppressing Sp1 binding in glial cells (24). The Cdk inhibitor, R-roscovitine, suppresses the proliferation and production of JCPyV by inhibiting the phosphorylation of T antigen (43).

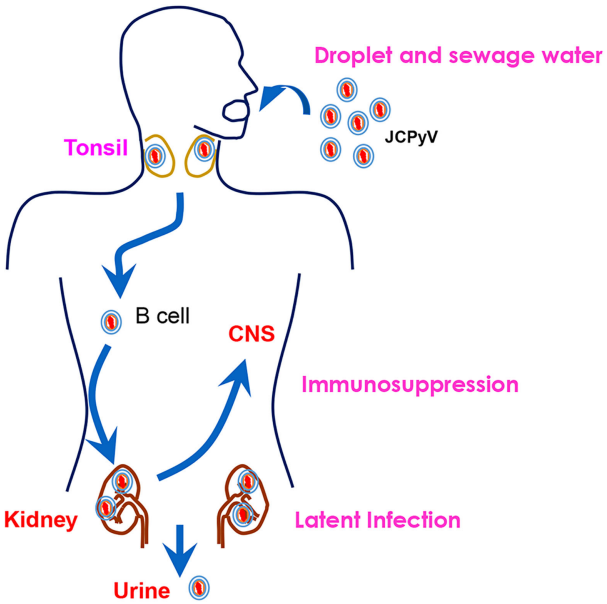

FIGURE 3
The natural history of JCPyV. JCPyV enters the human body through the intake of raw sewage or inhalation of air droplets. It is transported to the kidneys *via* B cells. It persists quiescently in the tonsil and renal tissues during latency. Upon immunosuppression, JCPyV enters the central nervous system (CNS) and undergoes lytic proliferation, resulting in the demyelinating brain disease, progressive multifocal leukoencephalopathy (PML). It can be excreted from the human body through the urine.

Hexadecyloxypropyl- cidofovir suppresses JCPyV replication in fetal brain SVG cells (44). JCPyV infection can be suppressed by nocodazole, cytochalasin D, or acrylamide in glial cells (45). Moreover, O'Hara et al. (46) found that teriflunomide could inhibit JCPyV infection and propagation in choroid plexus epithelial cells and glial cells. PARP-1 inhibitor, 3-aminobenzamide, could significantly suppress JCPyV replication and spread (47). In contrast, both trichostatin A (TSA) and butyrate can activate the JCPyV promoter and hyperacetylation of this promoter in non-glial cells. The enhancer and Sp1 element upstream of the TATA box are necessary for TSA-mediated activation (48). Some reagents are expected to prevent the infection and replication of JCPyV in the future.

The functions of JCPyV-encoded proteins

T antigen

T antigen is a multifunctional and oncogenic phosphoprotein essential for viral DNA replication in G_2-arrested cells *via* ATM- and ATR-mediated G2 checkpoint signaling (49). It binds to and breaks DNA to unwind the double helix and recruits helicase, ATPase, and polymerase (1, 50). T antigen primarily targets protein complexes that have PP4 and PP1 phosphatase, v-ATPase, and E3-ubiquitin ligase activities (51). Its N-terminal portion contains LXCXE and J domains, which are necessary for binding and inactivating the Rb family (52) and its N-terminal phosphorylation site at threonine 125 is critical to T-antigen-mediated replication *via* stabilizing T antigen, interaction with the Rb family members p107 and p130 and the release of E2F from RB-E2F complex (53). The origin-binding domain of T antigen contains a C-terminal pocket and interacts with the major groove of GAGGC sequences. The pocket residue increases T antigen expression, supporting JCPyV DNA replication (54).

Reportedly, AP-1 family (c-Fos and Jun) functionally interacts with T antigen, significantly diminishing T-antigen-mediated replication and transcription of JCPyV genes in glial cells. The c-Jun-binding domain for T antigen maps to the middle portion of the protein, while the T-antigen-binding domain for c-Jun is its basic-DNA binding region (55). In glial cells, T antigen interacts with Purα and serine/arginine-rich splicing factor 1 (SRSF1). T antigen promotes JCPyV gene expression by binding to the SRSF1 promoter and weakening SRSF1 transcription (56, 57). Purα and T antigen bind to the JCPyV early promoter *via* T-antigen, ameliorating SRSF1-mediated inhibition of JCPyV gene expression and replication (58). P53 can interact with T antigen, blocking viral DNA replication (59). However, neurofibromatosis type 2 could

induce proteasomal degradation of the T-antigen and suppress T-antigen protein expression in glioblastoma cells, weakening T-antigen-mediated regulation of the JCPyV promoter (60), and LIP (liver inhibitory protein) expression also induced the degradation of JCPyV T antigen in transgenic mouse tumor cells (32, 61). The partner proteins modulate the biological functions and protein instability of T antigen, which is involved in carcinogenesis and subsequent progression.

Agnoprotein

The JCPyV agnoprotein shares 50–60% homology with those of BK and SV40 viruses; however, its carboxyl-terminal region is relatively unique. It is firstly detected on day 3 of JCPyV post-infection, and its levels increase until the late stage of infection, and responsible for virion release and viral propagation (62). Agnoprotein localizes to the endoplasmic reticulum (ER) early in infection and then the plasma membrane late in infection (63). Agnoprotein is 71 amino acids (8kDa) and stably forms dimers and oligomers through its hydrophobic Leu/Ile/Phe-rich (aa 28–39) domain (64). Residues Lys22 to Asp44 may be the transmembrane domain, and the disulfide bond at Cys40 may trigger oligomerization (65). Its basic amino acid residues at positions 8 and 9 determine its viroporin activity (63). In agnoprotein, the major amphipathic α-helix conformation spans amino acids 23–39 of the Leu/Ile/Phe-rich region, while the minor α-helix consists of Leu6 to Lys13 (66). Leu29 and Leu36 of the major amphipathic α-helix are at the dimer interface, keeping the spatial structure and protein stability (67). All three Phe residues are localized to this amphipathic α-helix and mediate protein folding and stability (68). Moreover, agnoprotein primarily targets 501 cellular proteins containing "coiled-coil" motifs. The agnoprotein- host interactions were involved in protein synthesis and degradation, cellular transport, and organelles, including mitochondria, ER-Golgi, and the nucleus. Among the agnoprotein partners, Rab11B, importin, and Crm-1 have been biochemically validated (68).

In nucleus, agnoprotein promotes T antigen binding to the viral origin with indirect interactions with DNA. It contains several potential phosphorylation sites (ser7, ser11, and thr21) that can be phosphorylated by PKC (69). Small t antigen (aa 82-124) also interacts with agnoprotein and PP2A, suppressing the PP2A-mediated dephosphorylation of agnoprotein and promoting JCPyV replication (70). The amino-terminal of agnoprotein can bind to YB-1 and reduce YB-1-mediated gene transcription (71). The interaction of p53 with agnoprotein can lead to p21 expression, causing G_2/M arrest and sensitizing cells to cisplatin *via* chromosome fragmentation, micronuclei formation, and impaired double-strand DNA break repair activity by up-regulating the expression of the DNA repair proteins (e.g., Ku70 and Ku80) (72).

In cytosol, agnoprotein predominantly localizes to the perinuclear region of JCPyV-infected cells, and colocalizes with the cellular cytoskeletal protein tubulin (73), which is co-precipitated with phosphorylated agnoprotein (74). Suzuki et al. (75) also demonstrated that agnoprotein could directly interact with fasciculation and elongation protein zeta 1 (FEZ1) and microtubules. The interaction dissociated FEZ1 from the microtubules and inhibited FEZ1-facilitated neurite outgrowth. Saxena et al. (76) reported that the mitochondrial targeting sequence and dimerization domain of agnoprotein mediate mitochondrial localization, where agnoprotein decreased the respiration rate, mitochondrial membrane potential, and ATP production while increasing ROS production and Ca^{2+} uptake.

Caspids

Major coat protein VP1 couples with a minor coat protein (VP2 or VP3). VP2 and VP3 share DNA binding domain, VP1-binding domain, and nuclear localization signal (NLS). The 16 carboxy- terminal and 12 amino-terminal amino acids of VP1 are essential for the assembly of VLPs. Both minor coat proteins and the myristylation site on VP2 are important for properly packaging the genomic DNA of JCPyV (77). Furthermore, the cysteine residues of VP1 are dispensable for protein stability and oligomerization (78, 79). VP1 mediates VLP entry into the nucleus by importins α and β *via* its NLS (80). Point mutations in VP1 can influence virion binding to cellular glycan receptors and their recognition by polyomavirus-specific antibodies (81). Mutation 186G→C (Lys→Asp) in the VP1 gene could predispose MS patients undergoing treatment with natalizumab to PML (82). A deletion of the C-terminal 10 bp of VP1 is closely linked to lytic infection of granule cell neurons and atrophy in the cerebellum of an HIV/PML patient (83). VP1 mutations that are involved binding to sialic acid cell receptors favored PML onset (84). Hsp70 could interact with T antigen and VP2 or VP3, which accumulates in the nucleus of the infected cells and enhances viral DNA replication (85). VP2 binds to DNA through its DNA-binding domain between Lys332 and Lys336 (86). As for capsid expression, Ravichandran et al. (87) found that TGF-β1 activated MEK1/2 and subsequent phosphorylation of Smads, which bound to or increased binding to the JCPyV promoter for VP-1 synthesis.

The signal pathways of JCPyV

Multi-omics analysis has demonstrated that JCPyV-related carcinogenesis involves aberrant Forkhead box O, AMPK, p53, and PI3K/Akt signaling pathways. Moreover, T antigen can upregulate the expression of Akt, Rb, and survivin and downregulate p21 expression, indicating that it might activate the Akt/NF-κB/survivin pathway to block apoptosis and cause

Rb hyper-expression and p21 hypo-expression for cell cycle progression (88). The upregulated proteins are involved in signaling through Cyclin-CDK, TGF-β receptor 1, fibroblast growth factor family receptor and platelet-derived growth factor receptor and the inflammatory responses mediated by Cox-2 (89). T antigen might interact with ribosomal proteins, various keratins, G proteins, apolipoproteins, ubiquitin-related proteins, CCAAT enhancer-binding proteins, β-catenin, RPL19, β-TRCP, and p53 in lens tumor cells (88). T antigen knockdown could suppress glycolysis, mitochondrial respiration, proliferation, migration, and invasion in lens tumor cells; however, it promoted apoptosis. T antigen can also activate the Akt/NF-κB/survivin pathway, producing an anti-apoptosis effect and causing Rb hyperexpression and p21 hypoexpression to mediate cell cycle progression (88). These findings suggest that the T antigen can aggravate the cellular phenotype, possibly by inactivating tumor suppressors, activating oncogenes, or disrupting metabolism and cell adhesion.

As shown in Figure 4, p53 interacts with T antigen to repress transcription from the JCPyV early promoter and JCPyV DNA replication in non-glial cells (90, 91). The interaction between p53 and T-antigen up-regulated the p53 downstream target protein, p21/WAF1 (92). Additionally, E2F-1 dissociated from the pRb-E2F-1 complex and stimulated S phase-specific genes following the formation of a pRb-T antigen complex or Rb phosphorylation (93). T antigen can bind to pRb2/p130, p107, and pRb/p105, activating the E2F transcription factor family and promoting entry into S phase (94). As IRS1 signal pathway, T antigen also induces the nuclear translocation of IRS-1, and IRS-1 interacts with T antigen, which is independent of IRS-1 tyrosine phosphorylation and blocked by IRS-1 serine phosphorylation (95). After T-antigen-mediated nuclear translocation, IRS-1 binds to Rad51 at the site of damaged DNA to direct DNA repair, causing accumulation of mutations in the affected cells (96). IRS-1-Rad51 nuclear interaction also sensitizes JCV T-antigen positive medulloblastoma cells to cisplatin and γ-irradiation (97). T-antigen requires the presence of a functional insulin-like growth factor I receptor (IGF-IR) for transformation of fibroblasts and for survival of medulloblastoma cell line. IGF-IR is phosphorylated in medulloblastoma biopsies and JCV T-antigen inhibits homologous recombination-directed DNA repair, causing accumulation of mutations. In Wnt- β-catenin pathway, the interaction between the central domain (residues 82–628) of T-antigen and the C-terminal residues of β-catenin (aa 695-781) increases β-catenin levels and its nuclear entry, resulting in the upregulation of its downstream genes (c-myc, VEGF, and Cyclin D1). T antigen binds to the F-box proteins β-transducin repeat-containing protein-1 and 2 (βTrCP1/2) and recruits Rac1 to form the T antigen-Rac1-β-catenin complex that suppresses the ubiquitin- dependent degradation of β-catenin by proteasomes (98–100). T antigen downregulates

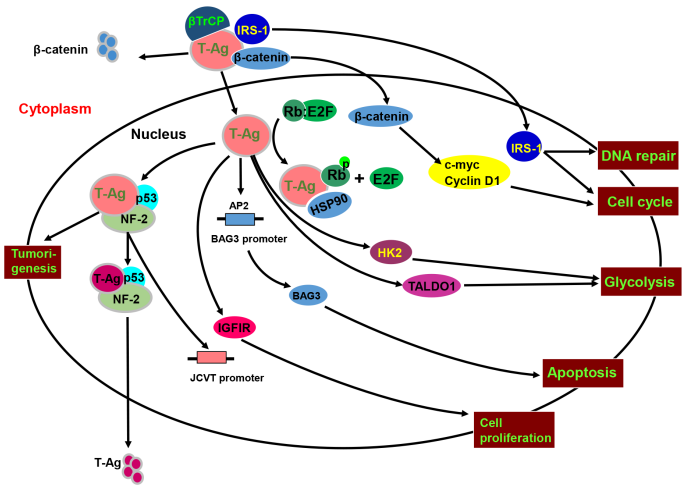

FIGURE 4
The biological function and signal pathways of the JCPyV T antigen. JCPyV T antigen binds to βTrCP1/2 protein to cause ubiquitin-mediated degradation of β-catenin, and binds to β-catenin to enhance its protein stability and facilitate its entry into the nucleus. Nuclear β-catenin promotes S-phase transformation by up-regulating c-myc and Cyclin D1 protein expression. T antigen interacts with p53 and neurofibromatosis-2 (NF-2) for the proteasome-mediated degradation of T antigen. The binding of T antigen to phosphorylated Rb protein results in the separation of Rb-E2F, resulting in an abnormal cell cycle. T antigen can promote the translocation of the insulin receptor substrate 1(IRS1) to the nucleus, induce cell cycle evolution, and participate in DNA repair. In addition, T antigen up-regulates the expression and phosphorylation of IRS1 and IGF1 receptor (IGF-1R), promoting cell proliferation and disrupting normal cell activity by binding to the transcription factor AP-1. The DNA binding domain of T antigen can bind to the AP2 sequence in the BAG3 promoter and CPG binding protein promoter of transcriptional regulatory methylation and regulate the expression of BAG3. T antigen can also stimulate the expression of transaldolase-1 (TALDO1) and hexokinase-2 (HK2) to induce glycolysis.

BAG-3 expression to inhibit apoptosis by blocking AP2 binding to the BAG3 promoter. Bag3 interacts with the T antigen, inducing its autophagic degradation (101). Additionally, T antigen binds to and activates the survivin promoter, upregulating its expression and mediating the nuclear translocation of suvivin *via* the T-antigen-survivin complex (102, 103). In contrast, T antigen can arrest G_1, sustain G_2, and block ROS induction and cytotoxicity during glucose deprivation. T antigen can also stimulate the expression of transaldolase-1 and hexokinase-2 (104).

The association between JCPyV and carcinogenesis

In transformed cells, JCPyV can cause anchorage-dependent growth, rapid division, prolonged life span, increased ploidy, unstable multicentric chromosomes, centric and acentric rings, dysregulated genomic stability and DNA repair, and increased micronuclei formation (105–107). Intracranially inoculated JCPyV caused glioblastoma in juvenile owl monkeys (108), grade 3-4 astrocytoma in adult owl monkeys (109), undifferentiated neuroectodermal tumors in the cerebrums of newborn Sprague-Dawley rats (110), cerebellar medulloblastoma, plexus tumors, medulloblastoma, and thalamic gliomatosis in hamsters (111), and neuroblastoma in the abdominal cavity, pelvis, mediastinum, and neck region of Syrian hamsters (112). Padgett et al. (113) demonstrated that malignant brain tumors developed in Syrian golden hamsters during a 6.5-month observation period following intracerebral inoculation of different JCPyV strains into newborns. The Mad-2 strain caused cerebellar medulloblastomas, whereas the MAD-3 strain induced extracranial neuroblastomas. In contrast, the Mad-4 strain produced pineal gland and cerebellar tumors.

The spontaneous tumors in the transgenic mice of JCV T antigen can provide direct evidences for the oncogenic role of JCPyV as shown in Table 1. The transgenic mouse with the early encoding region of the archetype strain was generated using its own promoter and developed neural crest tumors, such as primitive neuroectodermal tumors, adrenal neuroblastomas, medulloblastomas, pituitary tumors, glioblastomas, and malignant peripheral nerve sheath tumors (114). Krynska et al. (115) established the same transgenic mice and observed primitive tumors originating from the cerebellum and adjacent brain stem that were grossly and histologically similar to human medulloblastoma and primitive neuroectodermal tumors. However, Gordon et al. (92) used the same promoter to generate transgenic mice overexpressing T antigen, which developed large, solid pituitary masses. Shollar et al. (116) established transgenic mice expressing T-antigen under the control of the Mad-4 promoter and observed pituitary tumors by one year of age. Krynska et al. (93) found that transgenic mice harboring T antigen could develop massive abdominal tumors of neural crest origin. In our group, a transgene with the K19 promoter was generated and pulmonary tumors with T-antigen, p53, and CK19 expression and EGFR mutation were observed (117). We also established T antigen-expressing transgenic mice

TABLE 1 The JCPyV T antigen-induced spontaneous tumor in the transgenic mice.

Author and reference	Promoter name	Tissue and cellular specificity of promoter	Cancer types
Gordon et al. (92)	viral own promoter of Mad1	no	Pituitary tumors
Krynska (93)	viral own promoter of Mad1	no	Abdominal tumors of neural crest origin d
Del Valle et al. (114)	viral own promoter of Mad1	no	primitive neuroectodermal tumors, medulloblastomas, adrenal neuroblastomas, pituitary tumors, malignant peripheral nerve sheath tumors, and glioblastomas
Krynska et al. (115)	viral early region of Mad1	no	primitive invasive tumors originating from the cerebellum and the surrounding brain stem
Shollar et al. (116)	viral control region of the Mad-4 promoter	no	pituitary tumors, solid masses around the salivary gland, the sciatic nerve, and peripheral nerve sheath tumors
Noguchi et al. (117)	cytokeratin 19 promoter	gastric stem-like cells	lung adenoma and adenocarcinoma
Gou et al. (118)	α-crystallin A promoter	lens epithelium	lens tumors
Zheng et al. (119)	Albumin promoter	hepatocyte	Hepatocellular carcinoma
	villin promoter	intestinal epithelium	colorectal cancer
	cytokeratin 19 promoter	gastric stem-like cells	gastric cancer
	PGC promoter	gastric chief cells	gastric cancer, breast cancer
	Pdx1 promoter	pancreas and duodenum	pancreatic adenocarcinoma, insulinoma, vater's cancer, gastric tumors

PGC, pepsinogen C; Pdx1, pancreas/duodenum homeobox protein 1.

using α-crystallin A and observed lens tumors that were positive for T antigen, N-cadherin, p53, and β-catenin. Enlarged eyeballs were observed, and the tumors invaded the brain (118). Additionally, we generated CAG-loxp-LaZ-loxp T antigen transgenic mice with T antigen activation induced using matching tissue-specific cre transgenic mice. Gastric poorly-differentiated carcinoma was observed in gastric stem-like and chief cells following T antigen overexpression. Moreover, spontaneous hepatocellular and colorectal cancers developed in Alb-cre (hepatocytes)/T antigen and villin-cre (intestinal epithelium)/T antigen transgenic mice. Gastric, colorectal, and breast cancer were observed in PGC (Pepsinogen C)-cre/T antigen mice. Pancreatic insulinoma and ductal adenocarcinoma, gastric adenoma, and duodenal cancer were detected in Pdx1-cre/T antigen mice. There was alternative splicing of T antigen mRNA in all target organs of these transgenic mice and various cells transfected with pEGFP-N1-T antigen. It has been suggested that the JCPyV T antigen might induce gastroenterological carcinogenesis in a cell-specific manner (119).

It is important to detect and compare JCPyV DNA in cancer and adjacent normal tissues using either molecular or morphological approach. JCPyV detection might determine the etiology for JCPyV-related cancer. The correlation of JCPyV T antigen with carcinogenesis and subsequent progression was summarized in Table 2. Although JCPyV DNA was found in ependymomas and choroid plexus papilloma (138), Kutsuna et al. (120) found that glossitis and tongue dysplasia had significantly lower copies of JCPyV than tongue cancer. They observed T antigen DNA and protein in the nuclei of tongue cancer cells but not in normal or dysplastic epithelia. JCPyV DNA and T antigen were found in adenoid cystic carcinomas samples of the trachea, paranasal sinuses, and oral cavity by PCR and immunohistochemistry respectively (121). JCPyV DNA was more frequently detected in esophageal carcinomas than in normal, benign, or premalignant esophageal samples (122). JCPyV T antigen load is also higher in gastric cancer than in normal mucosa (123). Indeed, its DNA and protein were detected in the nuclei of gastric cancer cells. Moreover, T-antigen DNA is correlated with differentiation and the methylation of p14 and p16 in this cancer (124).

In colorectal cancer, the positivity rate of T antigen is decreased from colorectal adenocarcinoma to adenoma to mucosa (125, 126). Nosho et al. (127) reported that T antigen could inactivate wild-type p53, resulting in chromosomal instability. It was positively correlated with p53 expression, p21 loss, nuclear β-catenin, LINE-1 hypo-methylation and hyper-expression, and low MSI (microsatellite instability) levels. Link et al. (128) found that T antigen enhanced the migration and invasion of colorectal cancer cells *via* Akt and MAPK signaling. Indeed, T antigen could be detected by IHC in primary colorectal cancers and their corresponding liver metastases. The interaction between T-antigen and β-catenin and the nuclear detection of β-catenin in T-antigen-positive colorectal cancer cells demonstrates dys-regulation of the Wnt pathway (15, 129). Ripple et al. (130) found that T antigen and β-catenin were co-localized in the nuclei of colorectal cancer cells, resulting in the activation of TCF4-dependent promoters and the transcription of TCF4 downstream targets (e.g. c-myc, VEGF and Cyclin D1).

In the respiratory cancer, the positivity rate for the JCPyV T antigen in the respiratory system is lower in normal lung tissue than in tumors; T antigen DNA is strongly observed in lung adenocarcinoma (131). One study found a lower JCPyV copy number in normal lung cancer than in lung tumors (132). Moreover, the copy number was lower in lung adenocarcinomas compared to squamous, small, or large cell carcinomas. Lung cancers with a high JCPyV copy number were characterized by high proliferation and low β-catenin-mediated cell adhesion (132).

