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Noncoding RNA in disease: Diagnosis, etiology, progression, prognosis and treatment

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Noncoding RNA in disease: Diagnosis, etiology, progression, prognosis and treatment

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Circular RNAs in ferroptosis: regulation mechanism and potential clinical application in disease

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Ferroptosis, an iron-dependent non-apoptotic form of cell death, is reportedly involved in the pathogenesis of various diseases, particularly tumors, organ injury, and degenerative pathologies. Several signaling molecules and pathways have been found to be involved in the regulation of ferroptosis, including polyunsaturated fatty acid peroxidation, glutathione/glutathione peroxidase 4, the cysteine/glutamate antiporter system Xc-, ferroptosis suppressor protein 1/ubiquinone, and iron metabolism. An increasing amount of evidence suggests that circular RNAs (circRNAs), which have a stable circular structure, play important regulatory roles in the ferroptosis pathways that contribute to disease progression. Hence, ferroptosis-inhibiting and ferroptosis-stimulating circRNAs have potential as novel diagnostic markers or therapeutic targets for cancers, infarctions, organ injuries, and diabetes complications linked to ferroptosis. In this review, we summarize the roles that circRNAs play in the molecular mechanisms and regulatory networks of ferroptosis and their potential clinical applications in ferroptosis-related diseases. This review furthers our understanding of the roles of ferroptosis-related circRNAs and provides new perspectives on ferroptosis regulation and new directions for the diagnosis, treatment, and prognosis of ferroptosis-related diseases.

KEYWORDS

circular RNA, ferroptosis, regulation mechanism, clinical application, cancers

1 Ferroptosis

First described in 2012, ferroptosis is an iron- and reactive oxygen species (ROS)-dependent non-apoptotic form of regulatory cell death that differs from apoptosis, necrosis, and autophagy at the morphological, biochemical, and genetic levels (Figure 1A) (Dixon et al., 2012; Xie et al., 2016; Galluzzi et al., 2018). Morphologically, ferroptosis is characterized by marked mitochondrial contraction, increased membrane density, and the reduction or disappearance of mitochondrial cristae (Xie et al., 2016; Li et al., 2020). At the biochemical level, ferroptosis involves the accumulation of lipid peroxidation products and lethal ROS produced by iron metabolism, which can be inhibited by lipid peroxidation inhibitors and iron chelators, respectively (Xie et al., 2016; Galluzzi et al., 2018). Activation of mitochondrial voltage-dependent anion channels and mitogen-activated protein kinases, upregulation of endoplasmic reticulum (ER) stress, and inhibition of

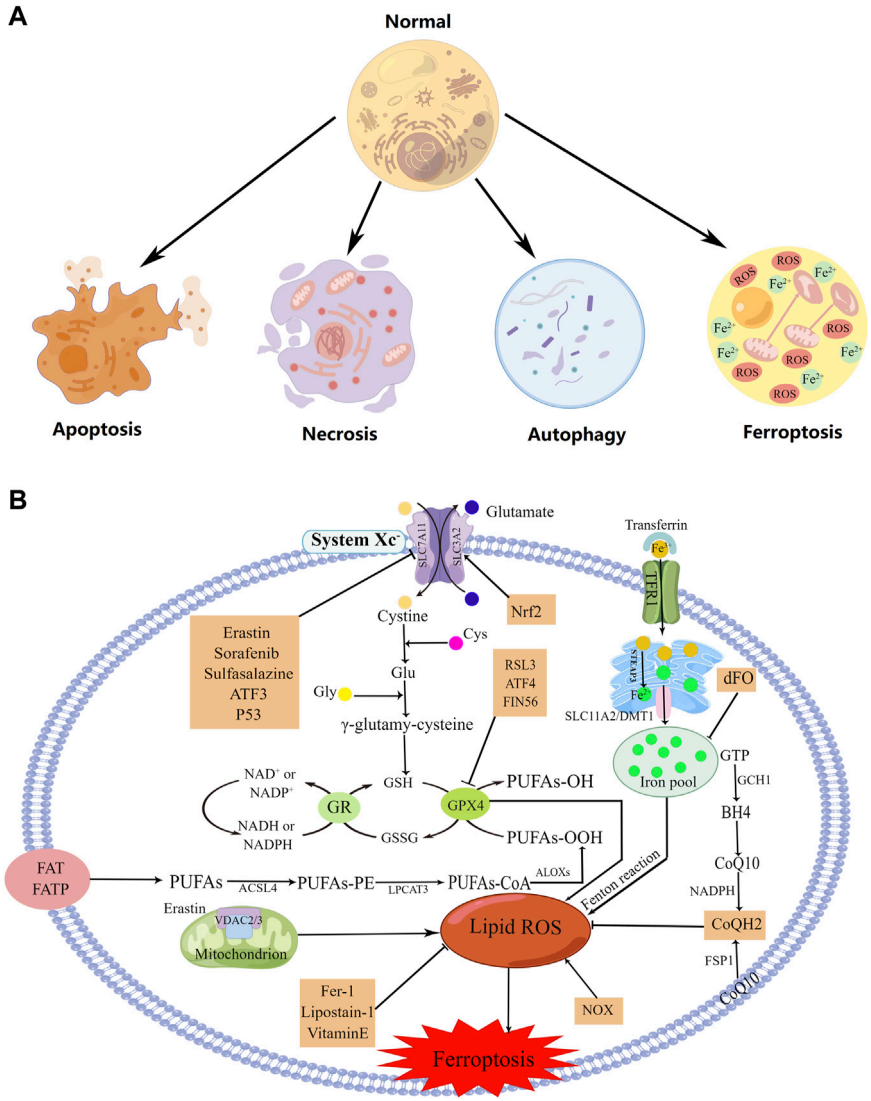

FIGURE 1
The regulation mechanism underlying cell ferroptosis. **(A)** Representations of ferroptosis, apoptosis, necrosis, and autophagy. **(B)** The regulation mechanism utilized during ferroptosis. Created using figdraw.

cystine/glutamate reverse transporters are all involved in the induction of ferroptosis (Xie et al., 2016).

Ferroptosis is involved in many physiological and pathological processes and is closely associated with many diseases, such as tumors, neurological disorders, ischemia-reperfusion injury, kidney injury, and blood diseases (Xie et al., 2016; Li et al., 2020). Several signaling molecules and pathways, such as polyunsaturated fatty acid (PUFA) peroxidation, glutathione (GSH)/glutathione peroxidase 4 (GPX4), the cysteine/glutamate antiporter (system Xc-), ferroptosis suppressor protein 1 (FSP1)/ubiquinone (CoQ10), and iron metabolism, have been found to be involved in ferroptosis regulation (Figure 1B) (Li et al., 2020). Also, four classes of ferroptosis inducers have been identified that drive cell death by either inhibiting system Xc-, inhibiting or degrading GPX4, consuming CoQ10, or inducing lipid peroxidation (Li et al., 2020).

1.1 Polyunsaturated fatty acid (PUFA) peroxidation

Lipid peroxidation is a hallmark of ferroptosis (Jiang et al., 2021). When subjected to oxidative or energy stress, cell membrane PUFAs—particularly arachidonic acid (AA) and adrenic acid—are oxidized to PUFA-OOH, inducing ferroptosis; the oxidation is catalyzed by acyl-CoA synthetase long-chain family member 4 (ACSL4), lysophosphatidylcholine acyltransferase (LPCAT), and lipoxygenases (ALOXs) (Figure 1B). PUFA peroxidation can cause the destruction of the lipid bilayer and damage cellular membranes, resulting in cellular dysfunction and cell death (Chen et al., 2021a).

Ferrostatin-1 (Fer-1) and lipostain-1 trap peroxides to reduce lipid peroxidation and alleviate ferroptosis (Ma et al., 2022a). In addition, fat-soluble vitamin E is adept at scavenging

free radicals due to its high affinity for unpaired electrons (Ma et al., 2022a) and thus can inhibit ferroptosis mediated by lipid peroxidation (Hu et al., 2021).

1.2 Glutathione (GSH)/glutathione peroxidase 4 (GPX4)

Glutathione peroxidases (GPXs) protect cells against oxidative damage, thus preventing ferroptosis (Jiang et al., 2021). As a member of the GPX family, GPX4 can directly reduce peroxidized phospholipids in the cell membrane and is a pivotal regulator of ferroptosis (Yang et al., 2014; Jiang et al., 2021). More specifically, GPX4 inhibits ferroptosis by reducing each PUFA-OOH to the corresponding PUFA-OH and oxidizing GSH (a reductive cofactor of GPX4) to GSSG (oxidized GSH) (Figure 1B) (Ma et al., 2022a).

Overexpression or knockdown of GPX4 has been shown to affect the lethality of 12 ferroptosis inducers (Yang et al., 2014). Also, given that a decline in the level of GPX4 can lead to the accumulation of lipid peroxides and lead to ferroptosis, it is often used as a marker of ferroptosis (Yang et al., 2014; Jiang et al., 2021).

Ras-selective lethal small molecule 3 (RSL3) directly inhibits the activity of GPX4 by covalently binding to selenocysteine, which is located at the active site of GPX4, thereby inducing ferroptosis (Ma et al., 2022a). FIN56, another specific ferroptosis inducer, triggers ferroptosis by promoting the degradation of GPX4 via the acetyl-CoA pathway (Sun et al., 2021; Ma et al., 2022a). Activating transcription factor 4 (ATF4), a critical mediator of metabolic and oxidative homeostasis and cell survival (Chen et al., 2017a), inhibits GPX4 by activating heat shock 70 kDa protein 5 to bind to GPX4, thereby promoting ferroptosis (Zhu et al., 2017). FINO2 promotes ferroptosis via GPX4 inactivation and iron oxidation (Gaschler et al., 2018).

1.3 System Xc-

System Xc-is an important intracellular antioxidant system that is composed of two subunits: SLC7A11 and SLC3A2 (Chen et al., 2021a; Jiang et al., 2021; Du et al., 2022). SLC7A11 is responsible for the main transport activity and is highly specific for cystine and glutamate (Du et al., 2022). System Xc- exchanges intracellular glutamate for extracellular cystine (Cys2) at a 1:1 ratio, and the subsequent cystine-to-GSH reaction is catalyzed by glutamate cysteine ligase (GCL) and glutathione synthetase (GSS) (Chen et al., 2021a). Inhibiting the activity of system Xc- prevents the absorption of cystine, affects GSH synthesis, and subsequently reduces GPX4 activity (the membrane lipid-repair enzyme), thus reducing the cellular antioxidant capacity and promoting ferroptosis (Figure 1B) (Chen et al., 2021a; Du et al., 2022).

Activating transcription factor 3 (ATF3), a common stress sensor, promotes lipid peroxidation by inhibiting system Xc- (Wang et al., 2020a). Sorafenib (SF) is an oral tyrosine kinase inhibitor that induces GPX4 inactivation by blocking system Xc- and promotes ferroptosis (Zheng et al., 2021a). It has been shown that p53 decreases cystine uptake and intracellular GSH and induces ferroptosis by transcriptionally suppressing the expression of SLC7A11 (Ou et al., 2016). In addition, sulfadiazine has been shown to inhibit system Xc-, promote the accumulation of ROS, and induce ferroptosis (Yu et al., 2019), and NRF2 inhibits ferroptosis by increasing SLC7A11 (Song and Long, 2020).

1.4 Ferroptosis suppressor protein 1 (FSP1)/ubiquinone (CoQ10)

FSP1 is a GSH-independent ferroptosis suppressor encoded by apoptosis-inducing factor mitochondria-associated 2 (*AIFM2*) (Doll et al., 2019). It can suppress ferroptosis by acting on CoQ10: FSP1 reduces CoQ10 to ubiquinol (CoQH2) on the cell membrane, which acts as a free radical-trapping antioxidant to prevent lipid peroxidation on the cell membrane (Bersuker et al., 2019; Ma et al., 2022a). FSP1 can also catalyze CoQ10 regeneration by utilizing NAD(P)H (Doll et al., 2019). This GSH-independent FSP1/CoQ10/NAD(P)H pathway works in cooperation with the GPX4/GSH mechanism to suppress ferroptosis (Figure 1B) (Doll et al., 2019).

GTP loop hydrolase 1 (GCH1) is one of the rate-limiting enzymes involved in the synthesis of tetrahydrobiopterin (BH4) (Cronin et al., 2022), and GCH1 promotes the formation of CoQ10 and inhibits ferroptosis (Ma et al., 2022a).

1.5 Iron metabolism

Transferrin present in the serum binds to Fe^{3+}, and the iron-loaded protein is recognized and bound by transferrin receptor protein 1 (TFR1) located on the cell membrane, forming a complex (Frazer and Anderson, 2014). Intracellular Fe^{3+} is reduced to Fe^{2+} by STEAP3 in the ER and then released by SLC11A2 into the cytoplasmic pool of free iron (Frazer and Anderson, 2014; Conrad et al., 2018). Fe^{2+} in the iron pool generates a considerable volume of hydroxyl radicals and ROS through the Fenton reaction, which causes ferroptosis (Figure 1B) (Frazer and Anderson, 2014; Conrad et al., 2018).

Deferoxamine (DFO) is an effective iron chelator (Zhu et al., 2022). After DFO enters the cell via endocytosis, it forms a stable octahedral coordination compound with Fe^{3+}, thereby reducing the unstable iron pool in the cell (Ma et al., 2022a).

1.6 Mitochondria and transmembrane channels

Mitochondria play a key role in ferroptosis. ROS are derived in part from mitochondrial metabolism, and transmembrane voltage-dependent anion channels (VDACs) transport ions and metabolites across the outer mitochondrial membrane (Ma et al., 2022a). Erastin reduces mitochondrial membrane permeability through activation of VDAC2/3, thereby generating ROS that promote ferroptosis (Figure 1B) (DeHart et al., 2018; Ma et al., 2022a).

1.7 Chemical inducers/inhibitors of ferroptosis

Several chemicals have been shown to act as ferroptosis inducers or inhibitors (Du and Guo, 2022). As mentioned above, erastin

TABLE 1 Ferroptosis-related circular RNAs (circRNAs) associated with disease conditions.

Disease	CircRNA	Expression	References
Breast cancer (BC)	CircGFRA1	Upregulated in HER2-positive BC cells and tissues	Bazhabayi et al. (2021)
	Circ-BGN	Upregulated in trastuzumab-resistant BC cells and tissues	Wang et al. (2022a)
	CircRHOT1	BC cells	Zhang et al. (2021b)
Glioma	CircCDK14	Upregulated in glioma tissues and cells	Chen et al. (2022a)
	Circ-TTBK2		Zhang et al. (2020a)
Thyroid cancer	CircKIF4A	Upregulated in papillary thyroid cancer	Chen et al. (2021b)
	Circ_0067934	Upregulated in clinical thyroid cancer samples	Wang et al. (2021a)
Gastric cancer (GC)	Circ_0000190	Downregulated in GC tissues and cell lines	Jiang et al. (2022)
Lung cancer	CircP4HB	Upregulated in LUAD tissues	Pan et al. (2022)
	CircDTL	Upregulated in NSCLC tissues	Shanshan et al. (2021)
	CircRNA_101093	Upregulated in LUAD tissue and plasma exosome	Zhang et al. (2022a)
Hepatocellular carcinoma (HCC)	Hsa_circ_0008367	Most upregulated in sorafenib-treated HCC cells	Liu et al. (2020)
	Circ0097009	Upregulated in HCC tissues and cell lines	Lyu et al. (2021)
	CircIL4R	Upregulated in HCC tissues and cell lines	Xu et al. (2020)
Cervical cancer	CircLMO1	Downregulated in cervical cancer tissues and cell lines	Ou et al. (2022)
	CircEPSTI1	Upregulated in cervical cancer cell lines	Wu et al. (2021)
Colorectal cancer	Circ_0007142	Upregulated in colorectal cancer tissues and cell lines	Wang et al. (2021b)
	CircABCB10	Upregulated in rectal cancer tissues	Xian et al. (2020)
Oral squamous cell carcinoma (OSCC)	CircFNDC3B	Upregulated in clinical OSCC tissues	Yang et al. (2021)
Acute lymphoblastic leukemia (ALL)	Circ_0000745	Upregulated in the peripheral blood samples from ALL patients	Yang et al. (2022)
Esophageal cancer	CircPVT1	Upregulated in ESCC cells resistant to 5-FU	Yao et al. (2021)
Myocardial infarction (MI)	CircRNA1615	Downregulated in myocardial tissue of mice with MI	Li et al. (2021a)
Heart failure	CircSnx12	Downregulated in myocardial tissues of mice with TAC	Zheng et al. (2021b)
Acute cerebral infarction (ACI)	Circ-Carm1	Upregulated in the serum of patients with ACI	Mao and Liu (2022)
Traumatic brain injury (TBI)	CircPtpn14	Upregulated in the brain of patients and mice with TBI	Wu et al. (2022a)
Polycystic ovary syndrome (PCOS)	CircRHBG	Upregulated in granular cells of PCOS patients	Zhang et al. (2021a)
Diabetic nephropathy (DN)	Mmu_circRNA_0000309	Downregulated in podocytes of mice with DN	Jin et al. (2022)
Diabetic retinopathy (DR)	Circ-PSEN1	Upregulated in high glucose-treated ARPE19 cells	Zhu et al. (2021a)

Notes: NSCLC, non-small cell lung cancer; LUAD, lung adenocarcinoma; ESCC, esophageal squamous cell carcinoma; TAC, transverse aortic constriction; ARPE19, adult retinal pigment epithelial cell line-19.

induces ferroptosis by blocking VDACs, which affects GSH formation and oxidation (Du and Guo, 2022). Temozolomide induces ferroptosis by enhancing DMT1 (Du and Guo, 2022).Tertiary-butyl hydroperoxide and SF induce ferroptosis by affecting lipid metabolism and producing lipid ROS directly (Du and Guo, 2022). Brequinar inhibits tumor growth by inducing tumor cell ferroptosis (Du and Guo, 2022). Mison promotes ferroptosis by upregulating a GSH metabolic pathway regulator called dipeptidase-1, which increases cell sensitivity to ferroptosis (Du and Guo, 2022). Ciclopirox olamine, desferrioxamine, DFO, and deferasirox inhibit ferroptosis by sequestering iron ions (Du and Guo, 2022). Fer-1 and hydroquinone inhibit ferroptosis by inhibiting lipid oxidation (Du and Guo, 2022). In addition, 2-amino-5-chloro-N, 3-dimethylbenzamide can inhibit degradation of GPX4 and protect cells from the effects of ferroptosis (Du and Guo, 2022). Finally, alpha-tocopherol, the main component of vitamin E, can inhibit ferroptosis (Du and Guo, 2022).

2 The role of circular RNAs (circRNAs) in the regulation of ferroptosis

Circular RNA (circRNA) is a novel type of RNA that forms a covalently closed continuous loop with neither 5′-to-3′ polarity nor

TABLE 2 The regulatory roles circular RNAs (circRNAs) play in disease progression via inhibiting ferroptosis.

Category	CircRNA	Mechanistic target	Function	Disease	References
GPX4 upregulation	CircKIF4A	miR-1231/GPX4	Inhibit ferroptosis and promote papillary thyroid cancer	Thyroid cancer	Chen et al. (2021b)
	CircDTL	miR-1287-5p/GPX4	Inhibit ferroptosis and promote non-small cell lung cancer	Non-small cell lung cancer	Shanshan et al. (2021)
	CircIL4R	miR-541-3p/GPX4	Inhibit ferroptosis and promote hepatocellular carcinoma	Hepatocellular carcinoma	Xu et al. (2020)
	Mmu_circRNA_0000309	miR-188-3p/GPX4	Inhibit ferroptosis and inhibit diabetic nephropathy	Diabetic nephropathy	Jin et al. (2022)
System Xc- upregulation	Circ-BGN	OTUB1/SLC7A11	Inhibit ferroptosis and promote HER-2-positive breast cancer	HER-2-positive breast cancer	Wang et al. (2022a)
	Circ_0067934	miR-545-3p/SLC7A11	Inhibit ferroptosis and promote thyroid cancer	Thyroid cancer	Wang et al. (2021a)
	CircP4HB	miR-1184/SLC7A11	Inhibit ferroptosis and promote lung adenocarcinoma	Lung adenocarcinoma	Pan et al. (2022)
	Circ0097009	miR-1261/SLC7A11	Inhibit ferroptosis and promote hepatocellular carcinoma	Hepatocellular carcinoma	Lyu et al. (2021)
	CircEPSTI1	miR-375/miR-409-3P/miR -515-5p/SLC7A11	Inhibit ferroptosis andpromote cervical cancer	Cervical cancer	Wu et al. (2021)
	CircFNDC3B	miR-520d-5p/SLC7A11	Inhibit ferroptosis and promote oral squamous cell carcinoma	Oral squamous cell carcinoma	Yang et al. (2021)
	CircRHBG	miR-515-5p/SLC7A11	Inhibit ferroptosis in polycystic ovary syndrome cells	Polycystic ovary syndrome	Zhang et al. (2021a)
FSP1 upregulation	CircGFRA1	miR-1228/AIFM2	Inhibit ferroptosis and promote HER-2-positive breast cancer	Breast cancer	Bazhabayi et al. (2021)
Lipid metabolism regulation	CircRNA_101093	Interacts with FABP3 and induce N-arachidonoyl taurine	Inhibit ferroptosis and Promote lung adenocarcinoma	Lung adenocarcinoma	Zhang et al. (2022a)
	Circ_0007142	miR-874-3p/GDPD5	Inhibit ferroptosis and promote colorectal cancer	Colorectal cancer	Wang et al. (2021b)
	CircRNA1615	miR-152-3p/LRP6	Inhibit ferroptosis and control pathological process of myocardial infarction	Myocardial infarction	Li et al. (2021a)
Inhibition of ferroptosis via other mechanisms	CircRHOT1	miR-106a-5p/STAT3	Inhibit ferroptosis and promote breast cancer	Breast cancer	Zhang et al. (2021b)
	CircCDK14	miR-3938/PDGFRA	Inhibit ferroptosis and promote glioma	Glioma	Chen et al. (2022a)
	Circ-TTBK2	miR-761/ITGB8	Inhibit ferroptosis and promote glioma	Glioma	Zhang et al. (2020a)
	CircABCB10	miR-326/CCL5	Inhibit ferroptosis and promote rectal cancer	Colorectal cancer	Xian et al. (2020)
	Circ_0000745	miR-494-3p/NET1	Inhibit ferroptosis and promote acute lymphoblastic leukemia	Acute lymphoblastic leukemia	Yang et al. (2022)
	CircPVT1	miR-30a-5p/FZD3	Inhibit ferroptosis and promote esophageal cancer	Esophageal cancer	Yao et al. (2021)

a polyadenylation tail (Chen and Yang, 2015; Qu et al., 2015). The unique circular structure of circRNA makes it more stable. It is formed by reverse splicing of pre-mRNA, and some circRNAs are abundant and evolutionarily conserved (Misir et al., 2022). *In vivo*, many circRNAs play important biological functions by acting as sponges for microRNAs, regulating protein functions, and self-translating (Gao et al., 2022; Misir et al., 2022). Increasing evidence suggests that circRNAs play important regulatory roles in the progression of many ferroptosis-related diseases and have great potential as novel diagnostic markers or therapeutic targets for such diseases (Zhang et al., 2020a; Liu et al., 2020; Xian et al., 2020; Xu et al., 2020; Li et al., 2021a; Wang et al., 2021a; Zhang et al., 2021a; Zhu et al., 2021a; Bazhabayi et al., 2021; Chen et al., 2021b; Wang et al., 2021b; Zhang et al., 2021b; Zheng et al., 2021b; Lyu et al.,

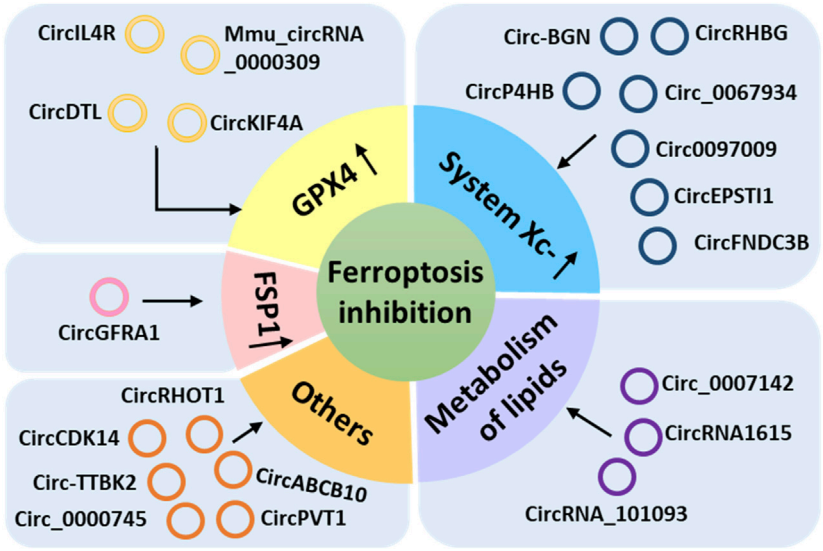

FIGURE 2
Ferroptosis-inhibiting circular RNAs classified according to the mechanism utilized to regulate ferroptosis.

2021; Shanshan et al., 2021; Wu et al., 2021; Yang et al., 2021; Yao et al., 2021; Chen et al., 2022a; Wang et al., 2022a; Wu et al., 2022a; Zhang et al., 2022a; Jiang et al., 2022; Jin et al., 2022; Mao and Liu, 2022; Ou et al., 2022; Pan et al., 2022; Yang et al., 2022). Therefore, in this review, we have summarized the recent research on ferroptosis-related circRNAs published prior to May 2022 in the PubMed and Web of Science databases (Table 1) to provide new perspectives on ferroptosis regulation and new directions for the diagnosis, treatment, and prognosis of ferroptosis-related diseases. The PubMed and Web of Science databases were searched using the keywords "ferroptosis" AND ("circRNA" OR "circular RNA" OR "non-coding RNA"). The resultant research studies were then manually collected and reviewed.