In urinary tract neoplasms, JCPyV has also been detected in renal pelvic urothelial carcinoma and renal cell carcinoma (133). Shen et al. (134) found that 90.1% of the urothelial carcinomas and all the renal cell carcinomas that they evaluated were positive for JCPyV using nested PCR. Prostate cancer is more susceptible to JCPyV infection than benign prostate hyperplasia. Tumors with both high prostate-specific antigen levels and high Gleason scores were associated with a high risk of JCPyV infection.

In addition, we reported that the positivity rate and expression levels of T antigen were lower in breast cancer than in normal breast tissue (135), in line with hepatocellular and pancreatic cancer (136). T antigen DNA positivity was inversely associated with E-cadherin expression and triple-negative breast cancer but positively associated with lymph node involvement and ER and PR expression. JCPyV copies were negatively linked to tumor size and E-cadherin expression in breast cancer but positively associated with histological grading. Additionally, we for the first time found that JCPyV was less detectable in cervical epithelium than dysplasia and carcinoma (137). We also observed T antigen DNA and protein in hepatocellular, pancreatic, breast and cervical cancer cells using *in situ* PCR and immunohistochemistry (134–136).

Conclusions and perspective

JCPyV enters eukaryotic cells and is inserted into genomic DNA. It induces tumorigenesis with tissue specificity by targeting the p53, β-catenin, IRS, Rb, TGF-β1, PI3K/Akt, and AMPK signal pathways. Pathological examination and animal experiments have demonstrated that the JCPyV T antigen might induce tumorigenesis in neural and gastroenterological systems and breast. Thus, JCPyV might be an etiological risk factor for

TABLE 2 The correlation of JCPyV T antigen with carcinogenesis and subsequent progression.

Author and references	Cancer type	DNA profile	Protein profile	Clinical and prognostic significances
Kutsuna et al. (120)	Oral squamous carcinoma	high	nd	ns
Hämetoja et al. (121)	adenoid cystic carcinoma of the oral cavity and the airways	nd	nd	ns
Del Valle et al. (122)	Esophageal squamous carcinoma	high	nd	ns
Murai et al. (123)	Gastric cancer	high	nd	ns
Ksiaa et al. (124)	Gastric cancer	high	nd	positively associated with elder age, differentiation, hypermethylation of p14 and p16 and poor prognosis.
Hori et al. (125)	Colorectal cancer	high	high	ns
Shavaleh e t al (126).*	Colorectal cancer	high	ns	ns
Nosho et al. (127)	Colorectal cancer	high	nd	negatively associated with proximal location, high grade, family history of colorectal cancer, and mucinous component and was associated with p53 expression, high CIN score, Cyclin D1 expression, LINE-1 hypomethylation, and BRAF mutation
Link et al. (128)	Colorectal cancer	high	nd	positively associated with clinical staging and liver metastasis
Vilkin et al. (129)	Colorectal cancer	nd	nd	positively associated with hMLH1 hypermethylation
Ripple et al. (130)	Colon cancer	nd	high	negatively associated with β-catenin expression**
Abdel-Aziz HO (131)	Lung cancer	high	nd	positively associated with lymph node metastasis, p53 and nuclear β-catenin expression, and high in adenocarcinoma than squamous carcinoma
Zheng et al. (132)	Lung cancer	high	high	positively associated with ki-67 and no membrane β-catenin expression, and high in adenocarcinoma than squamous carcinoma, small and large cell carcinoma
Antje et al. (133)	Renal clear cell carcinoma	nd	nd	ns
Shen et al. (134)	Prostate cancer	high	high	positively associated with PSA level and Gleason's scores
Zheng et al. (135)	Breast cancer	low	low	negatively correlated with tumor size, E-cadherin expression and triple-negative breast cancer, but positively correlated with lymph node metastasis, histological grading and ER and PR expression.
Zheng et al. (136)	Hepatocellular carcinoma	low	high	ns
Zheng et al. (136)	Pancreatic cancer	low	high	ns
Zheng et al. (137)	Cervical cancer	high	nd	ns

nd, not detection; ns, not significant; ER, estrogen receptor; PR, progestogen receptor; PSA, prostate-specific antigen; *meta-analysis; **protein level.

carcinogenesis and should be emphasized in tertiary prevention and treatment of cancer.

Because JCPyV infection rate reaches 80%, we should try our best to prevent the entry of JCPyV into the human body through the sewage and air droplet. In addition, it is better to block the endocytosis and nuclear transport of JCPyV virions by receptor antagonists. Finally, the agents to block the JCPyV infection or inhibit the JCPyV-related signal pathway should be developed to prevent and treat JCPyV-related cancers. In the future, we can realize the early diagnosis, finding and treatment of JCPyV-related cancers.

Author contributions

H-CZ conceived the review, and HX and CY-Z led its drafting and managed the editing of it. All authors contributed to the drafting of the review and approved the final manuscript.

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Conflict of interest

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The role of hepatocyte nuclear factor 4α (HNF4α) in tumorigenesis

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Hepatocyte Nuclear Factor 4 Alpha (HNF4α) is a master transcription factor mainly expressed in the liver, kidney, intestine and endocrine pancreas. It regulates multiple target genes involved in embryonic development and metabolism. HNF4α-related diseases include non-alcoholic fatty liver disease (NAFLD), obesity, hypertension, hyperlipidemia, metabolic syndrome and diabetes mellitus. Recently, HNF4α has been emerging as a key player in a variety of cancers. In this review, we summarized the role and mechanism of HNF4α in different types of cancers, especially in liver and colorectal cancer, aiming to provide additional guidance for intervention of these diseases.

KEYWORDS

HNF4α, tumorigenesis, liver cancer, colorectal cancer, transcription factor

Introduction

HNF4α is a critical transcription factor (TF) during development. Its silencing and dysfunction could lead to stunted development in gastrula formation (1), liver (2) and kidney (3). Interestingly, enforcing expression of HNF4α, in cooperation with Forkhead Box Protein A3 (FOXA3) and Hepatocyte Nuclear Factor 1-Alpha (HNF1α), could even reverse the hepatocellular carcinoma (HCC) cells into normal hepatocyte-like cells (4). Furthermore, HNF4α binds to different gene clusters between undifferentiated state and differentiated state during embryonic development (5), which may be a reason why HNF4α has opposite functions in different types of malignancies.

Another vital role of HNF4α is the regulation of metabolic homeostasis. Most HNF4α-related diseases have abnormal insulin secretion such as occurrence of diabetes mellitus (including Type I and Type II diabetes mellitus), while the

underlying molecular mechanism remains elusive (6, 7). It has been shown that HNF4α interacts with Circadian Locomoter Output Cycles Protein Kaput (CLOCK)/BMAL1 to regulate a series of metabolic genes involved in lipid, glucose and amino acid homeostasis. It was known that the circadian rhythm of metabolism was controlled by HNF4α through repression of transcriptional activity of CLOCK/BMAL1 (8). Knockout of BMAL1 attenuated the genome-wide binding of HNF4α in the liver possibly *via* transcriptional downregulation of HNF4α (9). In addition, HNF4α regulated energy metabolism and inflammation through recruiting glucocorticoid receptors (10).

Similarly, HNF4α has been shown to promote glycolysis, glucose uptake, lactic acid production and ATP levels in neuroblastoma cells, and the underlying mechanism involved hexokinase 2 (HK2) and Solute Carrier Family 2 Member 1 (SLC2A1) and the heterogeneous nuclear ribonucleoprotein U (hnRNPU) (11). In pancreatic cancer, HNF4α deletion led to a glycolytic energy metabolism transition from typical pancreatic adenocarcinoma to squamous pancreatic cancer, in which Fructose-Bisphosphate Aldolase A (ALDOA), Hexokinase 1 (HK), and Glycogen Synthase Kinase 3 Beta (GSK3β) genes are upregulated. The downstream Wingless-Type MMTV Integration Site Family, Member 7A (WNT7A) and Protein Kinase AMP-Activated Catalytic Subunit Alpha 1 (AMPK) signal activation further led to drug resistance in squamous pancreatic cancer (12).

Here, we review the role and the mechanism of HNF4α in various cancers, try to emphasize the importance of HNF4α in tumorigenesis and look forward to helping with the treatment and prevention of cancer.

The expression of HNF4α in different tumors

HNF4α has been demonstrated to be a tumor suppressor in certain types of tumors but act as an oncogene in other forms of cancers. We also analyzed the expression levels of HNF4α in different tumors from the TCGA database and found that HNF4α is upregulated in most gastrointestinal tumor tissues when compared to their matched normal tissues, including colon and rectal adenocarcinoma, esophageal cancer, stomach cancer and pancreatic cancer. However, HNF4α expression level is downregulated in cholangiocarcinoma and kidney chromophobe tissues compared to their normal counterparts (Figure 1). Although we did not see significant change of HNF4α expression in HCC and prostate cancer (Figure 1), HNF4α plays an important role in HCC and prostate cancer (see below).

HNF4α function in hepatocellular carcinoma

The important role of HNF4α in development and metabolism, especially in liver tissues, led to the initial research focusing on HNF4α in liver cancer. HNF4α was shown to play an inhibitory role in the development of liver cancer, and was significantly correlated with genes related to drug absorption, distribution, metabolism and excretion in patients with liver cancer (13). Mutations in the Catenin Beta 1 (*CTNNB1*) exon 3 region were detected in 54 of 59 samples (92%) of pediatric hepatoblastoma; In such tumors, Wnt

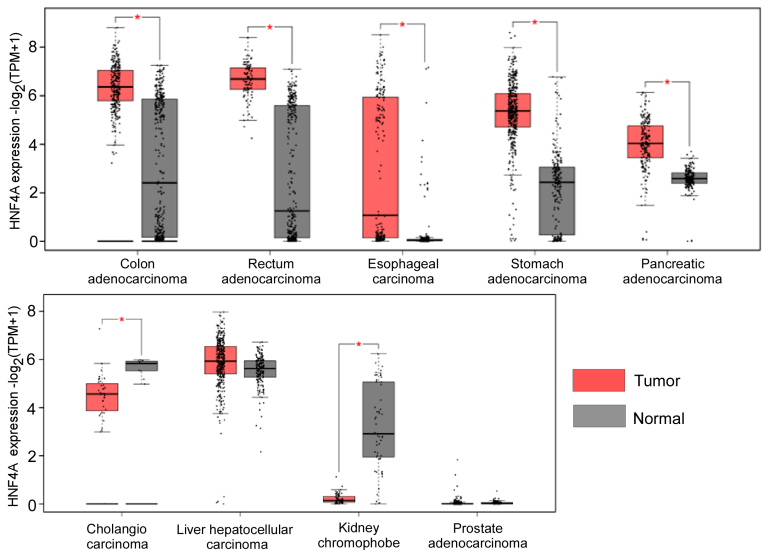

FIGURE 1
HNF4α expression levels in different types of cancer tissues and their matched normal counterparts. *indicates p<0.05. The raw data are from http://gepia2.cancer-pku.cn.

signaling and cell cycle pathways are usually upregulated. Moreover, in more malignant genotypes, HNF4α/CEBPα (CCAAT Enhancer Binding Protein Alpha) binding regions of the genome is highly methylated, and HNF4α/CEBPα transcriptional activity is inhibited (14).

HCC associated with hepatitis B virus (HBV) showed that E2F Transcription Factor 1(E2F1) acts as an active UR (upstream regulator), to positively regulate cell cycle and DNA replication, while HNF4α and HNF1α function as inhibitor URs. In alcoholic HCC, Erb-B2 Receptor Tyrosine Kinase 2 (ERBB2) is activated, while HNF4α and Nuclear Transcriptional Regulator Protein 1(NUPR1 are inhibited) (15).

Taniguchi, H. et al. provided evidence that the HNF4α gene mutations G79C, F83C and M125I (Zn DNA domain) are loss-of-function mutations that would lead to decreased expression of HNF1α and Apolipoprotein B (ApoB) genes and increased risk of liver tumor (16). HNF1α and HNF4α had positive feedback regulation, mutations of Y122C, R229Q and V259F in the POUC domain of HNF1α inhibited its activity and disrupted the binding to the HNF4α promoter, resulting in down-regulation of HNF4α and other HNF1α target genes, as well as disruption of HNF4α-HNF1α transcriptional network, thus triggering the development of HCC (17). In addition, Lysine Demethylase 8(KDM8) is a potential tumor suppressor downregulated in HCC and is a downstream target of HNF4α signaling (18). Furthermore, Mitochondrial Amidoxime Reducing Component 2 (MARC2)-HNF4α forms a positive feedback loop to inhibit the progression of HCC (19).

The expression of hydroxysteroid 17-β dehydrogenase 6 (HSD17B6) in HCC is lower than that in the normal liver and is associated with HCC stage and grade. HNF4α has been shown to bind to the enhancer and promoter regions of the HSD17B6 gene to activate its transcription, and the methylation of the HSD17B6 promoter negatively regulates its expression even in the presence of HNF4α (20).

Accumulating evidence indicates that ferroptosis is closely associated with liver cancer. Zhang, X. et al. showed that HNF4α is a controller of ferroptosis down-regulated factors (FDF), which inhibits iron death by affecting the synthesis of GSH. In response to ferroptosis, dissociation of histone acetyltransferase Lysine Acetyltransferase 2B (KAT2B) blocks the binding of HNF4α to the FDF promoter (21).

For HNF4α-based targeting therapeutics in HCC, HNF4α and HNF1α have been used to inhibit HCC cell proliferation and eliminate tumor-specific features. Takashima, Y et al. showed that the combined transduction of three liver TFs: HNF4α, HNF1α and FOXA3 could stably inhibit HCC cell proliferation and tumor stem cell renewal (22). Oligo-fucoidan, a sulfated polysaccharide, inhibited HCC growth by binding to the Asialoglycoprotein Receptor (ASGR) which led to Signal Transducer And Activator of Transcription 3 (STAT3) phosphorylation, and then p-STAT3 induced the transcription of the HNF4α (23). The mRNA of HNF4α encapsulated by lipid nanoparticles can reduce the liver damage (fibrosis and cirrhosis) in various mouse models. Paraoxonasel is a direct target of HNF4α and participates in the weakening of liver fibrosis mediated by HNF4α (24).

In conclusion, HNF4α maintains homeostasis of liver, and the mutations of HNF4α or epigenetic modifications of promoter regions of its targeting genes lead to the loss of function of HNF4α. Elevated expression of HNF4α has a good prognosis in patients with HCC. In the progression of liver cancer,HNF4α is regulated by factors such as STAT3 and KAT2B, and function as tumor suppressor through HSD17B6, HNF1α, FOXAs, MARC2. Some HCC phenotypes can even be reversed by overexpression of HNF4α.

Interestingly, not all studies support the point that HNF4α inhibits liver cancer. In a recent study on the effect of SNPs in liver cancer, the rs73613962 (T > G) site at the Protein Arginine Methyltransferase 7 (PRMT7) gene has allele-specific enhancer activity. HNF4α preferentially binds to this enhancer region with the risk allele G to activate PRMT7 transcription, and elevated PRMT7 promotes malignant phenotypes of HCC through inhibition of the p53 signaling cascade (25). Another study revealed that HNF4α bound to the -1409 to -1401 region of the circRNA_104075 promoter to induce its expression. Upregulated circRNA_104075 increased the expression of Yes1 Associated Transcriptional Regulator (YAP1), a target of mir-582-3p, by acting as a sponge of mir-582-3p, ultimately promoting the initiation of liver cancer (26).

HNF4α functions in colorectal cancer

HNF4α also plays a pivotal role in CRC through regulation of several major oncogenic pathways. In 2009, genomic-wide association scanning on 2361 cases of ulcerative colitis and 5417 control cases revealed that HNF4α was related to the progression of ulcerative colitis (27). The Cancer Genome Atlas (TCGA) analysis of colon and rectal tumors showed that HNF4α, TOMM34 (outer mitochondrial membrane translocation enzyme 34) and SRC (non-receptor tyrosine kinase) were overexpressed in colorectal cancer (28).

Expression of P1-/P2-promoter-driven nuclear HNF4α is significantly correlated with cytoplasmic β -catenin in colitis-associated tumor and sporadic CRC. Depletion of HNF4α reduces β -catenin expression (29). HNF4γ, a paralog of HNF4α, and Nuclear Receptor Subfamily 1 Group F Member 3 (RORC) along with HNF4α are also up-regulated in CRC tissues (30). Due to the compensatory role of HNF4γ in intestinal tissues, ablation of HNF4α did not cause changes in HDL level in serum or lipoprotein gene expression in ileum (31). Furthermore, transcription factors such as Heat Shock Transcription Factor 1 (HSF1) and Double-Strand-Break Repair Protein Rad21 Homolog (RAD21) play a regulatory role with HNF4α in colorectal cancer metastasis (32). In addition, HNF4α was identified as a positive

regulator of oxidoreductase related genes that involved in regulation of ROS level. Knockdown of HNF4α increases and ectopic expression of HNF4α reduces ROS production in CRC cells. Both HNF4α and oxidoreductase related genes are overexpressed in colorectal cancers (33). It has also been found that Nuclear Factor Kappa B Subunit 1 (NF-κB) regulates transcriptional activation of Carboxylesterase 1 (CES1) through HNF4α in invasive CRC (34). These findings suggest that HNF4α functions as an oncogene in CRC.

Contrary to the conclusion above, the expression of HNF4α in colon cancer leads to the decreased expression of oncogenic factors Lysine Demethylase 1 (ALSD1), SET Domain Containing 1A, Histone Lysine Methyltransferase (SETD1A), Protein Arginine Methyltransferase 1 (PRMT1), FOXM1, Protein Tyrosine Kinase 2 (FAK) and Snail Family Transcriptional Repressor 1(SNAI1), and inhibits the tumor-formation of HCT116 cells (35).

To sum up, HNF4α is a key player in CRC while the underlying mechanism is largely unknown. Further investigation of the role and the specific mechanism of HNF4α in the development and progression of CRC is of great significance for establishing HNF4α as a therapeutic target in CRC.

HNF4α functions in gastric cancer

HNF4α promotes gastrointestinal adenocarcinoma proliferation and survival in a genealogy-specific manner through transcriptional activation of many downstream targets, including HNF1α and interleukin signaling factors (36). The promoter and three distal enhancers of HNF4α are activated by four key transcription factors, ELF3, GATA-Binding Factor 4 (GATA 4), GATA6, and Kruppel Like Factor 5 (KLF5).

HNF4α is highly expressed in both primary gastric cancer and metastasis from gastric cancer to mammary gland, but not in breast cancer, which should be a good marker to distinguish primary and metastatic gastric cancer from breast cancer (37). The same conclusion was confirmed by Saad, DZ. HNF4α was overexpressed in 22 of 23 cases of primary gastric adenocarcinoma and in 15 of 16 cases of metastatic gastric adenocarcinoma, but not in 25 cases of primary breast cancer and 17 cases of metastatic breast cancer, suggesting HNF4α as a valuable biomarker (38). Moreover, HNF4α binds to Mucin 5AC, Oligomeric Mucus/Gel-Forming (MUC5AC) promoter and transcriptionally induces *MUC5AC* expression. Thus, HNF4α correlates with *MUC5AC* mucin expression during stomach development and in GC cells (39).

Furthermore, different HNF4α subtypes derived from two different promoters (P1 and P2) determine the malignancy degree in gastric cancer. Overexpression of P1-HNF4α rather than P2-HNF4α induces tumor growth, and Chemokine Ligand 15 (CCL15) was a direct target of P1-HNF4α in GC (40). In addition, X Inactive Specific Transcript (XIST), a long-strand non-coding RNA, increases enrichment of HNF4α in the promoter region of EPH Receptor A1 (EPHA1), contributing to the deterioration of GC (41).

In conclusion, HNF4α plays a role as a promoter in GC, but underlying mechanism remains elusive, and P1-HNF4α subtype could drive a more malignant phenotype than P2-HNF4α in GC.

HNF4α functions in other cancers

Apart from HCC, CRC, and GC, HNF4α has been shown to have significant role in many other cancer types.

Lung cancer

Activation of HNF4α in lung cancer leads to higher lung cancer grade and shorter survival (42). HNF4α has also been found to be elevated in lung adenocarcinoma (43) and induce mucin MUC3 expression in Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS) mutated lung mucinous adenocarcinoma (44). In addition, HNF4α recognizes the SNP site RS401681, which can interact with Telomerase Reverse Transcriptase (TERT) promoter to increase lung cancer risks (45).

Pancreatic cancer

Although the TCGA database showed that HNF4α expression level was elevated in pancreatic cancer tissues, Camolotto's results demonstrated that HNF4α inhibited tumor growth and promoted epithelial development through directly inhibiting expression of Sine Oculis Homeobox Homolog (SIX) 4 and SIX1, two markers of mesodermal/neuronal lineage expressed in basal-like subtypes (46). KRAS (G12D) -driven pancreatic tumors develop after GATA6 deletion, which is accompanied by the loss of HNF1α and HNF4α (47).

Prostate cancer

It has been revealed that HNF4α-mediated AMPK/mTOR pathway promotes prostate cancer progression (48). A previous study showed that selenium-binding protein 1(SBP1) inhibits prostate cancer growth by reducing oxygen consumption and increasing the activation AMPK, and that HNF4α binds to the promoter of SBP1 to restrain SBP1 transcription (49). In addition, exposure to bisphenol A (BPA) caused prostate preneoplasia, HNF4α-regulated gene networks were altered by BPA, which include nuclear factor-κB, ERK1/2 and insulin-related signaling (50). However, one report indicated HNF4α as a tumor suppressor of prostate cancer by promoting p21-driven senescence (51).

Renal cell carcinoma

HNF4α function as a tumor suppressor in clear cell renal cell carcinoma (ccRCC). HNF4α has been shown to regulate two metabolic enzymes ABAT and ALDH6A1 leading to inhibition of cell proliferation and migration, and impaired lactate production (52). In addition, HNF4α restrains the development of renal cell carcinoma by transcriptional activation of NR_023387 (53) and inhibition of E- cadherin (54). Moreover, ALDH2 can enhance anthracycline sensitivity of RCC and activates the transcription of HNF4α (55), and HNF4α also increased the chemosensitivity of RCC cells to oxaliplatin and 5-FU (56).

Cervical, bladder, esophageal and breast cancer

There are few reports about the roles of HNF4α in these tumors. In cervical cancer, HNF4α inhibits the tumorigenic potential *in vivo* and induces the tumor cell G0/G1 arrest through suppression of the Wnt/β-catenin pathway (57). A recent study showed that expression of HNF4α reduced cell proliferation and enhanced cisplatin sensitivity by activation of ALDH6A transcription in bladder cancer (58) and triggered malignant transformation in esophageal carcinoma (59). In addition, upregulation of HNF4α under hypoxia contributes to adriamycin resistance in breast cancer (60).

Conclusions and future perspectives

The important role of HNF4α in development and metabolism also directly reflect the tumor process. However, the regulation of HNF4α in tumor is not only dependent on metabolic and developmental pathways. In HCC, HNF4α has been demonstrated to inhibit malignant phenotype, while the effect of HNF4α in colon cancer is quite opposite. In addition, a few studies have shown that overexpression of HNF4α promoted the development of gastric and lung cancer and inhibited the development of pancreatic ductal carcinoma. Sporadic studies have shown that HNF4α played a certain regulatory role in all types of tumors. Further investigations of the role of the of HNF4α in different tumor types will greatly enhance the understanding HNF4α biological function and also will be important for development of HNF4α-based therapeutics.

Author contributions

LS and JWS were responsible for the study concept and design. LS, XW and WB drafted the manuscript. JLS, YZ, and JWS revised and edited the manuscript. All authors contributed to the article and approved the submitted version.

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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.

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Epidemiological evidence for associations between variants in CHRNA genes and risk of lung cancer and chronic obstructive pulmonary disease

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Background: Genetic studies have previously reported that single-nucleotide polymorphisms (SNPs) in *CHRNA* genes (such as *CHRNA3*, *CHRNA4*, *CHRNA5*, or *CHRNA3-CHRNA5-CHRNB4* clusters) are linked to the risk of neoplastic and non-neoplastic diseases. However, these conclusions were controversial and no systematic research synopsis has been available. We aimed to synthesize current knowledge of variants in the *CHRNA* genes on the risk of diseases.

Methods: We systematically searched for publications using PubMed, Medline, and Web of Science on or before 25 August 2021. A total of 1,818 publications were identified, of which 29 were deemed eligible for inclusion that could be used to perform meta-analysis based on at least three data sources to assess whether the morbidity associated with neoplastic and non-neoplastic diseases can be attributed to SNPs in *CHRNA* genes. To further evaluate the authenticity of cumulative evidence proving significant associations, the present study covered the Venice criteria and false-positive report probability tests. Through the Encyclopedia of DNA Elements (ENCODE) project, we created functional annotations for strong associations.

Results: Meta-analyses were done for nine genetic variants with two diseases {chronic obstructive pulmonary disease (COPD) and lung cancer (LC)}that had at least three data sources. Interestingly, eight polymorphisms were significantly related to changes in the susceptibility COPD and LC ($p < 0.05$). Of these, strong evidence was assigned to six variants (28 significant associations): *CHRNA3* rs1051730, *CHRNA3* rs6495309, and *CHRNA5* rs16969968 with COPD risk, and *CHRNA3* rs1051730, *CHRNA3* rs578776, *CHRNA3* rs6495309, *CHRNA3* rs938682, *CHRNA5* rs16969968, and *CHRNA5* rs588765 with LC risk; moderate evidence was assigned to five SNPs (12 total associations) with LC or COPD risk. Data from ENCODE and other public databases showed that SNPs with strong evidence may be located in presumptive functional regions.

Conclusions: Our study summarized comprehensive evidence showing that common mutations in *CHRNA* genes are strongly related to LC and COPD risk. The study also elucidated the vital function of *CHRNA* genes in genetic predispositions to human diseases.

KEYWORDS

CHRNA, genetic variants, lung cancer, COPD, susceptibility

1. Introduction

Worldwide, many diseases, including neoplastic and non-neoplastic illnesses, have become significant obstacles to the progress of human society. Although lung cancer (LC) has been surpassed by female breast cancer and is the second most commonly diagnosed cancer, the maximum number of deaths in cancerous people is attributed to LC (1). According to a report in 2020, the mortality rate of LC is 18%, with an estimated 1.8 million deaths per year, which exceeds other cancers by far (1). Chronic obstructive pulmonary disease (COPD) is another common global disease. The disease is considered preventable and remediable, and it is characterized by irreversible airway obstruction (2). According to various studies, environmental factors (such as tobacco smoking, ionizing radiation, occupational exposures, and air pollution for LC, and tobacco smoke, occupational dust, vapours, and fumes air pollutants for COPD) and variations in genes facilitate the advancement of LC and COPD (3, 4). Furthermore, more than 80% of LC patients have smoked, and about 50% of COPD cases are related to tobacco smoke worldwide (5–7). However, not everyone exposed to these risk factors develops LC, COPD, or other diseases, and only 20% of smokers are confirmed to have LC or COPD. In fact, previous studies have suggested that genetic variants may be responsible for susceptibility to LC and COPD (8, 9).

According to existing studies, the *CHRNA* gene can encode nicotine receptors that are expressed in many cells. These receptors can combine with their ligands (e.g., acetylcholine) to transmit biological information. Nicotine is an alkaloid found in tobacco that mimics acetylcholine (10). According to published articles, there are abundant nicotine receptors, which are thought to be the reason for nicotine addiction, in the brain core (11). In addition, because nicotine receptors consist of lung epithelial lung cells, tobacco carcinogens are presumed to act as risk factors for the onset of LC, and the receptors' signal transduction pathway may facilitate tumor metastasis (12–14). In previous papers, *CHRNA3* and *CHRNA5* have been proven to have significant correlations with smokers' susceptibility to LC for their polymorphisms (15). The hypomethylation in the promoter region of *CHRNB4* on 15q25 resulted in tumors' transcript overexpressing, and there were significant hypermethylation expression changes in *CHRNA3* and telomerase reverse transcriptase (*TERT*) and potential tumor suppressor genes that played out due to frequent methylation events (16, 17). Genome-wide association studies (GWAS) have also demonstrated that single-nucleotide polymorphisms (SNPs, such as CHRNA3, CHRNA5, and IREB2) in an area of chromosome 15q25 are closely related to COPD (18, 19). Cigarette smoking, the primary risk factor for the development of COPD, causes the chronic inflammatory process that promotes the structural changes in the small airways and parenchyma (20). The exchange of *CHRNA5* transcript expression may have influenced the inflammatory response to smoking (21).

In 2003, Chou et al. reported that neuronal nicotinic acetylcholine receptor subunit alpha 4 (*CHRNA4*) polymorphisms can play a role in febrile convulsions (22). In 2008, Amos et al. performed a GWAS in Caucasians and found that rs1051730 in *CHRNA3* was significantly related to LC susceptibility (23). In 2009, a study by Falvella et al. determined that both *CHRNA3* (a slight downgrade) and *CHRNA5* (a significant increase) expressed differently in lung adenocarcinoma tissue, which further explains the role of *CHRNA* SNPs in LC onset (24). Since then, studies have revealed the relationship between LC susceptibility and *CHRNA* SNPs, including rs12914385, rs3743073, rs578776, rs6495309, rs8042374, and rs938682 in *CHRNA3* and rs16969968 and rs588765 in *CHRNA5*. In 2012, Yang et al. declared that the rs6495309 CC genotype and rs6495309 CT/CC variant genotypes could increase the morbidity of LC and COPD in China (25). In the same year, Lee et al. reported that the CT or TT genotypes of rs6495309 in *CHRNA3* could significantly decrease the risk of COPD in the Korean population (26).

Although several studies have reported significant associations between *CHRNA* SNPs and the risk of cancerous or non-cancerous diseases, some studies have held controversial or disputed opinions about the same *CHRNA* SNPs. The possible reasons may have included small sample sizes or inauthentic positive associations. Because a comprehensive research synopsis with systematic functional annotation has

yet to measure the epidemiological evidence of genetic associations between *CHRNA* genes and susceptibility to cancerous or non-cancerous diseases, the present study aimed to account for the effect of studied *CHRNA* SNPs on the risk of all types of cancerous or non-cancerous diseases. First, we conducted a meta-analysis using data collected from all relevant existing studies. We then detected the statistical power of the generated significant evidence. Finally, we conducted a systematic functional annotation to detect the molecular mechanisms of the approved connections.

2. Methods

This study was performed under the guidance of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement guidelines (Supplementary Table S1) and the Human Genome Epidemiology Network for the systematic review of genetic association studies (27, 28).

2.1 Literature search

We used PubMed, Web of Science, and Medline to search for relevant papers delivered before 25 Aug 2021 by employing the following terms: *CHRNA3* or *CHRNA4* or *CHRNA5* or *CHRNA3-CHRNA5-CHRNB4* cluster and variant or variation or polymorphism or genotype or single-nucleotide polymorphism or SNP or mutation or rs. The published years ranged from 2008 to 2020. In addition, the references of the qualified articles were checked to acquire other related data.

2.2 Criteria for inclusion and exclusion

We selected qualified studies that met the following criteria: (i) studies that discussed relationships between *CHRNA* SNPs with the risk of neoplastic or non-cancerous disease with case–control or cohort designed studies of humanity; (ii) studies that provided the sample sizes of cases and controls, respectively; when necessary, studies could provide the amount of genotype and/or allelic distributions to compute the values of odds ratios (ORs) and 95% confidence intervals (95% CIs); and (iii) the full text of the journals was written completely in English. Any article that satisfied any of the following criteria were excluded: (i) studies without sufficient relevant data; (ii) studies in the form of conference abstracts, meetings, or letters to editors; and (iii) studies focused only on the prognoses and survival of cancer patients rather than on cancer morbidity.

2.3 Data extraction

We assigned two different authors to collect correlative information and cross-check each other. Any nonconformity was discussed with the corresponding author and eventually resolved. The following details would be recorded when the qualified SNPs were found: publication year, first author, SNP number, ethnicity, study design, gene name, gene variation, sample sizes of cases and controls, genotype counts, and minor allelic frequency. Ethnicity comprised four categories: Asians (East Asian descent), Caucasians (European descent), Africans (African descent), or others (including people from other countries, such as Indians, Native Hawaiians, Latinos, Hispanics, or mixed). More than 80% of the study's subjects belonged to one of the above-mentioned groups, and the overall population was composed of two or more of these groups. In addition, we selected the study that had been published most recently, which had the most complete sample of participants and the greatest amount of at least two studies that included the same study population. Because the presentation forms of results were usually inconsonant when identical genetic variants were studied in different research, we collated it at the NCBI site (https://www.ncbi.nlm.nih.gov/snp/) and then chose the most up-to-date and consistent one.

2.4 Statistical analysis

This study employed three models—the allelic model, the dominant model, and the recessive model (Supplementary Table S2)—to put the comprehensive meta-analyses into effect. An ethnicity-based subgroup analysis was also implemented as needed. The heterogeneity among the different publications was evaluated with the I^2 statistic and Cochran's Q test (29, 30). Three different ranges of I^2values were given different means: ≤25% (without or little heterogeneity), 25%–50% (middling heterogeneity), and ≥50% (abundant heterogeneity). A different kind of model was employed according to the p-value generated from the Q statistic. The random effect model was adopted if the p-value was < 0.1, and the fixed effect model was used if the p-value was > 0.1. In order to further evaluate the reliability of the significant ORs, we worked out sensitivity analyses for all SNPs with significant associations by excluding a single study (or dataset), the controls of studies that did not match the Hardy–Weinberg equilibrium (HWE), and the first published study. The study evaluated potential publication bias and small-study bias according to Begg's test and Egger's test, respectively (31, 32). The study also calculated the chance of collecting too many statistical findings for an independent meta-analysis (with a significance level of $p < 0.1$) (33). The small-study bias and potential publication bias were evaluated by adopting Begg's test and a modified version of Egger's test (with a significance level of $p < 0.1$, as recommended) (30, 31). Stata version 12 was used to conduct the statistical analyses (Stata, College Station, TX, USA).

2.5 Evaluation of cumulative evidence

To assess the epidemiological credibility of nominally statistical associations proved by meta-analyses, the present study used the Venice criteria to rate the cumulative evidence *via* three levels (strong, moderate, or weak) (34). The criteria consist of the amount of evidence, protection from bias, and replication of association (graded as A, B, or C, respectively). First, there were three levels for the quantity of evidence based on the total quantity of alleles or genotypes among the cases and controls. These levels were distinguished as follows: >1,000 (level A), 100–1,000 (level B), and <100 (level C). Similarly, the replication of association was evaluated by heterogeneity statistics (I^2) and was rated according to the following three levels: $I^2 \leq 25\%$ (level A), $25\% < I^2 < 50\%$ (level B), and $I^2 \geq 50\%$ (level C). Finally, we used sensitivity analysis, publication bias, the chance of collecting too many statistical findings, and small-study bias to assess the protection from bias. Importantly, associations without observable biases were assigned to grade A, and a grade A criterion meant that making an association clear was improbable. Grade B was chosen if an association lacked crucial information on identifying evidence without the presence of distinct bias (35). Finally, an association was assigned to grade C if the bias was explicit or the act of making the association clear was improbable.

The Venice criteria also cover an abundant checklist for checking the sources of bias in different options (see supplementary information notes). In addition, the confidence level of an association is related to the evaluation of protection from bias. To illustrate, a summary OR of an association of <1.15 (OR > 0.87 in a protection effect) was categorized as level C unless the relationship had been proven in other studies without obvious publication bias (primarily, GWAS or GWAS meta-analyses from collaborative studies). Finally, cumulative epidemiological evidence of nominally statistical associations was divided into three groups: strong associations (all three grades were A), weak associations (at least one grade of C), and moderate associations (apart from the above).

As Wacholder et al. recommended, a prior probability of 0.05 and a false-positive report probability (FPRP) cutoff value of 0.2 in the FPRP assay must be calculated to find potentially false-positive results among statistical associations and to discuss whether they are supposed to be excluded (36). We used the Excel spreadsheet acquired from the website to calculate FPRP values (35). We considered an association notable when the FPRP value was below the prespecified noteworthiness value of 0.2, indicating that the association might be true. The true evidence was graded by FPRP values of <0.05, 0.05–0.2, and >0.2, indicating strong, moderate, and weak, respectively. With a strong magnitude of FPRP, the credibility of the evidence would be upgraded from weak to moderate or from moderate to strong. If the FPRP was assigned as weak, we would downgrade the credibility of association.

2.6 Functional annotation

The underlying functional role of variants in *CHRNA* genes was evaluated with information from the Encyclopedia of DNA Elements (ENCODE) tool, HaploReg v. 4.1, and the UCSC Genome browser (http://genome.ucsc.edu/) (37). Furthermore, the present work explored genome-wide cis-eQTL data in multiple tissues from the Genotype-Tissue Expression Project and the Multiple Tissue Human Expression Resource Project databases to reveal whether these genes could account for the observed findings in these loci (38, 39).

3. Results

3.1 Characteristics of eligible studies

We initially searched for 1,818 studies using PubMed, Medline, and Web of Science (Figure 1). Of these, 1,706 papers were excluded because the titles and abstracts were duplicates or lacked correlation, and 92 papers were excluded due to insufficient information (such as about the number of variants in a genotype) in the full text. In addition, nine papers were included from meta-analyses, review articles, or references. The present study included a total of 29 publications with 70,960 cases and 124,838 controls to evaluate the relationship between nine *CHRNA* SNPs and LC or COPD susceptibility (eight SNPs with a relationship to LC and three SNPs with a relationship to COPD, respectively) after filtering out SNPs with no more than two datasets. The demographic characteristics of all available publications are summarized in Supplementary Table S3. Multiple diseases (such as epilepsy, Parkinson's disease, and Alzheimer's disease) were not evaluated by meta-analysis because there was only one dataset for each disease.

3.2 Main meta-analyses

Meta-analyses were performed to evaluate the associations between nine *CHRNA* SNPs and the risk of COPD or LC (Table 1). Of these, eight SNPs were statistically associated with the risk of LC or COPD risk; three SNPs were nominally statistically related to COPD risk (rs1051730 and rs6495309 in *CHRNA3* and rs16969968 in *CHRNA5*); and eight SNPs were nominally statistically associated with LC risk (rs1051730, rs12914385, rs578776, rs6495309, rs8042374, and rs938682 in *CHRNA3* and rs16969968 and rs588765 in *CHRNA5*).

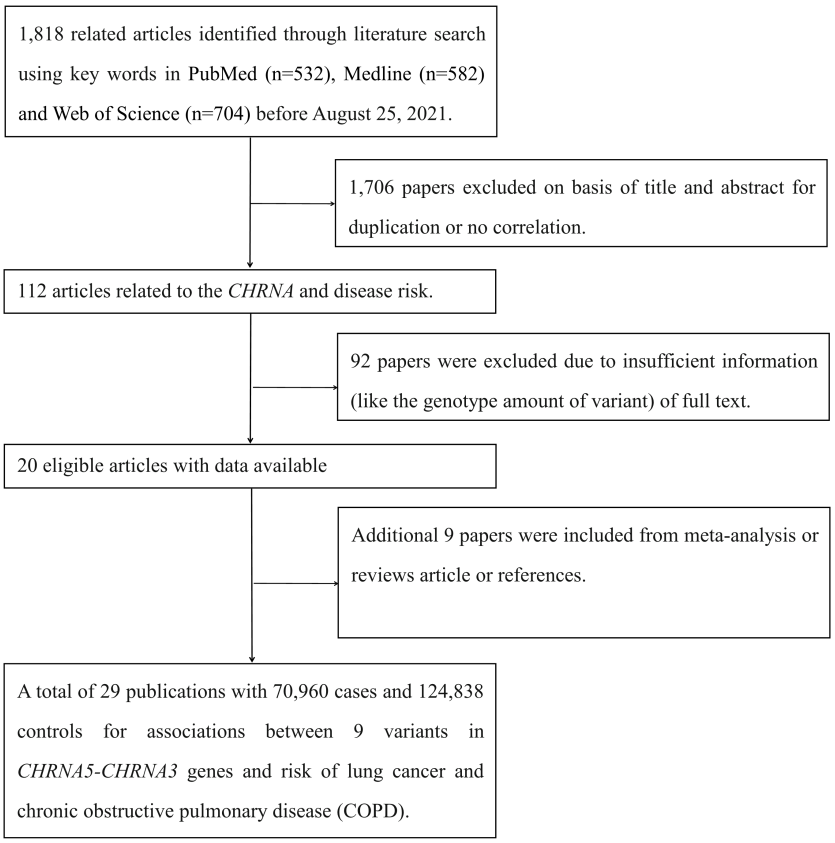

FIGURE 1
Flow diagram of literature search and study selection.

3.2.1 COPD

We identified a nominally statistical association between *CHRNA3* rs1051730 and COPD risk under the allelic and dominant models in all populations (allelic model: OR = 1.630, 95% CI = 1.293–2.054, $p < 0.001$; dominant model: OR = 1.662, 95% CI = 1.300–2.124, $p < 0.001$), a nominally significant association under the allelic and dominant models in Asians (allelic model: OR = 1.591, 95% CI = 1.204–2.103, $p = 0.001$; dominant model: OR = 1.625, 95% CI = 1.222–2.160, $p = 0.001$), and a null association under all three models in Caucasians. Regarding *CHRNA5* rs16969968, we found a nominally statistical association between SNP rs16969968 and COPD risk under the three genetic models in all populations (allelic model: OR = 1.307, 95% CI = 1.205–1.417, $p < 0.001$; dominant model: OR = 1.413, 95% CI = 1.268–1.573, $p < 0.001$; recessive model: OR = 1.370, 95% CI = 1.154–1.625, $p < 0.001$) and a nominal association under the allelic and dominant models in Asians (allelic model: OR = 1.591, 95% CI = 1.204–2.103, $p = 0.001$; dominant model: OR = 1.625, 95% CI = 1.222–2.160, $p = 0.001$). We discovered a null association between *CHRNA5* rs16969968 and Caucasians under the three genetic models. In addition, we found that SNP rs6495309 was statistically related to COPD risk under the allelic and dominant models in Asians (allelic model: OR = 0.830, 95% CI = 0.759–0.906, $p < 0.001$; dominant model: OR = 0.736, 95% CI = 0.644–0.842, $p = 0.001$).

3.2.2 Lung cancer

We detected a nominally statistical association between *CHRNA3* rs1051730 and LC risk under the three models in all populations (allelic model: OR = 1.348, 95% CI = 1.276–1.424, $p < 0.001$; dominant model: OR = 1.446, 95% CI = 1.342–1.559, $p < 0.001$; recessive model: OR = 1.519, 95% CI = 1.356–1.700, $p < 0.001$) and a nominally statistical association under the allelic and dominant models in Asians (allelic model: OR = 2.280, 95% CI = 1.626–3.197, $p < 0.001$; dominant model: OR = 2.329, 95% CI = 1.649–3.291, $p < 0.001$). There was also a nominally significant association under the three models in Caucasians (allelic model: OR = 1.313, 95% CI = 1.240–1.390, $p < 0.001$; dominant model: OR = 1.389, 95% CI = 1.283–1.504, $p < 0.001$; recessive model: OR = 1.506, 95% CI = 1.344–1.688, $p < 0.001$). For *CHRNA3* rs12914385, we presented no statistical association between

TABLE 1 Genetic variants showing summary ORs for different disease risks in main meta-analyses in all three genetic models.