2.1 Ferroptosis-inhibiting circRNAs

More than 20 circRNAs have been reported to inhibit ferroptosis by acting on GPX4, system Xc-, FSP1, or lipid metabolism or other pathways and play important regulatory roles in the progression of many diseases (Zhang et al., 2020a; Xian et al., 2020; Xu et al., 2020; Li et al., 2021a; Wang et al., 2021a; Zhang et al., 2021a; Bazhabayi et al., 2021; Chen et al., 2021b; Wang et al., 2021b; Zhang et al., 2021b; Lyu et al., 2021; Shanshan et al., 2021; Wu et al., 2021; Yang et al., 2021; Yao et al., 2021; Chen et al., 2022a; Wang et al., 2022a; Zhang et al., 2022a; Jin et al., 2022; Pan et al., 2022; Yang et al., 2022), such as thyroid cancer, lung cancer, hepatocellular carcinoma (HCC), breast cancer, cervical cancer, oral squamous cell carcinoma (OSCC), glioma, colorectal cancer, esophageal cancer, diabetic nephropathy (DN), polycystic ovary syndrome (PCOS), acute lymphoblastic leukemia (ALL), and myocardial infarction (MI; Table 2). We classified these ferroptosis-inhibiting circRNAs according to the mechanism by which they regulate ferroptosis (Figure 2).

2.1.1 CircRNAs that upregulate GPX4

Four circRNAs (circKIF4A, circDTL, circIL4R, and mmu_circRNA_0000309) inhibit ferroptosis by upregulating GPX4 (Figure 2; Table 2). CircKIF4A reportedly promotes the malignant progression of papillary thyroid cancer and inhibits ferroptosis by sponging miR-1231 and then upregulating its target gene *GPX4* (Chen et al., 2021b). Silencing of circKIF4A can downregulate GPX4, resulting in the proliferation and metastatic inhibition of papillary thyroid cancer cells and inhibition of tumor growth *in vivo* (Chen et al., 2021b). CircDTL inhibits ferroptosis and apoptosis of non-small cell lung cancer (NSCLC) cells through the circDTL/miR1287-5p/GPX4 axis (Shanshan et al., 2021). Downregulation of circDTL was found to increase cellular ROS, malondialdehyde (MDA; an endogenous genotoxic product of lipid peroxidation), and Fe^{2+} levels and reduce GSH levels, thus promoting ferroptosis of NSCLC cells (Shanshan et al., 2021). CircIL4R positively regulates the expression of GPX4 by adsorbing miR-541-3p, facilitates tumorigenesis, and inhibits ferroptosis in HCC cells (Xu et al., 2020). Knockdown of circIL4R can aggravate erastin-induced ferroptosis by increasing iron accumulation and oxidative stress in HCC cells, hindering the carcinogenesis process. Mmu_circRNA_0000309 was found to inhibit ferroptosis-dependent mitochondrial damage and podocyte apoptosis by competitively adsorbing miR-188-3p to promote GPX4 expression, thereby participating in the improvement of DN mediated by germacrone (Jin et al., 2022). Germacrone is the main bioactive component of turmeric, which has anti-inflammatory and antioxidant effects (Aggarwal et al., 2013). Silencing mmu_circRNA_0000309 or introducing miR-188-3p mimics was found to eliminate the anti-apoptotic and anti-injury effects of germacrone by aggravating mitochondrial damage and increasing the levels of ROS and iron deposition-related proteins (Jin et al., 2022). In the same study, overexpression of GPX4 was found to neutralize mitochondrial

damage and ferroptosis mediated by mmu_circRNA_0000309 silencing (Jin et al., 2022).

2.1.2 CircRNAs that upregulate system Xc-

SLC7A11, a core subunit of system Xc-, imports cystine into the cell for GSH biosynthesis and as an antioxidant defense (Koppula et al., 2021). Seven circRNAs (circ-BGN, circ_0067934, circP4HB, circ0097009, circEPSTI1, circFNDC3B, and circRHBG) have been reported to inhibit ferroptosis via upregulation of SLC7A11 (Figure 2; Table 2).

OTU deubiquitinase, ubiquitin aldehyde binding 1 (OTUB1) is a highly expressed cysteine protease and a member of the deubiquitinating enzyme family (Liu et al., 2014; Que et al., 2020). Circ-BGN was found to directly bind to OTUB1 and SLC7A11 and enhance OTUB1-mediated deubiquitination of SLC7A11, thereby inhibiting ferroptosis (Wang et al., 2022a). Downregulation of circ-BGN significantly increases the levels of lipid ROS, MDA, and Fe^{2+}, inhibits GPX4 activity, and leads to the inhibition of activity in breast cancer cells (Wang et al., 2022a). In addition, circ-BGN knockdown has been shown to enhance the significant inhibition of cell growth mediated by erastin on trastuzumab resistance breast cancer cells (Wang et al., 2022a).

Circ_0067934 reportedly upregulates the expression of SLC7A11 and thus promotes the progression of thyroid cancer and inhibits ferroptosis in thyroid cancer cells by adsorbing miR-545-3p (Wang et al., 2021a). Silencing circ_0067934 decreased the cell survival rate and enhanced ferroptosis and apoptosis in thyroid cancer cells (Wang et al., 2021a). Overexpression of an miR-545-3p inhibitor or SLC7A11 rescued the inhibitory effect of silencing circ_0067934 on thyroid cancer cells and resulted in a decrease in the levels of ferroptosis-associated markers, such as Fe^{2+}, iron, and ROS (Wang et al., 2021a).

CircP4HB, which is also called hsa_circ_0046263, is derived from the alternative transcription of the prolyl 4-hydroxylase subunit beta gene (Wang et al., 2019a). In lung adenocarcinoma (LUAD) cells, circP4HB was found to direct ferroptosis by regulating miR-1184/SLC7A11-mediated GSH synthesis (Pan et al., 2022). CircP4HB targeted and sponged miR-1184, and *SLC7A11* was found to be a target gene of miR-1184 (Pan et al., 2022). As an inhibitor of ferroptosis, circP4HB protects LUAD cells from ferroptosis by triggering GSH synthesis (Pan et al., 2022).

In HCC cells, SLC7A11 was found to be regulated by circ0097009 via the sponging of miR-1261. Ferroptosis is involved in HCC progression through the circ0097009/miR-1261/SLC7A11 axis (Lyu et al., 2021). Downregulation of circ0097009 has been shown to significantly inhibit cell growth, invasion, and metastasis and promote ferroptosis in HCC cells (Lyu et al., 2021).

CircEPSTI1, also known as hsa_circRNA_000479, is a cancer-associated circRNA (Peng et al., 2020; Tan et al., 2020; Xie et al., 2020). As a competing endogenous RNA (ceRNA), circEPSTI1 upregulates the expression of SLC7A11 by adsorbing miR-375, miR-409-3p, and miR-515-5p in cervical cancer cells (Wu et al., 2021). Silencing of circEPSTI1 inhibited cervical cancer cell proliferation and induced SLC7A11-mediated ferroptosis, and overexpression of SLC7A11 reversed this effect (Wu et al., 2021).

CircFNDC3B, also known as circ_0006156, has biological functions in a variety of cancers, such as papillary thyroid cancer (Wu et al., 2020), esophageal squamous cell carcinoma (ESCC) (Tang et al., 2022), and gastric cancer (GC) (Hong et al., 2019). A recent study found that circFNDC3B protects OSCC cells from ferroptosis and promotes malignant progression by regulating the miR-520d-5p/SLC7A11 axis (Yang et al., 2021). CircFNDC3B can enhance the accumulation of ROS, iron, and Fe^{2+} in cells to inhibit ferroptosis (Yang et al., 2021). Knockdown of circFNDC3B has been shown to enhance the inhibitory effect of erastin on OSCC cells, thereby inducing ferroptosis in OSCC cells (Yang et al., 2021).

CircRHBG is involved in the proliferation and ferroptosis of PCOS granulosa cells through the miR-515/SLC7A11 axis (Zhang et al., 2021a). In PCOS cells, circRHBG acts as a ceRNA for miR-515 and upregulates SLC7A11 (Zhang et al., 2021a). The downregulation of circRHBG was found to promote ferroptosis by causing a decrease in the GSH-to-GSSG ratio, leading to GPX4 inactivation (Zhang et al., 2021a).

2.1.3 CircRNAs that upregulate FSP1

CircGFRA1 acts as a ceRNA for miR-1228 and upregulates *AIFM2*, which encodes FSP1 (a ferroptosis suppressor that acts via CoQ10) (Bazhabayi et al., 2021). CircGFRA1 has been shown to promote the progression of HER2-positive breast cancer via the miR-1228/*AIFM2* axis (Bazhabayi et al., 2021). The silencing of circGFRA1 can enhance ferroptosis through the circGFRA1/miR-1228/*AIFM2* axis (Bazhabayi et al., 2021) and inhibit the proliferation, infiltration, and metastasis of HER2-positive breast cancer cells (Bazhabayi et al., 2021). In addition, circGFRA1 silencing also leads to a decrease in the GSH-to-GSSG ratio and downregulation of GPX4; the decrease in the GSH-to-GSSG ratio results in GPX4 inactivation, further promoting lipid ROS accumulation and ferroptosis (Bazhabayi et al., 2021).

2.1.4 CircRNAs that regulate lipid metabolism

Some circRNAs that are involved in lipid metabolism have been reported to inhibit ferroptosis (Li et al., 2021a; Wang et al., 2021b; Zhang et al., 2022a) (Figure 2). It was found that circRNA_101093 can desensitize LUAD cells to ferroptosis by upregulating fatty acid-binding protein 3 (*FABP3*), reducing global AA, and preventing AA incorporation into the plasma membrane (Zhang et al., 2022a). CircRNA_101093 integrated with and increased FABP3, which then transported AA and facilitated its reaction with taurine, thus reducing global AA and inducing production of N-arachidonoyl taurine (NAT; the product of AA and taurine) (Zhang et al., 2022a). NAT plays a role in desensitizing cells to ferroptosis by downregulating the expression of related enzymes (i.e., ACSL4, LPCAT3, and PLTP) and preventing the incorporation of AA into the plasma membrane of LUAD cells (Du et al., 2019; Cui et al., 2021; Jiang and Yu, 2021).

Altered choline phospholipid metabolism is a hallmark of cancer (Cao et al., 2012). Glycerophosphodiester phosphodiesterase domain containing 5 (*GDPD5*), the target gene of miR-874-3p, encodes a glycerophosphodiester phosphodiesterase that catalyzes the hydrolysis of deacylated glycerophospholipids to glycerol phosphate and an alcohol (Lang et al., 2008). Circ_0007142 has been identified as a carcinogenic factor due to its ability to regulate tumorigenesis and ferroptosis in colorectal cancer cells via the miR-874-3p/GDPD5 axis (Wang et al., 2021b). Low expression of circ_0007142 can inhibit proliferation and promote apoptosis and ferroptosis in colorectal cancer cells (Wang et al., 2021b).

Lipoprotein receptor-related protein-6 (LRP6) is involved in lipid homeostasis and is an essential co-receptor for canonical Wnt signaling (Li et al., 2010). It has been found that circRNA1615 regulates the expression of *LRP6* through the adsorption of miR-152-3p to prevent LRP6-mediated autophagy-related ferroptosis in cardiomyocytes, ultimately controlling the pathological process of MI (Li et al., 2021a). In addition, higher levels of MDA and Fe^{2+} observed in MI tissues have suggested that ferroptosis occurs in cardiomyocytes (Li et al., 2021a). LRP6 interference increased the expression of the autophagy-related proteins LC3-A/B (microtubule-associated protein 1 light chain 3-A/B) and autophagy related 5 and decreased the expression of sequestosome 1, resulting in induced ferroptosis in cardiomyocytes through autophagy (Li et al., 2021a).

2.1.5 CircRNAs that inhibit ferroptosis via other pathways

Some circRNAs have also been reported to inhibit ferroptosis via signal transducer and activator of transcription 3 (STAT3), platelet derived growth factor receptor alpha (PDGFRA), integrin subunit beta 8 (ITGB8), and other pathways and play important regulatory roles in the progression of various cancers, such as breast cancer, glioma, lung cancer, HCC, colorectal cancer, ALL, and esophageal cancer (Zhang et al., 2020a; Xian et al., 2020; Zhang et al., 2021b; Yao et al., 2021; Chen et al., 2022a; Yang et al., 2022) (Table 2; Figure 2).

CircRHOT1 has been found to play a key role in the development of multiple types of diseases, such as HCC (Wang et al., 2019b), osteoarthritis (Man et al., 2022), and NSCLC (Ren et al., 2021). In breast cancer cells, circRHOT1 functions by adsorbing miR-106a-5p, which targets STAT3 in this cell type (Zhang et al., 2021b). CircRHOT1 was found to promote the proliferation and migration of breast cancer cells and inhibit apoptosis and ferroptosis through the miR-106a-5p/STAT3 axis (Zhang et al., 2021b).

The transmembrane receptor PDGFRA is overexpressed, amplified, mutated, or truncated in gliomas and is the second most frequently mutated tyrosine kinase receptor in glioblastomas (Alentorn et al., 2012; Higa et al., 2022). It has been found that circCDK14 sponges miR-3938 and upregulates PDGFRA expression, resulting in resistance to ferroptosis and promotion of glioma progression (Chen et al., 2022a). In the same study, when circCDK14 was deleted, the SLC7A11 and GPX4 levels were significantly reduced and the Fe^{2+} and ROS levels were significantly increased (Chen et al., 2022a). In addition, circCDK14 has also been shown to promote epithelial-mesenchymal transition in glioma cells by regulating PDGFRA expression (Chen et al., 2022a).

Another study revealed that circ-TTBK2, also named has_circ_0000594, regulates glioma cell proliferation, invasion, and ferroptosis through the miR-761/ITGB8 axis (Liao et al., 2015; Zhang et al., 2020a). Knockdown of circ-TTBK2 or increased expression of miR-761 was found to delay the proliferation and invasion of glioma cells and promote ferroptosis (Zhang et al., 2020a). *ITGB8* encodes a beta subunit of integrin (integrin beta 8) (He et al., 2018) and is the target gene of miR-761; its overexpression can restore the inhibitory effect of miR-761 on cell proliferation (Zhang et al., 2020a).

CircABCB10, also known as circRNA-0008717 (Tian et al., 2019), plays a key role in the progression of many tumors, such as GC (Zhang et al., 2021c), HCC (Fu et al., 2019), and NSCLC (Tian et al., 2019). Xian et al. (Xian et al., 2020) found that circABCB10 acts as a sponge for miR-326, regulating C-C motif chemokine ligand 5 (CCL5) expression in rectal cancer cells (Xian et al., 2020). The deletion of circABCB10 significantly promoted the accumulation of intracellular lipid ROS and Fe^{2+}. CircABCB10 regulates ferroptosis and apoptosis in rectal cancer cells through the miR-326/CCL5 axis (Xian et al., 2020).

Oncogenic neuroepithelial cell transforming 1(*NET1*), which lacks the first 145 amino acids, is present in the cytosol and contributes to the efficient activation of RhoA and the formation of actin stress fibers in many tumor cell types (Wei et al., 2017). Circ_0000745 was found to inhibit ferroptosis and promote the progression of acute lymphoblastic leukemia via the miR-494-3p/NET1 axis (Yang et al., 2022). Circ_0000745 interference has also been shown to inhibit the cell cycle and glycolysis and increase the levels of intracellular iron and lipid ROS induced by erastin, thus accelerating ferroptosis (Yang et al., 2022). Silencing miR-4943p, the target of circ_0000745, largely reduced the antitumor effect induced by silencing circ_0000745 (Yang et al., 2022). It was also found that overexpression of NET1, the target of miR-494-3p, could partially reverse the antitumor effect induced by miR-494-3p overexpression (Yang et al., 2022).

5-fluorouracil (5-FU) is a typical antitumor drug, and circPVT1 has been found to inhibit the chemoresistance of ESCC cells to 5-FU by influencing ferroptosis and the Wnt/b-catenin pathway via the miR-30a-5p/Frizzled3 (FZD3) axis (Yao et al., 2021). Knockdown of circPVT1 can inhibit the Wnt/b-catenin pathway in ESCC cells, significantly increase the expression levels of ROS and ferroptosis-associated parameters, and significantly reduce the expression of GSH, GPX4, and SLC7A11; these effects can be significantly reversed by the addition of an miR-30a-5p inhibitor and by FZD3 overexpression (Yao et al., 2021).

2.2 Ferroptosis-stimulating circRNAs

Seven circRNAs have been identified that can stimulate ferroptosis via various pathways and that play important regulatory roles in the progression of many diseases, including cervical cancer, acute cerebral infarction (ACI), traumatic brain injury (TBI), heart failure (HF), diabetic retinopathy, HCC, and GC (Liu et al., 2020; Zhu et al., 2021a; Zheng et al., 2021b; Wu et al., 2022a; Jiang et al., 2022; Mao and Liu, 2022; Ou et al., 2022) (Table 3). We classified these ferroptosis-stimulating circRNAs according to the mechanism by which they regulate ferroptosis (Figure 3).

2.2.1 CircRNAs that upregulate acyl-CoA synthetase long-chain family member 4

ACSL4 is an isozyme of the long-chain fatty-acid-coenzyme A ligase family and preferentially activates PUFAs for phospholipid biosynthesis and for fueling ferroptosis; hence, it is a typical marker of ferroptosis (Zhang et al., 2022b). CircLMO1 and circ_Carm1 have been reported to stimulate ferroptosis by upregulating ACSL4 (Figure 3) (Mao and Liu, 2022; Ou et al., 2022). CircLMO1, also known as hsa_circ_ 0021087, acts as a ceRNA and upregulates

TABLE 3 The regulatory roles circular RNAs (circRNAs) play in disease progression via stimulating ferroptosis.

Category	CircuRNA	Mechanistic target	Function	Disease	References
ACSL4 upregulation	CircLMO1	miR-4291/ACSL4	Promote ferroptosis and inhibit cervical cancer	Cervical cancer	Ou et al. (2022)
	Circ-Carm1	miR-3098-3p/ACSL4	Promote ferroptosis in acute cerebral infarction	Acute cerebral infarction	Mao and Liu (2022)
5-lipoxygenase upregulation	CircPtpn14	miR-351-5p/5-LOX	Promote ferroptosis and reverse the effects of melatonin	Traumatic brain injury	Wu et al. (2022a)
Ferritin heavy chain 1 upregulation	CircSnx12	miR-224-5p/FTH1	Promote ferroptosis and lead to cardiomyocyte death	Heart failure	Zheng et al. (2021b)
Cofilin-2 upregulation	Circ-PSEN1	miR-200b-3p/CFL2	Promote ferroptosis and involved in Diabetic retinopathy	Diabetic retinopathy	Zhu et al. (2021a)
Inhibition of ALKBH5-mediated autophagy inhibition	Hsa_circ_0008367	Interacts with ALKBH5	Promote ferroptosis and inhibit hepatocellular carcinoma	Hepatocellular carcinoma	Liu et al. (2020)
ZNRF3 upregulation	Circ_0000190	miR-382-5p/ZNRF3	Promote ferroptosis and inhibit gastric cancer	Gastric cancer	Jiang et al. (2022)

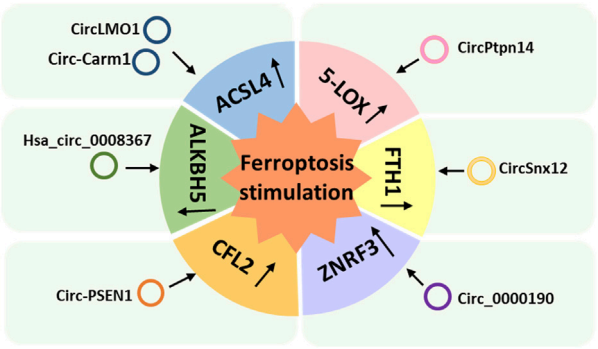

FIGURE 3
Ferroptosis-stimulating circular RNAs classified according to the mechanism used to regulate ferroptosis.

ACSL4 expression by adsorbing miR-4192, which decreases GSH and MDA and increases ROS levels, stimulating ferroptosis (Ou et al., 2022). Overexpression of circLMO1 inhibits cervical cancer growth and metastasis both *in vitro* and *in vivo*, whereas circLMO1 depletion promotes cervical cancer cell proliferation and invasion (Ou et al., 2022).

Circ-Carm1 is involved in the progression of ACI; it induces miR-3098-3p to upregulate the expression of ACSL4 *in vitro* (Mao and Liu, 2022). Knockdown of circ-Carm1 was shown to restore cell viability and inhibit ferroptosis; however, downregulation of miR-3098-3p could reverse the inhibitory effect of circ-Carm1 by promoting the secretion of Fe^{2+} and MDA (Mao and Liu, 2022). ACSL4 is the target of miR-3098-3p (Mao and Liu, 2022). Upregulation of ACSL4 inhibited the effect of miR-3098-3p on cell viability and ferroptosis (Mao and Liu, 2022).

2.2.2 A circRNA that upregulates 5-lipoxygenase

5-lipoxygenase (5-LOX), a member of the lipoxygenase gene family, plays an important role in producing toxic lipids; thus, induces ferroptosis. A study showed that melatonin reduced ferroptosis and ER stress in TBI by regulating the expression of ferroptosis-related 5-LOX through the circPtpn14/miR-351-5p/5-LOX signaling pathway (Wu et al., 2022a). Overexpression of circPtpn14 can partially abolish the inhibitory effect of melatonin on ferroptosis and reverse the anti-lipid peroxidation and anti-ER stress effects of melatonin (Wu et al., 2022a). The introduction of miR-351-5p (the target of circPtpn14) was found to reverse the 5-LOX upregulation and ER stress signaling activation caused by circPtpn14 overexpression alone and to rescue the decreased cell viability, inhibition of GPX activity, and increased blood-brain barrier permeability *in vitro* caused by circPtpn14 Wu et al. 2022a.

2.2.3 A circRNA that upregulates ferritin heavy chain 1

Zheng et al. (2021b) proposed that circSnx12 is involved in ferroptosis during HF by targeting the miR-224-5p/ferritin heavy chain 1 (FTH1) axis. FTH1 is a ferritin complex that catalyzes the conversion of Fe^{2+} into Fe^{3+} to protect the cell from oxidative damage (Zhang et al., 2017). CircSnx12 acts as a sponge for miR-224-5p, and *FTH1* is a target gene of miR-224-5p. Low expression of circSnx12 and high expression of miR-224-5p can downregulate FTH1 expression, which can directly induce ferroptosis in cardiomyocytes and eventually lead to cardiomyocyte death (Zheng et al., 2021b).

2.2.4 A circRNA that upregulates cofilin-2

Circ-PSEN1, also known as circ_0008521, regulates ferroptosis in retinal pigment epithelial cells of patients with diabetic retinopathy (DR) via the miR-200b-3p/cofilin-2 (CFL2) axis (Zhu et al., 2021a). CFL2 is a small actin-binding protein and a member of the AC group of proteins, which is predominantly expressed at sarcomeres in skeletal and cardiac muscles (Agrawal et al., 2012). Circ-PSEN1 acts as a sponge for miR-200b-3p, and *CFL2* is a target gene of miR-200b-3p (Zhu et al., 2021a). Knockdown of circ-PSEN1 was found to increase cell viability and inhibit ferroptosis, and CFL2 was found to abolish the inhibitory effect of miR-200b-3p

on ferroptosis (Zhu et al., 2021a). Overexpression of *CFL2* resulted in a decrease in GSH and an increase in MDA and ferrous iron, which decreased cell viability (Zhu et al., 2021a).

2.2.5 A circRNA that induces ferroptosis by interacting with AlkB homologue 5

Hsa_circ_0008367, also known as cIARS, is a promoter of ferroptosis in HCC cells treated with SF (Liu et al., 2020). SF has been approved by the US Food and Drug Administration for the treatment of HCC. However, its clinical application is limited by its poor water solubility and adverse side effects (Thapa et al., 2015). Nevertheless, cIARS expression was found to be significantly upregulated in SF-treated HCC cells, and cIARS positively regulates SF-induced ferroptosis by inhibiting AlkB homologue (ALKBH) 5-mediated autophagy inhibition (Liu et al., 2020). AlkB homologues are a specific family of demethylases that depend on Fe^{2+} and α-ketoglutarate to catalyze demethylation of different substrates (Xu et al., 2021). ALKBH5 is a negative regulator of autophagy in HCC cells, and cIARS can inhibit the activity of ALKBH5 in the regulation of autophagy (Liu et al., 2020).

2.2.6 A circRNA that induces ferroptosis by upregulating zinc and ring finger 3 (ZNRF3)

The tumor suppressor circ_0000190 sponges miR-382-5p and suppresses cell proliferation and motility and promotes cell death by targeting ZNRF3 in GC cells (Jiang et al., 2022). ZNRF3 is a transmembrane E3 ubiquitin ligase that inhibits endogenous Wnt-mediated activation of the β-catenin signaling pathway (Hao et al., 2012). Circ_0000190 induces apoptosis and ferroptosis in GC cells (Jiang et al., 2022). Overexpression of circ_0000190 was found to significantly increase the levels of iron and Fe^{2+} in GC cells treated with erastin or RSL3 (Jiang et al., 2022). Additionally, with the accumulation of circ_0000190, the production of MDA and lipid ROS was found to increase, and the activity of caspase-3 and the rate of apoptosis also increased significantly (Jiang et al., 2022). As a target of circ_0000190, miR-382-5p has a negative regulatory relationship with circ_0000190 (Jiang et al., 2022). Meanwhile, ZNRF3 is the target of miR-382-5p, and overexpression of it can also counteract the effect of miR-382-5p accumulation on GC cells (Jiang et al., 2022).

2.3 CircRNAs that are potential biomarkers of ferroptosis

Several studies have reported that a range of circRNAs exhibit abnormal expression levels in cells treated with ferroptosis inducers (Liu et al., 2020; Wang et al., 2022a; Hou et al., 2022; Mao and Liu, 2022). For example, compared with untreated HCC cell lines, 102 significantly upregulated circRNAs were identified in cells treated with the ferroptosis inducer SF (Liu et al., 2020). The circRNA that recorded the highest level of upregulation in that study, hsa_circ_0008367, has great potential as a biomarker of ferroptosis induced by SF. In another study, circ-Carm1 was highly expressed in HT22 cells after treatment with erastin, a ferroptosis activator (Mao and Liu, 2022). Yet another study found that erastin-treated HER2-positive breast cancer cells presented significantly high expression levels of circ-COL1A2, circ-SC5D, circ-MSH2, circ-ACRBP, and circ-DTL compared with untreated cells (Wang et al., 2022a). Furthermore, RNA sequencing was used to identify 17 downregulated and 18 upregulated circRNAs in human coronary artery endothelial cells after hydrogen peroxide treatment, and the five most upregulated circRNAs were hsa_circ_0001558, hsa_circ_0002665, hsa_circ_0000530, hsa_circ_0005871, and hsa_circ_0009353 (Hou et al., 2022).