Gene	Variant	Alleles[a]	Ethnicity	Number evaluated		Genetic model	Effect model	Risk of meta-analysis		Heterogeneity		Venice criteria[b]	FPRP values[c]	Credibility of evidence[d]
				Datasets	Cases/ Controls			OR (95% CI)	*p*-value	I^2 (%)	P_Q			
Chronic obstructive pulmonary disease (COPD)														
CHRNA3	rs1051730	A vs. G	Overall	4	1,618/1,909	Allelic	Fixed	1.630 (1.293–2.054)	<0.001	0.0	0.897	BAA	<0.001	Strong
CHRNA3	rs1051730	A vs. G	Overall	4	1,618/1,909	Dominant	Fixed	1.662 (1.300–2.124)	<0.001	0.0	0.877	BAA	<0.001	Strong
CHRNA3	rs1051730	A vs. G	Overall	4	1,618/1,909	Recessive	Random	2.433 (0.737–8.033)	0.144	0.0	0.425			
CHRNA3	rs1051730	A vs. G	Asian	3	1,501/1,717	Allelic	Fixed	1.591 (1.204–2.103)	0.001	0.0	0.780	BAA	0.001	Strong
CHRNA3	rs1051730	A vs. G	Asian	3	1,501/1,717	Dominant	Fixed	1.625 (1.222–2.160)	0.001	0.0	0.748	BAA	<0.001	Strong
CHRNA3	rs1051730	A vs. G	Asian	3	1,501/1,717	Recessive	Random	1.017 (0.106–9.793)	0.988	0.0	0.341			
CHRNA3	rs6495309	T vs. C	Asian	3	1,917/2,068	Allelic	Fixed	0.830 (0.759–0.906)	<0.001	25.9	0.259	ABA	<0.001	Strong
CHRNA3	rs6495309	T vs. C	Asian	3	1,917/2,068	Dominant	Fixed	0.736 (0.644–0.842)	<0.001	0.0	0.892	AAA	<0.001	Strong
CHRNA3	rs6495309	T vs. C	Asian	3	1,917/2,068	Recessive	Random	0.830 (0.637–1.081)	0.166	64.7	0.059			
CHRNA5	rs16969968	A vs. G	Overall	6	3,126/7,685	Allelic	Fixed	1.307 (1.205–1.417)	<0.001	0.0	0.471	AAC	<0.001	Moderate
CHRNA5	rs16969968	A vs. G	Overall	6	3,126/7,685	Dominant	Fixed	1.413 (1.268–1.573)	<0.001	0.0	0.687	AAC	<0.001	Moderate
CHRNA5	rs16969968	A vs. G	Overall	6	3,126/7,685	Recessive	Fixed	1.370 (1.154–1.625)	<0.001	0.0	0.713	BAA	<0.001	Strong
CHRNA5	rs16969968	A vs. G	Asian	3	1,501/1,717	Allelic	Fixed	1.591 (1.204–2.103)	0.001	0.0	0.780	BAA	0.001	Strong
CHRNA5	rs16969968	A vs. G	Asian	3	1,501/1,717	Dominant	Fixed	1.625 (1.222–2.160)	0.001	0.0	0.748	BAA	<0.001	Strong
CHRNA5	rs16969968	A vs. G	Asian	3	1,501/1,717	Recessive	Fixed	1.017 (0.143–7.229)	0.987	0.0	0.341			
Lung cancer														
CHRNA3	rs1051730	A vs. G	Overall	11	7,657/7,515	Allelic	Fixed	1.348 (1.276–1.424)	<0.001	37.0	0.103	ABA	<0.001	Strong
CHRNA3	rs1051730	A vs. G	Overall	11	7,657/7,515	Dominant	Fixed	1.446 (1.342–1.559)	<0.001	10.0	0.349	AAA	<0.001	Strong

(Continued)

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TABLE 1 Continued

Gene	Variant	Alleles[a]	Ethnicity	Number evaluated		Genetic model	Effect model	Risk of meta-analysis		Heterogeneity		Venice criteria[b]	FPRP values[c]	Credibility of evidence[d]
				Datasets	Cases/ Controls			OR (95% CI)	*p*-value	I^2 (%)	P_Q			
CHRNA3	rs1051730	A vs. G	Overall	11	7,657/7,515	Recessive	Fixed	1.519 (1.356–1.700)	<0.001	0.0	0.582	AAA	<0.001	Strong
CHRNA3	rs1051730	A vs. G	Asian	3	1,834/1,460	Allelic	Fixed	2.280 (1.626–3.197)	<0.001	0.0	0.978	BAA	<0.001	Strong
CHRNA3	rs1051730	A vs. G	Asian	3	1,834/1,460	Dominant	Fixed	2.329 (1.649–3.291)	<0.001	0.0	0.982	BAA	<0.001	Strong
CHRNA3	rs1051730	A vs. G	Asian	3	1,834/1,460	Recessive	Random	2.249 (0.234–21.659)	0.483	Na	Na			
CHRNA3	rs1051730	A vs. G	Caucasian	6	3,492/5,434	Allelic	Fixed	1.313 (1.240–1.390)	<0.001	0.0	0.611	AAA	<0.001	Strong
CHRNA3	rs1051730	A vs. G	Caucasian	6	3,492/5,434	Dominant	Fixed	1.389 (1.283–1.504)	<0.001	0.0	0.886	AAA	<0.001	Strong
CHRNA3	rs1051730	A vs. G	Caucasian	6	3,492/5,434	Recessive	Fixed	1.506 (1.344–1.688)	<0.001	8.0	0.365	AAA	<0.001	Strong
CHRNA3	rs12914385	T vs. C	Overall	4	10,037/4,443	Allelic	Random	1.096 (0.799–1.505)	0.569	96.7	< 0.001			
CHRNA3	rs12914385	T vs. C	Overall	4	10,037/4,443	Dominant	Random	1.101 (0.712–1.703)	0.665	96.6	< 0.001			
CHRNA3	rs12914385	T vs. C	Overall	4	10,037/4,443	Recessive	Random	1.206 (0.839–1.733)	0.312	89.4	< 0.001			
CHRNA3	rs12914385	T vs. C	Caucasian	3	8,514/2,900	Allelic	Random	1.264 (1.053–1.517)	0.012	84.4	0.002	ACA	0.012	Moderate
CHRNA3	rs12914385	T vs. C	Caucasian	3	8,514/2,900	Dominant	Random	1.458 (1.331–1.596)	0.014	81.5	0.004	ACA	<0.001	Moderate
CHRNA3	rs12914385	T vs. C	Caucasian	3	8,514/2,900	Recessive	Random	1.449 (1.134–1.851)	0.003	66.5	0.051	ACA	0.003	Moderate
CHRNA3	rs3743073	T vs. G	Overall	3	1,391/1,500	Allelic	Random	0.785 (0.546–1.129)	0.191	91.3	< 0.001			
CHRNA3	rs3743073	T vs. G	Overall	3	1,391/1,500	Dominant	Random	0.755 (0.410–1.392)	0.368	90.7	< 0.001			
CHRNA3	rs3743073	T vs. G	Overall	3	1,391/1,500	Recessive	Random	0.729 (0.504–1.056)	0.094	80.0	0.007			
CHRNA3	rs578776	A vs. G	Overall	3	1,254/2,009	Allelic	Fixed	0.868 (0.773–0.976)	0.018	0.0	0.908	AAA	0.018	Strong

(Continued)

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TABLE 1 Continued

Gene	Variant	Alleles[a]	Ethnicity	Number evaluated		Genetic model	Effect model	Risk of meta-analysis		Heterogeneity		Venice criteria[b]	FPRP values[c]	Credibility of evidence[d]
				Datasets	Cases/ Controls			OR (95% CI)	*p*-value	I^2 (%)	P_Q			
CHRNA3	rs578776	A vs. G	Overall	3	1,254/2,009	Dominant	Fixed	0.841 (0.722–0.979)	0.026	0.0	0.937	AAA	0.025	Strong
CHRNA3	rs578776	A vs. G	Overall	3	1,254/2,009	Recessive	Random	0.839 (0.655–1.073)	0.162	0.0	0.944			
CHRNA3	rs6495309	T vs. C	Asian	3	1,865/1,983	Allelic	Fixed	0.770 (0.704–0.843)	<0.001	0.0	0.400	AAA	<0.001	Strong
CHRNA3	rs6495309	T vs. C	Asian	3	1,865/1,983	Dominant	Fixed	0.736 (0.642–0.843)	<0.001	0.0	0.749	AAA	<0.001	Strong
CHRNA3	rs6495309	T vs. C	Asian	3	1,865/1,983	Recessive	Fixed	0.680 (0.580–0.797)	<0.001	0.0	0.373	BAA	<0.001	Strong
CHRNA3	rs8042374	G vs. A	Caucasian	3	8,501/2,920	Allelic	Random	0.814 (0.703–0.941)	0.006	67.7	0.045	ACC	0.005	Moderate
CHRNA3	rs8042374	G vs. A	Caucasian	3	8,501/2,920	Dominant	Fixed	0.763 (0.697–0.834)	<0.001	47.3	0.150	ABC	<0.001	Moderate
CHRNA3	rs8042374	G vs. A	Caucasian	3	8,501/2,920	Recessive	Random	0.715 (0.503–1.016)	0.061	62.6	0.069			
CHRNA3	rs938682	A vs. G	Overall	3	4,958/2,575	Allelic	Fixed	1.239 (1.131–1.357)	<0.001	0.0	0.384	AAC	<0.001	Moderate
CHRNA3	rs938682	A vs. G	Overall	3	4,958/2,575	Dominant	Random	1.233 (0.829–1.834)	0.301	61.8	0.073			
CHRNA3	rs938682	A vs. G	Overall	3	4,958/2,575	Recessive	Fixed	1.295 (1.158–1.447)	<0.001	0.0	0.932	AAA	<0.001	Strong
CHRNA5	rs16969968	A vs. G	Overall	14	22,794/82,907	Allelic	Fixed	1.293 (1.260–1.328)	<0.001	25.1	0.183	ABA	<0.001	Strong
CHRNA5	rs16969968	A vs. G	Overall	14	22,794/82,907	Dominant	Fixed	1.374 (1.324–1.426)	<0.001	34.4	0.100	ABA	<0.001	Strong
CHRNA5	rs16969968	A vs. G	Overall	14	22,794/82,907	Recessive	Fixed	1.445 (1.372–1.522)	<0.001	0.0	0.568	AAA	<0.001	Strong
CHRNA5	rs16969968	A vs. G	Caucasian	12	21,035/80,599	Allelic	Fixed	1.298 (1.264–1.333)	<0.001	0.0	0.575	AAC	<0.001	Moderate
CHRNA5	rs16969968	A vs. G	Caucasian	12	21,035/80,599	Dominant	Fixed	1.384 (1.333–1.437)	<0.001	0.0	0.495	AAA	<0.001	Strong
CHRNA5	rs16969968	A vs. G	Caucasian	12	21,035/80,599	Recessive	Fixed	1.447 (1.374–1.524)	<0.001	0.0	0.540	AAC	<0.001	Moderate
CHRNA5	rs588765	C vs. T	Caucasian	4	5,851/7,321	Allelic	Fixed	1.124 (1.069–1.182)	<0.001	0.0	0.890	AAC	<0.001	Moderate

(Continued)

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TABLE 1 Continued

Gene	Variant	Alleles[a]	Ethnicity	Number evaluated		Genetic model	Effect model	Risk of meta-analysis		Heterogeneity		Venice criteria[b]	FPRP values[c]	Credibility of evidence[d]
				Datasets	Cases/ Controls			OR (95% CI)	*p*-value	I^2 (%)	P_Q			
CHRNA5	rs588765	C vs. T	Caucasian	4	5,851/7,321	Dominant	Fixed	1.122 (1.020–1.234)	0.018	0.0	0.724	AAC	0.018	Moderate
CHRNA5	rs588765	C vs. T	Caucasian	4	5,851/7,321	Recessive	Fixed	1.192 (1.109–1.280)	<0.001	0.0	0.898	AAA	<0.001	Strong

OR, odds ratio; A, adenine; T, thymine; G, guanine; C, cytosine.
[a]Minor alleles vs. major alleles (reference).
[b]Strength of epidemiological evidence based on the Venice criteria.
[c]FPRP values at prior probability of 0.05 at power OR of 1.5, and the FPRP level of noteworthiness is 0.20.
[d]Degree of epidemiological credibility based on the combination of results from Venice guidelines and FPRP tests.

rs12914385 and LC risk under the three models in all populations but a nominally conspicuous relationship with LC risk under the three models in Caucasians (allelic model: OR = 1.264, 95% CI = 1.053–1.517, p = 0.012; dominant model: OR = 1.458, 95% CI = 1.331–1.596, p = 0.014; recessive model: OR = 1.449, 95% CI = 1.134–1.851, p = 0.003) and a null relationship between *CHRNA3* rs12914385 and LC risk under the three models in Asians.

Regarding *CHRNA3* rs578776, we identified a nominally statistical relationship between SNP rs578776 and LC risk under two models in all populations, including Asians and Caucasians (allelic model: OR = 0.868, 95% CI = 0.773–0.976, p = 0.018; dominant model: OR = 0.841, 95% CI = 0.722–0.979, p = 0.026). We also demonstrated that *CHRNA3* rs6495309 likely had a statistical relationship with LC risk under the three models in Asians (allelic model: OR = 0.770, 95% CI = 0.704–0.843, p < 0.001; dominant model: OR = 0.736, 95% CI = 0.642–0.843, p < 0.001; recessive model: OR = 0.680, 95% CI = 0.580–0.797, p < 0.001). For *CHRNA3* rs8042374, we found a nominally statistical association between SNP rs8042374 and LC risk under two models in Caucasians (allelic model: OR = 0.814, 95% CI = 0.703–0.941, p = 0.006; dominant model: OR = 0.763, 95% CI = 0.697–0.834, p < 0.001). For *CHRNA3* rs938682, we detected a nominally statistical association between SNP rs938682 and LC risk under two models in all populations (allelic model: OR = 1.239, 95% CI = 1.131–1.357, p < 0.001; recessive model: OR = 1.295, 95% CI = 1.158–1.447, p < 0.001). In addition, *CHRNA3* rs3743073 was revealed to have no statistical relationship with LC risk under the three models in all populations, though this finding was not verified in Asians or Caucasians.

A nominally statistical association between *CHRNA5* rs16969968 and LC risk was shown under the three models in all populations (allelic model: OR = 1.293, 95% CI = 1.260–1.328, p < 0.001; dominant model: OR = 1.374, 95% CI = 1.324–1.426, p < 0.001; recessive model: OR = 1.445, 95% CI = 1.372–1.522, p < 0.001), and a nominally statistical association between *CHRNA5* rs16969968 and LC risk was identified under the three models in Caucasians (allelic model: OR = 1.293, 95% CI = 1.264–1.333, p < 0.001; dominant model: OR = 1.384, 95% CI = 1.333–1.437, p < 0.001; recessive model: OR = 1.447, 95% CI = 1.374–1.524, p < 0.001). In addition, a nominally statistical association was identified between SNP rs588765 and LC risk under the three models in Caucasians (allelic model: OR = 1.124, 95% CI = 1.069–1.182, p < 0.001; dominant model: OR = 1.122, 95% CI = 1.020–1.234, p = 0.018; recessive model: OR = 1.192, 95% CI = 1.109–1.280, p < 0.001).

Moreover, our study also found that some SNPs had no association with risk of disease. For example, our study found that rs12914385 in *CHRNA3* gene had a non-significant association with risk of LC in all populations under any

genetic model, but had significant associations in Caucasians. rs12914385 in *CHRNA3* gene had no association with risk of LC in all populations.

3.3 Cumulative evidence of association

We initially used the Venice criteria to assess the cumulative epidemiological evidence for the eight SNPs that showed significant relationships to LC or COPD risk. More information on this evidence is listed in Supplementary Table S4 and Table 1. For the Venice criteria test, we first assigned 30 A grades, 10 B grades, and 0 C grades to further assess the authenticity of evidence according to the amount of evidence. We then assigned 31 A grades, 5 B grades, and 4 C grades to evaluate their replication. Finally, we assigned 31 A grades, 0 B grades, and 9 C grades to assess protection from bias. Ultimately, the relationship to COPD risk could be rated according to three groups: strong (included *CHRNA3* rs6495309 under the dominant model in Asians), moderate (reflected eight associations, including *CHRNA3* rs1051730 under the allelic and dominant models in all populations and the allelic and dominant models in Asians, *CHRNA3* rs6495309 under the allelic model in Asians and *CHRNA5* rs16969968 under the recessive model in all populations, and the allelic and dominant models in Asians), and weak (represented two associations, including *CHRNA5* rs16969968 under the allelic and dominant models in all populations).

In terms of LC risk, the strong group was rated for 13 associations (including *CHRNA3* rs1051730 under the dominant and recessive models in all populations and under all three models in Caucasians, *CHRNA3* rs578776 under the allelic and dominant models in all populations, *CHRNA3* rs6495309 under the allelic and dominant models in Asians, *CHRNA3* rs938682 under the recessive model in all populations, *CHRNA5* rs16969968 under the recessive model in all populations and under the dominant model in Caucasians, and *CHRNA5* rs588765 under the recessive model in Caucasians); the moderate group was rated for 6 associations (including *CHRNA3* rs1051730 under the allelic model in all populations and in Asians, *CHRNA3* rs6495309 under the recessive model in Asians, and *CHRNA5* rs16969968 under the allelic and dominant models in all populations); and the weak group was rated for 10 associations (including *CHRNA3* rs12914385 under all three models in Caucasians, *CHRNA3* rs8042374 under the allelic and dominant models in Caucasians, *CHRNA3* rs938682 under the allelic model in all populations, *CHRNA5* rs16969968 under the allelic and recessive models in Caucasians, and *CHRNA5* rs588765 under the allelic and dominant models in Caucasians). In addition, we calculated the FPRP values of these nominally statistical associations to evaluate the probability of a precisely significant relationship between SNPs and LC or COPD risk. Of note, the *p*-values of the FPRP assay above all nominally significant associations between SNPs and LC or COPD risk were less than 0.05.

Finally, strong evidence was assigned to six variants with LC or COPD risk (28 significant associations). A strong association was identified between *CHRNA3* rs1051730 and COPD risk under the allelic and dominant models in all populations and in Asians; between *CHRNA3* rs6495309 and COPD risk under the allelic and dominant models in Asians; between *CHRNA5* rs16969968 and COPD risk under the recessive model in all populations and under the allelic and dominant models in Asians; between *CHRNA3* rs1051730 and LC risk under all three models in all populations and in Caucasians and under the allelic and dominant models in Asians; between *CHRNA3* rs578776 and LC risk under the allelic and dominant models in all populations; between *CHRNA3* rs6495309 and LC risk under all three models in Asians; between *CHRNA3* rs938682 and LC risk under the recessive model in all populations; between *CHRNA5* rs16969968 and LC risk under all three models in all populations and under the dominant model in Caucasians; and between *CHRNA5* rs588765 and LC risk under the recessive model in Caucasians. Moderate evidence was assigned to five SNPs (12 associations) with risk of LC or COPD. A moderate relationship was identified between *CHRNA5* rs16969968 and COPD risk under the allelic and dominant models in all populations; between *CHRNA3* rs12914385 and LC risk under all three models in Caucasians; between *CHRNA3* rs8042374 and LC risk under the allelic and dominant models in Caucasians; between *CHRNA3* rs938682 and LC risk under the allelic model in all populations; between *CHRNA5* rs16969968 and LC risk under the allelic and dominant models in Caucasians; and between *CHRNA5* rs588765 and LC risk under the allelic and dominant models in Caucasians.

3.4 Heterogeneity, bias, and sensitivity analyses

We performed assessments of heterogeneity, bias, and sensitivity analyses (Supplementary Table S4 and Table 1). For nominally significant associations between SNPs and COPD risk, low heterogeneity was found for associations of *CHRNA3* rs1051730 in all populations (allelic model: $I^2 = 0.0\%$, $p = 0.897$; dominant model: $I^2 = 0.0\%$, $p = 0.877$) and in Asians (allelic model: $I^2 = 0.0\%$, $p = 0.780$; dominant model: $I^2 = 0.0\%$, $p = 0.748$); for associations of *CHRNA3* rs6495309 in Asians (dominant model: $I^2 = 0.0\%$, $p = 0.892$); and for associations of *CHRNA5* rs16969968 in all populations (allelic model: $I^2 = 0.0\%$, $p = 0.471$; dominant model: $I^2 = 0.0\%$, $p = 0.687$; recessive model: $I^2 = 0.0\%$, $p = 0.713$) and in Asians (allelic model: $I^2 = 0.0\%$, $p = 0.780$; dominant model: $I^2 = 0.0\%$, $p = 0.748$). Moderate heterogeneity was detected only for associations of *CHRNA3* rs6495309 (allelic model: $I^2 = 25.9\%$, $p = 0.259$). In addition, we found little proof of publication bias for nominally

significant associations between SNPs and COPD risk ($p > 0.10$), except in the case of *CHRNA5* rs16969968 under the allelic and dominant models in all populations ($p < 0.10$). Furthermore, we evaluated the robustness of these nominally significant associations by performing a sensitivity analysis that removed single studies (or datasets). The removal of studies that had been published first or studies deviating from HWE in COPD control groups did not alter the summary ORs. We did not test the excess of significant findings due to the unavailability of genotype amounts in most studies.

For nominally significant associations between SNPs and LC risk, the following associations were considered to have low heterogeneity: *CHRNA3* rs1051730 in all populations (dominant model: $I^2 = 10.0\%$, $p = 0.349$; recessive model: $I^2 = 0.0\%$, $p = 0.582$), in Asians (allelic model: $I^2 = 0.0\%$, $p = 0.978$; dominant model: $I^2 = 0.0\%$, $p = 0.982$), and in Caucasians (allelic model: $I^2 = 0.0\%$, $p = 0.611$; dominant model: $I^2 = 0.0\%$, $p = 0.886$; recessive model: $I^2 = 8.0\%$, $p = 0.365$); *CHRNA3* rs578776 in all populations (allelic model: $I^2 = 0.0\%$, $p = 0.908$; dominant model: $I^2 = 0.0\%$, $p = 0.937$); *CHRNA3* rs6495309 in Asians (allelic model: $I^2 = 0.0\%$, $p = 0.400$; dominant model: $I^2 = 0.0\%$, $p = 0.749$; recessive model: $I^2 = 0.0\%$, $p = 0.373$); *CHRNA3* rs938682 in all populations (allelic model: $I^2 = 0.0\%$, $p = 0.384$; recessive model: $I^2 = 0.0\%$, $p = 0.932$); *CHRNA5* rs16969968 in all populations (recessive model: $I^2 = 0.0\%$, $p = 0.568$) and in Caucasians (allelic model: $I^2 = 0.0\%$, $p = 0.575$; dominant model: $I^2 = 0.0\%$, $p = 0.495$; recessive model: $I^2 = 0.0\%$, $p = 0.540$); and *CHRNA5* rs588765 in Caucasians (allelic model: $I^2 = 0.0\%$, $p = 0.890$; dominant model: $I^2 = 0.0\%$, $p = 0.724$; recessive model: $I^2 = 0.0\%$, $p = 0.898$). Four associations were found to have moderate heterogeneity: *CHRNA3* rs1051730 in all populations (allelic model: $I^2 = 37.0\%$, $p = 0.103$); *CHRNA3* rs8042374 in Caucasians (dominant model: $I^2 = 47.3\%$, $p = 0.150$); and *CHRNA5* rs16969968 in all populations (allelic model: $I^2 = 25.1\%$, $p = 0.183$; dominant model: $I^2 = 34.4\%$, $p = 0.100$). Another four associations were found to have large heterogeneity: *CHRNA3* rs12914385 in Caucasians (allelic model: $I^2 = 84.4\%$, $p = 0.002$; dominant model: $I^2 = 81.5\%$, $p = 0.004$; recessive model: $I^2 = 66.5\%$, $p = 0.051$) and *CHRNA3* rs8042374 in Caucasians (allelic model: $I^2 = 67.7\%$, $p = 0.045$).

As with the associations connected to COPD risk, we also performed a publication bias test. The test showed little confidence for nominally significant associations between SNPs and LC risk ($p > 0.10$), except in *CHRNA3* rs8042374 under the allelic and dominant models in Caucasians, *CHRNA3* rs938682 under the allelic model in all populations, *CHRNA5* rs16969968 under the allelic and recessive models in Caucasians, and *CHRNA5* rs588765 under the allelic and dominant models in Caucasians ($p < 0.10$). Regarding sensitivity analysis, the summary ORs were not modified by removing studies that had been published first or studies deviating from HWE in LC control groups, except *CHRNA5* rs588765 under the dominant model in Caucasians due to the removal of a study that had been published first. We did not test the excess of significant findings due to the unavailability of genotype amounts in most studies.

3.5 Functional annotation

By referring to the data gained from the ENCODE tool, HaploReg v. 4.1, we analyzed the functional roles of the six variants strongly associated with LC or COPD risk (Table 2). The results showed that rs938682 and rs588765 mapped to intronic regions, rs578776 mapped to 3'UTR, rs1051730 was annotated as a synonymous variant, and rs16969968 was annotated as missense. Six SNPs could be considered expression quantitative trait loci (eQTLs) for numerous genes in various tissue types, six SNPs could be situated in the histone modification regions of enhancers, four SNPs could be located in promoters, and one SNP could be found in sites exhibiting DNase I hypersensitivity. We also found that four SNPs (rs1051730, rs6495309, rs578776, and rs938682) may be involved in transcriptional regulatory element activity. Moreover, linkage disequilibrium (LD) plots showed that regions delegated by significant SNPs had distinct genetic structures among European,

TABLE 2 Summary of functional annotations for six SNPs in CHRNA genes with diseases risk (strong epidemiological credibility).