CircRNAs that are highly expressed in cells after treatment with a ferroptosis inducer have potential as biomarkers of ferroptosis. Their identification also provides new avenues for the detection of ferroptosis *in vivo* or *in vitro*. However, further studies are needed to confirm the potential applications of ferroptosis-related circRNAs as biomarkers *in vivo* and *in vitro*.

3 Potential clinical applications of circRNAs in the diagnosis and treatment of ferroptosis-related diseases

3.1 Breast cancer

In 2020, breast cancer was the most commonly diagnosed cancer in women, and it is the fifth leading cause of cancer deaths worldwide (Sung et al., 2021). Early diagnosis and timely treatment are vital for improving the prognosis of breast cancer patients. Several studies have suggested that ferroptosis-related circRNAs can be used as biomarkers for the diagnosis, treatment, and prognosis of breast cancer (Bazhabayi et al., 2021; Zhang et al., 2021b; Wang et al., 2022a) (Table 4).

CircGFRA1 has great potential as a diagnostic marker and therapeutic target for HER2-positive breast cancer. The expression of circGFRA1 is significantly upregulated in HER2-positive breast cancer tissues compared with non-malignant tissues (Bazhabayi et al., 2021). Furthermore, deletion of circGFRA1 could delay tumor growth *in vivo* (Bazhabayi et al., 2021). Circ-BGN has potential as a therapeutic target and a prognostic biomarker for trastuzumab-resistant breast cancer (Wang et al., 2022a). The expression of circ-BGN is significantly increased in trastuzumab-resistant breast cancer cells and tissues compared to parental cells, and its increase is associated with poor overall survival (Wang et al., 2022a). In addition, circRHOT1 promotes tumor growth by inhibiting ferroptosis in breast cancer cells and is thus a promising therapeutic target for the development of future breast cancer treatment strategies (Zhang et al., 2021b).

3.2 Glioma

Glioma is the most common type of primary intracranial tumor in adults; it can occur anywhere in the central nervous system and is associated with high mortality and morbidity rates (Morgan, 2015). The identification of ferroptosis-related circRNAs is providing new directions for research on the diagnosis and treatment of gliomas (Table 4).

CircCDK14 resists ferroptosis and promotes tumor progression; thus, it may form part of a therapeutic strategy

TABLE 4 Potential therapeutic target and diagnostic and prognostic biomarkers of diseases.

Disease	Diagnostic biomarker	Therapeutic target	Prognostic biomarker
Breast cancer	CircGFRA1	CircGFRA1;	Circ-BGN
		Circ-BGN;	
		CircRHOT1	
Glioma	CircCDK14;	CircCDK14;	CircCDK14
	Circ-TTBK2	Circ-TTBK2	
Thyroid cancer	CircKIF4A;	CircKIF4A;	
	Circ_0067934	Circ_0067934;	
Gastric cancer	Circ_0000190	Circ_0000190	Circ_0000190
Lung cancer	CircDTL;	CircDTL;	CircP4HB;
	CircP4HB;	CircP4HB;	
	CircRNA_101093	CircRNA_101093	
Hepatocellular carcinoma	CircIL4R;	CircIL4R;	CircIL4R
	Circ0097009	Circ0097009;	
		Hsa_circ_0008367	
Cervical cancer	CircEPSTI1;	CircEPSTI1;	CircLMO1
	CircLMO1;	CircLMO1;	
Colorectal cancer	Circ_0007142;	Circ_0007142;	
	CircABCB10	CircABCB10	
Oral squamous cell carcinoma	CircFNDC3B	CircFNDC3B	CircFNDC3B
Esophageal cancer	CircPVT1	CircPVT1	
Acute lymphoblastic leukemia	Circ_0000745	Circ_0000745	
Myocardial infarction		CircRNA1615	
Heart failure	CircSnx12	CircSnx12	
Acute cerebral infarction	Circ-Carm1	Circ-Carm1	
Traumatic brain injury		CircPtpn14	
Polycystic ovary syndrome	CircRHBG	CircRHBG	
Diabetic nephropathy		Mmu_circRNA_0000309	
Diabetic retinopathy		Circ-PSEN	

and holds promise as a diagnostic and prognostic biomarker for glioma (Chen et al., 2022a). Glioma tissues have significantly higher levels of circCDK14 expression than normal tissues, and the expression level is inversely related to the overall survival time of glioma patients: the higher the circCDK14 expression, the worse the prognosis of the glioma patient. Grade III–IV glioma tissues have significantly higher levels of circCDK14 than grade I–II glioma tissues (Chen et al., 2022a). CircCDK14 silencing has been found to reduce the growth of tumors *in vivo* (Chen et al., 2022a). Furthermore, circ-TTBK2 is upregulated in glioma tissues (Zhang et al., 2020a), and it regulates glioma cell proliferation, invasion, and ferroptosis, which means that it could form the basis of a therapeutic strategy and potentially be used as a diagnostic biomarker for glioma as well. The deletion of circGFRA1 can also delay the growth of tumors *in vivo* (Zhang et al., 2020a).

3.3 Thyroid cancer

Thyroid cancer is the most common type of endocrine malignant cancer worldwide, and early diagnosis and treatment are critical for improving the prognosis of thyroid cancer patients (Schneider and Chen, 2013; Hao et al., 2021). The identification of ferroptosis-related circRNAs is providing new directions for the early diagnosis and treatment of thyroid cancer (Table 4).

CircKIF4A has been reported to inhibit ferroptosis and promote the malignant progression of papillary thyroid cancer; hence, this

circRNA could be targeted in a therapeutic strategy and/or potentially be used as a diagnostic biomarker for thyroid cancer (Chen et al., 2021b). CircKIF4A was found to be highly expressed in papillary thyroid cancer cells, and deletion of circKIF4A inhibited the growth of tumors *in vivo* (Chen et al., 2021b). Similarly, circ_0067934 is known to be elevated in thyroid cancer tissues and inhibits ferroptosis and promotes the progression of thyroid cancer, making it a candidate target and prognosis biomarker for thyroid cancer (Wang et al., 2019c; Wang et al., 2021a). Silencing of circ_0067934 was found to inhibit the growth of tumors *in vivo*, and elevated circ_0067934 was found to be associated with a poor prognosis in thyroid cancer (Wang et al., 2019c). Therefore, targeting circ_0067934 may be a potential therapeutic strategy for regulating ferroptosis in thyroid cancer cells.

3.4 Gastric cancer

GC is one of the most harmful cancers in world; it ranks fifth in terms of morbidity rate and fourth in terms of mortality rate (Karimi et al., 2014; Sung et al., 2021). Circ_0000190 induces apoptosis and ferroptosis in GC cells and thus has great potential as a diagnostic and prognostic marker for GC. The expression of circ_0000190 is significantly decreased in GC tissues, and low expression of circ_0000190 was found to be related to the advanced tumor, node, metastasis (TNM) stages of GC (Jiang et al., 2022). In one study, the area under a receiver operating characteristic (ROC) curve of circ_0000190 in GC tissues and plasma was reported to be up to 0.75 and 0.60, respectively (Chen et al., 2017b). Low expression of circ_0000190 is associated with poor survival in GC patients and can be used as a poor prognostic indicator for GC patients (Jiang et al., 2022). Circ_0000190 suppresses GC tumor growth *in vivo*, so restoration of circ_0000190 or ZNRF3 expression may be an effective strategy for GC treatment (Jiang et al., 2022).

3.5 Lung cancer

Lung cancer is the second most commonly diagnosed cancer and the leading cause of cancer deaths (Zappa and Mousa, 2016). NSCLC comprises 85% of all lung cancer cases and includes three types of cancer: squamous cell carcinoma, LUAD, and large-cell carcinoma (Zappa and Mousa, 2016).

As inhibitors of ferroptosis, circDTL and circP4HB may prove to be useful diagnostic biomarkers and therapeutic targets for NSCLC. The expression levels of circDTL and circP4HB are significantly increased in NSCLC tissues (Shanshan et al., 2021; Pan et al., 2022). Silencing of circDTL has been shown to improve the sensitivity of NSCLC to chemotherapeutic drugs and inhibit the growth of tumors *in vivo* (Shanshan et al., 2021), and overexpression of circP4HB has been shown to promote tumor growth *in vivo* (Pan et al., 2022). In addition, circP4HB expression is related to the prognosis of patients: the higher the expression of circP4HB, the lower the overall survival rate of patients (Pan et al., 2022).

CircRNA_101093 also has great potential as a diagnostic marker for LUAD. The expression of circRNA_101093 in LUAD tissues and in the plasma exosome of LUAD patients is significantly increased compared to that of healthy individuals, and reducing the exosome improved the outcome of a ferroptosis-based treatment in preclinical *in vivo* models (Zhang et al., 2022a). Improving the efficacy of ferroptosis by blocking exosomal biosynthesis may prove to be a useful strategy for developing ferroptosis-based therapy, and it may also provide a new direction for the future treatment of LUAD (Wang et al., 2022b).

3.6 Hepatocellular carcinoma

HCC is one of the most common cancers in the world. It can rapidly develop into a malignant form and has a low 5-year survival rate of <5% (Forner et al., 2012; Lu et al., 2016). Fortunately, circIL4R, an inhibitor of ferroptosis, has potential as a therapeutic target and as a diagnostic and prognostic biomarker for HCC. CircIL4R is significantly upregulated in HCC cells, and deletion of circIL4R has been shown to inhibit tumor growth *in vivo* (Xu et al., 2020). Also, circIL4R has clinical significance in the prognosis of HCC patients: compared with patients with lower expression of circIL4R, patients with higher expression of circIL4R tend to have a lower overall survival rate (Xu et al., 2020).

Circ0097009 is another potential diagnostic biomarker and therapeutic target for HCC. It has been shown that circ0097009 is significantly upregulated in HCC cells and that inhibition of circ0097009 suppresses tumor growth and reduces the number of lung metastases (Lyu et al., 2021). In addition, hsa_circ_0008367, a promoter of ferroptosis in HCC cells treated with SF, is another promising target for improving the cellular sensitivity to SF during HCC treatment (Liu et al., 2020).

3.7 Cervical cancer

Cervical cancer is the fourth most common type of malignant tumor in females, and the identification of ferroptosis-related circRNAs provides new opportunities for early diagnosis and treatment of cervical cancer (Li et al., 2021b).

CircEPSTI1, a ferroptosis inhibitor, is a potential therapeutic target and an ideal biomarker for monitoring and treating cervical cancer. CircEPSTI1 expression was found to be upregulated in cervical cancer cell lines, and circEPSTI1 knockdown was found to reduce tumor weight and tumor volume and thus affect the proliferation of cervical cancer cells *in vivo* (Wu et al., 2021).

The identification of circLMO1 as a ferroptosis promotor is also providing new opportunities to develop a therapeutic strategy and a diagnostic and prognostic biomarker for cervical cancer. CircLMO1 has been shown to be downregulated in cervical cancer tissues and to have a negative relationship with the international federation of gynecology and obstetrics (FIGO) stages of cervical cancer (Wu et al., 2021). In addition, overexpression of circLMO1 inhibits cervical cancer cell growth and metastasis both *in vitro* and *in vivo* (Wu et al., 2021).

3.8 Colorectal cancer

Globally, colorectal cancer is the third most commonly diagnosed malignancy and the second leading cause of death.

Colorectal cancer is a heterogeneous disease that exhibits distinct molecular characteristics in different patient populations (Pawlik, 2022).

Circ_0007142, as a ferroptosis inhibitor, is a promising therapeutic target and potential diagnostic biomarker for colorectal cancer. In colorectal cancer tissues, circ_0007142 has been found to be significantly upregulated, and silencing circ_0007142 has been shown to repress tumorigenesis *in vivo* (Wang et al., 2021b). In addition, higher circ_0007142 expression is associated with larger tumor size, higher TNM classification, distant metastasis, and lymph node metastasis in colorectal cancer patients (Wang et al., 2021b).

CircABCB10 also has great potential as a diagnostic biomarker and therapeutic target for rectal cancer. In a study that involved rectal cancer tissue, circABCB10 was found to be upregulated (Xian et al., 2020). Furthermore, knockdown of circABCB10 promoted ferroptosis and apoptosis in rectal cancer cells *in vitro* and inhibited tumor growth *in vivo* (Xian et al., 2020).

3.9 Oral squamous cell carcinoma

OSCC is a very aggressive form of cancer (most patients die within three to 5 years of diagnosis) that affects more than 275,000 people worldwide each year (Pena-Oyarzun et al., 2020). CircFNDC3B is an inhibitor of ferroptosis and promotes the malignant progression of OSCC by regulating the miR-520d-5p/SLC7A11 axis; hence, studies of this circRNA have revealed several potential therapeutic targets and diagnostic and prognostic markers for OSCC (Yang et al., 2021). The expression of both circFNDC3B and *SLC7A11* is enhanced in clinical OSCC tissues, whereas the expression of miR-520d-5p is reduced, and the silencing of circFNDC3B inhibits tumor growth *in vivo* (Yang et al., 2021). In addition, the expression of circFNDC3B in clinical OSCC tissues was found to be negatively correlated with the prognosis of OSCC patients (Yang et al., 2021).

3.10 Esophageal cancer

Esophageal cancer is the seventh most frequently diagnosed cancer, and due to its poor prognosis, it is the sixth leading cause of cancer-related death worldwide (Yu et al., 2018; Ajani et al., 2019). Therefore, the discovery of susceptibility genes or new biomarkers is of great significance for the treatment of patients.

CircPVT1 regulates the chemosensitivity of ESCC cells by influencing ferroptosis and the Wnt/b-catenin pathway via the miR-30a-5p/FZD3 axis (Yao et al., 2021). It has been found that circPVT1 expression is enhanced in clinical ESCC tissues (Zhong et al., 2019) and that knockdown of circPVT1 enhances the chemosensitivity of 5-FU-resistant ESCC cells *in vivo* and *in vitro* (Frazer and Anderson, 2014). Thus, circPVT1 is a potential biomarker for ESCC diagnosis and treatment.

3.11 Acute lymphoblastic leukemia

ALL occurs in both children and adults, and the prognosis is poor in elderly patients and those with relapsed or refractory ALL (Malard and Mohty, 2020). Therefore, there is a need to develop and implement new diagnostic and therapeutic strategies for this condition. As an inhibitor of ferroptosis that acts via the miR-494-3p/NET1 axis, circ_0000745 is a potential biomarker for the diagnosis and treatment of ALL (Yang et al., 2022). Circ_0000745 expression was found to be significantly upregulated in the peripheral blood samples of patients with acute lymphoblastic leukemia (Yang et al., 2022).

3.12 Myocardial infarction

MI is the main cause of sudden cardiac death (Feng and Feng, 2021). It has been found that ferroptosis inhibitors can reverse the effect of ferroptosis in an MI mouse model and improve the survival rate of myocardial cells (Li et al., 2021a). Hence, ferroptosis is a new potential target in the prevention and treatment of MI. CircRNA1615 prevents LRP6-mediated autophagy-related ferroptosis in cardiomyocytes via adsorption of miR-152-3p and controls the pathological process of MI (Li et al., 2021a), providing a potential target for the treatment of MI.

3.13 Heart failure

HF is a complex syndrome with a high mortality rate (Zhang et al., 2017). The prognosis of patients with HF is generally poor (Zhang et al., 2017). Therefore, it is necessary to identify and develop appropriate treatment strategies to improve the prognosis and quality of life of HF patients (Zhang et al., 2017). Using an HF mouse model, it has been shown that decreased expression of GPX4 and increased expression of NADPH oxidase 1 and ACSL4 are indicative of lipid peroxidation in cardiomyocytes (Zheng et al., 2021b). Hence, studying circSnx12, a ferroptosis-related circRNA present in cardiomyocytes, may provide new insights into HF and new directions for the development of diagnostic markers or treatments.

3.14 Acute cerebral infarction

ACI, also known as ischemic stroke, is the second leading cause of death globally (He et al., 2022). Timely diagnosis and treatment after disease onset, as well as evaluation of the treatment, is the key to saving patients who have experienced an ACI. Despite the progress that has been made in ACI diagnosis and treatment, there is still a need for new methods to increase diagnostic and therapeutic accuracy and efficiency.

Circ-Carm1, which is highly expressed in the serum of ACI patients, promotes the development of ACI via ferroptosis (Xiao et al., 2021). Thus, inhibition of ferroptosis and induction of a circ-Carm1 deficiency may be a promising approach for the prevention and treatment of ACI.

3.15 Traumatic brain injury

Globally, TBI is the leading cause of death, and more than 60 million people experience TBI each year (Dewan et al., 2019).

Moreover, TBI has been associated with a long-term risk of neurological disease (Turner et al., 2021). CircPtpn14 is a ferroptosis promoter and opposes the therapeutic effect that melatonin has in TBI cases via the miR-351-5p/5-LOX signaling pathway. Hence, circPtpn14 is a potential target in TBI treatment strategies.

3.16 Polycystic ovary syndrome

PCOS is one of the most common endocrine and metabolic disorders in premenopausal women. It is characterized by a series of signs and symptoms, namely, clinical or biochemical hyperandrogenism, oligoovulation, and polycystic ovarian morphology (Azziz, 2018; Escobar-Morreale, 2018). CircRHBG inhibits ferroptosis in PCOS cells and thus should be investigated as a potential diagnostic molecular marker and therapeutic target for PCOS (Zhang et al., 2021a). In the granulosa cells of PCOS patients, circRHBG expression was found to be significantly upregulated, and circRHBG knockdown can inhibit cell proliferation and decrease cell viability (Zhang et al., 2021a).

3.17 Diabetic nephropathy

About 40% of people with diabetes develop DN (Gross et al., 2005). Extensive innovations are urgently needed to improve the health outcomes of patients with DN. In terms of the use of circRNAs, the efficacy of exogenous mmu_circRNA_0000309 in combination with germacrone should be examined as a potential DN treatment. Given that germacrone inhibits ferroptosis-dependent mitochondrial damage and podocyte apoptosis by regulating the miR-188-3p/GPX4 axis in combination with exogenous mmu_circRNA_0000309, such studies would provide insight into the potential of this combination as a treatment for DN (Jin et al., 2022).

3.18 Diabetic retinopathy

More than 45% of people with type 2 diabetes have DR, which is the leading cause of blindness in adults (Calderon et al., 2017). In most cases, DR is not noticed until it irreversibly damages the eye and causes blurred vision and eventual blindness (Adki and Kulkarni, 2020). Therefore, early diagnosis is vital for the treatment of patients with DR. Circ-PSEN1 regulates ferroptosis in retinal pigment epithelial cells of patients with DR via the miR-200b-3p/CFL2 axis and thus may be a novel therapeutic target for DR.

3.19 The incorporation of circRNA data into machine learning models to identify therapeutic targets and diagnostic and prognostic biomarkers

Machine learning is an indispensable tool for identifying relevant biomarkers and classifying samples in the validation of biomarkers (Zhang et al., 2020b; Chen et al., 2022b). CircRNAs, as potential biomarkers of various diseases, have been widely incorporated into machine learning models for disease diagnosis, treatment, and prognosis prediction. As a result, machine learning classification models have identified several circRNAs as potential disease biomarkers, such as circERBB2 and circCHST12 for intracerebral hemorrhage diagnosis (Bai et al., 2022), circ-0080695 for liver cancer diagnosis (Zhu et al., 2021b), circ_0059706 for acute myeloid leukemia prognosis (Ma et al., 2022b), and hsa_circ_0007919, hsa_circ_0002419, and hsa_circ_0005521 for pulmonary tuberculosis diagnosis (Yuan et al., 2022).

In addition to conventional logistic regression, gradient boosting, deep neural networks, and K-means clustering algorithms, some useful new models and frameworks have also been used to predict circRNA–disease associations, such as SGANRDA (Wang et al., 2021c), MRLDC (Xiao et al., 2019) and MSFCNN (Fan et al., 2020), GCNCDA (Wang et al., 2020b), MDGF-MCEC (Wu et al., 2022b), CLCDA (Wang et al., 2023), and GBDTCDA (Lei and Fang, 2019).

In terms of the statistical tools used, ROC curve analysis has typically been used to examine the potential diagnostic value and investigate the specificity and sensitivity of the identified circRNAs as diagnostic biomarkers. Kaplan–Meier survival curve analysis has generally been used to examine the potential prognostic value of the identified circRNAs.

Using machine learning tools to further predict the associations among the abovementioned ferroptosis-related circRNAs, diseases, and ferroptosis may provide researchers in the field with an effective and efficient method for generating reliable classification criteria for the clinical application of these potential disease biomarkers.

4 Perspective

Ferroptosis is a lipid peroxidation-driven and iron-dependent form of cell death (Chen et al., 2021a). This unique form of cell death is regulated by a variety of cellular metabolic pathways, such as redox homeostasis, iron treatment, mitochondrial activity, and metabolism of amino acids, lipids, and sugars (Jiang et al., 2021). Many organ injuries and degenerative lesions are driven by ferroptosis (Jiang et al., 2021).

CircRNA is a newly identified class of non-coding single-stranded RNA without free 3′poly (A) tails or 5′caps (Ren et al., 2020). CircRNA is abundant in eukaryotes, conserved in evolution, highly stable, and tissue-specific; it also plays crucial roles in many tissue types (Xu et al., 2017; Kristensen et al., 2019; Chen, 2020). Due to their characteristics, circRNAs have great potential as biomarkers in tumor diagnosis and as targets in tumor treatment.

In this review, we have outlined the recent progress made in understanding the roles of circRNAs in the molecular mechanisms and regulatory networks of ferroptosis and the potential clinical applications of circRNAs in ferroptosis-related diseases. More than 20 circRNAs have been reported to inhibit ferroptosis by acting on GPX4, system Xc-, FSP1, lipid metabolism, and other pathways and play important regulatory roles in the progression of many diseases, including various cancers, diabetic nephropathy, polycystic ovary syndrome, and myocardial infarction. Seven circRNAs have been reported to stimulate ferroptosis and play important regulatory roles in the progression of cervical cancer, acute cerebral infarction, traumatic brain injury, diabetic retinopathy, hepatocellular carcinoma, and gastric cancer. These ferroptosis-related circRNAs

have great potential as biomarkers in the diagnosis, treatment, and prognosis of diseases. This review furthers our understanding of the roles of ferroptosis-related circRNAs and provides new perspectives on ferroptosis regulation and new directions for the diagnosis, treatment, and prognosis of ferroptosis-related diseases.

Notably, the research on circRNAs in ferroptosis is still incomplete. Most of the recently published studies on ferroptosis-related circRNAs were conducted with tumor tissues and cells; therefore, using blood, urine, or tear samples in future studies may provide new insights and ideas for further research. It is also likely that there are many more ferroptosis-related circRNAs that have not yet been discovered. The circRNAs that are found to be biomarkers of ferroptosis may provide new perspectives for the detection of ferroptosis. However, the notion that ferroptosis-related circRNAs can be used as biomarkers of ferroptosis must also be further interrogated.

The ultimate goal of conducting all the studies described in this review is to improve clinical disease diagnosis and treatment. However, most of the studies have been conducted under experimental conditions. Thus, there is a need to undertake a large number of clinical studies and experiments to ensure the safety and efficacy of the tested molecules and methods.

Although there are still many obstacles hindering our efforts to explore the potential of ferroptosis-related circRNAs in the diagnosis and treatment of diseases, we believe that understanding the interactions between circRNAs and ferroptosis will help us to address these barriers. Based on the progress made to date, it is clear that circRNAs related to ferroptosis will be widely used in the diagnosis, treatment, and prognosis of diseases and in research on drug resistance in the future. These advances will greatly reduce mortality rates and improve cure rates, alleviating the pain of patients and bringing happiness to their lives.

Author contributions

FL and XDH performed the literature search wrote and revised the paper. PFL participated in the revision of the paper. 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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Circular RNAs in gynecologic cancers: mechanisms and implications for chemotherapy resistance

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Chemotherapy resistance remains a major challenge in the treatment of gynecologic malignancies. Increasing evidence suggests that circular RNAs (circRNAs) play a significant role in conferring chemoresistance in these cancers. In this review, we summarize the current understanding of the mechanisms by which circRNAs regulate chemotherapy sensitivity and resistance in gynecologic malignancies. We also discuss the potential clinical implications of these findings and highlight areas for future research. CircRNAs are a novel class of RNA molecules that are characterized by their unique circular structure, which confers increased stability and resistance to degradation by exonucleases. Recent studies have shown that circRNAs can act as miRNA sponges, sequestering miRNAs and preventing them from binding to their target mRNAs. This can lead to upregulation of genes involved in drug resistance pathways, ultimately resulting in decreased sensitivity to chemotherapy. We discuss several specific examples of circRNAs that have been implicated in chemoresistance in gynecologic cancers, including cervical cancer, ovarian cancer, and endometrial cancer. We also highlight the potential clinical applications of circRNA-based biomarkers for predicting chemotherapy response and guiding treatment decisions. Overall, this review provides a comprehensive overview of the current state of knowledge regarding the role of circRNAs in chemotherapy resistance in gynecologic malignancies. By elucidating the underlying mechanisms by which circRNAs regulate drug sensitivity, this work has important implications for improving patient outcomes and developing more effective therapeutic strategies for these challenging cancers.

KEYWORDS

circular RNAs (circRNAs), gynecologic cancers, chemoresistance, malignant cancer, drug rsesistance

Abbreviations: AUC, area under the ROC curve; Beclin1 and p62, autophagy-related genes; CEBPG, CCAAT enhancer binding protein γ; CC, cervical cancer; CDDP, Cisplatin; ciRNA, intron-derived circRNA; circRNA, Circular RNA; CSCC, cervical squamous cell carcinoma; CSCs, tumor stem cells; EC, Endometrial cancer; EC, endometrial cancer; EcirRNA, exon-derived circRNA; ElciRNA, exon- and intron-derived circRNA; FMNL3, formin like 3; HPVhuman, papillomavirus; IRES, Internal Ribosome Entry Site; miRNAs, microRNAs; MPA, Medroxyprogesterone acetate; ncRNAs, non-coding RNAs; OC, Ovarian cancer; ORF, open reading frame; Pol II, polymerase II; pre-mRNAs, precursor mRNAs; PTX, Paclitaxel.