Variant	Gene	Position[a]	Annotation	Promoter histone marks[b]	Enhancer histone marks[c]	DNase[d]	Proteins bound[e]	Motifs changed[f]
rs1051730	CHRNA3	78601997	Synonymous	LNG, SPLN	SPLN			AP-2, Foxl1, Foxo
rs6495309	CHRNA3	78622903		THYM	4 tissues	THYM		7 altered motifs
rs578776	CHRNA3	78596058	3'-UTR		ESDR			Hdx, Pou1f1
rs938682	CHRNA3	78604205	Intronic	ESDR, BLD, CRVX	ESC, ESDR, HRT			6 altered motifs
rs16969968	CHRNA5	78590583	Missense		ESC, IPSC			
rs588765	CHRNA5	78573083	Intronic	IPSC, HRT	ESC, LNG			

[a]The chromosome position is based on NCBI Build 37.
[b]Histone modification of H3K4me1 and H3K27ac (tissue types: if >3, only the number is included).
[c]Histone modification of H3K4me3 (tissue types: if >3, only the number is included).
[d]Levels of DNase I hypersensitivity (tissue types: if >3, only the number is included).
[e]Alteration in transcription factor binding (disruptions: if >3, only the number is included).
[f]Alteration in regulatory motif (disruptions: if >3, only the number is included).

Asian, and African ancestries (Figure 2). The Genotype-Tissue Expression Project revealed that rs1051730, rs16969968, and rs588765 are eQTLs for *CHRNA3* and *CHRNA5*. In brief, while rs1051730, rs16969968, and rs588765 are associated with a decrease of *CHRNA3* and *CHRNA5* gene expression in lung tissue, rs6495309, rs6495309, and rs938682 are associated with an increase in *IREB2* gene expression in lung tissue (Supplementary Table 5).

4. Discussion

Although numerous existing studies have confirmed potential associations between *CHRNA* SNPs and LC or COPD risk, their results are inconsistent and controversial. To our knowledge, this is the first study to comprehensively elucidate whether the studied variants of *CHRNA* genes are related to the risk of cancerous or non-cancerous diseases and to evaluate the credibility of significant epidemiological evidence using the Venice criteria and FPRP tests. This meta-analysis assessed 29 papers with 70,960 cases and 124,838 controls. The study also evaluated the associations between nine SNPs and the risk of LC or COPD. Among these SNPs, eight were found to be statistically related to the risk of LC or COPD. In addition, two approaches (the Venice guidelines and FPRP tests) were used for the first time to assess these significant associations. The cumulative evidence for associations between six variants and LC or COPD risk was shown to be strong (28 significant associations: *CHRNA3* rs1051730, *CHRNA3* rs6495309, and *CHRNA5* rs16969968 with COPD risk and *CHRNA3* rs1051730, *CHRNA3* rs578776, *CHRNA3* rs6495309, *CHRNA3* rs938682, *CHRNA5* rs16969968, and *CHRNA5* rs588765 with LC risk), and the cumulative evidence for associations between five SNPs (12 associations) and LC or COPD risk was moderate. The current study also constructed functional annotations for the six SNPs with strong evidence using information from the ENCODE project and other public databases, revealing that these mutations may lie in several putative regulatory areas. In brief, this research provided comprehensive epidemiological evidence that familiar variants in *CHRNA* genes show an association with a predisposition to LC or COPD.

The *CHRNA3* gene (Gene ID: 1136) and *CHRNA5* gene (Gene ID: 1138), which are located in chromosome 15q25.1, were found to be related to the risk of LC and COPD (14, 15, 18). Many published papers, including GWAS, have demonstrated a significant relationship between *CHRNA* and COPD risk (15, 18). Some studies have indicated that the nicotine receptors expressed in lung epithelial cells can facilitate cancer cell proliferation and metastases (11–13). In the present study, six variants were shown to present with strong cumulative evidence in their associations with LC or COPD risk (*CHRNA3* rs1051730, *CHRNA3* rs6495309, and *CHRNA5* rs16969968 with COPD risk, and *CHRNA3* rs1051730, *CHRNA3* rs578776, *CHRNA3* rs6495309, *CHRNA3* rs938682, *CHRNA5* rs16969968, and *CHRNA5* rs588765 with LC risk).

For the evaluation of COPD, three SNPs showed strong associations with the risk of COPD. Phase 3 of the 1000 Genomes Project (Supplementary Table 6) suggested that CHRNA3 rs1051730 is uncorrelated with CHRNA3 rs6495309 in both Asians and Africans ($r^2 < 0.05$), and these SNPs showed weak LD in Europeans ($r^2 = 0.1751$) (40). Furthermore, *CHRNA5* rs16969968 was uncorrelated with *CHRNA3* rs6495309 in both Asians and Africans ($r^2 < 0.05$), and these SNPs were weak in Europeans ($r^2 = 0.1729$). According to the results, different causal mutations and functional mechanisms may exist in the relationships between COPD risk and mutations in the *CHRNA3* and/or *CHRNA5* genes. Moreover, while *CHRNA5* rs16969968 showed strong LD with *CHRNA3* rs1051730 in both Asians and Europeans ($r^2 > 0.9$), it showed weak LD in Africans ($r^2 = 0.2520$). Based on the results, the functional mechanisms of the two variants may change among different ethnic groups and partially answer to ethnic differences among variants related to disease, such as COPD risk.

For the assessment of LC, six SNPs were strongly related to LC risk (rs1051730, rs578776, rs6495309, and rs938682 in the *CHRNA3* gene and rs16969968 and rs588765 in the *CHRNA5* gene). Phase 3 of the 1000 Genomes Project (Supplementary Table 6) suggested that CHRNA3 rs6495309 showed a strong LD with CHRNA3 rs938682 in both Asians and Europeans ($r^2 > 0.8$) and a moderate LD in Africans ($r^2 = 0.5143$). While CHRNA3 rs578776 showed a strong LD with CHRNA3 rs938682 in Europeans ($r^2 > 0.8$), these SNPs showed weak LD in both Asians ($r^2 = 0.2185$) and Africans ($r^2 = 0.2991$). Furthermore, CHRNA3 rs6495309 showed moderate LD with CHRNA3 rs578776 in Europeans ($r^2 = 0.7556$), but these SNPs showed weak LD in both Asians ($r^2 = 0.1698$) and Africans ($r^2 = 0.1289$). Finally, although CHRNA5 rs16969968 showed moderate LD with CHRNA5 rs588765 in Europeans ($r^2 = 0.3601$), these SNPs were uncorrelated in both Asians and Africans ($r^2 < 0.05$). Based on the results, the functional mechanisms of the three variants related to the risk of LC may be different across ethnic groups and may partially answer the ethnic differences of some variants related to disease. Finally, rs1051730 showed weak LD or was uncorrelated with three SNPs (rs6495309, rs578776, and rs938682), indicating that different causal mutations and functional mechanisms may exist in the relationships between LC risk and *CHRNA3* gene mutations.

Current evidence has indicated that both *CHRNA3* rs1051730 and *CHRNA5* rs16969968 have excellent responses to nicotinic agonists *in vitro* (41). When compared to smokers without these two SNPs, these polymorphisms are generally present in heavy smokers who have higher levels of nitrosamines and other derivatives due to the combustion of tobacco, which can trigger an inflammatory response to COPD and elevated cellular proliferation in lung tissue, resulting in the development of LC or COPD (14). *CHRNA3* rs6495309 can change the binding ability of the transcriptional factor *Oct-1*, which has been shown to repress gene transcription, leading to alterations in *CHRNA3* RNA expression. This influences the ability of cells to progress into apoptosis, thereby impacting LC risk (17).

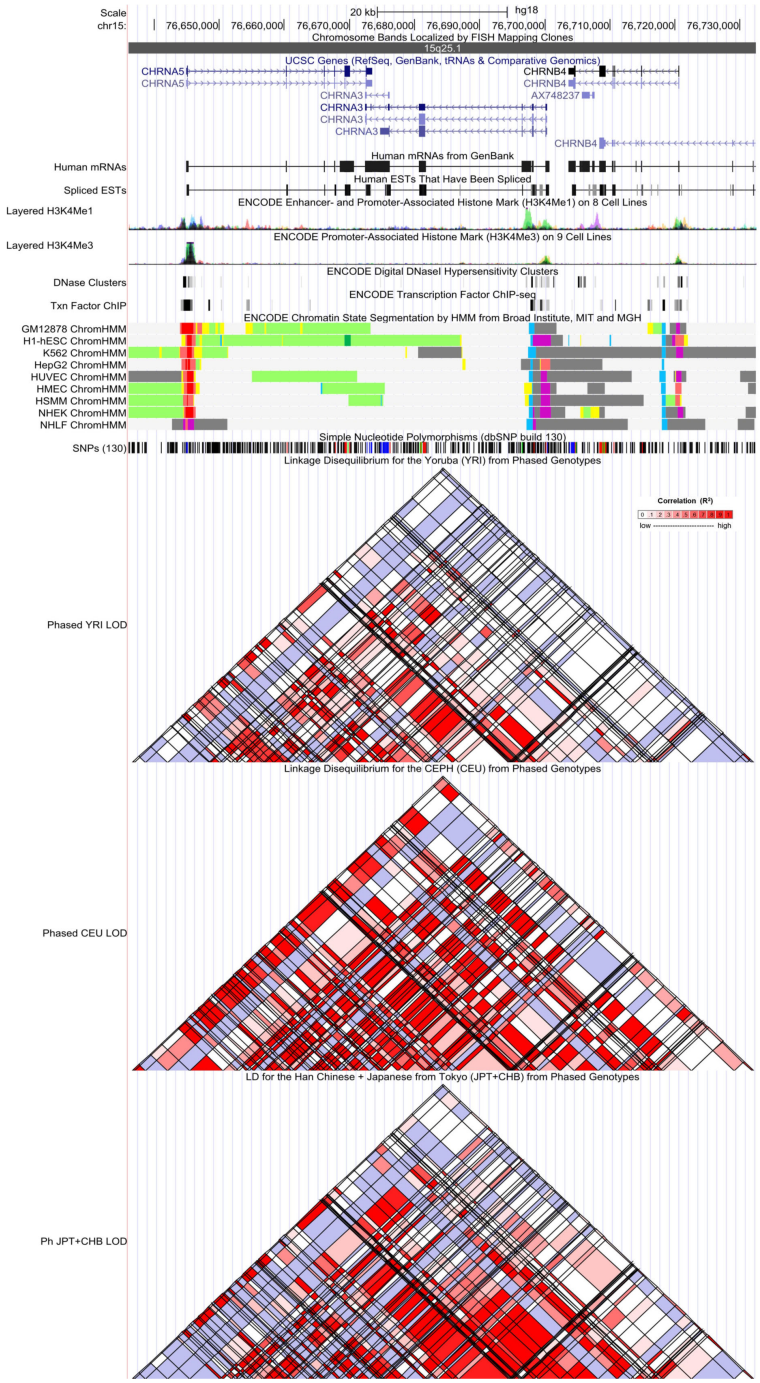

FIGURE 2
Evidence from the Encyclopedia of DNA Elements (ENCODE) data for the regulatory function of variants in 15q25.1 using the UCSC Genome Browser. The plot represents 15q25.1 within a 20-kb window centered on the CHRNA5–CHRNA3 gene region. Tracks (from top to bottom) in each of the plots are Genome Base Position, Chromosome Bands, UCSC Genes, Human messenger RNAs from GenBank, Human expressed sequence tag (ESTs) That Have Been Spliced, ENCODE Enhancer and Promoter-Associated Histone Mark (H3K4Me1) on 8 Cell Lines, ENCODE Promoter-Associated Histone Mark (H3K4Me3) on 9 Cell Lines, ENCODE Digital DNaseI Hypersensitivity Clusters, ENCODE Transcription Factor ChIP-seq, ENCODE Chromatin State Segmentation by Hidden Markov Model (HMM) from Broad Institute (bright red, active promoter; light red, weak promoter; purple, inactive/poised promoter; orange, strong enhancer; yellow, weak/poised enhancer; blue, insulator; dark green, transcriptional transition/elongation; light green, weak transcribed; gray, polycomb-repressed; light gray, heterochromatin/low signal/repetitive/copy number variation), Simple Nucleotide Polymorphisms (dbSNP build 130), Linkage Disequilibrium for the Yoruba (YRI) from Phased Genotypes, Linkage Disequilibrium for the CEPH (CEU) from Phased Genotypes, and LD for the Han Chinese + Japanese from Tokyo (JPT+CHB) from Phased Genotypes.

Interestingly, the rs6495309 T allele has a decreased susceptibility to COPD due to reduced promoter activity, which diminishes *CHRNA3* expression and the inflammatory response to smoking exposure (25). A previous study showed that the rs578776 A allele could reduce the risk of nicotine dependence and the risk of LC in Caucasians (42). Moreover, the SNP rs588765 was reported to be linked to changes in *CHRNA5* mRNA expression in lung tissue and to show a strong relationship with nicotine dependence (23, 43). In European, Asian, and African populations, four variants in the *CHRNA3* gene were shown to be uncorrelated or show weak LD with two mutations in the *CHRNA5* gene. According to the results, different causal mutants and functional mechanisms exist in the relationships between mutations in the *CHRNA3* and *CHRNA5* genes and LC susceptibility.

Moreover, this study showed that some SNPs had no association with LC risk. Briefly, our study analyzing the same SNP from different groups yielded inconsistent results due to the selection of association models, ethnicity, and variations in sample size. For the inconsistent results in different genetic models, the existence of different genetic backgrounds such as age and gender of patients, subtypes of cancers, and environmental factors were not taken into consideration and may present as sources of variation in the result. The minor allele frequency of SNP had differences among different races, and studies with smaller sample sizes had low statistical power, which may explain why these associations produced inconsistent results in different ethnicities.

While this study provides the largest sample and a comprehensive evaluation of variants related to the risk of cancerous and non-cancerous diseases, it contains several limitations. First, although comprehensive research was conducted on databases, some publications may have been missed, and certain papers may have lacked sufficient data, such as the genotype amount, resulting in an incomplete assessment of other malignancies and non-cancerous diseases. Second, sufficient data could not be provided for assessments of the interactions between different variants and the adjusted effect of environmental factors, including smoking and *H. pylori* infection. Third, a detailed subgroup analysis of cancer types ascribed to the heterogeneity of cancer typing among eligible studies was not completed. Fourth, the excess of significant findings was not alternatively evaluated due to insufficient data. Finally, some of the significant findings were identified with moderate credibility. Because this was partially due to the small sample of subgroups related to ethnicity under different genetic models, studies with sufficient subgroups are recommended to validate the current research's findings.

In this extensively updated meta-analysis, eight SNPs were proven to be significantly related to LC and COPD risk; of these, six variants were graded to show strong cumulative evidence for LC or COPD predisposition (*CHRNA3* rs1051730, *CHRNA3* rs6495309, and *CHRNA5* rs16969968 with COPD risk and *CHRNA3* rs1051730, *CHRNA3* rs578776, *CHRNA3* rs6495309, *CHRNA3* rs938682, *CHRNA5* rs16969968, and *CHRNA5* rs588765 with LC risk), and five SNPs were graded to show moderate cumulative evidence for LC or COPD risk. This study also provides a basis for further understanding of the genetic predisposition of LC and COPD susceptibility. Our findings could inspire further studies to elucidate the cause of LC and COPD and may lead to the development of screening and prevention strategies for clinical management.

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.

Author contributions

LY and HC designed this work. LY and XL integrated and analyzed the data. LY and HC wrote this manuscript. LY, CZ, XL, TL, ZY and CJ finished the related tables and figures. LY and HC edited and revised the manuscript. All authors contributed to the article and approved the submitted version.

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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.

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Supplementary material

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Clinical significance of MMP-9 overexpression in endometrial cancer: A PRISMA-compliant meta-analysis

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Objective: Several studies have found that MMP-9, one of the extracellular matrix-degrading proteinases, was involved in EC's (endometrial cancer) clinical progression and prognosis. However, the results involving the associations of MMP-9 expression with risk, clinical features and prognosis of EC were conflicting. Therefore, we performed a systematic review and meta-analysis to clarify the correlation of MMP-9 expression with EC.

Methods: Relative studies involving the associations between MMP-9 expression and EC were retrieved from PubMed, Embase, Web of Science and CNKI (China National Knowledge Infrastructure) electronic databases. OR (odds ratio) with 95% CI (confidence interval) was applied to evaluate the associations of MMP-9 expression with risk and clinical features of EC. Furthermore, we evaluated the role of MMP-9 expression in prognosis of EC using HR and 95% CI. The funnel plots and Begg test were used to assess the publication bias.

Results: A total of 28 eligible studies were acquired from Pubmed, Embase, Web of science and CNKI databases. We found MMP-9 overexpression was significantly associated with the risk of EC (OR = 11.02, 95% CI = 7.51-16.16, $P < 0.05$). In the meantime, MMP-9 overexpression was significantly associated with the tumor grade, FIGO stage, lymph node metastasis and myometrial invasion (Tumor grade: OR = 1.68, 95% CI = 1.09-2.58, $P < 0.05$; FIGO stage: OR = 3.25, 95% CI = 1.73-6.08, $P < 0.05$; Lymph node metastasis: OR = 2.98, 95% CI = 1.27-7.03, $P < 0.05$; Myometrial invasion: OR = 2.42, 95% CI = 1.42-4.12, $P < 0.05$) in Asians. In addition, the overall results showed that MMP-9 overexpression predicted a worse prognosis of EC (OR = 1.82, 95% CI = 1.01-2.62, $P < 0.05$).

Conclusions: MMP-9 overexpression might be a potential predictor of poor clinical progression and prognosis of EC.

KEYWORDS

MMP-9, endometrial cancer, clinical progression, prognosis, meta-analysis

Introduction

EC is the most common gynecologic malignancy in developed countries, while its incidence and mortality are rising (1). Most of the patients were diagnosed at 70 years or older (2). In the past several years, estrogen therapy, tamoxifen therapy, and surgical treatment significantly improved the survival rates of EC patients. However, 42,000 women still died of EC (3). Early menstruating, late menopause, infertility, polycystic ovary syndrome, increased age, hypertension and diabetes increased the risk of EC. It has been reported that obesity and conditions associated with metabolic syndrome were significantly linked with the development of EC. Obesity rates continued to rise in developed countries, which might aggravate the occurrence of EC (4). In addition, racial disparity in death rates of EC patients was found in genetic studies (4). These studies suggested that many risk factors increased EC mortality. Although the 5-year survival of EC patients with early stage was estimated to be 90%, those patients with advanced stage had a worse prognosis of EC (5). Therefore, identification of novel and reliable markers for the diagnosis, prediction for clinical progression and prognosis of EC were urgently needed.

Endometrial carcinoma could invade the basement membrane and myometrium through gelatinase, penetrating the lymphatic vascular lumen and spreading (6). MMP-9 gene was located at chromosome 20q13.12 which encoded Gelatinase B. Gelatinase B could degrade gelatin, collagen and elastin through proteolytic cleavage to regulate extracellular matrix (ECM) remodeling (7). Furthermore, Gelatinase B could directly cleave polypeptides after MMP-9 was secreted into the extracellular space (8). Therefore, MMP-9 was involved in many biological processes such as proteolytic degradation of ECM, cleavage of cell surface proteins and alteration of cell-cell or cell-ECM interactions (9). Published studies showed that MMP-9 significantly affected tumor invasion, metastasis, angiogenesis and tumor microenvironment (9–37). Therefore, MMP-9 might be a potential biological target for prediction and treatment of EC. However, the expression of MMP-9 in EC patients at different stages was still controversial in published studies (10–37). Therefore, the meta-analysis carried out a quantitative analysis to explore whether the high MMP-9 expression predicted EC's risk, clinical progression and prognosis.

Methods

Search strategy

The meta-analysis was conducted according to the PRISMA 2015 statement (38). The search strategy of "("Matrix Metalloproteinase 9"[Mesh]) AND "Endometrial Neoplasms"[Mesh]" were used to searched all studies involving the associations of MMP-9 expression with risk, clinical outcome and prognosis of EC from PubMed, Embase, Web of Science, and CNKI databases until April 2022. The following search terms were also used: "MMP-9", "matrix metallopeptidase 9", "prognosis", "survival", "neoplasms", "EC", "endometrial carcinoma" and "carcinoma of endometrium". In addition, references in the eligible literature were reviewed to obtain the relevant articles.

Study selection criteria

The literature's inclusion and exclusion criteria were established to select and eliminate retrieved literature. All included articles should meet the inclusion criteria: 1) Studies evaluating the role of MMP-9 in the risk, clinical progression, and prognosis of EC; 2) Articles providing enough data to calculate the ORs and 95% CI; 3) Literature containing HRs with 95% CI or survival curve about the prognosis of EC. 4) Studies that the detection method of MMP-9 protein expression was IHC. Studies were excluded if they met the following exclusion criteria: 1) studies with insufficient data for calculating the OR, HR and 95% CI; 2) publications with duplicate data; 3) studies carried out in cells or animals. Two authors independently identified the eligible studies according to the inclusion and exclusion criteria.

Data extraction

Two reviewers independently extracted relevant data from the included studies. The following information was extracted: first author's name, year of publication, country, ethnicity, disease type, time of follow-up, the detection method of MMP-9 protein expression, cut-off values of MMP-9 protein expression and HRs with 95% CI about overall survival time of EC. If studies only provided a survival curve about the overall survival time of EC patients, we used Engauge Digitizer 4.1 software (http://digitizer.sourceforge.net/) to extract the HRs and 95% CI (39). The quality of included studies was assessed with the Newcastle Ottawa Scale (NOS) table (40). The scores of eligible studies were from 0 to 9, while 7 to 9 scores were considered as high-quality.

Statistical analysis

Chi-squared test and I^2 statistic were applied to assess the heterogeneity among studies, and $I^2 > 50$ or P-value < 0.05 presented significant heterogeneity (41, 42). The random-effects model was adopted if significant heterogeneity existed, and the fixed-effects model was used when heterogeneity was not found (43). We drew the funnel plots by conducting the Begg's test to evaluate the publication bias (44). Sensitivity analysis was performed to test the stability of the pooled results. In

addition, subgroup analysis was used to examine the source of heterogeneity among the included studies. All statistical analysis of the present study were performed with STATA 12.0 software (Stata Corp., College Station, TX, USA). All statistical tests were two-sided, and a P-value < 0.05 was considered statistical significance.

Results

Study inclusion and characteristics

The initial search based on the inclusion and exclusion criteria identified 190 articles from electronic databases. Among them, 70 reports were duplicated and therefore were removed. Moreover, three articles were review types, so they were excluded. After titles and abstracts were read, 23 studies were excluded since they were unrelated to the associations of MMP-9 expression and risk, clinical features or prognosis of EC. In addition, 13 articles were excluded because of insufficient data. Finally, 28 eligible studies were included in the meta-analysis (10–37) (Figure 1 and Table 1). In the included studies, 8 reports were carried out in Caucasians and 20 articles were performed in Asians. In addition, the information of included studies for the analysis of associations between MMP-9 expression and EC clinical features was presented in Supplementary Table 1.