1 Introduction

The increasing incidence of gynecological tumors poses a significant concern, particularly in the cases of cervical cancer (CC), ovarian cancer (OC) and endometrial cancer (EC), which are considered widespread malignancies and gravely threaten women's health (Diaz-Padilla et al., 2012; Lõhmussaar et al., 2020). Malignant gynecologic cancer is a significant contributor to the global burden of disease, accounting for three out of every ten deaths. As expected, cancer exerts a substantial impact on the economy, with the direct costs of cancer-related medical care in Australia amounting to approximately 0.5% of the country's gross domestic product (GDP) (Goldsbury et al., 2018). Besides, the economic consequences of premature loss of life results in lost productivity valued at over $4 billion annually in Australia (Carter et al., 2016). Globally, cervical cancer is the fourth most prevalent malignancy, with an annual mortality of 270,000 individuals. This disease primarily impacts younger women, and its highest burden is observed in low- and middle-income countries, where the mortality rate is 18 times greater than in high-income countries (Sung et al., 2021). Ovarian cancer, on the other hand, is the seventh most common cancer among women worldwide, accounting for 3.3% of all female cancers. It is also the leading cause of death from gynecologic malignancies and the fifth highest among all cancers affecting women (Passarello et al., 2019). Variation in the incidence and mortality rates of ovarian cancer are observed worldwide, with the highest rates noted in developed countries such as Europe and North America (paragraph 3). Despite advancements in diagnosis and treatment, ovarian cancer continues to have a high case-fatality rate, with a 5-year survival rate of only approximately 30% for advanced-stage ovarian cancer (Webb and Jordan, 2017). Among these CC is primarily caused by persistent human papillomavirus (HPV) infection, with HPV types 16 and 18 responsible for 71% of cases worldwide (Choi et al., 2023; Reich and Regauer, 2023). Prevention and treatment of high-risk HPV cervical infections remain the main approach in combating CC, with the introduction of CC vaccines being a major development in recent years, together with screening technologies (Rahangdale et al., 2022; Rimel et al., 2022; Sivars et al., 2022; Sun et al., 2022; Sabeena, 2023). OC, as the seventh most commonly diagnosed female cancer worldwide, poses as the fifth leading cause of cancer-related deaths in women and the most lethal of all gynecological malignancies (Chen et al., 2023; Ye et al., 2023). Relatively few conventional screening tools exist for early detection, resulting in over 70% of the cases being diagnosed at advanced stages (Armbrister et al., 2023; Brown et al., 2023; Terp et al., 2023). The three main types of OC are epithelial, germ cell, and interstitial gonadal carcinoma, with epithelial carcinomas constituting the majority at about 90% of all OCs (Devlin and Miller, 2023; Zwimpfer et al., 2023). EC, on the other hand, is one of the most widespread malignancies occurring in the female reproductive tract, with inchoate phases typically being asymptomatic, while terminal phases feature symptoms akin to those of OC, including pelvic and abdominal pain, anemia, abdominal distention, wasting, and cachexia (Gordhandas et al., 2023). The current understanding of EC oncogenesis is still incipient, with most cases being sporadic and the few familial inherited cases resulting from mismatch repair protein gene mutations (Kalampokas et al., 2022; Tronconi et al., 2022). Predisposing risk factors for EC include obesity, infertility, and irregular menstrual cycles (Chiu et al., 2022; Jamieson and McAlpine, 2023). Furthermore, overexposure to endogenous or exogenous estrogens augments the risk of both endometrial hyperplasia and carcinogenesis, with conditions such as polycystic ovary syndrome, estrogen-secreting tumors, or the medical use of estrogen replacement therapy with inadequate progestin antagonism being implicated (Gjorgoska and Rizner, 2022; Yu et al., 2022). The tumor microenvironment plays a crucial role in modulating the malignant phenotype of various gynecological cancers, including enhancing their radiotherapy- and chemotherapy-tolerant properties, as well as their proliferative and metastatic potentials. Figure 1 illustrates the interaction between immune and cancer cells in the microenvironment of gynecological cancers. The currently available treatment of gynecologic tumors entails surgery, radiotherapy, and chemotherapy, there is a pressing need to explore alternative modalities that may yield more effective outcomes in the treatment of gynecologic tumors.

Significant advances in medical science have greatly improved anti-tumor therapy. However, drug resistance of tumor cells remains a major factor leading to high mortality rates (Gjorgoska and Rizner, 2022; Ming et al., 2023). Chemotherapy drug-sensitive tumors are present in only about 50% of cases, whereas acquired drug resistance is pervasive during treatment and a major contributor to chemotherapy failure (Liu et al., 2022a; Pang et al., 2023). Additionally, natural resistance of some tumor cells to multiple chemotherapeutic agents is prevalent, and drug resistance is estimated in no less than 90% of cancer deaths (Li et al., 2023a). Figure 2 describes the mechanisms of chemotherapeutic drug resistance in cancer cells. Although the mechanisms of drug resistance in gynecologic malignancies remain unknown, numerous studies have indicated a strong correlation between the development of gynecologic drug resistance and enhanced proliferation and migration of tumor cells, suppression of apoptosis, and immunosuppression (Alatise et al., 2022). Increasing evidence suggests that drug sensitivity in ovarian cancer (OC) is significantly influenced by non-coding RNAs (ncRNAs), tumor stem cells (CSCs), immune mechanisms, autophagy, and tumor heterogeneity (Cen et al., 2023; Tau and Miller, 2023). Additionally, it is evident that drug resistance in tumor cells is not solely dependent upon the sensitivity of individual tumor cells, but is tightly linked to the microenvironment in which the tumor cells reside (Li et al., 2022a; Parma et al., 2022). Further, the activation of given signaling pathways can regulate cell growth and differentiation, suppress apoptosis, and contribute to the development of drug resistance in tumor cells (Wang et al., 2022a; Yang et al., 2022a). The standard course of treatment for cervical, ovarian, and endometrial cancers is multifactorial and dependent upon several clinical criteria, including the stage, grade, and histologic type of the tumor, as well as the individual's overall health and medical preferences. Treatment modalities generally entail surgical intervention, radiation therapy, and chemotherapy, typically administered in varying combinations. Surgery and radiation therapy represent the primary therapeutic options for cervical cancer, and chemotherapy may be given concurrently with radiation. Drug regimens currently recommended for cervical cancer may consist

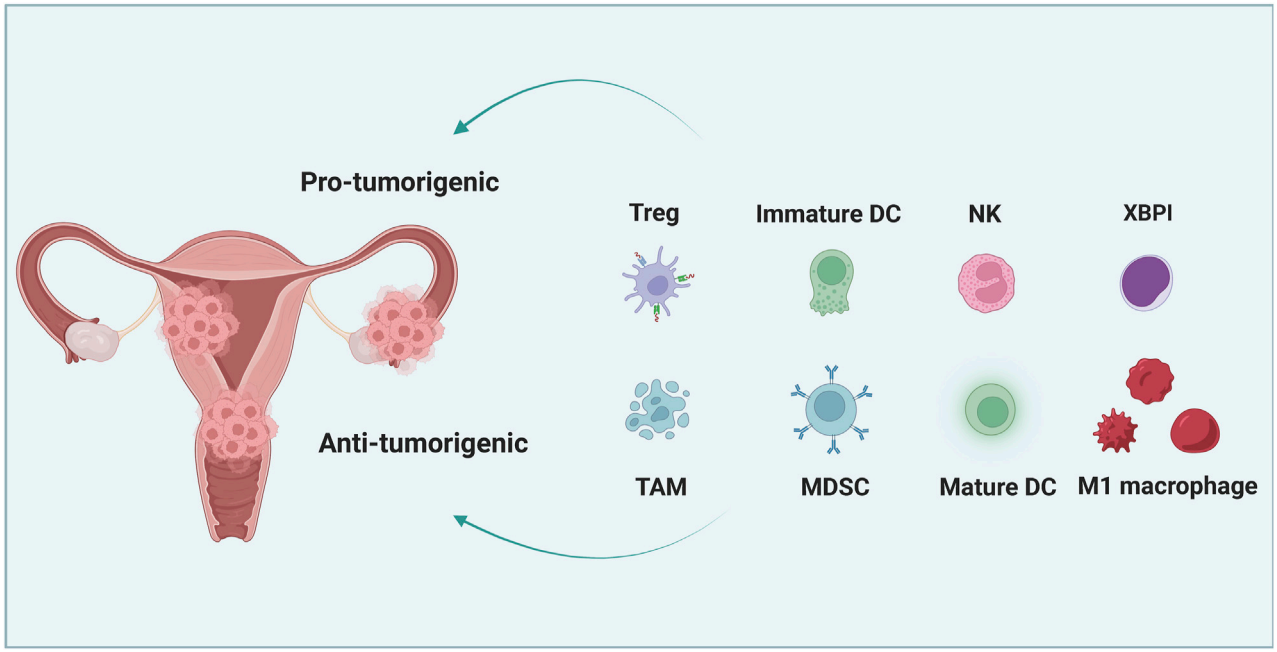

FIGURE 1
The interaction between cancer cells and immune cells in the microenvironment of gynecological cancer. Immature dendritic cells (DC), tumor-associated macrophages (TAM), regulatory T cells (Tregs) and myelogenous inhibitory cells (MDSCs) can promote the immune resistance and therapeutic resistance of gynecological cancer cells. However, mature DC, M1 macrophages, natural killer (NK) cells and cytotoxic T lymphocytes (CTL) can significantly inhibit tumor growth and increase the susceptibility of tumor cells to treatment.

of cisplatin, paclitaxel, and carboplatin, among others. Ovarian cancer typically requires debulking surgery followed by chemotherapy. Chemotherapy for ovarian cancer generally involves a combination of agents, such as carboplatin and paclitaxel, delivered via intravenous or intraperitoneal routes. In the case of endometrial cancer, surgical resection is the mainstay of management, with chemotherapy reserved for advanced or recurrent disease. Standard chemotherapy regimens for endometrial cancer may incorporate drugs such as paclitaxel and carboplatin (Armstrong et al., 2021). It is essential to recognize that these treatments are not prescriptive and must be individualized based on patient and disease-specific features. Collaboration between the patient, medical oncologist, and gynecologic oncologist is crucial for determining appropriate therapeutic interventions. The choice of chemotherapy agents is ultimately influenced by the discretion of the treating physician, patient preference, and individual case intricacies.

The circRNAs are a type of small RNA molecules characterized by their closed-loop structure that is formed by the exon skipping or reverse splicing of pre-mRNA transcripts, rendering them resistant to enzymatic degradation and thus highly stable within living organisms (Lee et al., 2022; Ren et al., 2022). Initially, circRNAs were deemed to be non-functional within the human body; however, the advent of high-throughput sequencing techniques has identified their extensive presence in various organs and tissues of the body, where they play crucial biological roles (Yuan et al., 2022; Zhou et al., 2022). Multiple studies have proposed that circRNAs contribute to essential physiological processes, such as tumorigenesis and development, and are inextricably linked to cancer cell proliferation, invasiveness, and metastasis (Chen et al., 2022a; Kim et al., 2023). More recent studies have demonstrated that circRNAs can modulate and influence drug resistance in different ways. For example, CircRNA_0067717 has been shown to facilitate paclitaxel (PTX) resistance in nasopharyngeal carcinoma, acting as a scaffold for TRIM41 and p53 (Cheng et al., 2023), whereas CircPOFUT1 enhances malignant traits and chemoresistance related to autophagy by binding to miR-488-3p and activating the PLAG1-ATG12 axis in cancer cells (Luo et al., 2023). CircPTK2 promotes epithelial-mesenchymal transition (EMT)-mediated bladder cancer metastasis and gemcitabine resistance by regulating the PABPC1/SETDB1 axis (Meng et al., 2023). To provide new insights into the management of drug resistance in gynecologic malignancies, this paper reviews the role and underlying mechanisms of circRNAs in chemoresistance in such cancers. CircRNAs were first detected in viruses in the 1970s, and at the time, due to limited understanding of circRNAs, they were thought to be splicing errors. The biogenesis and functions of circRNAs are demonstrated in Figure 3.

1.1 The biogenesis of circRNAs

CircRNAs are a unique class of RNA molecules generated from mRNA splicing events. Depending on their origin, CircRNAs are classified into three categories: exon-derived CircRNA (EcirRNA), intron-derived CircRNA (ciRNA), and exon- and intron-derived CircRNA (EIciRNA) (Caba et al., 2021; Huang and Zhu, 2021; Chen et al., 2022a; Liu et al., 2022b; Gao et al., 2022; Nielsen et al., 2022).

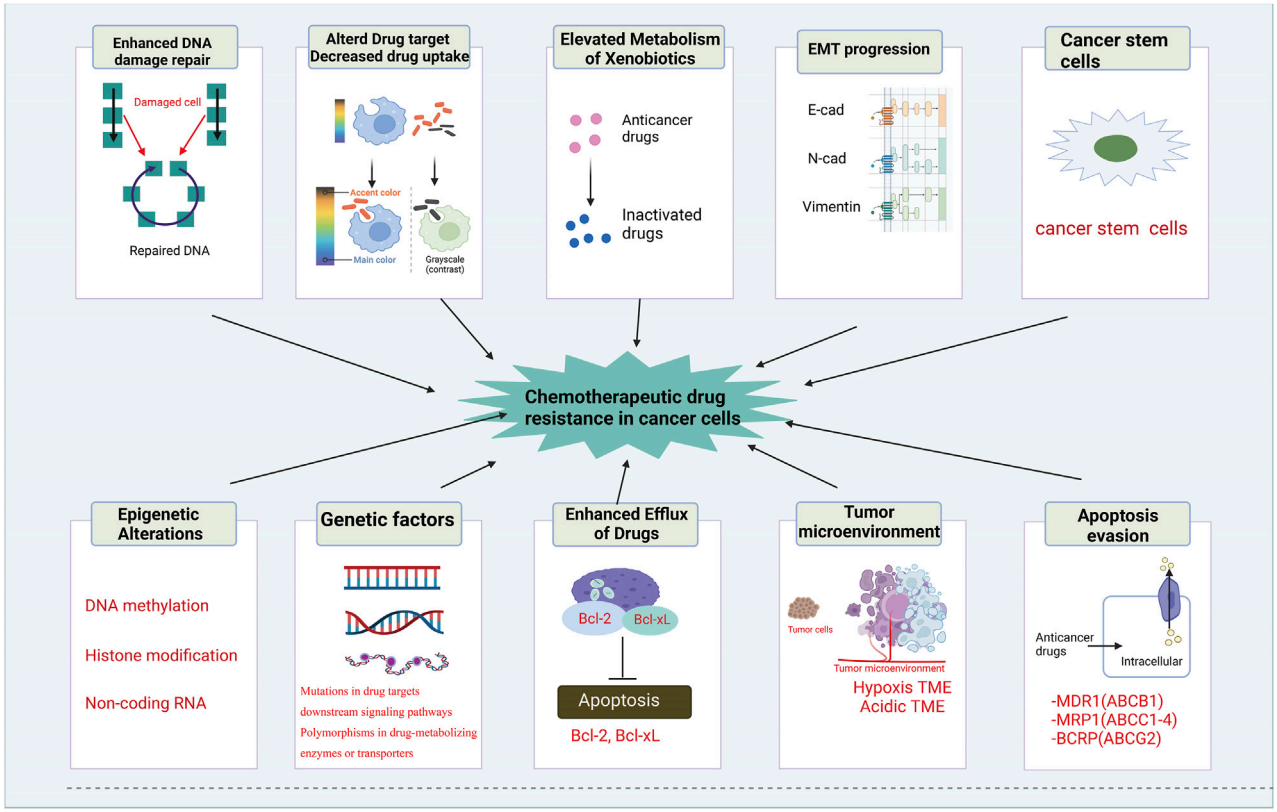

FIGURE 2
Mechanisms of chemotherapeutic drug resistance in cancer cells. The mechanisms of chemotherapeutic drug resistance in cancer cells includes enhanced DNA damage repair, altered drug target and decreased drug uptake, elevated metabolism of xenobiotics, EMT progression, cancer stem cells, epigenetic alterations, genetic factors, enhanced efflux of drugs, tumor environment and apoptosis evasion.

Intron removal, a necessary step in mRNA splicing, leads to the formation of multiple mature mRNAs, each containing a unique combination of exons. The splicing complex mediates the nucleophilic site by using a branching site 2′-OH adenosine residues located between 20–50 nucleotides, leading to the formation of a lariat structure. This process involves the 3′ end of the upstream exon engaging in a nucleophilic attack on the 5′ splice site, resulting in the fusion of two exonic regions by breaking the phosphodiester backbone of the RNA molecule. In contrast to conventional splicing, the circularization of RNA can result from a process known as trans-splicing, though the exact mechanism is still under investigation. Two hypotheses have been proposed to explain the formation of CircRNAs by trans-splicing. The exon-skipping hypothesis suggests that two joining events are required to form the circular RNA structure, while in the direct trans-splicing hypothesis only one splicing event is involved in joining the 2′-OH branching point and the donor site of the intron[119]. The free 3′OH of the exon is then hypothesized to be responsible for the looping process leading to the formation of a closed looped structure.

1.2 Biological functions of circRNAs

CircRNAs refer to a class of RNA molecules that are generated through non-canonical splicing such as back-splicing or exon skipping of pre-mRNAs. These processes result in the formation of a continuous closed loop structure known as back-splicing, which is primarily induced via the junction of a downstream 3′ splice site with an upstream 5′ splice site (head-to-tail splicing) resulting in resistance of these molecules to exonucleolytic degradation by RNase R. Exon skipping can also lead to a restricted lariat structure promoting cyclization. Direct back-splicing often results in the generation of exonic circRNA (ecircRNA), while exon-skipping generates intronic circRNA. Currently, there are four categories of circRNAs, namely, ecircRNAs, circular intronic RNAs (ciRNAs), exon–intron circRNAs (EIciRNAs), and tRNA intronic circular RNAs (tricRNAs). ecircRNAs constitute over 80% of the identified circRNAs and are primarily located in the cytoplasm. ciRNAs and EIciRNAs, on the other hand, are predominantly located in the nucleus, suggesting a potential role in the regulation of gene transcription. Recently, a novel type of circular transcript called the read-through circRNA has been identified, which is formed through back-splicing of exons flanking a gene (Geng et al., 2020). According to recent studies, circRNAs are involved in pathophysiological processes *in vivo* through various mechanisms (Wang et al., 2022b). One of their more pervasive functions is that they can competitively bind microRNAs (miRNAs) and thus affect pathological processes such as tumor proliferation, aggression, and metastasis (Cheng et al., 2021; Zhou et al., 2021) (Zhang et al., 2021). Additionally, circRNAs can sponge-bind proteins, which may alter the transcription of parental genes, change the subcellular localization of

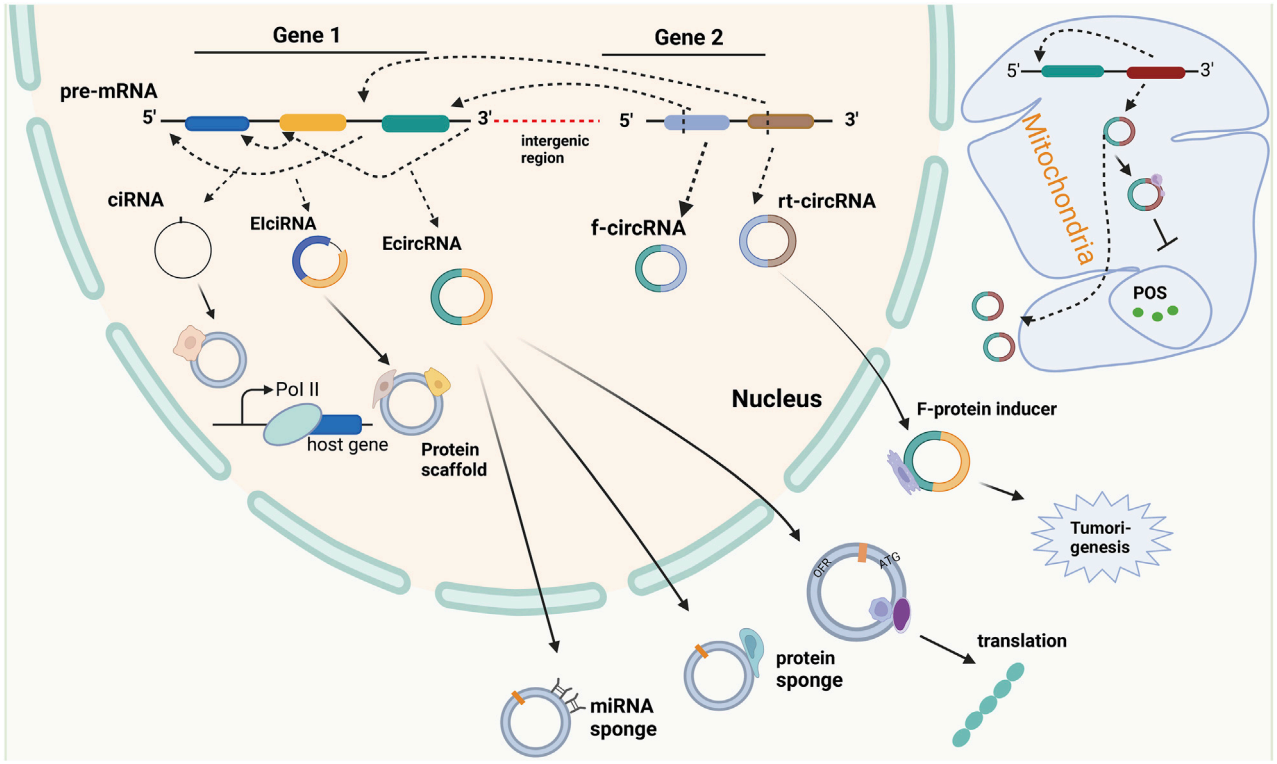

FIGURE 3
The biogenesis and function of circular RNA. Circular RNA (circRNA) is the product of reverse splicing of pre-messenger RNA (pre-mRNA), mainly including intron circRNA (ciRNA) from intron, exon and intron cirRNA (ElciRNA) from exon covering intron region, and exon circRNA (EcircRNA) from exon gene in nucleus and mitochondrial genome (MecciRNA). In addition, it also includes reading circRNA (rt-cicRNA) from the exon between adjacent genes on the same chain, and fusing circRNA (f-circRNA) from the exon between two distant genes. CircRNAs from different sources have different functions. CiRNA can interact with small ribonucleoprotein (snRNP) to improve the transcription rate of its host gene. ElciRNA can be used as a scaffold for recruiting functional molecules. ECIRcRNA can combine microRNAs and proteins to regulate the expression of downstream genes, and can also be used as a template for translation into new proteins and output to the cytoplasm. In addition, MecciRNA may be related to the inhibition of ROS. The combination of F-circRNA and fusion protein promotes tumorigenesis.

proteins, and enable the interaction of multiple proteins among other effects (Zhou et al., 2020; Wu et al., 2021a; Das et al., 2021; Xu et al., 2022a). Interestingly, some circRNAs possess Internal Ribosome Entry Site (IRES) activity and open reading frame (ORF), which enable their translation into proteins *in vitro* or in cells (Sinha et al., 2021; Wen et al., 2022), (Yang et al., 2022a). Moreover, studies have demonstrated that elciRNA and ciRNA can adjust and control the transcriptional activity of RNA polymerase II (Pol II) and other transcription factors, which in turn regulate the expression of parental genes (Kim et al., 2021; Shao et al., 2021; Tang and Lv, 2021). Of course, additional regulatory mechanisms for circrna may require further investigation.

2 Circular RNAs and gynecologic cancer chemoresistance

2.1 CircRNA regulates cisplatin resistance in gynecologic cancer cells

Cisplatin (CDDP) is a commonly employed first-line treatment for gynecologic cancer. However, despite its effectiveness over years, repeated rejection of cis-CDDP frequently results in the death of these patients. Initially, CDDP was believed to interfere with DNA repair mechanisms by cross-linking with purine bases on DNA, leading to DNA damage and triggering apoptosis in cancer cells (Barman et al., 2023; Li et al., 2023b; Wang et al., 2023). Recent studies have revealed that CDDP also has harmful effects on various elements of the cell membrane and cytoplasm. Nonetheless, prolonged CDDP exposure leads tumor cells to activate a variety of mechanisms to obstruct cisplatin, which is manifested at the molecular, organelle, and cellular levels (Lugones et al., 2022; Romani, 2022; Tang et al., 2023). These mechanisms involve reducing platinum compound accumulation through active efflux/isolation or suppression of endocytosis; increasing oncogene mutagenesis; detoxifying through metallothionein, GSH conjugates, and other antioxidants; modulating DNA methylation status; increasing DNA-damage repair levels; altering protein post-translational modifications; over-expressing chaperone molecules; reinforcing compensatory signaling communication between organelles; suppressing apoptotic pathways; and activating the EMT pathway, among others (Ali et al., 2022; Domingo et al., 2022; Tsvetkova and Ivanova, 2022). Numerous studies have now demonstrated that certain circular RNAs (circRNAs) are also involved in drug resistance of gynecologic cancer cells to CDDP

TABLE 1 Potential roles of circRNAs in the cisplatin-resistance of gynecologic cancer.

Cancer	CircRNAs	Expression	Biological function	Targets	References
Cervical cancer	CircEPSTI1	Up	Promote cell proliferation and cisplatin resistance	miR-370-3p-MSH2	Wu et al. (2022)
	CircMTO1	Up	Promote cisplatin resistance and malignant progression	miR-6893/S100A1/Beclin1/p62	Chen et al. (2019b)
	CircARHGAP5	Down	Inhibit cell proliferation and cisplatin resistance, and promote cell apoptosis	AUF1/BIM	Deng et al. (2023)
	Hsa_circ_0023404	Up	Promote cell invasion, lymphatic formation and cisplatin resistance	miR-5047/VEGFA	Guo et al. (2019a)
				Beclin1/p62	
	Circ_0074269	Up	Promote cisplatin resistance and malignant progression	miR-485-5p/TUFT1	Chen et al. (2022b)
Ovarian cancer	Circ-Cdr1as	Down	Inhibit cell proliferation and cisplatin resistance, and promote cell apoptosis	miR-1270/SCAI	Zhao et al. (2019)
	CircHIPK2	Up	Promote cisplatin resistance and malignant progression	miR-338-3p/CHTOP	Cao et al. (2021)
	Circ-Cdr1as	Up	Promote cisplatin resistance and malignant progression	miR-1299/PPP1R12B	Wu et al. (2021b)
	circ_0063804	Up	Promote cell proliferation and cisplatin resistance, and inhibit cell apoptosis	miR-1276/CLU	You et al. (2022)
	Circ-TYMP1	Up	Promote cell proliferation, invasion and cisplatin resistance	miR-182A-3p/TGF1B/Smad2/3	Rao et al. (2022)
	Circ_0026123	Up	Promote cisplatin resistance and malignant progression	miR-543/RAB1A	Wei et al. (2022)
	Circ-PIP5K1A	Up	Promote cisplatin resistance and malignant progression	miR-942-5p/NFIB	Sheng and Wang (2023)
	CircITGB6	Up	Promote cisplatin resistance and induce polarization of TAMs towards M2 phenotype	IGF2BP2/FGF9	Li et al. (2022b)
	CircPBX3	Up	Promote cell colony formation and tumor growth and reduce cell apoptosis under cisplatin treatment	IGF2BP2/ATP7A	Fu et al. (2022)
	CircFoxp1	Up	Promote cell proliferation and cisplatin resistance	miR-22-miR-150-3p/CEBPG-FMNL3	Luo and Gui (2020)

(Table 1). In particular, circEPSTI1 expression was significantly increased in both tissues and cells of cervical cancer (CC). Suppression of circEPSTI1 decreased the proliferative capability of CC cells and increased the sensitivity to cisplatin. Mechanistic experiments revealed that circEPSTI1 contributes to the malignant progression of CC by modulating the miR-370-3p-MSH2 axis, thereby leading to cisplatin resistance in CC (Wu et al., 2022). Similarly, studies have reported that the expression of circ-Cdr1as is significantly decreased in CDDP-resistant ovarian cancer (OC) tissues and cells. Overexpression of Cdr1as suppresses OC cell proliferation and promotes CDDP-induced apoptosis by modulating the miR-1270/SCAI signaling pathway (Zhao et al., 2019). Also, circHIPK2 expression was identified to be increased in CDDP-resistant OC tissues and cells. Suppression of circHIPK2 significantly suppressed the proliferation, cell cycle, migration, and invasion of SKOV3/CDDP and A2780/CDDP cells and promoted apoptosis. Mechanistic experiments showed that silencing circHIPK2 can regulate the miR-338-3p/CHTOP axis to suppress DDP resistance and malignant progression of OC (Cao et al., 2021). Compared to CDDP-sensitive OC cells, CDR1as expression was significantly reduced in CDDP-resistant OC cells. The downregulated expression of CDR1as suppressed OC tumorigenesis and predicted CDDP resistance and a poor prognosis in OC patients. Additionally, tumor xenograft data indicated that knockdown of CDR1as increased tumor growth and enhanced cell resistance to CDDP treatment (Wu et al., 2021b). CDR1as, also known as ciRS-7 (circular RNA sponge for miR-7), is a circular RNA molecule that has been shown to be involved in the pathogenesis of various cancers, including gynecologic malignancies such as endometrial cancer and ovarian cancer. CDR1as, also known as ciRS-7 (circular RNA sponge for miR-7), is a circular RNA molecule that has been shown to be involved in the pathogenesis of various cancers, including gynecologic malignancies such as cervical cancer and ovarian cancer. CDR1as upregulation was observed after TGF-β activation, which was positively correlated with lymph node metastasis and reduced survival duration, as evidenced by *in situ* hybridization. Overexpression of CDR1as was found to enhance cervical cancer metastasis both *in vitro* and *in vivo*. Furthermore, CDR1as was found to promote the orchestration of IGF2BP1 on the SLUG mRNA and to maintain its stability, thereby contributing to

cervical cancer metastasis. Silencing IGF2BP1 hindered CDR1as-mediated metastasis in cervical cancer. Finally, it was found that CDR1as could activate TGF-β signaling factors, including P-Smad2 and P-Smad3, which promote EMT, demonstrating its potential role in EMT-related pathological processes (Zhong et al., 2023). The expression of CDR1as in ovarian tissues showed a significant difference between ovarian cancer patients and non-cancer controls, where the former exhibited lower levels of CDR1as expression. Overexpression of CDR1as significantly impeded the proliferation, invasion, and migration of ovarian cancer cells. In contrast, knockdown of CDR1as resulted in increased expression of miR-135b-5p and decreased levels of HIF1AN expression, ultimately elevating the proliferative potential of ovarian cancer cells (Chen et al., 2019a). Results of mechanistic experiments showed that CDR1as contributes to malignant progression of OC and CDDP resistance by regulating the miR-1299/PPP1R12B axis (Wu et al., 2021b). Additionally, it was found that circ_0063804 expression was remarkably upregulated in OC patients and predicts a poor prognosis. The overexpression of circ_0063804 in OC cells heightened resistance to cisplatin and decreased apoptosis. Results indicated that circ_0063804 can increase clusterin expression and thus lead to malignant phenotype and resistance to cisplatin in OC by sponging miR-1276 (You et al., 2022). Similarly, TYMP1 expression was also remarkably increased in OC tissues. Circ-TYMP1 functions as a sponge for miR-182A-3p and thus improves TGF1B expression, promoting proliferation, migration, aggression, and cisplatin resistance in A2780-Res cells and reducing Smad2/3 phosphorylation (Rao et al., 2022). Furthermore, circ_0026123 expression was increased significantly in both CDDP-resistant OC tissues and cells. Inhibition of circ_0026123 led to decreased cell growth, angiogenesis, invasion, and migration. It significantly increased the sensitivity of CDDP-resistive OC cells to CDDP, showing circ_0026123 could act as a sponge for miR-543 and thus increase the expression of RAB1A, thereby contributing to CDDP resistance and tumorigenesis in OC (Wei et al., 2022). Lastly, circ-PIP5K1A was highly expressed in CDDP-resistant OC tissues and cells. Suppression of circ-PIP5K1A restrained proliferation, migration, and invasion of CDDP-resistant OC cells, increased apoptosis, and sensitivity to CDDP. Mechanistically, circ-PIP5K1A could serve as a sponge for miR-942-5p and thus facilitate NFIB expression (Sheng and Wang, 2023). Sun et al. (2019), demonstrated a significant association between circPIP5K1A and the progression of ovarian cancer through its interaction with the miR-661/IGFBP5 axis. Silencing circPIP5K1A resulted in a downregulation of IGFBP5 due to an increase in miR-661 levels, which revealed that overexpression of IGFBP5 efficiently reversed the circPIP5K1A depletion effects. The conglomeration of these results suggests that circPIP5K1A is implicated in ovarian cancer's progression by affecting the miR-661/IGFBP5 axis, and therefore, it may represent a viable target for therapeutic intervention of the disease (Sun et al., 2019). CircMTO1 expression was conspicuously increased in CC tissues and cell lines. It could improve migration, aggression, and CDDP resistance in CC cells and restrain apoptosis by regulating the miR-6893/S100A1/Beclin1/p62 signaling axis (Chen et al., 2019b).

In addition to their function as ceRNAs in regulating downstream gene expression, certain circular RNAs (circRNAs) have been demonstrated to regulate resistance to cisplatin (CDDP) in several ways including through protein binding and direct regulation of gene expression (as demonstrated in Table 1). For instance, the expression of circARHGAP5 is reduced in cervical squamous cell carcinoma (CSCC) tissues and overexpression of circARHGAP5 was found to hinder cisplatin-induced apoptosis in CSCC cells, ultimately leading to the progression of CSCC. Mechanistically, experiments indicated that under direct binding conditions, circARHGAP5 can inhibit the interaction between AUF1 and BIM mRNA, which enhances cisplatin resistance and the malignant transformation of CSCC (Deng et al., 2023). Similarly, it was reported that the expression of circITGB6 is conspicuously increased in tissues and sera of CDDP-resistant ovarian cancer (OC) patients and predicts poor prognosis. Overexpression of circITGB6 was found to promote M2 macrophage-dependent resistance to CDDP. Mechanistically, circITGB6 can directly interact with IGF2BP2 and FGF9 mRNA to form circITGB6/IGF2BP2/FGF9 RNA-protein ternary complexes in the cytoplasm, leading to increased stability of FGF9 mRNA and thereby inducing TAMs to polarize toward the M2 phenotype (Li et al., 2022b). Additionally, the expression of circPBX3 was significantly increased in both OC tissues and cisplatin-resistant OC cells, and overexpression of circPBX3 strongly promoted OC cell colony formation, tumor xenograft growth, and decreased apoptosis under cisplatin treatment. Mechanistic experiments suggested that circPBX3 can interact with IGF2BP2 to increase the stability of ATP7A mRNA and strengthen the level of ATP7A protein (Fu et al., 2022). Similarly, hsa_circ_0023404 was shown to be significantly increased in cervical cancer (CC) and its overexpression was found to facilitate VEGFA expression by binding miR-5047 and resulting in increased aggression of CC cells and lymphatic vessel formation in HDLEC cells. Furthermore, this circRNA also regulates the expression of autophagy-related genes (Beclin1 and p62), improving cisplatin resistance in CC cells (Guo et al., 2019a).

Moreover, it has been demonstrated that some circRNAs present in exosomes are also involved in regulating CDDP resistance (as outlined in Table 1). For example, circ-PIP5K1A is highly expressed in CDDP-resistant OC tissues and cells, and its inhibition results in the inhibition of proliferation, migration, and aggression of CDDP-resistant OC cells, as well as an increase in apoptosis and susceptibility to CDDP. The underlying mechanism involves circ-PIP5K1A acting as a sponge for miR-942-5p, which facilitates NFIB expression. Additionally, circ-PIP5K1A can be packaged into exosomes and internalized by surrounding cells to mediate intercellular communication between OC cells (Sheng and Wang, 2023). Similarly, circ_0074269 is overexpressed in CDDP-resistant CC tissues and cells, and its silencing strengthens CDDP sensitivity, inhibiting proliferation, migration, and the induction of apoptosis in CDDP-resistant CC cells. Moreover, circ_0074269 is enriched in the exosomes of CDDP-resistant CC cells and can be transmitted between CC cells (Chen et al., 2022b). Finally, it was reported that circulating exosome circFoxp1 was significantly more highly expressed in epithelial ovarian cancer (EOC) patients, particularly those with CDDP resistance. High expression of circFoxp1 predicts a worse prognosis in EOC patients, and its overexpression in EOC cells promotes cell proliferation and confers CDDP resistance. Mechanistically, circFoxp1 positively regulates the expression of

TABLE 2 Potential roles of circRNAs in the paclitaxel-resistance of gynecologic cancer.

Cancer	CircRNAs	Expression	Biological function	Targets	References
Cervical cancer	CircMYBL2	Up	Enhance PTX resistance and promote tumor growth	miR-665/EGFR	Dong et al. (2021)
	Circ-CEP128	Up	Promote cell growth, migration and invasion and inhibit PTX sensitivity	miR-432-5p/MCL1	Zhao et al. (2022b)
	Circ_0004488	Up	Promote cell proliferation, invasion, and spheroid formation and inhibits PTX sensitivity	miR-136/MEX3C	Yi et al. (2022a)
Ovarian cancer	CircCELSR1	Up	Enhance PTX resistance and promote tumor growth	miR-1252/FOXR2	Zhang et al. (2020)
	CircTNPO3	Up	Inhibit cell apoptosis and promote PTX resistance	miR-1299/NEK2	Xia et al. (2020)
	CircEXOC6B	Down	Inhibit cell proliferation and movement and reduce PTX resistance	miR-376c-3p/FOXO3	Zheng et al. (2020)
	CircNRIP1	Up	Enhance PTX resistance	miR-211-5p/HOXC8	Li et al. (2020)
	Hsa_circ_0000714	Up	Enhance PTX resistance and promote tumor growth	miR-370-3p/CDK6/RB/RAB17	Guo et al. (2020)
	Circ_CELSR1	Up	Enhance PTX resistance and promote tumor growth	miR-149-5p/SIK2	Wei et al. (2021)
	Circ_0061140	Up	Enhance PTX resistance and promote tumor growth	miR-136/CBX2	Zhu et al. (2021)
	CircSETDB1	Up	Enhance PTX resistance	miR-508-3p/ABCC1	Huang et al. (2023)
	CircANKRD17	Up	Promote cell viability, PTX resistance and inhibit cell apoptosis	FUS/FOXR2	Liang et al. (2022)
Endometrial cancer	Circ_0007534	Up	Promote cell proliferation, invasion, EMT and PTX resistance	miR-625/ZEB2	Yi et al. (2022b)
	Circ_0039569	Up	Promote cell growth and invasion and	miR-1271-5p/PHF6	Li et al. (2022c)

CCAAT enhancer binding protein gamma (CEBPG) and formin-like 3 (FMNL3) by binding miR-22 and miR-150-3p (Luo and Gui, 2020).

2.2 CircRNA regulates paclitaxel resistance in gynecologic cancer cell resistance

Paclitaxel (PTX), or tamsulosin, is a novel terpenoid compound that has been approved by the FDA for clinical use as an anti-leukemia and anti-tumor drug (Xu et al., 2022b; Smith et al., 2022). PTX exerts its antitumor effects by inducing and promoting microtubule polymerization, preventing depolymerization, suppressing spindle formation, and blocking mitosis (Zhao et al., 2022a; Rubinstein et al., 2022). While most patients with gynecologic cancer respond well to paclitaxel chemotherapy during their first treatment, paclitaxel resistance often occurs as the number of chemotherapy sessions increases (Ortiz et al., 2022). enhanced efflux of drugs by overexpression of drug efflux pumps, such as P-gp and MRP1 (Kamazawa et al., 2002), appears to be the major mechanism contributing to paclitaxel resistance in gynecologic cancers. While alterations in tubulin expression or stability, activation of prosurvival signaling pathways, and deregulation of mitotic checkpoints can all contribute to paclitaxel resistance, the overexpression of drug efflux pumps has been identified as a key contributor to resistance in paclitaxel-resistant ovarian and endometrial cancer cells (Guo et al., 2019b). Other mechanisms, such as altered drug target and decreased drug uptake, may also play a role in paclitaxel resistance, but the evidence suggests that enhanced efflux of drugs via overexpression of drug efflux pumps is the most prevalent mechanism. Drug resistance is a critical factor leading to the mortality of patients. Recent studies have shown that circular RNAs (circRNAs) play a crucial role in PTX resistance in patients with gynecologic cancer and can act as competitive endogenous RNAs (ceRNAs) by binding to miRNAs and regulating downstream target genes (Table 2). CircMYBL2 is upregulated in cervical cancer (CC) tissues and cells, particularly in PTX-resistant CC tissues and cells. Overexpression of circMYBL2 enhances PTX resistance in CC cells, resulting in CC tumor growth. Mechanistic experiments demonstrate that circMYBL2 facilitates epidermal growth factor receptor (EGFR) expression, leading to PTX resistance by binding to miR-665 (Dong et al., 2021). Circ-CEP128 is conspicuously overexpressed in both CC tissues and cells, and its silencing in CC cells suppresses cell growth, migration, and aggression and heightens paclitaxel sensitivity by regulating the miR-432-5p/MCL1 axis (Zhao et al., 2022b). In another study, circ_0004488 is significantly increased in paclitaxel-resistant CC cells and highly expressed in cancer stem cell (CSC)-rich CC cell line subpopulations. Knockdown of circ_0004488 reduces cell proliferation, invasion, and spheroid formation in CC cells, thereby suppressing paclitaxel sensitivity. The outcomes of mechanistic experiments suggest that circ_0004488 enhances MEX3C expression by binding miR-136, thereby leading to CC malignancy progression and PTX resistance (Yi et al., 2022a). In ovarian cancer (OC), circCELSR1 is highly expressed in OC tissues and correlates with

TABLE 3 Potential of chemoresistance related circRNAs as dianostic and prognostic tools in gynecologic cancer.

Cancer	CircRNA	Detection method	*p*-value	Diagnosis	FIGO (*p*-value)	LNM (*p*-value)	DM (*p*-value)	OS (*p*-value)	DFS (*p*-value)	Follow-up (months)	References
Ovarian camcer	CircTNPO3	Specific qRT-PCR	$p < 0.001$	AUC = 0.910	$p = 0.008$	$p = 0.57$	$p = 0.082$	$p = 0.030$	/	60	Xia et al. (2020)
	CircFoxp1	Specific qRT-PCR	$p < 0.001$	AUC = 0.914	$p = 0.0312$	$p = 0.0009$	$p = 0.0394$	$p < 0.0001$	$p < 0.0001$	60	Luo and Gui (2020)
	CircEXOC6B	Specific qRT-PCR	$p < 0.05$	/	$p < 0.05$	$p < 0.05$	/	$p = 0.012$	/	60	Zheng et al. (2020)
	CircITGB6	Specific qRT-PCR	$p < 0.001$	/	/	/	/	$p = 0.006$	$p < 0.001$	60	Li et al. (2022b)
	CircANKRD17	Specific qRT-PCR	$p < 0.001$	/	/	/	/	$p = 0.033$	/	60	Liang et al. (2022)
	CircSETDB1	Specific qRT-PCR	$p < 0.001$	/	/	/	/	$p = 0.012$	/	60	Huang et al. (2023)
	Circ_0063804	Specific qRT-PCR	$p < 0.001$	/	$p < 0.05$	/	$p = 0.508$	$p = 0.0197$	/	60	You et al. (2022)
	CircPBX3	Specific qRT-PCR	$p < 0.001$	/	$p < 0.001$	$p = 0.010$	$p = 0.783$	/	/	/	Fu et al. (2022)
Cervical cancer	Circ_0004488	Specific qRT-PCR	$p < 0.001$	/	/	/	/	$p < 0.001$	/	60	Yi et al. (2022a)
Endometrial cancer	Circ_0007534	Specific qRT-PCR	$p < 0.001$	/	$p < 0.001$	/	$p < 0.001$	$p = 0.012$	/	60	Yi et al. (2022b)

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PTX resistance. Additionally, its expression is higher in PTX-resistant OC cells compared to PTX-sensitive cells. Suppression of circCELSR1 heightens PTX-induced cytotoxicity in OC cells, restraining tumor growth and promoting apoptosis by regulating miR-1252-FOXR2 (Zhang et al., 2020). CircTNPO3 expression is remarkably higher in OC samples and correlates with PTX resistance. Suppression of circTNPO3 in OC cells promotes PTX-induced apoptosis and strengthens cellular sensitivity to PTX by binding to miR-1299 and facilitating the expression of NEK2 (Xia et al., 2020). Alternatively, the overexpression of circEXOC6B in OC cells inhibits OC proliferation and motility, reducing OC resistance to PTX. The mechanistic outcomes suggest that circEXOC6B upregulates forkhead box O3 (FOXO3) expression by sponging miR-376c-3p, leading to PTX sensitivity in OC cells (Zheng et al., 2020). Moreover, circNRIP1 is highly expressed in PTX-resistant OC tissues and cells. Its suppression in OC cells restricts PTX resistance by regulating the miR-211-5p/HOXC8 axis (Li et al., 2020). Similarly, circ_0061140 facilitates chromobox 2 (CBX2) expression by binding to miR-136, leading to malignant OC progression and PTX resistance (99). On the other hand, circSETDB1 regulates PTX resistance in OC cells by targeting the miR-508-3p/ABCC1 axis (Huang et al., 2023). In endometrial cancer (EC), circ_0007534 is highly expressed and associated with poor prognosis in EC patients. Overexpression of circ_0007534 in EC cells enhances cell proliferation, aggression, epithelial-mesenchymal transition (EMT), and PTX resistance. The outcomes of mechanistic experiments show that circ_0007534 promotes EC invasiveness, progression, and PTX resistance by sponging miR-625 and promoting zinc finger E-box binding homeobox 2 (ZEB2) expression (Yi et al., 2022b). In contrast, the knockdown of circ_0039569 in EC cells restrains cell growth and invasion, leading to PTX sensitivity. Mechanistically, circ_0039569 promotes PTX resistance in EC by binding to miR-1271-5p and regulating plant homeodomain finger protein 6 (PHF6) (Li et al., 2022c).

In addition to binding miRNAs to regulate downstream gene expression, some circRNAs also adjust and control PTX resistance by binding proteins (Table 3). CircANKRD17 is highly expressed and prognostic of poor outcomes in PTX-resistant OC tissues and cells. Its knockdown suppresses PTX resistance in OC cells by suppressing cell viability and inducing apoptosis. Mechanistically, circANKRD17 stabilizes forkhead box R2 (FOXR2) by interacting with fused in sarcoma (FUS), leading to PTX resistance in OC through the circANKRD17/FUS/FOXR2 signaling axis (Liang et al., 2022).

2.3 CircRNAs regulate resistance of gynecologic cancer cells to other chemotherapeutic agents

Several research studies have demonstrated that circular RNAs (circRNAs) have the potential to regulate the resistance of gynecologic cancer cells to other chemotherapeutic agents, as depicted in Table 3. Several research studies have demonstrated that circular RNAs (circRNAs) have the potential to regulate the resistance of gynecologic cancer cells to other chemotherapeutic agents, such as docetaxel (DTX), as depicted in Table 3. Treatment of SKOV3-R cells with DTX led to a significant decrease in the expression of circRNA_0006404, while an upregulation in circRNA_0000735 expression was observed. circRNA_0000735 was found targeted by miR-526b, which subsequently regulated the expression of DKK4 and p-GP, leading to chemotherapy resistance in SKOV3-R cells treated with DTX (Chen and Tai, 2022). Medroxyprogesterone acetate (MPA) constitutes one of the most commonly administered progesterone treatments for endometrial cancer (EC), whereas hsa_circ_0001860 expression was noted to be significantly decreased in MPA-resistant tissues and cells, with a negative correlation noted with lymph node metastasis and histological grading of EC. Observation of the downstream effects of inhibiting hsa_circ_0001860 in EC cells included a conspicuous promotion of cell proliferation, migration, invasion and a suppressed apoptosis. The results obtained from mechanistic experiments have established that hsa_circ_0001860 promotes the expression of Smad7 when it binds to miR-520h (Yuan et al., 2021).

3 The diagnostic and prognostic value of drug resistance-associated circRNAs in gynecologic cancer

Drug-resistant related circular RNAs (circRNAs) are valuable in the early diagnosis and prognostic assessment of gynecologic cancers (GC). Certain circRNAs have diagnostic significance in GC, such as circTNPO3 which is highly expressed in ovarian cancer (OC) tissues and significantly correlates with the terminal Federation of Gynecology and Obstetrics (FIGO) stage and histological type of OC patients (Xia et al., 2020). ROC curve analysis of samples ranging from normal ovarian tissues to paclitaxel (PTX)-sensitive OC tissues ($n = 20$) to PTX-resistant OC tissues ($n = 28$) showed that circTNPO3 effectively distinguishes between PTX-sensitive and PTX-resistant OC tissues with an area under the ROC curve (AUC) of 0.910. Furthermore, Kaplan-Meier survival curve analysis revealed that OC patients with low circTNPO3 expression experienced significantly longer overall survival than those with high circTNPO3 expression. Another circRNA, exosomal circFoxp1, displayed conspicuously higher expression in the serum of epithelial OC (EOC) patients, showing an AUC value of 0.914 in ROC curve analysis. Additionally, serum exosome circFoxp1 expression is associated with FIGO stage, primary tumor size, lymph node metastasis, distal metastasis, residual tumor diameter, clinical response, and histological type and grade. The aforementioned results suggest that exosomal circFoxp1 can serve as a valuable biomarker for EOC patients, as lower overall survival and disease-free survival were observed in patients with higher expression levels of circFoxp1 (Luo and Gui, 2020).

The study highlights the prognostic significance of the expression levels of some circRNAs in gynecological tumors. Specifically, in PTX-resistant cervical cancer (CC) tissues, it was found that the expression of circ_0004488 was remarkably higher than in PTX-sensitive CC tissues. Moreover, the Kaplan-Meier survival curves showed that increasing levels of circ_0004488 were associated with a decrease in overall survival of CC patients (Yi et al., 2022a). Similarly, in ovarian cancer (OC), the expression of circEXOC6B was observed to decrease and was negatively correlated with tumor progression. Furthermore, high expression of circEXOC6B was linked to long-term survival time in OC patients (Zheng et al., 2020). Conversely, in CDDP-resistant OC patients, the expression levels of circITGB6 were significantly upregulated as

compared to those in CDDP-sensitive OC patients and normal controls. Notably, OC patients with high levels of circITGB6 had a relatively low overall survival rate and a higher relapse rate, as determined by survival analysis (Li et al., 2022b). Additionally, the expression of circANKRD17 was significantly upregulated in OC tissues, with patients with higher circANKRD17 expression demonstrating a shorter overall survival time compared to those with low expression (Liang et al., 2022). The expression of circSETDB1 was found to be notably higher in PTX-resistant ovarian cancer tissues than in normal tissues. Importantly, OC patients with high circSETDB1 expression had a worse prognosis, according to Kaplan-Meier survival curve analysis (Huang et al., 2023).

Furthermore, some circRNAs were found to be associated with clinical features of gynecologic cancer. For instance, circ_0007534 expression levels were significantly higher in endometrial cancer (EC) tissues, and high expression of circ_0007534 predicted worse tumor differentiation, more terminal pathological phase, deeper infiltration, and stronger cancer metastasis. Importantly, patients with high circ_0007534 expression level had a significantly shorter survival time (Yi et al., 2022b). Similarly, it was observed that in OC tumor tissues, the expression of circ_0063804 was remarkably higher than in normal control tissues. Additionally, high expression of circ_0063804 was strongly correlated with lower survival, terminal FIGO stage and grade, and larger tumor size, as determined by various analyses (You et al., 2022) Finally, the expression of circPBX3 was found to be highly upregulated in OC, and high expression of circPBX3 was positively correlated with larger tumor size, terminal FIGO stage, and lymph node metastasis, as determined by analysis (Fu et al., 2022).