Meta-analysis results

The pooled results revealed that there was a significant association between MMP-9 overexpression and risk of EC (OR = 11.02, 95% CI = 7.51 – 16.16, P < 0.05). Small heterogeneity was observed (I^2 = 50.5, P = 0.006), and a random-effects model was applied. Moreover, subgroup analysis based on ethnicity or cut-off values was performed. The results showed that MMP-9 expression was significantly correlated with risk of EC in Asians (OR = 10.55, 95% CI = 7.27 – 15.30, P < 0.05). In the meantime, the subgroup analysis based on cut-off values indicated that high expression of MMP-9 was still an increased risk for EC (cut-off value: 0%, OR = 11.62, 95% CI = 5.28 – 25.60, P < 0.05; 5%, OR = 8.32, 95% CI = 4.91 – 14.08, P < 0.05; 10%, OR = 9.28, 95% CI = 5.27 – 16.36, P < 0.05). Moreover, heterogeneity among the included studies significantly decreased in the subgroup analysis. Thus, ethnicity and cut-off values of MMP-9 expression might contribute to the heterogeneity (Figure 2 and Table 2).

Then we performed a meta-analysis to explore the role of MMP-9 overexpression in the clinical characteristics of EC. The

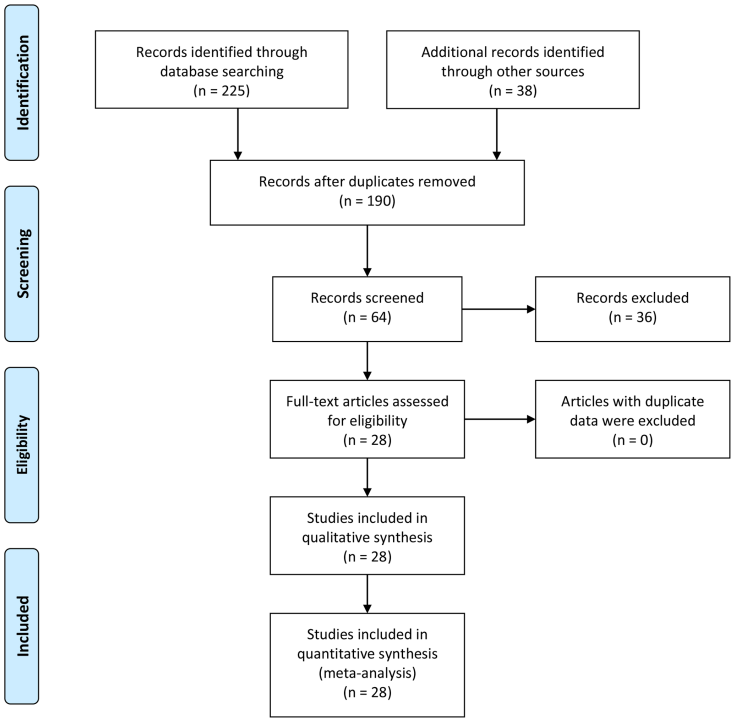

FIGURE 1
Flow chart of eligible studies selection process.

TABLE 1 Study characteristics of the included studies for the risk and prognosis of EC.

Author	Reference	Time	Country	Ethnicity	Method	Histology	Number of NT	Number of CT	NT		CT		NOS	Cut-off
									MMP9-	MMP9+	MMP9-	MMP9+		
Ma	17	2020	China	Asians	IHC	EC	18	49	15	3	14	35	7	5%
Assaf	18	2018	Egypt	Mixed	IHC	EC	10	25	10	0	2	23	7	None
Gan	19	2018	China	Asians	IHC	EC	100	100	96	4	32	68	7	0%
Miao	20	2017	China	Asians	IHC	EC	60	60	49	11	13	47	8	30%
He	21	2016	China	Asians	IHC	EC	20	39	12	8	11	28	8	5%
Wu	22	2016	China	Asians	IHC	EC	45	60	37	8	14	46	7	25%
Wang	23	2015	China	Asians	IHC	EC	40	70	24	16	25	45	7	0%
Zhang	24	2013	China	Asians	IHC	EC	15	56	12	3	22	34	7	5%
Zhang	25	2013	China	Asians	IHC	EC	10	37	8	2	9	28	7	10%
Gao	26	2013	China	Asians	IHC	EC	27	73	20	7	12	61	7	0%
Yu	27	2012	China	Asians	IHC	EC	30	128	25	5	74	54	7	0%
Wang	28	2012	China	Asians	IHC	EC	20	43	17	3	10	33	7	5%
Liu	29	2012	China	Asians	IHC	EC	26	42	19	7	13	29	7	5%
Lu	30	2011	China	Asians	IHC	EC	18	60	15	3	13	47	7	5%
Wu	31	2011	China	Asians	IHC	EC	27	73	20	7	12	61	7	0%
Meng	32	2010	China	Asians	IHC	EC	80	180	57	23	39	141	7	10%
Gao	33	2009	China	Asians	IHC	EC	22	74	20	2	24	50	7	0%
Zhang	34	2008	China	Asians	IHC	EC	10	70	8	2	16	54	7	0%
Zhang	35	2006	China	Asians	IHC	EC	12	40	6	6	2	38	7	0%
									Num.	Survival analysis	Source of HR	HR	95% CI	
Yu	27	2012	China	Asians	IHC	EC	108	1.68	108	OS	Survival cure	1.68	0.77-3.71	0%
Aglund	41	2004	Finland	Caucasians	IHC	EC	90	2.97	90	OS	Survival cure	2.97	1.01-8.70	50%
Liu	37	2014	China	Asians	IHC	EC	120	1.8	120	OS	Survival cure	1.8	1.06-3.06	0%

IHC, immunohistochemistry; NOS, Newcastle Ottawa Scale; OS, overall survival; Num, number; HR, hazard ratio; EC, endometrial cancer; NT, normal tissue; CT, cancer tissue; EC, endometrial cancer.

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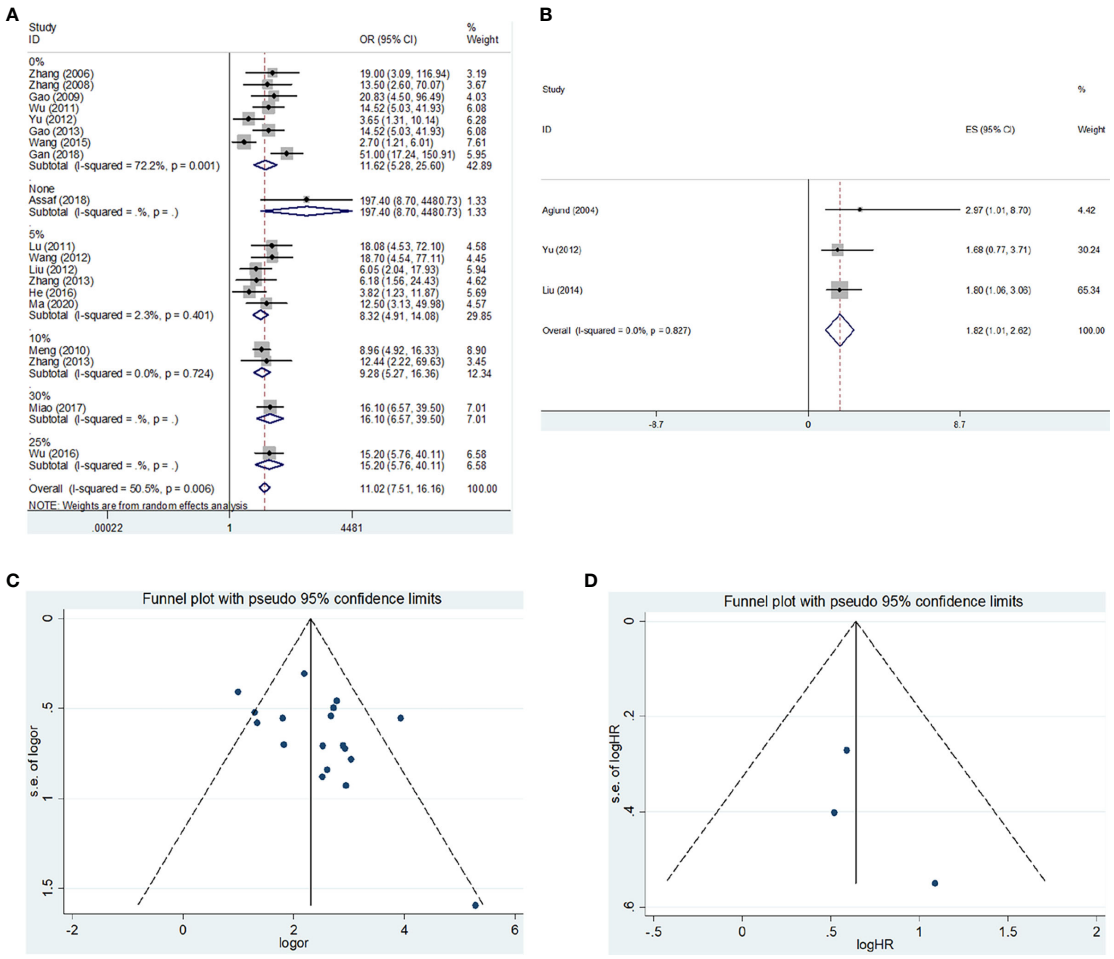

FIGURE 2
The forest plot and funnel plots for the correlations of MMP-9 overexpression with risk and prognosis of EC. **(A)** Forest plot for the risk of EC. **(B)** Forest plot for the overall survival of EC. **(C)** Funnel plot for the risk of EC. **(D)** Funnel plot for the overall survival of EC. OR, odds ratio; HR, hazard ratio; EC, endometrial cancer.

results indicated that patients with high G2-G3 grade had higher MMP-9 expression than that with G1 grade in Asians (OR = 1.68, 95% CI = 1.09 – 2.58, $P < 0.05$). And, high MMP-9 expression might represent the III-IV FIGO stage in Asians (OR = 3.25, 95% CI = 1.73 – 6.08, $P < 0.05$). In addition, high MMP-9 expression was significantly associated with lymph node metastasis (OR = 2.98, 95% CI = 1.27 – 7.03, $P < 0.05$), myometrial invasion (OR = 2.42, 95% CI = 1.42 – 4.12, $P < 0.05$) and vascular invasion (OR = 2.67, 95% CI = 1.27 – 5.60, $P < 0.05$) of EC in Asians. Some heterogeneities were found in the analysis of the associations of MMP-9 expression with lymph node metastasis, myometrial invasion and vascular invasion of EC, so the random-effects model was used. Moreover, the subgroup analysis based on the cut-off values significantly reduced the heterogeneity among studies and significant associations were still found (Figures 3, 4 and Tables 2, 3). Therefore, the pooled results were convincing. According to the results of the present meta-analysis, some studies have suggested that MMP-9 expression was associated with the development of EC, while others have obtained opposite results which can be seen in Figure 3.

The HR and 95% CI values were extracted from included studies and combined to evaluate the correlation between MMP-9 expression and overall survival of EC. The outcome showed that higher MMP-9 expression represented a worse overall survival of EC (OR = 1.82, 95% CI = 1.01 – 2.62, $P < 0.05$). No significant heterogeneity was found in the meta-analysis for the overall survival of EC ($I^2 = 0$, $P = 0.827$). In the three included studies for the overall survival of EC, two studies believed MMP-9 expression affected the survival of EC, while negative result obtained in the study of Yu et al. (Figure 2 and Table 2).

TABLE 2 Meta-analysis of the correlations of MMP-9 expression with risk, clinical features and prognosis of EC.

Characteristics	Studies	Forest plot analysis		Heterogeneity analysis		Begg test
		OR (95% CI)	*P*	I^2 (%)	*P*	*P*
Risk (Normal vs EC)	19	11.02 (7.51-16.16)	< 0.05	50.50	0.006	0.234
Caucasian	1	197.0 (8.70-4480.73)	< 0.05	–	–	–
Asian	18	10.55 (7.27-15.30)	< 0.05	48.10	0.012	0.544
Tumor grade (G1 vs G2+G3)	21	1.55 (1.12-2.15)	< 0.05	42.90	0.020	0.027
Caucasian	4	1.33 (0.88-2.01)	> 0.05	0.00	0.911	0.497
Asian	17	1.68 (1.09-2.58)	< 0.05	53.60	0.005	0.127
Tumor stage (I vs II+III)	14	2.30 (1.35-3.92)	< 0.05	40.90	0.055	0.033
Caucasian	4	1.03 (0.61-1.74)	> 0.05	0.00	0.485	0.042
Asian	10	3.25 (1.73-6.08)	< 0.05	24.10	0.222	0.089
Lymph node metastasis (No vs Yes)	19	2.66 (1.20-5.90)	< 0.05	77.60	0.000	0.726
Caucasian	2	0.88 (0.13-5.86)	> 0.05	35.10	0.214	–
Asian	17	2.98 (1.27-7.03)	< 0.05	79.10	0.000	0.711
Myometrial invasion (< 1/2 vs > 1/2)	14	2.20 (1.36-3.57)	< 0.05	55.00	0.007	0.298
Caucasian	2	1.19 (0.40-3.56)	> 0.05	17.50	0.271	–
Asian	12	2.42 (1.42-4.12)	< 0.05	57.50	0.007	0.217
Vascular invasion (No vs Yes)	4	1.76 (0.61-5.07)	> 0.05	56.70	0.074	1.000
Caucasian	2	1.05 (0.09-11.79)	> 0.05	58.90	0.119	–
Asian	2	2.67 (1.27-5.60)	< 0.05	0.00	0.617	–
Menopausal status (Premenopause vs Postmenopause)	4	1.14 (0.77-1.68)	> 0.05	45.80	0.137	0.497
Survival	3	1.82 (1.01-2.62)	< 0.05	0.00	0.827	0.602

OR, odds ratio; CI, confidence interval; EC, endometrial cancer; EC, endometrial cancer.

Publication bias and sensitivity analysis

No significant publication bias was found in the meta-analysis of the present study. In the meantime, the results of sensitivity analysis suggested that the overall results were stable (Figure 2 and Tables 2, 3).

Discussion

It has been documented that MMP-9 protein plays a pivotal part in various diseases. For instance, MMP-9 could degrade components of the extracellular matrix and numerous nonmatrix proteins in fibrosis disease. Surprisingly, although MMP-9 levels were increased in the alveolar lavage fluid of idiopathic pulmonary fibrosis patients, MMP-9 promoted abnormal epithelial cell migration and lung tissue repair (45). In the lung fibrosis model of MMP-$9^{-/-}$ mice, deficiency of MMP-9 protected mice from alveolar bronchiolization (46). On the contrary, some studies have shown that MMP-9 overexpression in liver tissue was a risk factor for advanced T category, tumor stage and poor outcome (47, 48). Therefore, MMP-9 might have different roles in diverse diseases. Previous studies have reported overexpression of MMP-9 was associated with the clinical progression of EC (10, 13, 29, 32, 37, 49). However, other studies found no significant associations between MMP-9 overexpression and the clinical stage of EC (50, 51). Furthermore, many inconsistent results involving the associations between MMP-9 expression and clinical features of EC were reported (10–37). Therefore, the role of MMP-9 overexpression in EC needs to be studied urgently. In fact, functional studies have showed that MMP-9 was expressed in proliferative phase endometrium, hyperplastic endometrium and EC (52, 53). In the peritoneal endometriotic lesions, positive cells (59.1%) were more than colorectal endometriosis (44.4%). Nevertheless, EC patients had the highest levels of MMP-9 expression (54). Therefore, the expression level of MMP-9 might increase with the development of endometrial disease.

This was the first meta-analysis assessing the associations of MMP-9 overexpression with EC risk, clinicopathological features and prognosis. The results indicated the significant associations between MMP-9 expression and EC risk. However, only one included study was performed on Caucasians for the analysis of risk. So, the overall results for the risk of EC might be more applicable to Asian population. Furthermore, stratified analysis based on the cut-off values of MMP-9 expression significantly decreased the heterogeneity among studies and a significant correlation for risk was still found in the stratified analysis, which showed that the results of the meta-analysis were convinced. It's worth noting that MMP-9 expressed highly in EC patients with G2-G3 grade, III-IV FIGO

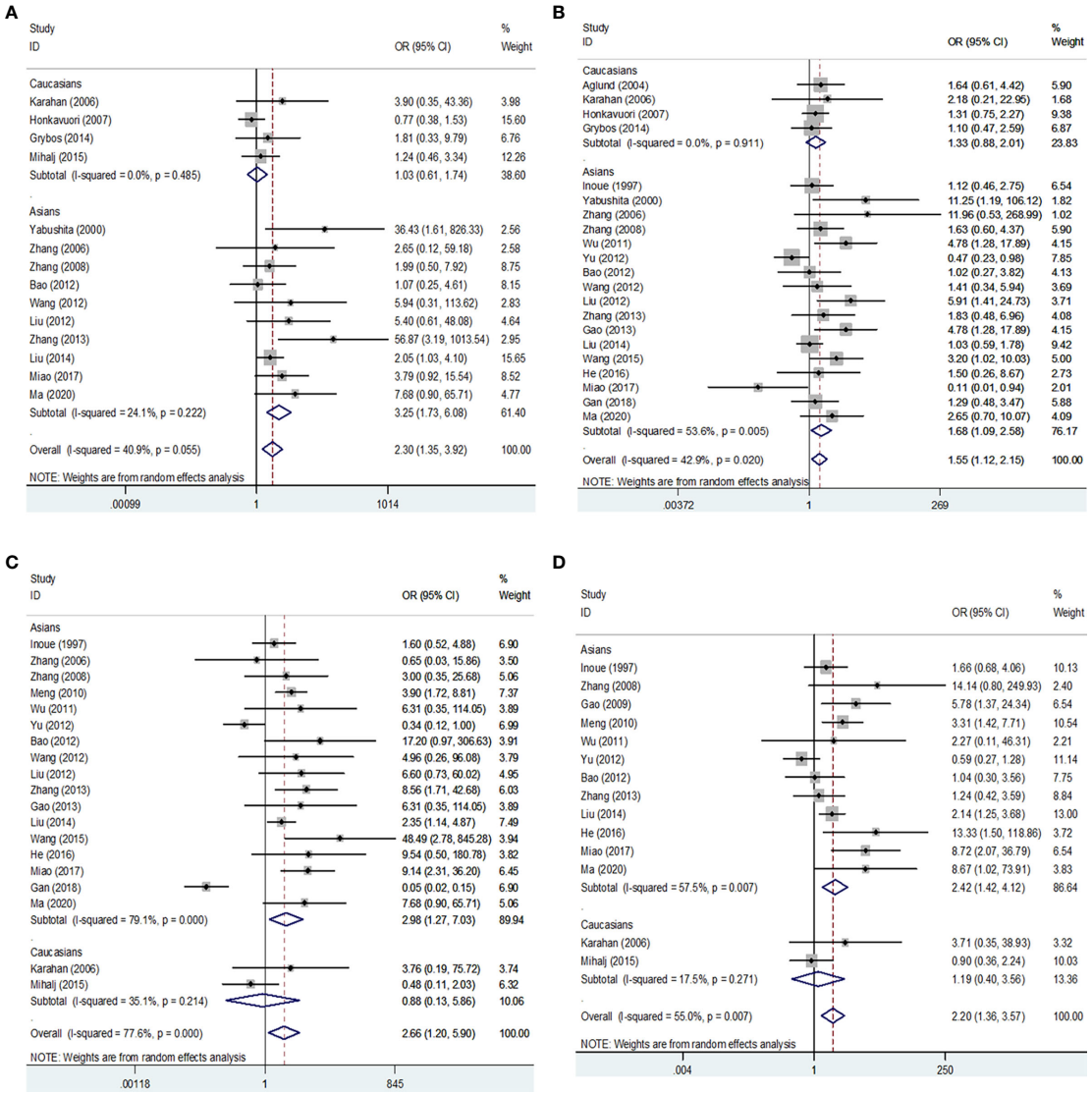

FIGURE 3
Forest plots for the associations of MMP-9 overexpression with clinical features of EC in Asians and Caucasians. **(A)** Forest plot for FIGO stage of EC. **(B)** Forest plot for endometrial tumor grade. **(C)** Forest plot for lymph node metastasis of EC. **(D)** Forest plot for myometrial invasion of EC. OR, odds ratio; EC, endometrial cancer.

stage, lymph node metastasis, myometrial invasion, vascular invasion or postmenopausal, indicating that high MMP-9 expression promoted clinical progress of EC. According to our results, ethnicity might be an important influencing factor because MMP-9 overexpression mainly promoted the clinical progression of EC in Asians. One meta-analysis has also found MMP-9 expression of bladder cancer tissue presented significant race diversity (55). Therefore, MMP-9 expression might have a potential association with ethnicity. In the meantime, stratified analysis based on the cut-off values of MMP-9 expression also significantly reduced the heterogeneity among studies for clinical features of EC, which hinted different cut-off values of MMP-9 expression were used in the included studies of the meta-analysis. Besides, the overexpression of MMP-9 was significantly associated with a worse prognosis for EC, which demonstrated MMP-9 overexpression reduced the survival time of EC patients. Therefore, the expression level of MMP-9 should be reduced to improve the prognosis in the clinical treatment of EC patients. Moreover, no significant heterogeneity among included studies for the association of EC survival with MMP-9 overexpression was found, which indicated that the pooled result was convincing. Sensitivity analysis revealed that no individual study significantly affected the overall results which showed that the pooled results were stable.