4 Conclusion and perspective

Chemotherapy has long been considered one of the most effective treatments for cancer. Despite this, the development of drug resistance has proved to be a major obstacle to successful patient outcomes (Wang et al., 2022c; Karami Fath et al., 2022; Pastwińska et al., 2022). Chemotherapy exerts its cytotoxic effects by inhibiting cellular synthesis of DNA and RNA, suppressing cell proliferation, and promoting apoptosis (Abdelaal and Haffez, 2022; Yang et al., 2022b; Li et al., 2022d). However, the efficacy of chemotherapy is limited by drug resistance, which leads to tumor progression and ultimately patient mortality. Initial studies on drug resistance in tumors identified several protein-encoding genes that are closely associated with chemoresistance development, including the drug transport proteins MDR1, MRP, and ABCG2 (Chimento et al., 2022; Yang et al., 2022c; Zhao et al., 2022c; Vaghari-Tabari et al., 2022). Recent advances in molecular analysis and high-throughput sequencing techniques have enabled rapid and accurate identification of the expression profiles of non-coding RNAs associated with drug resistance (Sánchez-Marín et al., 2022). Due to the chemotherapy resistance and early-stage metastasis of gynecological cancer, the prognosis for patients is unfavorable, and the 5-year survival rate remains low despite aggressive treatment. Consequently, identifying reliable biomarkers and gaining insight into the molecular mechanisms of chemoresistance in gynecological cancer is critical to developing new anti-gynecological cancer strategies. High-throughput RNA sequencing has proven useful in identifying circRNAs that are dysregulated in association with gynecological cancer chemoresistance and elucidating their potential mechanisms. This paper presents the circRNAs associated with chemoresistance identified in the mentioned research, which are involved in the regulation of drug metabolism, DNA damage repair, apoptosis and EMT signaling pathways. Some of these circRNAs may even serve as valuable prognostic markers.

The search for circRNAs associated with drug resistance in gynecologic cancers has the potential to minimize the "experimental" use of drugs and enable more rational selection of treatment regimens. Furthermore, combining circRNA inhibitors or enhancers with chemotherapeutic drugs can enhance chemotherapy sensitivity. For patients who are dose-limited, adding circRNAs to targeted therapy, while decreasing the dose of chemotherapeutic drugs, could significantly reduce the adverse effects of dose limitation and alleviate the discomfort caused by treatment. Nonetheless, the development and clinical application of related circRNAs remain inadequate. Tumor drug resistance is a multifactorial trait, and the complexity of the tumor microenvironment may result in differences in *ex vivo* research. This complexity makes targeting circRNAs to enhance chemotherapy sensitivity challenging and uncertain.

Our manuscript provides a comprehensive review of the role of circular RNAs (circRNAs) in chemotherapy resistance in gynecologic malignancies and their mechanisms. While there have been some previous studies on this topic, our review offers several novel and innovative contributions to the literature. Firstly, we have identified specific circRNAs that are involved in regulating chemotherapy resistance for different chemotherapeutic agents used in the treatment of gynecologic malignancies. This information can be used to develop more targeted and effective treatment strategies. Secondly, we have discussed the mechanisms by which these circRNAs regulate chemotherapy resistance, including drug metabolism, DNA injury repair, apoptosis and EMT signaling pathways. By understanding these mechanisms, researchers and clinicians can develop new approaches to overcome drug resistance. Thirdly, we have highlighted the potential clinical applications of circRNAs as biomarkers for predicting chemotherapy response and as therapeutic targets for improving treatment outcomes in patients with gynecologic malignancies. Overall, our manuscript offers a unique perspective on the role of circRNAs in chemotherapy resistance in gynecologic malignancies and provides valuable insights into potential new approaches for improving treatment outcomes.

Author contributions

Original draft preparation, allocation: CZ and MQ manuscript revision, supplement and edition: YL. All authors contributed to the 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.

Publisher's note

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Identification of a novel lncRNA prognostic signature and analysis of functional lncRNA AC115619.1 in hepatocellular carcinoma

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Background: Hepatocellular carcinoma (HCC) is the deadliest malignancy. Long non-coding RNAs (lncRNAs) are involved in the development of multiple human malignancies. This study aimed to establish a reliable signature and identify novel biomarkers for HCC patients.

Methods: Differentially expressed lncRNAs (DElncRNAs) were identified from Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) databases. Univariate, LASSO, and multivariate Cox regression analyses were applied to screen the prognostic lncRNAs and establish a prognostic model. Receiver operating characteristic (ROC) curves and Kaplan–Meier analyses were conducted to validate the prognostic value of this model. The association between lncRNAs and differential m6A genes was analyzed by Spearman's analysis. A series of bioinformatic and *in vitro* experiments were applied to explore the function of hub lncRNA.

Results: A total of 32 DElncRNAs were identified, and 12 DElncRNAs were associated with the prognosis of HCC patients. A prognostic signature comprising six prognostic lncRNAs (LINC02428, LINC02163, AC008549.1, AC115619.1, CASC9, and LINC02362) was constructed, and the model exhibited an excellent capacity for prognosis prediction. Furthermore, 12 differential m6A regulators were identified, and RBMX was found to be correlated negatively with the hub lncRNA AC115619.1. The expression level of AC115619.1 was lower in HCC tissues than that in normal tissues and was significantly related to clinicopathologic features, survival rate, and drug sensitivity. Overexpression of AC115619.1 notably inhibited the proliferation, migration, and invasion of HCC cells.

Conclusion: This study provided a promising prognostic signature for HCC patients and identified AC115619.1 as a novel biomarker, which plays an essential role in regulating the progression of HCC.

KEYWORDS

long non-coding RNA, hepatocellular carcinoma, biomarker, diagnosis, prognosis

1 Introduction

Primary liver cancer is one of the most common malignancies worldwide, with about 906,000 new cases and 830,000 deaths reported in 2020. Hepatocellular carcinoma (HCC) accounts for the majority of incidence and mortality with a 75%–85% constitution statistically (Sung et al., 2021). Despite the great efforts and huge improvement in diagnosis and treatment therapies, the prognosis of HCC is still poor with a 5-year survival rate of approximately 12% (Petrowsky et al., 2020). The poor outcome of HCC poses a tremendous burden to social economy and public health. With the obscure symptom of early-stage HCC, most HCC patients are diagnosed at a late stage and lose their opportunity to receive radical resection (Anwanwan et al., 2020; Demir et al., 2021). In addition, recurrence, metastasis, and chemoresistance present major barriers to a satisfactory effect for HCC treatment (Kim et al., 2017). Therefore, highly efficient and specific biomarkers are still needed for diagnosis and prognostic prediction, which could improve the poor prognosis and individualized treatments for HCC patients.

Accumulating evidence has demonstrated that long non-coding RNA (lncRNA), which is defined as RNA transcripts of more than 200 base pairs in length (Esteller, 2011), has contributed to tumorigenesis, progression, and metastasis (Schmitt and Chang, 2016; Calle et al., 2018). LncRNAs exert various biological effects to participate in the biological and pathological processes by regulating multiple processes including transcription, epigenesis, and mRNA expression (Wang and Chang, 2011; Dykes and Emanueli, 2017; Ransohoff et al., 2018; Herman et al., 2022). The aberrant expression of lncRNA has been identified as "oncogenes" or "tumor suppressors," as well as a prognostic factor of cancer patients. Several lncRNAs have been identified to play a role in HCC. For example, lncRNAs MALAT1, PVT1, and HOTAIR have been found to contribute to the prognosis and different cellular phenotypes such as proliferation and metastasis in human malignancies (Zhao et al., 2018; Rajagopal et al., 2020; Shigeyasu et al., 2020; Goyal et al., 2021). Ni et al. proposed that lncRNA uc.134 was downregulated in HCC and directly conferred with the patient's prognosis. LncRNA uc.134 inhibited proliferation and metastasis by suppressing CUL4A-mediated ubiquitination of LATS1 (Ni et al., 2017).

Although many previous studies focused on the functions of lncRNAs, exploring a novel biomarker of lncRNA is still needed. Given the promising role of lncRNAs in HCC, we aimed to identify a lncRNA-related prognosis biomarker and elucidate its function in HCC. In this work, we identified lncRNAs expressed differentially in multiple public databases and constructed a prognostic prediction model by bioinformatics analysis. Systematic analysis showed that lncRNA AC115619.1 contributed excellent value in patients' survival, but its function has never been explored previously. A ceRNA network and functional enrichment of lncRNA AC115619.1 were also applied, as well as analysis of drug sensitivity. Additionally, we verified the downregulation of AC115619.1 in local HCC samples. Functional experiments revealed that overexpression of AC115619.1 inhibited the progression of HCC. Our study might develop a novel biomarker and provide more insights to better understand the molecular mechanism of HCC.

2 Materials and methods

2.1 Data acquisition

The expression level of the lncRNA microarray was obtained from the Gene Expression Omnibus (GEO) database. Studies were chosen from GEO according to the following criteria: 1) studies with HCC tissue and adjacent normal tissue samples; 2) studies with information on the technology and platform utilized for studies. Based on these criteria, nine microarray datasets (GSE138178, GSE93789, GSE101728, GSE115018, GSE70880, GSE67260, GSE58043, GSE55191, and GSE84004) were downloaded from the GEO repository. Details of each microarray study are provided in Table 1. Meanwhile, the RNA sequencing data (374 tumor samples and 50 normal liver samples; type: FPKM), and the corresponding clinical and prognostic information on HCC patients were obtained from The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/) database. Samples with gene expression of "0" value and insufficient clinical and survival information were excluded.

TABLE 1 Details of the lncRNA microarray from the GEO database.

GEO ID	Platform	Sample	Numbers (tumor)	Numbers (normal)
GSE138178	GPL21827	HCC	49	49
GSE93789	GPL16956	HCC	5	5
GSE101728	GPL21047	HCC	7	7
GSE115018	GPL20115	HCC	12	12
GSE70880	GPL19748	HCC	16	16
GSE67260	GPL19072	HCC	5	5
GSE58043	GPL13825	HCC	7	7
GSE55191	GPL15314	HCC	3	3
GSE84004	GPL22109	HCC	38	38

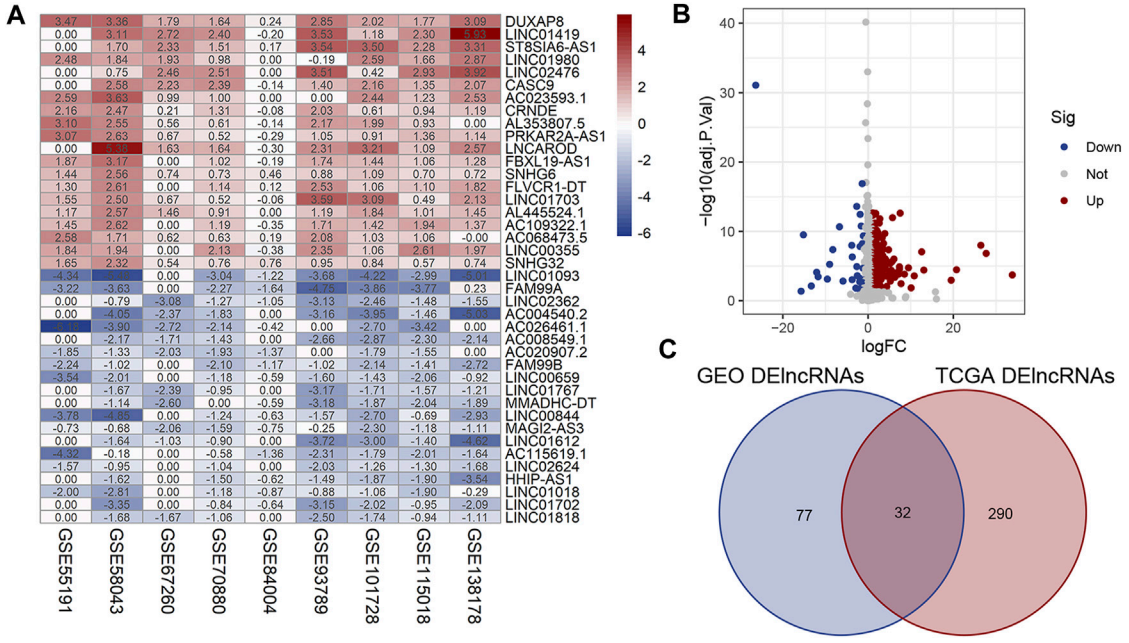

FIGURE 1
Identification of DElncRNAs in HCC. **(A)** Heatmap of DElncRNAs in HCC and normal liver tissues from the nine GSE microarrays. **(B)** Volcano plot for the DElncRNAs identified from TCGA dataset. **(C)** Intersection of DElncRNAs from GEO and TCGA databases. Row and column represent DElncRNAs/DEmRNAs and tissue samples, respectively. The color scale indicated the expression level of DElncRNAs. Red and blue represent up- and downregulation, respectively.

2.2 Reannotation of microarray probes

LncRNA expression profiles were downloaded from the GEO database with probe ID and sequences. A custom pipeline was performed to re-annotate the probes of the lncRNA microarray. The corresponding sequences of the re-annotated probes were uniquely mapped to the human genome with no mismatch, and the chromosomal position of the retained probes was subsequently matched to the chromosomal position of lncRNAs or protein-coding genes from the GENCODE project (https://www.gencodegenes.org).

2.3 Identification of robust DElncRNAs

Batch normalization and the R package "limma" were utilized to generate differentially expressed lncRNAs. The aforementioned nine GSE datasets were then integrated and filtrated using robust rank aggregation (RRA). eBayes was used for identifying DElncRNAs in HCC samples compared with adjacent normal tissues with the criteria of $|\log_2 FC| > 1$, adjusted $p < 0.05$. The dysregulated lncRNA lists from the GEO and TCGA platforms were converged for further analysis.

2.4 Construction of a prognostic model

Corresponding survival information on HCC patients was obtained from TCGA dataset. To filter the potential prognostic lncRNAs in HCC patients, we performed univariate regression analysis and subsequent least absolute shrinkage and selection operator (LASSO) regression to carry out prognostic analysis. Multivariate Cox regression analyses were then used to determine which lncRNA was an independent prognostic factor for HCC patients. All HCC patients from TCGA dataset were randomly divided into training and test cohorts for further prognostic prediction for lncRNAs. Both training and test cohorts were then implemented with a risk score model calculated with the formula, risk score $= \sum_{1}^{6} \text{coefficient of lncRNA} * \text{expression of lncRNA}$. LncRNA represented the six lncRNAs screened from multivariate Cox regression. Kaplan–Meier analysis and the log-rank test were applied to compare the low- and high-risk subgroups and additional subgroups based on the median values of the risk score. A receiver operating characteristic (ROC) analysis was performed to estimate the value of the prognostic model. Finally, univariate and multivariate Cox regression analyses were applied to evaluate whether the risk score was an independent prognostic factor when combined with other clinical characteristics.

2.5 Association analysis between prognostic lncRNAs and m6A regulators

The expression profile of m6A-related regulators was obtained from TCGA database, as well as the corresponding survival information on HCC patients. Then, the univariate Cox regression analysis was applied to estimate the prognostic value of m6A-related regulators using the "survival" R package. Pearson's

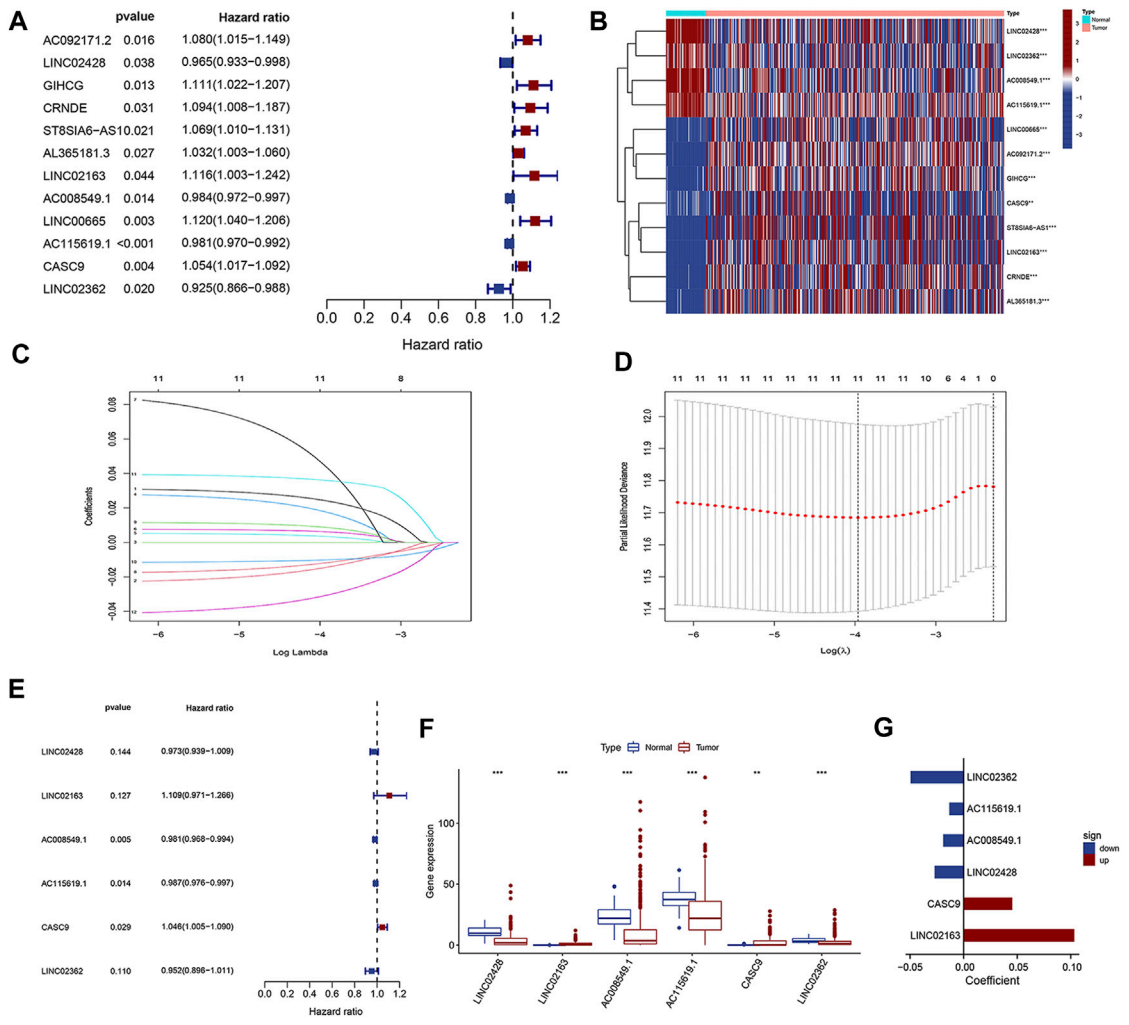

FIGURE 2
Identification of prognosis-related DElncRNAs. **(A)** Forest plot of univariate Cox regression analysis. **(B)** Heatmap of DElncRNAs from univariate Cox regression. **(C, D)** LASSO Cox analysis for DElncRNAs from univariate Cox regression. **(E)** Forest plot of multivariate Cox regression analysis. **(F)** Box plot and **(G)** forest plot for the six prognostic lncRNAs from multivariate Cox regression analysis.

correlation analysis was subsequently implemented to investigate the correlation of HCC prognostic-related lncRNAs and m6A-related regulators.

2.6 Validation of the expression and evaluation of the clinical significance of lncRNA AC115619.1 for HCC patients in public databases

To further validate the expression of the identified hub lncRNA AC115619.1 in HCC tumor tissues compared to adjacent normal tissues, the expression profiles of several GSE datasets were downloaded and analyzed. The clinicopathologic and prognostic information on patients from TCGA database was then used to determine the clinical significance of lncRNA AC115619.1. The differences among the clinicopathologic factors, including tumor grade, tumor invasion, and TNM stage, were evaluated. Kaplan–Meier survival curve analysis was conducted to demonstrate the overall survival (OS) of patients with different expression levels of AC115619.1 using the survival R package.

2.7 CeRNA regulatory network and functional enrichment analysis

A ceRNA network was constructed to explore the regulatory relationship. The miRcode database (http://www.mircode.org/) was applied to predict the target miRNAs of AC115619.1. Potential target mRNAs of the miRNAs were then screened using miRDB (http://www.mirdb. org/), miRTarBase (https://mirtarbase.cuhk.edu.cn/), and TargetScan databases together. To acquire more accurate target mRNAs, target mRNAs were subsequently filtered by intersecting with HCC-related differentially expressed genes from TCGA

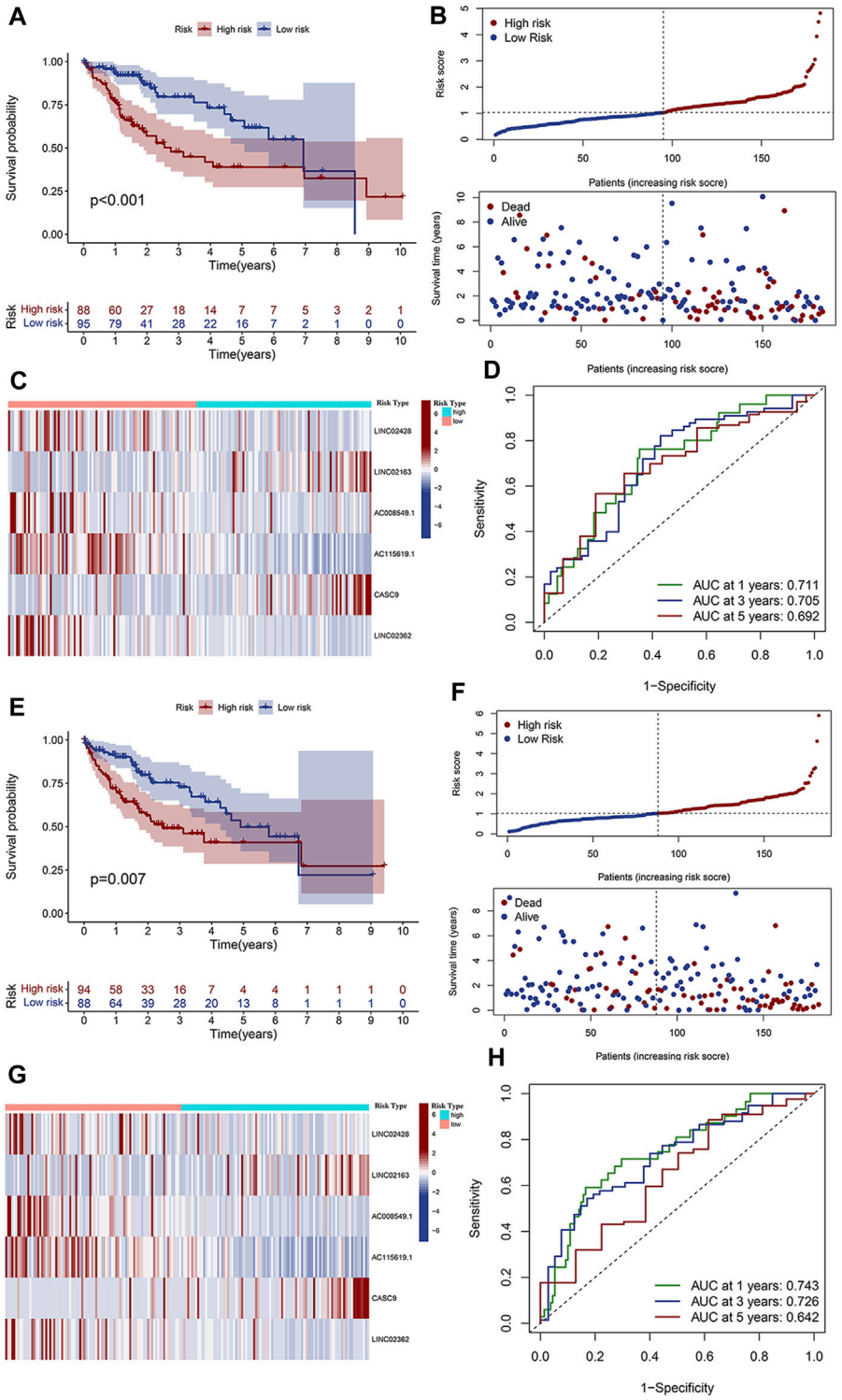

FIGURE 3
Validation of the clinical significance of the prognostic risk model. **(A)** Kaplan–Meier curves depicted the overall survival of patients in the training cohort from TCGA databases. Patients were divided into low- and high-risk groups based on the median value of the risk score. **(B)** Distributions of risk scores (upper) and survival status (lower) of HCC patients in the training cohort. **(C)** Expression heatmap of the six prognostic lncRNAs with low- and high-risk scores in the training cohort. **(D)** ROC curve of the prognostic signature for predicting the 1/3/5-year survival in the training cohort. **(E)** Overall survival curve of patients with low- and high-risk scores in the test cohort. **(F)** Distributions of risk scores (upper) and survival status (lower) of HCC patients in the test cohort. **(G)** Expression heatmap of the six prognostic lncRNAs with low- and high-risk scores in the test cohort. **(H)** ROC curve of the prognostic signature for predicting the 1/3/5-year survival in the test cohort.

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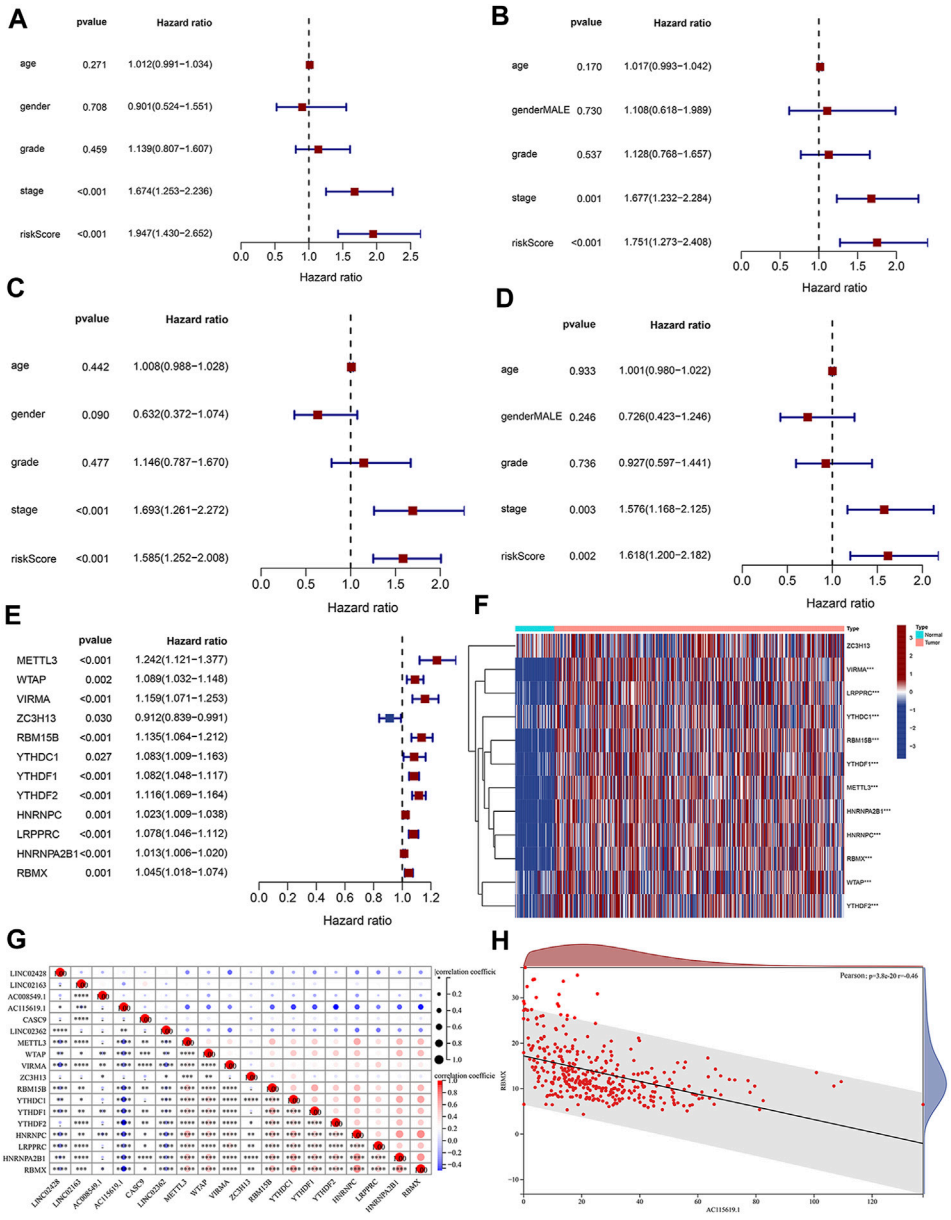

FIGURE 4
Clinical significance analysis of the risk score and the correlation of m6A and lncRNAs. Forest map of **(A)** univariate and **(B)** multivariate Cox regression analyses in the training cohort. Forest map of **(C)** univariate and **(D)** multivariate Cox regression analyses in the test cohort. **(E)** Univariate Cox regression analysis of the prognostic value of m6A-related regulators. **(F)** Heatmap of prognostic m6A regulators in tumor tissues and normal liver tissues. **(G)** Expression correlation between the six prognostic DElncRNAs and 12 m6A regulators. **(H)** Expression correlation between the lncRNA AC115619.1 and RBMX determined by Pearson's coefficient analysis.

database ($|\log_2 FC| > 2, p < 0.01$). The lncRNA–miRNA–mRNA ceRNA network was visualized using Cytoscape software. Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis were performed to investigate the functions and potential signaling pathways of differentially expressed lncRNAs using the R package "clusterProfiler." GO includes the following three major groups: biological processes, cellular components, and molecular functions.

2.8 Estimation of immunocyte infiltration and analysis of drug sensitivity

The CIBERSORT algorithm was applied to estimate the proportion of immune cell infiltration in 22 human hematopoietic cell phenotypes between high- and low-AC115619.1 groups. To improve the clinical application of AC115619.1, the pRRophetic (https://github.com/paulgeeleher/

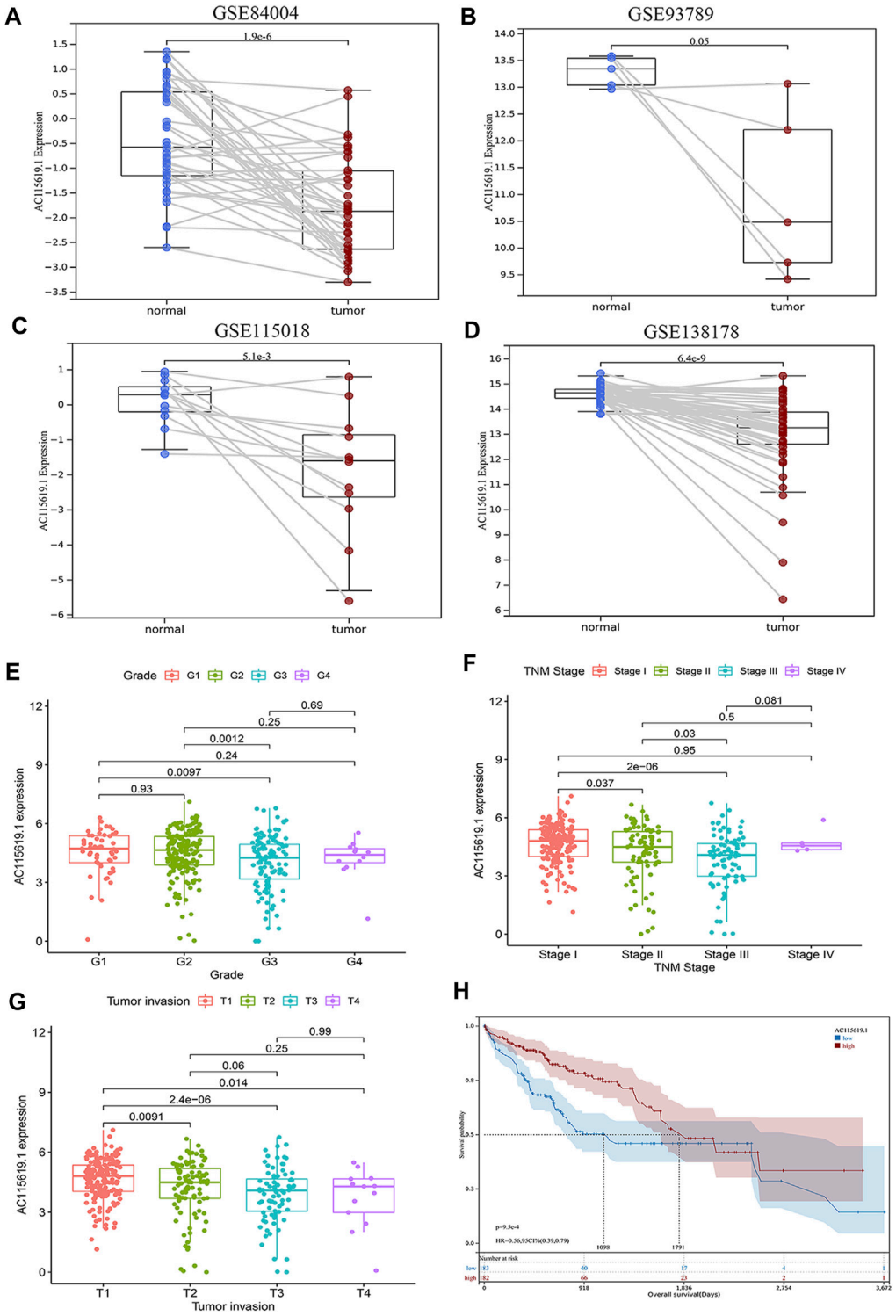

FIGURE 5
Validation of the expression and clinical value of lncRNA AC115619.1. **(A–D)** Validation of the low expression of lncRNA AC115619.1 in the public GSE microarray of the GEO database. **(E–G)** Correlations between AC115619.1 expression and tumor grade, stage, and invasion. **(H)** Overall survival Kaplan–Meier curves of patients with low and high expression of AC115619.1.

pRRophetic) R package was used to predict the sensitivity of chemotherapeutic and targeted agents between high- and low-AC115619.1 patients. The half-maximal inhibitory concentration (IC_{50}) of the targeted and chemotherapeutic agents for each patient were predicted using the R package based on the pretreated gene expression and drug sensitivity data on cancer cell lines.

TABLE 2 Correlation of AC115619.1 expression in HCC tissues with patients' clinicopathologic features from TCGA dataset.

Clinicopathologic variable		AC115619.1 expression (n = 363)		
		Low	High	*p*-value
Age (years)	≤61	95	91	0.714
	>61	87	90	
Gender	Men	121	123	0.765
	Women	61	58	
Grade	G1~2	105	125	0.013
	G3~4	76	52	
Tumor invasion	T1~2	119	151	<0.001
	T3~4	63	27	
Lymph node metastasis	No	125	121	0.974
	Yes	2	2	
Distant metastasis	No	129	132	0.553
	Yes	2	1	
TNM stage	I ~ II	111	142	<0.001
	III ~ IV	58	28	

2.9 Tissue collection

A total of 43 tumor tissue samples and paired adjacent normal tissue samples were randomly collected from HCC patients who were admitted to The First Affiliated Hospital of Guangxi Medical University. All specimens were routinely processed for a pathological diagnosis of surgeries according to the WHO classification. No patients received radiotherapy, chemotherapy, or immunotherapy before the samples were collected. The study was approved by the Research Ethics Committee of Guangxi Medical University. Informed consent was obtained from all participating patients.

2.10 Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

The total RNA was isolated using the TRIzol reagent (Invitrogen), and cDNA was synthetized using the PrimeScript™ Kit (TaKaRa Bio Inc., Dalian, China), following the manufacturer's instructions. qRT-PCR was performed in triplicate using SYBR Green fluorescent-based assay (GeneCopoeia, Guangzhou, China) on a ViiATM6 RT-PCR system (Applied Biosystems, Carlsbad, CA). The primers used for real-time PCR are as follows: AC115619.1 forward: 5′-TGATGATATCGACGTGAGGTTCC-3′, reverse: 5′-ATCAAA CACGTTATCCTTGAGTCC-3'; GAPDH forward: 5′-CGG AGTCAACGGATTTGGTCGTAT-3′, reverse: 5′-AGCCTT CTCCATGGTGGTGAAGAC-3'. Relative mRNA expression levels were calculated by the $2^{-\Delta\Delta Ct}$ method and were normalized to the internal control of GAPDH.

2.11 Immunohistochemistry

Tissues were fixed in 10% formalin, dehydrated using graded concentrations of ethanol, and embedded in paraffin. Then, 4-μm-thick sections were processed for analyses. Dewaxing, hydrating, and heat-mediated antigen retrieval with pH 9.0 Tris/EDTA buffer were performed. Subsequent antigen–antibody reactions and IHC staining were performed, according to the protocol of a commercial detection kit (ZSGB-BIO, Beijing, China). Antibodies for RBMX were diluted to recommended concentrations, following the manufacturer's protocol (Abcam, Cambridge, United Kingdom). The immunoreactivity-tested protein was scored according to the percentage of positive staining cells and staining intensity, as described previously (Deng et al., 2020).

2.12 Cell culture and transfection

HCC cell lines SNU-449 and HepG2 were obtained from Procell Life Science and Technology (Wuhan, China) and cultured in RPMI-1640 or Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum (Procell Life Science and Technology). The cells were grown in a cell incubator with 5% CO_2 at 37°C. Full-length AC115619.1 was amplified by PCR and cloned into the expression vector pcLV3 for AC115619.1 overexpression. The pcLV3–AC115619.1 plasmid or an empty vector was transfected into SNU-449 and HepG2 cells using the Lipofectamine 3000 reagent (Invitrogen). The cells were harvested 48 h after transfection for further analysis.

2.13 Cell viability assay

The cells were equivalently pipetted into a 96-well plate 48 h after transfection, and the cell viability was determined using the Cell Counting Kit-8 (CCK-8) (Dojindo Molecular Technologies, Inc., Tokyo, Japan) for different time points. Briefly, 10 μL of CCK-8 was added to each well, and the absorbance was measured at 450 nm.

2.14 5-Ethynyl-20-deoxyuridine (EdU) assay

We used an EdU kit (RiboBio, Guangzhou, China) to detect the proliferation ability of HCC cells. The cells were seeded and grown in a 96-well plate with a density of 5×10^3 cells. Then, the cells were incubated with 50 μM EdU buffer at 37°C for 2 h, and then fixed and washed at room temperature. The cells were then permeabilized with 0.5% Triton X-100 for 10 min and subsequently reacted with the Apollo® staining solution for 0.5 h in the dark. The cells were washed, and then, Hoechst 33342 was added to stain the nuclei. Images were visualized and captured using a fluorescence microscope.

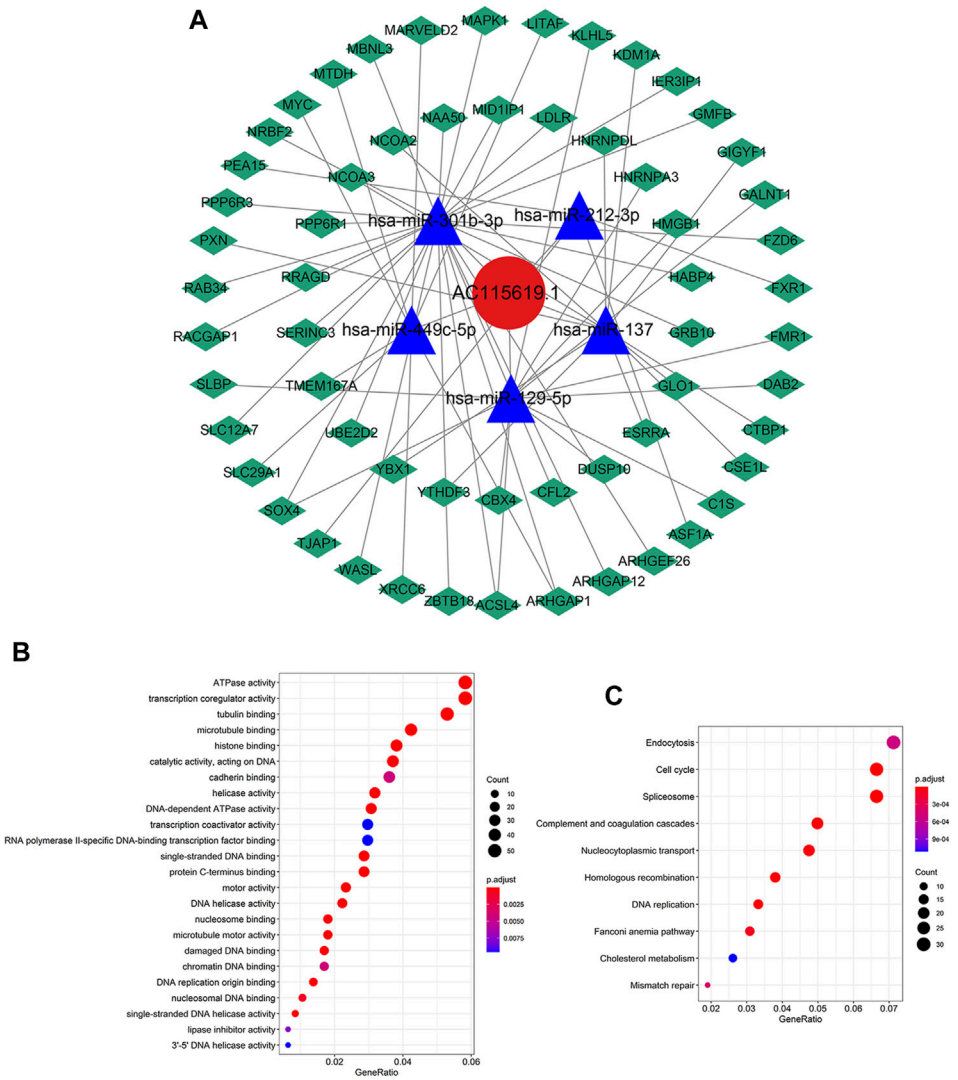

FIGURE 6
Functional enrichment analysis of AC115619.1. **(A)** ceRNA network of AC115619.1 (red), its target miRNAs (blue), and corresponding target mRNAs (green). **(B)** Gene Ontology (GO) enrichment and **(C)** KEGG pathway analyses of AC115619.1. Dot size represents the count of relative genes, and the color represents the p-value.

2.15 Cell migration assay

Wound healing assay was used to detect the migration ability. Cells were seeded into 6-well plates after transfection with the plasmid for 48 h. Then, the confluent cell monolayers were scratched straightly using a 200-μL pipette tip. The cells were washed with PBS and cultured in fresh medium containing 1% FBS and 1% BSA for 72 h. Images of the scratched cells were captured using an inverted microscope.

2.16 Transwell invasion assay

Matrigel-coated upper inserts containing polycarbonate filters with a pore size of 8 μm (Corning, Tewksbury, MA) were used to assess the cell migration ability. The cells were suspended in 200 μL of serum-free DMEM or RPMI 1640 and cultured in the upper chambers and incubated at 37°C for 48 h, while the lower chambers were covered with DMEM or RPMI 1640 containing 10% FBS. The cells which penetrated the filter were fixed with methanol and then stained with 0.1% crystal violet hydrate solution. Images of the invaded cells were captured using an inverted microscope.

2.17 Statistical analysis

Statistical analysis was performed using SPSS software (version 21.0, SPSS Inc., Chicago, IL). The continuous variable data are presented as the means ± standard deviations (SDs). Median survival time, log-rank *p*-value, adjusted *p*-value, 95% confidence interval (CI), and hazard ratio (HR) were calculated using the Kaplan–Meier and Cox proportional hazard regression models. The χ^2 test was performed to compare the categorical variables assessing the pathological and clinical

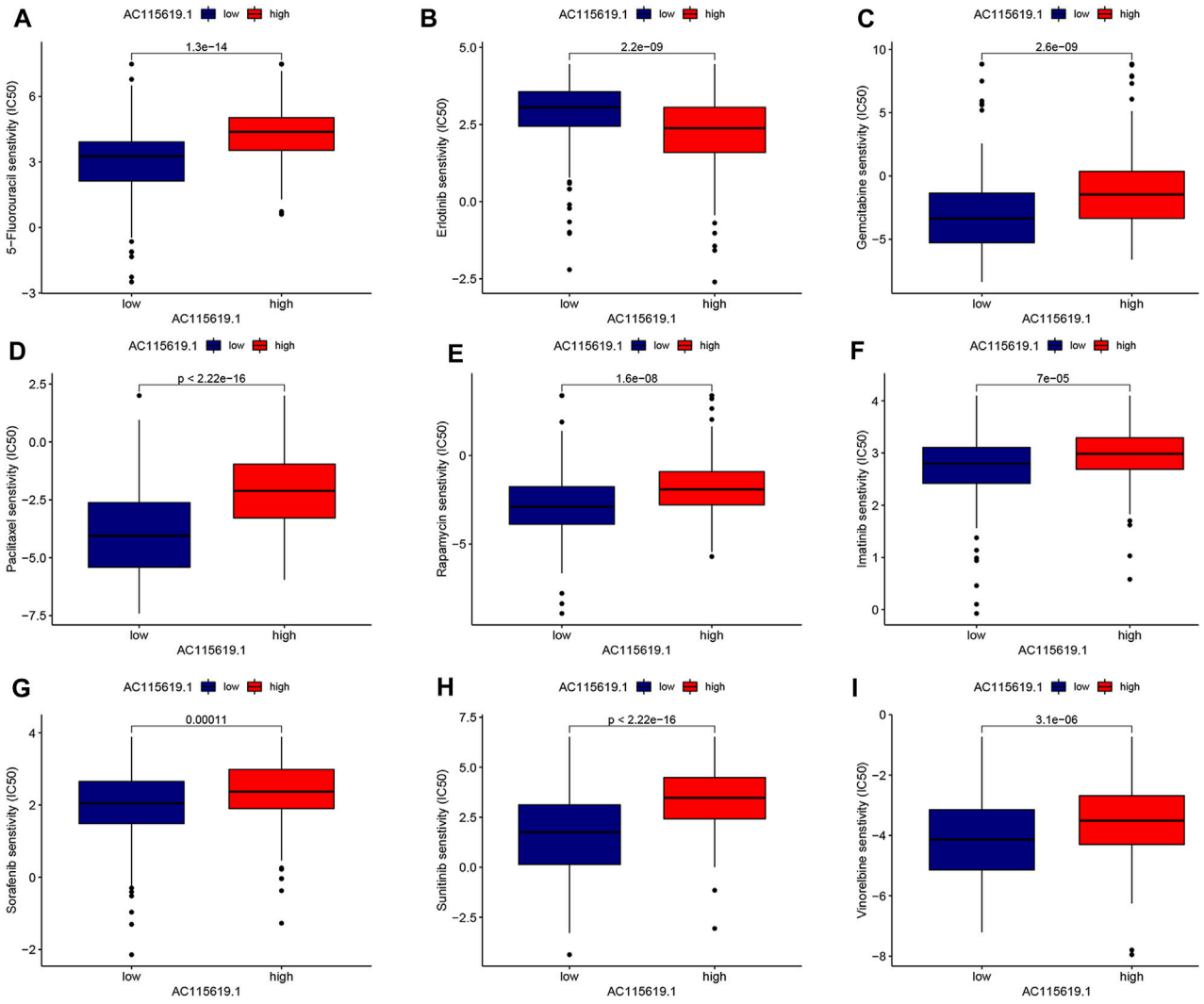

FIGURE 7
Prediction for the drug IC_{50} value of AC115619.1 in HCC. **(A–I)** Differential IC_{50} values of chemotherapy and targeted therapy responses in high and low expression of AC115619.1. The high expression of AC115619.1 was related to a higher IC_{50} value for most chemotherapy and targeted drugs.

characteristics. The differences between the experimental groups were analyzed using Student's t-test or one-way ANOVA. $p < 0.05$ was considered to be statistically significant.

3 Results

3.1 Identification of DElncRNAs in HCC patients

A total of 142 pairs of samples of HCC patients from nine GEO microarray datasets were enrolled to determine the expression level of lncRNAs in HCC tumor tissues and adjacent normal liver tissues. As shown in the heatmap given in Figure 1A, 51 upregulated lncRNAs and 58 downregulated lncRNAs in the GEO database were identified ($|\log_2 FC|>1$, $p < 0.05$). Meanwhile, we identified 322 dysregulated lncRNAs (35 upregulation and 287 downregulation) in 374 HCC tumor samples and 50 normal liver tissues obtained from TCGA database (Figure 1B, $|\log_2 FC|>1$, $p < 0.05$). DElncRNAs from the two platforms were then converged, and we finally obtained 32 lncRNAs which were significantly dysregulated in both GEO and TCGA databases (Figure 1C).

3.2 Prognostic analysis of DElncRNAs

Combining with the prognostic information, univariate Cox regression analysis was then performed to screen prognosis-related lncRNAs from the aforementioned 32 dysregulation lncRNAs in both datasets. Finally, 12 lncRNAs were found to be correlated with the prognosis of HCC patients in both datasets (Figure 2A, $p < 0.05$). As shown in the forest plot (Figure 2A), LINC02428, AC008549.1, AC115619.1, and LINC02362 were protective factors with HR < 1 in HCC patients, while AC092171.2, GIHCG, CRNDE, ST8SIA6-AS1, AL365181.3, LINC02163, LINC00665, and CASC9 were risk factors

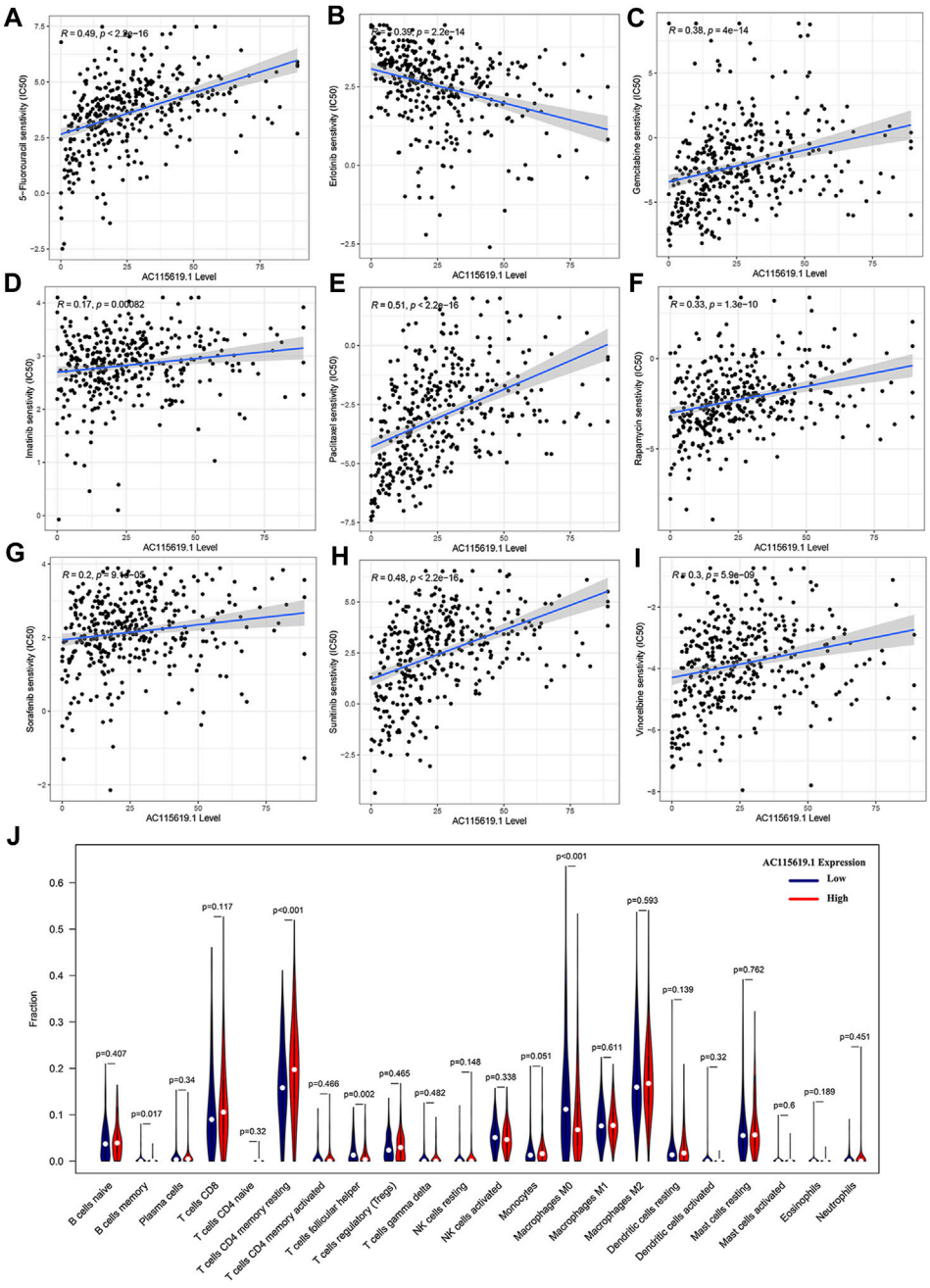

FIGURE 8
Drug sensitivity and immunocyte infiltration analysis of AC115619.1. **(A–I)** Detailed correlation of drug sensitivity and the expression of AC115619.1. **(J)** Box plot of 22 hematopoietic cell phenotypes between differential expression of AC115619.1.

with HR > 1 (Figure 2A). The heatmap showed the altered expression of the aforementioned 12 prognosis-related lncRNAs in TCGA dataset (Figure 2B). We performed LASSO Cox analysis based on the 12 prognostic lncRNAs to identify the prognostic lncRNAs more accurately and filtered six key lncRNAs (i.e., LINC02428, LINC02163, AC008549.1, AC115619.1, CASC9, and LINC02362) (Figures 2C,D) using a dimensionality reduction method. Then, a multiple stepwise Cox regression analysis was further conducted to assess which lncRNA contributed most to the prognosis of HCC patients when combined together. The results showed that AC008549.1, AC115619.1, and CASC9 were suggested to be an independent prognostic factor of HCC patients ($p < 0.05$, Figure 2E). Expression levels of these lncRNAs in tumor tissues compared to normal tissues were also displayed (Figures 2F,G).