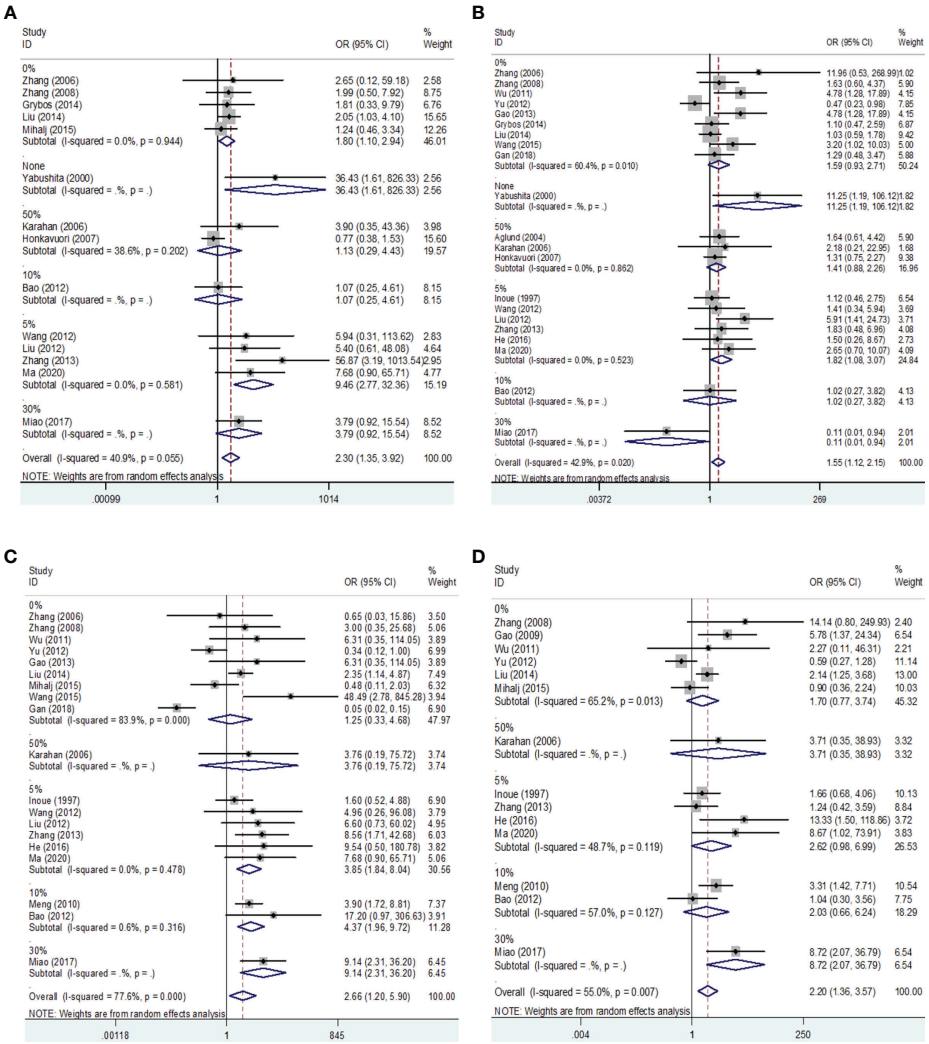

FIGURE 4
Forest plots for the associations of MMP-9 overexpression with clinical features of EC in different cut-off values of IHC. **(A)** Forest plot for FIGO stage of EC. **(B)** Forest plot for endometrial tumor grade. **(C)** Forest plot for lymph node metastasis of EC. **(D)** Forest plot for myometrial invasion of EC. IHC, immunocytochemistry. OR, odds ratio; EC, endometrial cancer.

TABLE 3 The subgroup analysis based on cut-off values of MMP-9 expression for the risk and clinical features of EC.

Characteristics	Studies	Forest plot analysis		Heterogeneity analysis		Begg's test
		OR (95% CI)	*P*	I^2 (%)	*P*	*P*
Risk (Normal vs EC)						
0% (Cut-off value)	8	11.62 (5.28-25.60)	< 0.05	72.200	0.001	0.266
5% (Cut-off value)	6	8.32 (4.91-14.08)	< 0.05	2.300	0.401	0.039
10% (Cut-off value)	2	9.28 (5.27-16.36)	< 0.05	0.000	0.724	–
Tumor grade (G1 vs G2+G3)						
0% (Cut-off value)	9	1.59 (0.93-2.71)	> 0.05	60.400	1.010	0.009

(Continued)

TABLE 3 Continued

Characteristics	Studies	Forest plot analysis		Heterogeneity analysis		Begg's test
		OR (95% CI)	*P*	I^2 (%)	*P*	*P*
5% (Cut-off value)	6	1.82 (1.08-3.07)	< 0.05	0.000	0.523	0.851
50% (Cut-off value)	3	1.41 (0.88-2.26)	> 0.05	0.000	0.862	
Tumor stage (I vs II+III)						
0% (Cut-off value)	5	1.80 (1.10-2.94)	< 0.05	0.000	0.944	1.000
5% (Cut-off value)	4	9.46 (2.77-32.36)	< 0.05	0.000	0.581	1.000
50% (Cut-off value)	2	1.13 (0.29-4.43)	> 0.05	38.600	0.202	–
Lymph node metastasis (No vs Yes)						
0% (Cut-off value)	9	1.25 (0.33-4.68)	> 0.05	83.900	0.000	0.602
5% (Cut-off value)	6	3.85 (1.84-8.04)	< 0.05	0.000	0.478	0.573
10% (Cut-off value)	2	4.37 (1.96-9.72)	< 0.05	0.600	0.316	–
Myometrial invasion (< 1/2 vs > 1/2)						
0% (Cut-off value)	6	1.70 (0.77-3.74)	> 0.05	65.200	0.013	0.851
5% (Cut-off value)	4	2.62 (0.98-6.99)	> 0.05	48.700	0.119	0.174
10% (Cut-off value)	2	2.03 (0.66-6.24)	> 0.05	57.000	0.127	–
Vascular invasion (No vs Yes)						
0% (Cut-off value)	1	0.43 (0.12-1.49)	> 0.05	–	–	–
5% (Cut-off value)	2	2.67 (1.27-5.60)	< 0.05	0.000	0.617	–
50% (Cut-off value)	1	5.33 (0.27-106.24)	> 0.05	–	–	–
Menopausal status (Premenopause vs Postmenopause)						
0% (Cut-off value)	2	1.02 (0.66-1.55)	> 0.05	35.800	0.212	–
5% (Cut-off value)	1	1.01 (0.25-4.17)	> 0.05	–	–	–
30% (Cut-off value)	1	4.18 (1.11-15.79)	< 0.05	–	–	–

OR, odds ratio; CI, confidence interval; EC, endometrial cancer; cut-off: cut-off values of MMP-9 protein detection with immunohistochemistry; EC, endometrial cancer.

Several limitations of the present study should be pointed out. First, the number of included studies conducted on Caucasians was too small for the meta-analysis of EC risk. Second, cut-off values of MMP-9 expression were evaluated dependent on included studies for the associations of EC clinical features with MMP-9 overexpression, which significantly affected the heterogeneity among the eligible studies. Third, other factors might be involved in the heterogeneity among the included studies. However, these factors were not quantified or provided in the eligible studies. Thus, more multi-center studies with more clinical information were warranted to verify the role of MMP-9 overexpression in EC.

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.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.925424/full#supplementary-material

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Functional properties of circular RNAs and research progress in gastric cancer

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Circular RNAs (circRNAs) are a class of closed circular non-coding RNAs widely exist in eukaryotes, with high stability and species conservation. A large number of studies have shown that circRNAs are abnormally expressed in various tumor tissues, and are abundant in plasma with long half-life and high specificity, which may be served as potential tumor biomarkers for early diagnosis, treatment and prognosis of malignant tumors. However, the role of circRNAs is still poorly understood in gastric cancer. This article reviews the research progress of circRNAs in gastric cancer in recent years so as to explore the relationship between circRNAs and the occurrence and the development of gastric cancer, and provide new ideas for the diagnosis and treatment of gastric cancer.

KEYWORDS

Circular RNA, gastric cancer, marker, therapeutic target, molecular mechanisms

The formation and regulatory mechanism of circRNAs

Formation of circRNA

CircRNAs are mainly divided into four categories according to their sources: exonic circRNAs (ecircRNAs) (1), intronic circRNAs (ciRNAs) (2), exon-intron circular RNAs (EIciRNA) (3) and intergenic circRNA (4). The biosynthesis of circRNA is different from the traditional canonical splicing mode of linear mRNA, but is formed by back splicing (5). Although the efficiency of circRNA reverse splicing is much lower than that of linear RNA, circRNA maintains high abundance in various species on accout of its high stability and long half-life. At present, the two formation mechanisms studied thoroughly are exon cyclization mechanism and intron cyclization mechanism.

Exonic circRNAs can be formed by reverse splicing of a single exon or multiple exons. The specific splicing methods are mainly divided into the following two models: the lasso-driven circularization model (6) and the intron pairing-driven circular model (7). The efficiency of the lasso-driven circularization model to form circRNAs is significantly higher than the intron pairing-driven circularization model, and it is a more common form of splicing in organisms (8).

Most exon-derived circRNAs are mainly formed through a lasso-driven circularization model. The mRNA precursor (pre-mRNA) is partially folded during transcription, bringing the distance between originally non-adjacent exons closer, resulting in exon skipping, thereby the crossed region forms the circRNA intermediates, and then forms the so-called exon circRNA through splicing.

During primary transcription, the intron regions on both sides of the primary mRNA(pri-mRNA) transcription product are complementary to each other due to the presence of reverse complementary sequences, such as ALU repeat elements, resulting in complementary pairing of introns and mediating the reverse splicing of exons and folding them together, and then partially cutting off introns to form some exonic circular RNAs.

The formation of intronic circRNAs is formed by the splicing of introns, and can be divided into group I introns, group II introns, and nuclear pre-mRNA introns (spliceosomal intron) according to different splicing methods. Most commonly, intron splicing during exonic circRNA formation is mediated by nuclear pre-mRNA introns, whereas intron circRNA formation is associated with class I introns and class II introns (9).

CircRNAs formed by intron cyclization can be produced in two ways: one is that an exogenous guanine nucleotide attacks the 5' splice site, excises the 5' exon and connects to the intron, and then the 3' hydroxyl end of the excised 5' exon attacks the 3' splice site, releases the linear intron and joins the exons, and finally, the linear intron releases the 3' tail to form a 2'-5' junction intronic circular RNAs. The other is to first release the 3' exon, and then the 2' hydroxyl end of the intron attacks the 5' splice site to generate circRNA.

An exogenous guanine nucleotide is used as a nucleophile to attack the 5 ' end splicing site, the exon at the 5' end is excised by transesterification, and the exogenous guanine and intron are connected to each other, while the 3' hydroxyl (-OH) above the cleaved 5' terminal exon attacks the 3' terminal splice site, resulting in the release of the linear intron by excision, the exons are connected to each other. Then the released linear intron removes its own 3'-terminal tail, and the remaining 2'-OH and 5'-terminal splice sites are connected to form a phosphodiester bond to generate an intronic circRNA (10, 11) (Figure 1).

The regulatory mechanism of circRNA

Mechanistic studies have shown that the flanking intron regions of circRNA circularized splice junctions usually contain reverse complementary sequences of varying lengths, including a repeat sequence of about 30-40 nucleotides that exists in primate genomes, called ALU element, and this structure can

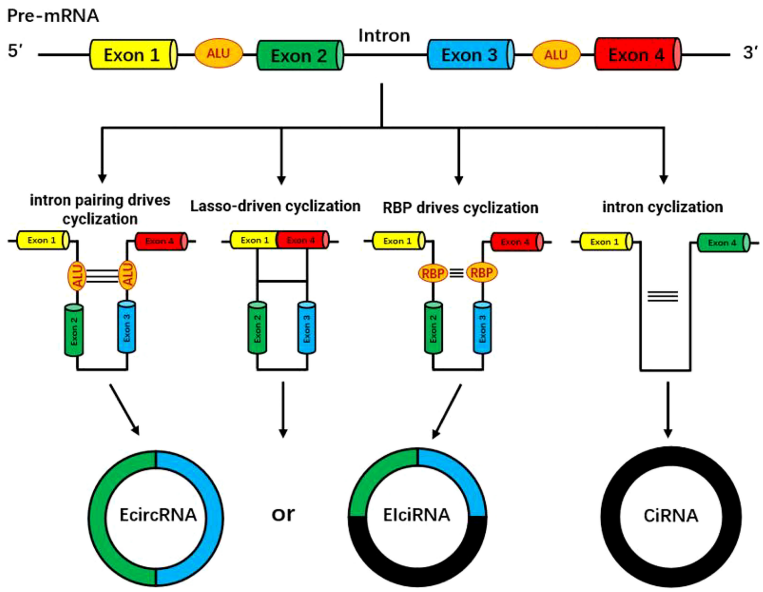

FIGURE 1
The formation and regulatory mechanism of circRNA.

significantly promote the formation of circRNA (12). In addition to the above-mentioned Alu elements, it is reported that the complementary sequences within certain exons and their flanking introns can promote RNA reverse splicing to form circRNAs through base pairing (13).

The formation of circRNAs may also be affected by RNA-binding proteins (RBPs). On the one hand, RBPs can promote the formation of circRNAs by binding to target sites in the flanking intronic regions of pre-mRNA. For example, the splicing factor Quaking promotes circRNA formation by binding to targets upstream and downstream of circularized flanking intron regions on SMARCA5 pre-mRNA (14). The immune factors NF90 and/or NF110 promote circRNA production by binding to the inverted repeat Alus (IRAlus) of pre-mRNA (15). On the other hand, RBPs can also inhibit the formation of circRNAs by affecting the RNA pairing process. For example, the adenosine deaminase ADAR1 affects RNA pairing that A-to-I editing circularized exon flanks, reducing the complementarity and stability of the RNA pairing and inhibiting circRNA formation (16). The RNA helicase DHX9 can bind to IRAlus and inhibit circRNA formation by unraveling the paired RNAs flanking the circular exons (17).

In addition, circRNA formation occurs in synergistic transcription and post-transcriptional coupling with Pol II transcription (18, 19). A study on Pol II transcription elongation (TER) of genes that promote circRNA formation (20) found that the average TER of circRNA was higher than that of non-circRNA, and the change of TER had a significant effect on the formation of circRNA. They believed that this might because higher TER can allow the transcription of downstream intron complementary sequences (ICS), increasing the possibility of ICS cross-exon pairing, thereby increasing the possibility of reverse splicing circRNA formation. At the same time, higher TER-related linear splicing reduction may also promote the formation of circRNA. There are also quite a few circRNAs that are formed after transcription. A large number of nascent circRNAs are detected only after the transcription of their host pre-mRNAs has been completed (20). Further Mechanistic studies have shown that mRNA 3 ‘ -end processing is required for circRNA production (2), and inhibition of co-transcription 3 ‘ -end processing can increase circRNA formation (21), which can be attributed to increased transcription of Pol II upstream polyadenylation signals.

Biological functions of circRNAs

Interaction between circRNA and miRNA

Some circRNAs contain miRNA binding sites, and act as competing endogenous RNAs (ceRNAs) to negatively regulate miRNA activity by competing miRNA binding sites, thereby reducing the inhibitory effect of miRNAs on their downstream target genes. The most representative example is the antisense transcript of cerebellar degeneration-related protein-1 (CDR1as), also known as ciRS-7, which contains 74 selectively conserved miRNA-binding sites and acts as a molecular sponge for miR-7. Another circular RNA circSRY, exists specifically in mouse testis tissue, contains 16 binding sites for miR-138 (22, 23). Another study found that circHIPK3 could act as a molecular sponge for miR-124, upregulate the expression of miR-124 target genes IL6R and DXL2, and then promote the proliferation of liver cancer cells (24). But it is worth noting that the concentration of circRNA and corresponding miRNA should be at a similar level to facilitate effective competitive binding of miRNA, and only in this way, circRNAs can effectively bind miRNAs and inhibit their functions. However, many circRNAs in organisms are usually expressed in low abundance and lack multiple targets for the same miRNA molecule, so the function of miRNA sponge is limited (25) (Figure 2A).

Besides acting as miRNA sponges and specific inhibitors of target miRNAs, circRNAs can also act as miRNA reservoirs to stabilize or activate miRNA functions. For example, ciRS-7 can be considered as miR-7 reservoir. After miR-671 cleaves ciRS-7, miR-7 is released in large quantities and its activity increases rapidly, consequently resulting in the effective inhibition of miR-7 targets. At this time, ciRS-7 becomes a “miR-7 reservoir” and activates miR-7 function (26). ERβ represses the circular RNA circATP2B1, which acts as a reservoir of miR-204-3p by transcription. The decreased circATP2B1 cannot stabilize the expression of miR-204-3p, resulting in the decrease of miR-204-3p, which in turn increases its downstream target FN1 and enhanced the invasive ability of ccRCC cells (27). Other studies have shown that circ-HIAT1 can act as a reservoir for miR-195-5p, miR-29a-3p and miR-29c-3p to stabilize the expression of these miRNAs, thereby inhibiting the activity of the downstream target gene CDC42 (28).

circRNA interacts with RBP

RBP is a class of proteins widely involved in gene transcription and translation in organisms, and the interaction with RBP can be considered as an important part of the function of circRNA. RBPs function in splicing, processing, folding, stability and localization of circRNAs by interacting with the circularized splice junctions of circRNAs (29). For example, circ-Foxo3 can form a Foxo3-p21-CDK2 ternary complex by interacting with cyclin-dependent kinase 2 (CDK2) and p21, resulting in the inhibition of CDK2 function and the blockage of cell cycle progression, thereby regulating tumor development (30). In addition, circ-Foxo3 was also found to bind to two proteins, MDM2 and p53, promoting MDM2-induced p53 ubiquitination and subsequent degradation (31). Another

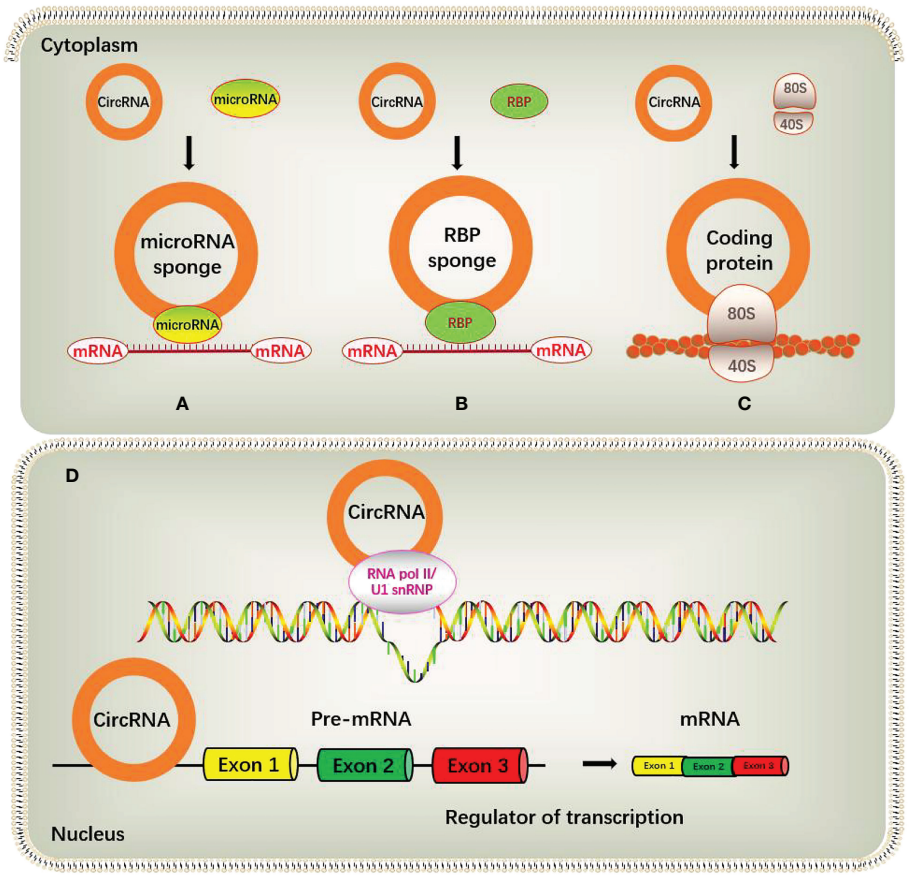

FIGURE 2
Biological functions of circRNAs.

study found that the circular RNA circPABPN1 inhibited the combine of HuR with linear PABPN1 mRNA by competitively interacting with HuR, thereby affecting the translation of its cognate transcript PABPN1 (32) (Figure 2B).

circRNAs can regulate gene transcription

Nuclear EIciRNAs and ciRNAs may be involved in the transcriptional regulation of genes. Studies shown that the transcription of the parental gene was inhibited after knockdown of EIciRNA. Mechanistic studies have shown that EIciRNAs such as circEIF3J and circPAIP2 form EIciRNA-U1 snRNP complexes by interacting with U1 small nuclear ribonucleoprotein (U1snRNP), and interacte with RNA polymerase II (Pol II) on the promoters of EIciRNA parent genes to promote transcription of the parental gene. In addition, when the above RNA-RNA interaction is blocked, the interaction between EIciRNA and Pol II is disrupted, resulting in a subsequent decrease in the transcription of the EIciRNA parental gene (3). Another study found that circSEP3, a circular RNA derived from exon 6 of SEPALLATA3 (SEP3) in Arabidopsis thaliana, could tightly bind to its cognate DNA locus to form a circRNA : DNA complex, which could cause transcriptional pause, and exon-skipping alternatively spliced SEP3 mRNA (33). Besides, abundant ciRNAs such as ci-ankrd52 in the nucleus may positively regulate Pol II transcription by extending the Pol II mechanism. When ci-ankrd52 is knocked down, the transcription of its parental gene is also reduced (2) (Figure 2D).

circRNAs can encode polypeptides

Although circRNAs have always been defined as non-coding RNAs, some circRNAs have internal ribosome entry site elements (IRES) (34) or prokaryotic ribosome binding sites (35), so these circRNAs are no longer non-coding RNA in the traditional sense, but a special circular RNA capable of encoding polypeptides. Given circRNAs lack caps and poly(A) tails, thus,

translation of circRNAs may occur in a cap-independent manner. At present, with the continuous development of circRNA research, many online databases can be referenced for researchers. Such as, the circRNADb database contains the specific information of 32,914 human exonic circRNAs, including the genome sequence, open reading frame (ORF) and IRES. This information can help us to assess whether circRNAs have coding potential (36). For example, circZNF609 can encode functional polypeptides and participate in regulating the proliferation of myoblasts during muscle differentiation (37). Another circular RNA, circMbl, derived from the muscleblind (Mbl) locus, encodes a protein that has been detected in fly head extracts by mass spectrometry (38).

Interaction between circRNA and mRNA

Mechanistic studies have shown that CircRNA can not only bind to mRNA, but also act as a regulator of mRNA translation and stability (39, 40). For example, CircIPO11, a regulator necessary for liver cancer stem cells (CSCs) to maintain self-renewal, can recruit the TOP1 to the GLI1 promoter to trigger its transcription, thereby activating the Hedgehog signaling to promote liver CSC self-renewal and HCC progression (40). circYAP is a circRNA that regulates the translation of mRNA. In the translation initiation complex, circYAP can specifically recognize and bind to YAP mRNA, eliminating the interaction between PABP on the poly (A) tail and eIF4G on the 5 ' -cap of Yap mRNA, resulting in the inhibition of Yap translation initiation (41). CircRNA can regulate mRNA stability. When circXPO1 binds to IGF2BP1 and enhances the stability of CTNNB1 mRNA, the inhibitory effect of CTNNB1 is enhanced, thereby accelerating the progression of lung tumors (42). Similarly, circARHGAP12 enhances the stability of EZR mRNA by binding to the 3'UTR of EZR mRNA, thereby promoting the progression of nasopharyngeal carcinoma (NPC) (39, 43).

In addition to the above mechanisms, circRNAs may also initiate the translation process through N6-methyladenosine (m6A) modification. CircRNAs are rich in m6A consensus motifs, and a single m6A site is sufficient to drive translation initiation. One study found that m6A-driven circRNA translation was widespread through ribosome sequencing analysis and mass spectrometry detection, and many endogenous circRNAs had translation potential (44) (Figure 2C).

The relationship between circRNA and gastric cancer

With the deepening of circRNA research in recent years, the relationship between circRNA and various human diseases has been discovered one after another, including difficult-to-treat tumors. Gastric Cancer (GC) is one of the most common malignant tumors of the digestive system in the world, with higher morbidity and mortality in our country (45). Although great progress has been made in the treatment of gastric cancer, the five-year overall survival rate of patients with gastric cancer is still low due to the high clinical heterogeneity and the variable progression of gastric cancer (46). Therefore, there is an urgent need to find effective biomarkers for early diagnosis, early treatment and prognostic of gastric cancer, so as to provide more timely and precise treatment options for gastric cancer patients. Recently, some studies have emerged on the abnormal expression and mechanism of circRNAs in gastric cancer, which may have important implications for the diagnosis, treatment and prognosis of gastric cancer.

Differential expressions of circRNAs in gastric cancer

The expression of some circRNAs is up-regulated in gastric cancer (Table 1). For example, a study detected by RT-PCR technology found that the expression level of circPVT1 was significantly higher than that in the corresponding adjacent normal tissue as a proliferation factor and prognostic marker in gastric cancer tissue (47). circHIPK3 is also found to be up-regulated in gastric cancer tissues and cells, and the expression level of circHIPK3 is significantly correlated with the TNM stage of gastric cancer patients (48). Similar results are as follows, the expression of circ_0006282 in gastric cancer tissues is significantly higher than that in its adjacent non-cancerous tissues, and the high expression of circ_0006282 was associated with tumor size, lymph node metastasis and TNM stage (49). The expression of hsa_circ_0010882 is significantly up-regulated in gastric cancer patient plasma and gastric cancer cell lines, and its expression level is significantly correlated with the tumor size and histological grade of the patients (50). The expression level of hsa_circ_0000467 in gastric cancer tissues is significantly higher than that in the corresponding adjacent tissues, and it is correlated with the histological grade of gastric cancer (51). A study quantitatively detected the expression of circRBM33 in 79 pairs of GC tissues and paracancerous tissues and 4 GC cell lines (MGC-803, BGC-823, SGC-7901 and AGS) by RT-PCR. It was significantly up-regulated in GC tissue specimens and cell lines, and the expression level of circRBM33 is observed to be closely related to the clinical characteristics of GC patients (52). In the analysis of the expression levels of circURI1 in GC and adjacent normal tissues, and found that circURI1 is generally and significantly up-regulated in GC compared with adjacent tissues, and the decreased expression level of circURI1 was associated with TNM stage (III-IV) and distant metastasis (53). CircNHSL1 is the most up-regulated circRNA in gastric cancer metastatic tissues through RNA-seq analysis in 3 gastric cancer tissues with metastasis and 2 gastric cancer tissues without metastasis, and the high expression of circNHSL1 is positively correlated with UICC stage, pathological

TABLE 1 Summary of some tumor-related CircRNAs.

CircRNA ID	Expression	Tumor size	Differentiation grade	T stage	TNM stage	Lymphatic metastasis	Distant metastasis	Drug resistance	Mechanisms
CircRNA ciRS-7	↑								CircRNA ciRS-7/MiR-7/PTEN/PI3K/AKT
Hsa_circ_0007507	↑			✓	✓	✓			Unknown
Hsa_circ_0110389	↑		✓	✓	✓	✓	✓		HsacircOl l0389/MiR-l27-5p or miR-l36-5p/SORT **l**
CircTMEM87A	↑	✓				✓			CircTMEM87A/MiR-l42-5p/ULKl
CircRNA UBE2Q2	↑	✓				✓			CircRNA UBE2Q2/MiR-370-3p/STAT3
CircDNA2	↑								CircDNA2/miR-l49-5p/CCDC6
CircLM07	↑			✓	✓				CircLMO7/MiR30a-3p/WNT2/|3-catenin
CircURIl	↑					✓	✓		CircUIl/hnRNPM
CircHIPK3	↑								CircHIPK3/miR-637/AKT **l**
Hsa_circ_0001020	↑						✓		Unknown
CircAG02	↑		✓		✓	✓			CircAG02/HURR
Circ-DONSON	↑				✓	✓			Circ-DONSON/NURF/SOX4
CircNRIPl	↑	✓				✓			CircNRIPl/MiRl49-5p/AKT l/mTOR
Circ-RanGAPl	↑	✓			✓	✓			Circ RanGAP1/MiR-877-3p/VEGFA
CircSHKBPl	↑	✓	✓		✓				CircSHKBP **l** *M* iR582-3p /HUR/VEGF/H SP90
CircNHSLl	↑		✓		✓	✓	✓		CircNHSLl/MiR-l3063p/SIXl/vimentin
CircAXINl	↑		✓		✓	✓			CircAXIN **l** /AXIN **l** -295aa/Wnt/|3-catenin
EBV-CircLMP2A	↑				✓	✓	✓		EBV-CircLMP2A/KHSRP/VHL/HIFla/VEGFA
Circ_SMAD4	↑								Circ_SMAD4/wnt/|3-catenin
CircHAS2	↑			✓	✓	✓			CircHAS2/MiR-944/PPM **I**E
CircRNA_l 00290	↑				✓	✓			CircRNA_l00290/MiR-29b-3p/lTGAl **l**
CircLMTK2	↑			✓	✓	✓			CircLMTK2/MiR-l505p/c-Myc
circ_0006282	↑	✓			✓	✓			Circ_0006282/MiR-l55/FBXO22
hsa_circ_0010882	↑	✓	✓						Hsa_circ_00l0882/PI3K/Akt/mTOR
circRBM33	↑								CircRBM33/M lR-149/1L-6
CircDLST	↑				✓			5-FU resistance	CircDLST/MiR-502-5p/NRAS/MEKl/ ERKl/2
CircRNA AKT 3	↑	✓	✓		✓			cisplatin resistance	CircRNA AKT3/MrR-l98/PIK3Rl
CircFAM73A	↑	✓			✓			cisplatin resistance	CircFAM73A/MiR-490-3p/HMGA2/hnrnpk/|3-cateniri
CircPVT 1	↑							cisplatin resistance	CircP V**I**1/M lR-152-3p/HDGF
Circ_ASAP2	↑							cisplatin resistance	Crrc_ASAP2/MiR-330-3p/NT5E
CircCPM	↑							5-FU resistance	Circular CPM/MiR-21-3p/PRKAA2

(Continued)

TABLE 1 Continued

CircRNA ID	Expression	Tumor size	Differentiation grade	T stage	TNM stage	Lymphatic metastasis	Distant metastasis	Drug resistance	Mechanisms
CircFNl	↑			✓	✓			cisplatin resistance	CrrcFNl/MiR-182-5p
CircOl 10805	↑							cisplatin resistance	Circ_0110805/MiR-299-3p/ ENDOPDI
Circ_0026359	↑							cisplatin resistance	Crrc_0026359/MiR-1200/ POLD4
Circ_0000260	↑							cisplatin resistance	Circ_0000260/M lR-129-5p MMP11
CircMCTP2	↓	✓		✓	✓			cisplatin resistance	CircMCTP2/MiR-99a-5p/ MTMR3
Circul2	↓	✓	✓		✓	✓		cisplatin resistance	Crrcul2/MiR142-3p/ROCK2
CircRNA YAP1	↓	✓			✓			5-FU sensitive	CircRNA YAPl/MiR-367-5p/ p27 Kip 1
Circ_0001017	↓							cisplatin resistance	Circ_0001017/MrR-543/ PHLPP2
Hsa_circ_0009172	↓		✓		✓				Hsa_circ_0009172/MiR-485-3p/NTRK3
CircDIDOl	↓	✓					✓		CrrcDIDO 1/DIDO1-529aa/ PRDX2
Circ0007360	↓								Crrc0007360/MiR-762/[RF7
CircGSK3B	↓	✓			✓	✓			CircGSK3B/EZH2/RORA
Circ-HuR	↓				✓	✓	✓		Crrc-HuR/CNBP/HuR
CircMRPS35	↓	✓			✓	✓			CircMRPS35/KAT7
CircCCDC9	↓	✓			✓	✓			CrrcCCDC9/MiR-67923p/ CAVl
CircFATl(e2)	↓				✓	✓	✓		CircFATl(e2)/MiR-548gYBXl
CircRNA ST3GAL6	↓	✓			✓				CircRNA ST3GAL6/FOXP2/ MET/mTOR
CircPTK2	↓					✓			CrrcPT K2/M rR-196a-3p /AAT K
CircMTOl	↓								CircM TOl/MiR-3200-5p /PEBP1
Hsa_crrc_0004872	↓	✓		✓		✓			Hsa_crrc_0004872/M rR-224/ Smad4/ADAR1
CircRNA_LARP4	↓								CircRNA_LARP4/MiR-424-5p/LAT S**I**
CircPSMC3	↓				✓	✓			CrrcPSM C3/M rR-296-5p
CircST3GAL6	↓	✓							CircST3GAL6/FOXP2/MET/ mTOR
CircRAB31	↓	✓			✓	✓			CircRAB31/MiR-885-5p/ PTEN/PI3K/AKT
Hsa_crrc_00001649	↓								Unknown
Hsa_circ_0003159	↓								Hsa_circ_0003159 / MiR-223-3p/NDRGl
Circ-KIAA1244	↓				✓	✓			Unknown
Circ-SMAD7	↓								Unknown

↑/↓, up-regulation and down-regulation; ✓, CircRNAs are associated with clinicopathological features.

T stage, lymphatic metastasis, distant metastasis and histological grade. Meanwhile, the expression level of circNHSL1 in M1 stage tissues is higher than that in M0 stage tissues, and it is associated with progression and poor prognosis (54). In analyzing the expression of ebv-circLMP2A in 69 EBVa GC patients. It is found that high expression of ebv-circLMP2A is significantly associated with lymph node metastasis, distant metastasis and TNM advanced stage (55). The expression level of circDLST is

significantly increased in gastric cancer tissues compared with adjacent tissues, and it is an independent prognostic factor for poor survival of gastric cancer patients. Among patients receiving chemotherapy (oxaliplatin plus 5-Fu), patients with high circDLST expression have shorter overall survival than those with low expression (56). Circ_ASAP2 is overexpressed in DDP-resistant gastric cancer tissues and cells, the down-regulation of circ_ASAP2 promote the sensitivity and apoptosis of DDP-resistant gastric cancer cells and inhibite cell proliferation, migration and invasion (57).

Down-regulated circRNAs in gastric cancer

Some circRNAs are down-regulated in gastric cancer. For example, circRNA LARP4 is lowly expressed in gastric cancer tissue, and its expression level is significantly correlated with the pathological stage and overall survival rate of gastric cancer patients (58). Hsa_circ_00001649 is significantly down-regulated in tumor tissue and serum of patients with gastric cancer, and its expression may be related to the type of gastric cancer, with relatively high sensitivity and specificity (59). hsa_circ_0003159 is lowly expressed in GC tissues and cells, and low expression of hsa_circ_0003159 is associated with lower overall and disease-free survival in gastric cancer patients (60). Similar results are as follows, a study showed that circ-KIAA1244 in GC tissues, plasma and cells was significantly lower compared with normal controls by analysing the circRNA expression profiles in plasma samples from 10 GC patients with different TNM stages and 5 healthy people, and further clinical data analysis showed that the low expression of circ-KIAA1244 in plasma was negatively correlated with TNM stage, lymph node metastasis and overall survival time in GC patients (61). Another study showed that circ-SMAD7 expression was significantly reduced in GC tissues compared with adjacent normal tissues (62). circYAP1 was significantly lower in gastric cancer tissues than in adjacent normal tissues. In addition, the treatment outcome of gastric cancer patients with high circYAP1 expression was better than those with low circYAP1 expression by observing 75 gastric cancer patients who received adjuvant chemotherapy (oxaliplatin and 5-Fu) (63). The expression level of circ_0001017 in GC tissues of recurrent patients was lower than that of primary patients by analysing 26 patients with primary gastric cancer (sensitive) and 33 patients with recurrent gastric cancer (resistant), and low expression of circ_0001017 was associated with CDDP resistance in GC (64).

Relationship between circRNA and the occurrence and development of gastric cancer

Studies have found that some circRNAs may act as oncogenes to promote the occurrence and development of gastric cancer. For example, circ_0006282 can promote the proliferation and metastatic capacity of GC cells *in vitro* by acting as a miR-155 molecular sponge, and can promote tumor growth *in vivo* (49). The research results also showed that knockdown of hsa_circ_0010882 inhibited the proliferation, migration and invasion of gastric cancer cell lines, and increased apoptosis. In addition, the overexpression of hsa_circ_0010882 can enhance the proliferation, migration and invasion of gastric cancer cell, without changing apoptosis (50). Hsa_circ_0000467 promotes the proliferation and invasion ability of gastric cancer cells and the number of cells entering G2/M phase by regulating the expression of miR-326-3p (51). On the other hand, some circRNAs may act as tumor suppressor genes to inhibit the occurrence and development of gastric cancer. For example, circRBM33 inhibits gastric cancer cell apoptosis and promotes cell proliferation, migration and invasion through the circRBM33/miR-149/IL-6 axis (52). Hsa_circ_0003159 inhibits the proliferation, migration and invasion but induces apoptosis of GC cells by regulating miR-223-3p and NDRG1 (60). CircRHOBTB3 affects p21 protein expression by acting as a sponge for miR-654-3p, and then inhibits gastric cancer cell proliferation *in vitro* and xenograft tumor growth *in vivo* (65).

circRNA as a diagnostic marker for gastric cancer

Although the current treatment technology for gastric cancer has been greatly improved, the overall survival of gastric cancer patients is still unsatisfactory. The main reason is that many patients do not undergo early surgical resection in time, and the gastric cancer is found at a later stage and miss the best opportunity for surgery, resulting in a greatly reduced treatment effect. Therefore, the early screening and diagnosis of gastric cancer has become the key to improve the prognosis of patients (66). Histopathology is currently the gold standard for the diagnosis of gastric cancer, but it requires invasive procedures to obtain gastric tissue samples. In recent years, gastroscopic examination has been widely used in clinical practice. It can visually check the tissue lesions of the stomach and biopsy suspected tissues. However, because it is an invasive examination, it may cause some physical discomfort to the examiner, and has contraindications, so the application is still limited. Tumor marker detection is also a commonly used method to assist the diagnosis of gastric cancer. Carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), carbohydrate antigen 19-9 (CA19-9) and carbohydrate antigen 72- 4 (CA72-4) and other substances are of great significance for the diagnosis of tumors, but due to the lack of specificity, they can only be used as auxiliary diagnosis. Based on the above analysis, in order to improve the current status of early diagnosis of gastric cancer, it is of great urgent to explore less or even non-invasive, high-sensitivity and specific detection methods.

The inherent closed-loop structure of circRNA makes its expression relatively stable in tissues and blood, which makes it more likely to be a diagnostic marker for tumors. For example, circRNAs are highly stable in mammalian cells, and one specific circRNA hsa_circ_002059 may be a potential novel and stable biomarker for the diagnosis of gastric cancer (67). The expression level of hsa_circ_0000190 is significantly reduced in gastric cancer tissue and plasma, and its sensitivity and specificity in diagnosing gastric cancer are significantly better than those of CEA and CA19-9 (68). Besides, the expression level of hsa_circ_0000745 in gastric cancer tissue is related to the degree of tumor differentiation, and its expression level in plasma was related to the stage of tumor lymph node metastasis. At the same time, the combination of its plasma expression level and CEA level may be an effective marker for the diagnosis of gastric cancer (69). Likewise, hsa_circ_0001020 is significantly up-regulated in gastric cancer patient plasma compared with normal plasma and is significantly lower in both the postoperative group and the healthy group than the preoperative group by analysing plasma samples from preoperative and postoperative gastric cancer patients and healthy volunteers, subsequently ROC curve is constructed to determine the potential screening value of hsa_circ_0001020 in plasma. The AUC, sensitivity, specificity are 0.738, 46.55% and 97.83%, respectively. When CEA combined with hsa_circ_0001020 are used as plasma biomarkers, their AUC, sensitivity and specificity are 0.852, 66.7%, and 91.3%, respectively (70). CircPSMC3 is significantly downregulated in gastric cancer and correlated with poor prognosis, and the down-regulation of circPSMC3 is negatively correlated with TNM stage and lymph node metastasis, meanwhile, the area under the ROC curve (AUC) of circPSMC3 in differentiating GC-MS from normal GC-MS was 0.9326, with a sensitivity of 85.85% and a specificity of 95.24% (71). In addition, Gastric cancer patients with high expression of circYAP1 had better therapeutic effect than those with low expression of circYAP1 by observing 75 gastric cancer patients who received adjuvant chemotherapy (oxaliplatin and 5-Fu) (63). Therefore, the circRNAs have important guiding significance for the early diagnosis of gastric cancer and chemotherapy of advanced gastric cancer.

circRNAs as therapeutic targets for gastric cancer

With the deepening of the concept of precision medicine and the rapid development of molecular biology technology, molecular targeted therapy has been widely used in clinical anti-tumor work. Molecular targeted therapy, also known as “bio-missile”, is targeted at a certain protein molecule or gene fragment in the discovered tumor cells to specifically kill tumor cells, thereby inhibiting tumor growth and control tumor recurrence and metastasis (72). At present, the therapeutic targets of gastric cancer mainly include HER-2, EGFR, PI3K, mTOR and c-Met, etc., and targeted drugs targeting these receptors and kinases have achieved certain results in the clinical application of gastric cancer treatment (73). With the continuous development of circRNA research in recent years, its regulatory mechanism has been increasingly discovered in gastric cancer. Among them, circRNA function in a variety of cancer pathways by regulating the expression of some tumor-related genes, providing new potential molecular targets for targeted therapy of gastric cancer (74). For instance, circPVT1 exhibits frequent gene fragment amplification in gastric cancer, and can upregulate the expression of target gene E2F2 by acting as a molecular sponge of miR-125b, promoting the growth and proliferation of gastric cancer cells, and the stability of its expression makes it possible as a potential therapeutic target for gastric cancer (47). Synthetic anti-nuclease digested circRNA sponges have been reported to inhibit the proliferation of gastric cancer cells and reduce the activity of miR-21 against downstream targets, including the tumor protein DAXX. This finding suggests that synthetic circRNA sponges represent a simple, effective, and convenient therapeutic strategy to target miRNA loss-of-function *in vitro*, and is expected to gain potential therapeutic applications in human patients (75).

circRNA as a prognostic marker for gastric cancer

Medical technology continues to advance, the incidence and mortality of gastric cancer have been decreasing year by year in our country, but the prognosis of patients with advanced gastric cancer still needs to be improved (76). At present, tumor recurrence and metastasis after surgical treatment are the main factors affecting the prognosis of gastric cancer patients. A study screened 4 circRNAs significantly associated with postoperative recurrence in patients with stage III gastric cancer by gene chip technology, namely circRNA_101308, circRNA_104423, circRNA_104916 and circRNA_100269. Based on the above four circRNAs, a new risk prediction model was constructed for the recurrence of stage III gastric cancer patients after radical resection, and the model was more accurate than the traditional TNM staging and could better reflect the prognosis of patients (77). Another study found that the survival rate of gastric cancer patients with high expression of hsa_circ_0081143 was significantly lower than that of gastric cancer patients with low expression, indicating that hsa_circ_0081143 might be a potential prognostic marker for gastric cancer (78). In addition, the aforementioned circRNA_LARP4 could also be used as an independent prognostic factor for gastric cancer patients, and the overall survival time of patients with high circRNA_LARP4 expression was significantly longer than that of patients with low expression (58). At the same time, circPVT1 molecule can also be considered as a prognostic indicator of gastric cancer, the survival rate of gastric cancer patients with

high expression of circPVT1 and low expression of PVT1 is significantly higher compared with gastric cancer patients with low expression of circPVT1 and high expression of PVT1, this joint detection of circPVT1 and PVT1 has greater application value for the prognosis evaluation of gastric cancer patients (47).

Advantages and disadvantages of circRNA as a diagnostic and prognostic biomarker

Through the whole transcriptome analysis of human peripheral blood, a large number of repeatable circRNAs were identified, laying the foundation for the study of the potential of circRNA as a tumor biomarker (39, 79). Compared with traditional biomarkers, circRNA has the following characteristics. Firstly, circRNA has a closed ring structure and can resist the degradation of linear mRNA decay mechanism, thus showing excellent stability and long half-life (80, 81). Secondly, circRNA is highly conserved and highly expressed, making it easier to detect. In addition, circRNA is widely present in various body fluids (blood, saliva, urine and gastric juice) (82–85), making detection more convenient. Thirdly, the expression pattern of circRNA is highly specific, including cell specificity, tissue specificity and developmental specificity (39). The expression patterns and their diversity in different cell and tissue types and developmental stages are highly recognizable (86, 87). In summary, circRNA is expected to become an ideal clinical biomarker and therapeutic target and has been proved in many diseases. However, although circular RNAs have good application prospects, the research of circRNAs in cancer is still in its infancy. Compared to traditional markers, circRNAs lack common standards for reporting and naming circRNAs, available cancer-related RNA sequence data sets, and new technologies for detecting circRNAs (88).

Discussion

This review systematically summarizes the formation and regulatory mechanism of circRNA, its biological function and its relationship with gastric cancer. As a class of non-coding RNA molecules that are stably expressed in eukaryotes, circRNAs have rich biological functions. CircRNAs can interact with miRNAs and RBPs to regulate gene expression, and many nuclear-localized circRNAs can also regulate gene transcription. Although circRNAs have always been classified as non-coding RNAs, with in-depth research in recent years, circRNAs have been found to have coding potential, and its encoded polypeptides have important biological effects. These findings not only deepen our understanding of circRNAs, but also remind us that many unknown areas in circRNA research worth exploring.

Gastric cancer is a complex disease caused by multiple factors. Its exact pathogenesis has not been elucidated, and the treatment strategy still needs to be further improved. Given its closed loop structure, circRNA can exist stably in tissues and plasma, and the expression of circRNA has obvious tissue specificity. Therefore these characteristics make circRNA more potential to be an effective disease diagnosis, treatment and prognosis marker. Studies have found that many circRNAs are up-regulated or down-regulated in gastric cancer tissue or plasma, and these abnormally expressed circRNA molecules can regulate the expression of certain tumor-related proteins through different mechanisms, thus affecting the occurrence and development of gastric cancer. The expression level of some circRNAs may have a certain correlation with the clinicopathological indicators and survival time of gastric cancer patients. The above findings reveal that circRNAs function in the early diagnosis, lesion progression, treatment and prognosis of gastric cancer. Nevertheless, the action mechanism of circRNAs is still unclear, and some circRNAs with lower expression levels have not been discovered due to the limitations of detection methods in gastric cancer. In short, our current understanding of circRNAs is still at the primary level, and many experimental techniques and research strategies are still immature. Therefore, the transition from basic theory to clinical practice is worth studying and discussing. However, it is believed that more valuable circRNA molecules will be discovered through continuous innovation and exploration of scientific researchers, providing more effective molecular targets for the diagnosis, treatment and prognosis of gastric cancer.

Author contributions

(I) Conception and design: QZ. (II) Administrative support: QZ. (III) Provision of study materials or patients: P'AD, PY, PL, YT. (IV) Collection and assembly of data: P'AD, PY. (V) Data analysis and interpretation: P'AD. (VI) Manuscript writing: All authors. (VII) Final approval of manuscript: All authors.

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Conflict of interest

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