3.3 Establishment of a prognostic risk model

Based on each coefficient of the six prognostic lncRNAs in the multivariate Cox regression model, a risk score was calculated for

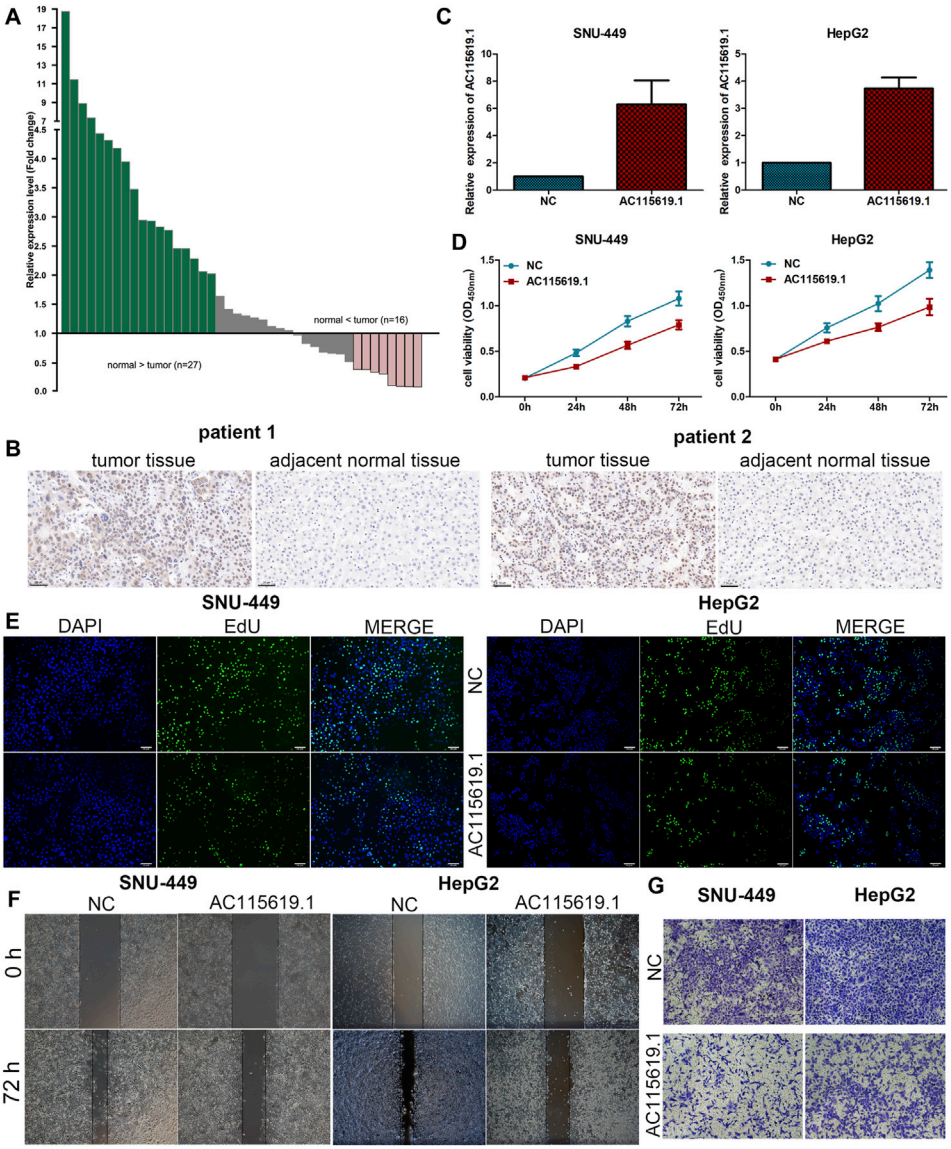

FIGURE 9
AC115619.1 was negatively related to RBMX and inhibited the progression of HCC. **(A)** Downregulation of AC115619.1 was validated by qRT-PCR in local HCC samples. Fold changes are analyzed using the formula, $2^{-(\Delta\Delta CT\ (tumor/adjacent\ normal\ tissue))}$. **(B)** Representative IHC pictures of RBMX expression in HCC tumor and adjacent normal liver tissues (original magnification: ×200). **(C)** Transfected efficiency of the AC115619.1 plasmid in HCC cells detected by qRT-PCR. **(D)** Cell viability of HCC cells after AC115619.1 overexpression detected by CCK8 assay. **(E)** EdU assay displayed the proliferating ability of HCC cells. **(F)** Migration ability determined by wound healing assay. **(G)** Invasion ability determined by transwell assay.

each HCC patient in TCGA dataset. We then established a novel prognostic signature with the six lncRNAs. Patients in both training and test cohorts were divided into low- and high-risk subgroups, according to the median value of risk scores. Kaplan–Meier survival curves showed that HCC patients with high-risk scores had poor prognosis (Figures 3A,E). Survival status distributions suggested that patients in the high-risk score group suffered from higher mortality rates than low-risk score patients (Figures 3B,F). The heatmap revealed that the expression levels of prognosis-related lncRNAs were higher than those in patients with a low-risk score (Figures 3C,G). ROC curves were also performed to evaluate the predictive accuracy of this prognostic risk model, which demonstrated that prognostic lncRNAs harbored a potential ability to predict the OS (training cohort: 1-year AUC = 0.711 and test cohort: 1-year AUC = 0.743; Figures 3D,H). Univariate Cox regression was also utilized to analyze the prognostic value of the risk score and the patient's clinical characteristics, including age, gender, grade, and stage. The results revealed that the stage and risk score of our model were positively correlated with poor prognosis in HCC patients (HR > 1, $p < 0.05$; Figures 4A,C). Moreover, multivariate Cox regression indicated that the stage and risk score were independent prognostic factors for HCC patients ($p < 0.05$; Figures 4B,D).

3.4 Correlation analysis of prognostic lncRNAs and m6A-related regulators

Emerging evidence has shown that RNA modification plays an important role in the expression and function of lncRNA. N6-methyladenosine (m6A) is the most abundant modification of RNA, and m6A regulators contribute to HCC by regulating various biological processes. To elucidate the relativity of m6A methylation and prognosis-related lncRNAs, we performed univariate Cox regression to screen the prognostic m6A regulators in HCC patients from TCGA dataset. The results showed that 12 m6A regulators were significantly correlated with the prognosis of HCC patients (Figure 4E, $p < 0.05$), and the heatmap depicted their expression alteration in tumor tissues and normal tissues (Figure 4F). Pearson's correlation coefficient was further conducted to analyze the relationship between prognostic m6A-related regulators and six prognostic lncRNAs obtained from the prognostic signature (Figure 4G). The m6A-related regulator RBMX was found to be significantly correlated with the prognostic lncRNA AC115619.1 (Figure 4H; cor = −0.46, p = 3.8e-20).

3.5 Validation of the potential significance of the hub lncRNA AC115619.1 in public databases

Since AC115619.1 has significantly contributed to HCC prognosis, is correlated with the m6A-related regulator RBMX tightly, and has never been reported in HCC, we selected AC115619.1 as a hub lncRNA for further investigation. Then, four datasets from the GEO database were used to validate the expression of lncRNA AC115619.1 in HCC patients. GSE84004, GSE93789, GSE115018, and GSE138178 datasets all showed that the expression of lncRNA AC115619.1 in HCC tumor tissues was significantly lower than that in normal tissues (Figures 5A–D; $p < 0.05$). We further analyzed the association between high and low AC115619.1 expression and the clinicopathologic characteristics of HCC patients obtained from TCGA database. As shown in Table 2, AC115619.1 expression was associated with tumor grade ($p = 0.013$), tumor invasion ($p < 0.001$), and TNM stage ($p < 0.001$). However, AC115619.1 expression was not associated with age ($p = 0.714$), gender ($p = 0.765$), lymph node metastasis ($p = 0.974$), and distant metastasis ($p = 0.553$, Table 2). We also verified the clinical significances in HCC patients with different subsets obtained from TCGA database. The expression of lncRNA AC115619.1 was found to be correlated negatively with tumor grade, tumor invasion, and, partly, TNM stage (Figures 5E–G). Additionally, the prognostic value of lncRNA AC115619.1 in predicting the patient's OS was estimated, as shown in Figure 5H. The Kaplan–Meier curve showed that HCC patients with high AC115619.1 expression had a better OS obviously (Figure 5H; $p < 0.05$; HR = 0.56, 95% CI: 0.39–0.79). These data indicated a tumor suppressor role of AC115619.1 in HCC.

3.6 Construction of a ceRNA network and functional enrichment analysis

We also constructed a ceRNA network through the miRcode database to explore the potential interaction miRNAs of AC115619.1. We found that there were 11 miRNAs that possessed interaction positions with lncRNA AC115619.1 (Supplementary Figure S1A). The target mRNAs of miRNAs which potentially interact with AC115619.1 were further screened by combining the miRDB, miRTarBase, and TargetScan databases together. For predicting the target mRNAs more accurately, these screened mRNAs were further intersected with HCC differentially expressed mRNAs (DEmRNAs) obtained from TCGA database. A total of five miRNAs, namely, miR-212-3p, miR-129-5p, miR-301b-3p, miR-449c-5p, and miR-137, which might regulate the 60 DEmRNAs, were finally identified (Figure 6A, $|\log_2 FC|>2$, $p < 0.01$). To further investigate the biological insights and pathway of lncRNA AC115619.1, we performed GO and KEGG analyses. GO annotation revealed that the biological processes of AC115619.1 were primarily associated with ATPase activity, transcription co-regulator activity, and tubulin binding (Figure 6B). The KEGG pathway analysis showed that AC115619.1 was involved in the pathway of endocytosis, cell cycle, and spliceosome (Figure 6C). Additionally, genes/enzymes from the most remarkable enrichment cell cycle pathway were negatively correlated with the expression of AC115619.1 in HCC (Supplementary Figure S1B).

3.7 Patient responses to chemotherapy and targeted therapy, and the immunocyte infiltration landscape of AC115619.1

To promote the potential clinical application, we predicted the IC_{50} value of commonly used chemotherapeutic and targeted agents in high and low AC115619.1 expression groups based on the algorithm provided in the pRRophetic R package. The IC_{50} values of 5-fluorouracil, gemcitabine, paclitaxel, rapamycin, imatinib, sorafenib, sunitinib, and vinorelbine were higher in the high AC115619.1 expression group of HCC patients, indicating that HCC patients with low AC115619.1 expression were more sensitive to these eight drugs (Figures 7A–I). The detailed correlation was also provided (Figures 8A–I). Emerging evidence indicates that the immune microenvironment plays an important role in tumor progression. We also investigated the tumor immunocyte infiltration proportion between high and low AC115619.1 expression patients using the CIBERSORT algorithm. The results showed that memory B cells ($p = 0.017$), CD4 memory resting T cells ($p < 0.001$), T follicular helper cells ($p = 0.002$), and M0 macrophages ($p < 0.001$) were significantly enriched in the high and low AC115619.1 subgroups (Figure 8J).

3.8 Validation of the lncRNA AC115619.1 and RBMX in the local cohort

To validate the expression of AC115619.1 in local samples, we detected its expression level in our collected 43 pairs of HCC samples using the qRT-PCR assay. Our results demonstrated that AC115619.1 was downregulated in most HCC tumor samples compared to adjacent normal tissues (Figure 9A). Among the 43 pairs of HCC patient samples, the expression

of AC115619.1 in 27 normal liver tissues was higher than that in HCC samples (Figure 9A). To further evaluate the correlation between AC115619.1 and m6A-related regulator RBMX, immunohistochemistry staining was performed in these 43 pairs of HCC samples. As shown in Figure 9B, typical pictures of IHC staining revealed that RBMX was localized in the cell nucleus and the expression of RBMX in tumor tissues was higher than that in adjacent normal liver tissues, which was negatively correlated with the AC115619.1 expression in HCC (Figure 9B).

3.9 Overexpression of AC115619.1 inhibited the proliferation, migration, and invasion of HCC cells

Since AC115619.1 expression was downregulated in HCC tissues and low AC115619.1 expression is closely related to a poor prognosis of HCC patients, we used SNU-449 and HepG2 cell lines for further experiments. After transfection with the plasmid, the expression level of AC115619.1 was significantly upregulated, as confirmed by qRT-PCR (Figure 9C). CCK-8 and EdU assays demonstrated that overexpression of AC115619.1 inhibited the proliferation of HCC cells (Figures 9D,E). In addition, the wound healing migration and transwell invasion experiments revealed that overexpression of AC115619.1 repressed the migration and invasion abilities in both SNU-449 and HepG2 cells (Figures 9F,G). Taken together, our results suggested that AC115619.1 inhibited the progression of HCC.

4 Discussion

HCC is characterized with a low diagnosis rate and rapid progression at the early stage. Most patients are diagnosed at an advanced stage and lost the opportunity to receive curative surgery treatment. Due to the insidiousness and heterogeneity of HCC, there is no appropriate biomarker to accurately predict clinical prognosis. Therefore, developing novel biomarkers is important to improve the clinical outcomes of HCC patients.

In recent years, many biomarkers have been identified, owing to the great development of microarray and high-throughput sequencing technologies. LncRNAs have been investigated and proposed to be potential diagnostic and therapeutic biomarkers in human malignancies (Iaccarino and Klapper, 2021). In HCC, the aberrant expression of lncRNAs has been reported to be a potential biomarker for early diagnosis and predicting the prognosis (Dickson, 2016; Huang et al., 2020). For instance, lncRNA-D16366 was found to be downregulated in both tumor tissues and serum samples of HCC patients, which implied a significant value of diagnostic and prognosis prediction with its aberrant expression (Liu et al., 2014; Chao and Zhou, 2019). The lncRNA AC099850.3 was reported to be overexpressed and accurately predicted the prognostic outcomes of HCC patients (Wang et al., 2022). The present study identified 32 HCC-related DElncRNAs overlapping from 516 HCC patients obtained from GEO and TCGA datasets. Six lncRNAs (LINC02428, LINC02163, AC008549.1, AC115619.1, CASC9, and LINC02362) were screened by univariate Cox and LASSO regression and were then selected to construct a novel prognostic signature. Our results showed that patients with a high-risk score in the prognostic signature had a better survival rate, and the risk score was an independent prognostic factor for HCC patients in both training and test cohorts. This might provide a sensitive and specific model in predicting the patient's outcomes. Among the six lncRNAs in the prognostic model, LINC02163 and CASC9 were found to be upregulated in various cancers including HCC. LINC02163 and CASC9 were closely associated with the patient's survival and acted as a candidate prognostic biomarker with their significant values (Dong et al., 2018; Qin et al., 2020; Qi et al., 2021; Tian et al., 2021). LINC02362 and AC008549.1 were identified to be tumor-inhibitory lncRNAs and contributed to the patient's survival of HCC, while the lncRNA AC008549.1 was classified as a pyroptosis-related signature (Wang et al., 2022; Li et al., 2022). AC115619.1 was reported to be a ferroptosis-related lncRNA and showed to be an independent prognostic factor in gastric adenocarcinoma (Fu et al., 2020; Cai et al., 2022). Consistently, our results supported the aberrant expressions of the six lncRNAs in previous reports. Our multivariate Cox regression identified three lncRNAs (AC008549.1, AC115619.1, and CASC9) to be independent factors in HCC.

Since there is no report of AC115619.1 in HCC yet, we selected it as a hub lncRNA for further exploration. The clinical significance and expression level of AC115619.1 were validated both in public datasets and local HCC samples. To analyze its targeted miRNAs and potential pathways, a suite of bioinformatics methods was executed subsequently. Meanwhile, we analyzed the correlation between the differential expression of AC115619.1 and immune cell infiltration. The drug sensitivity of AC115619.1 was also provided to predict the IC_{50} value of chemotherapeutic and targeted agents for each patient. The results showed that the high expression of AC115619.1 had higher IC_{50} values in most common drugs used in HCC. In addition, we explored and validated an inhibitory biological function of AC115619.1 in HCC cells. Overexpression of AC115619.1 by the plasmid inhibited the proliferation, migration, and invasion *in vitro*. Combined with the aforementioned results, AC115619.1 exerted its potential therapeutic value by serving as an independent prognostic factor and tumor suppressor in HCC.

N^6-Methyladenosine (m6A), the most popular and common modification of mRNA, exerts a tremendous effect on posttranscriptional regulation (Zhang et al., 2020). In recent years, evidence revealed that m6A modification exists on non-coding RNA, including lncRNAs, and plays a critical role in deciding the fate of lncRNAs (Chen et al., 2020). The regulation effects of m6A modification might be attributed to the m6A regulators, which include methyltransferases, demethylases, and binding proteins. M6A regulators have been found to be the key component of m6A modification and play an essential role in the progression of human malignancies (Wang et al., 2017; Gao et al., 2021). It is reported that overexpression of METTL3 (m6A writer) increased the m6A level of colon cancer by enhancing the expression and protein binding effect of lncRNA RP11. As a result, METTL3 promoted the metastasis of colon cancer (Wu et al., 2019). The abnormal increase in m6A regulators has been revealed to be involved in the progression, drug sensitivity, and immune response of HCC, suggesting that targeting m6A-modified

lncRNAs might be a potential therapy strategy for HCC (Chen et al., 2020; Lin et al., 2020). METTL3 upregulated the expression of LINC00958 and LNCAROD to regulate the malignant phenotype of HCC (Zuo et al., 2020; Jia et al., 2021). KIAA1429, a component of the m6A methyltransferase complex, promoted the growth and metastasis of HCC by mediating m6A modification on GATA3 pre-mRNA (Lan et al., 2019). Herein, we identified 12 m6A regulators which were significantly related to the prognosis of HCC patients by univariate Cox regression analysis. Then, we revealed the correlation between the 12 m6A regulators and the six lncRNAs in the prognostic model and found that RBMX was the most correlated with the prognostic lncRNA AC115619.1. RBMX has been reported to be overexpressed in HCC tissues and cell lines, favoring malignant behavior and sorafenib resistance of HCC (Song et al., 2020). Similarly, our analysis showed that RBMX was highly expressed in HCC tumor tissues and negatively associated with the expression of lncRNA AC115619.1. Consistently, our results from the local cohort also confirmed the high expression of RBMX and the negative association with AC115619.1. Then, we hypothesized that the abnormal expression of lncRNA AC115619.1 might result from an m6A modification pattern through RBMX. However, the detailed relationship and whether m6A modification affects AC115619.1 need to be explored in the future.

In conclusion, this study identified and established a risk signature of HCC-related lncRNAs systematically, which could be applied to predict the prognosis of HCC patients. A novel lncRNA AC115619.1 was first identified as an independent prognostic factor for HCC patients, revealing an inhibitory effect of AC115619.1 and its correlation with RBMX. Our comprehensive evaluation of lncRNA provides a new therapeutic strategy for HCC patients.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University. The patients/participants provided their written informed consent to participate in scientific research.

Author contributions

GD designed and directed the study. BG and YH conducted most of the experiments and collected the data. YM and LM analyzed the data and statistical analyses. CL helped in the data analysis and literature search. GD drafted the manuscript. LM provided critical intellectual revision. 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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The landscape of lncRNAs in gastric cancer: from molecular mechanisms to potential clinical applications

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Gastric cancer (GC) is a highly prevalent and deadly malignant neoplasm worldwide. Currently, long non-coding RNAs (lncRNAs) have recently been identified as crucial regulators implicated in GC development and progression. Dysregulated expression of lncRNAs is commonly associated with enhanced tumor migration, invasiveness, and therapy resistance, highlighting their potential as promising targets for clinical applications. This review offers a comprehensive historical overview of lncRNAs in GC, describes the molecular mechanisms, and discusses the prospects and challenges of establishing lncRNAs as precision biomarkers.

KEYWORDS

long non-coding RNAs, gastric cancer, gastric carcinogenesis, historical overview, molecular mechanisms, biomarkers

1 Introduction

Gastric Cancer (GC) is a significant public health challenge due to its high incidence and mortality rates. The frequency of GC is correlated with biological sex, ethnicity, and geographic regions. In 2020, the estimated number of new cases exceeded 1 million, with approximately 768,793 associated deaths, encompassing both men and women. Table 1 provides an overview of the prevalence of GC relative to other cancer types, emphasizing its significance in the global burden of disease (Sung et al., 2021).

The leading causes established for the development of GC are replication errors, environmental and hereditary factors. Among environmental factors, nutritional habits, and infections by *Helicobacter pylori* and Epstein-Barr virus stand out (Tomasetti and Vogelstein, 2015; Ashktorab et al., 2017; Tomasetti et al., 2017; Assumpção et al., 2020).

The advances in next-generation sequencing technologies have suggested that aberrant expression of non-coding RNAs (ncRNAs) plays a critical role in GC. The discovery of ncRNAs has revolutionized cancer research, opening paths for novel insights into tumor biology. Previously, ncRNAs were thought to be by-products of transcription without important biological significance. However, in the 1960s, the first speculations on the regulatory function of RNA molecules emerged, and since then, they have been identified as key players in various physiological and pathological processes.

NcRNAs can be classified into two categories based on their length: short ncRNAs and long ncRNAs (lncRNAs). Short ncRNAs in the context of GC have been extensively studied, while there is a growing interest in exploring the potential clinical applications of lncRNAs (Denaro et al., 2019; Ahmad et al., 2021).

Based on data obtained from PubMed from 2010 to 2023, 2,456 articles were published investigating the relationship between GC and lncRNAs. GC stands out among the top five cancer types frequently associated with lncRNAs, as indicated in Table 1. These studies have made significant advancements in establishing the connections between lncRNAs and essential biological processes in GC, including cell proliferation, metabolic alterations, metastasis, and therapy resistance (Cao et al., 2021; Chen Y. et al., 2021; Ding et al., 2021).

This review explores the fundamental characteristics and historical perspective of lncRNAs in GC pathogenesis. Specifically, we focus on their regulatory roles in proliferation, invasion, epithelial-mesenchymal transition, and therapeutic response. Furthermore, we address the prospects and challenges associated with the clinical implementation of lncRNAs as precision biomarkers. By thoroughly examining these aspects, we aim to provide new insights into the potential use of lncRNAs as therapeutic targets and promising biomarkers for the effective GC management.

2 Key biological features and mechanisms of LncRNAs

The lncRNAs represent the most abundant group of ncRNAs, comprising transcripts longer than 200 nucleotides with minimal or absent protein-coding potential (Dahariya et al., 2019; Hartford and Lal, 2020). According to the manually curated GENCODE v41 database, the total estimated number of human lncRNA genes is 19,095 (54,291 transcripts). Other lncRNA databases such as NONCODE and LNCipedia proved higher estimates. NONCODE reports 96,411 human lncRNA genes and 173,112 transcripts, while LNCipedia suggests 56,946 and 127,802 transcripts (Volders et al., 2019; Zhao et al., 2021).

Initially, the description of lncRNAs was limited to those transcribed from intergenic regions. However, it is now understood that lncRNAs can originate from various regions within the genome, including the mitochondrial genome, DNA regulatory elements, 3′and 5′untranslated regions (UTRs), and nuclear genomic loci in both sense and antisense orientations relative to protein-coding genes (Dahariya et al., 2019; Mattick et al., 2023).

Similar to messenger RNA, most lncRNAs are transcribed by RNA polymerase II (RNAPII) and undergo splicing, polyadenylation, and 5′cap addition. Furthermore, lncRNAs typically exhibit a reduced number of exons and are expressed at lower levels than coding RNAs (Mattick et al., 2023). LncRNAs can undergo diverse processing mechanisms, such as non-sequential intron splicing (back splicing) to form circular RNAs (circRNAs) or capping at both ends by small nuclear RNAs (snoRNAs) (Xing and Chen, 2018). In some instances, the lncRNAs undertake post-transcriptional cleavage, leading to the formation of a helix at the 3′end as an alternative mechanism to protect against nucleolytic cleavage (Schmitz et al., 2016; Schlackow et al., 2017; Wang et al., 2017; Dahariya et al., 2019).

The primary sequences of lncRNAs show limited conservation across different species or even within the same species, making functional characterization difficult. Proteins are commonly categorized according to conserved domains and functional mechanisms, but this does not apply to lncRNAs. An example is observed in the lncRNAs Xist and Kcnq1ot1, which both suppress gene expression in cis by recruiting the Polycomb Repressive Complex (PRC). Despite their shared mechanism, these lncRNAs display significant nucleotide sequence differences. This divergence suggests that factors beyond nucleotide sequences are crucial in controlling their regulatory activities (Fang and Fullwood, 2016; Kirk et al., 2018; Dahariya et al., 2019).

In contrast, the structural features of lncRNAs are highly conserved and considered relevant to determine their biological function. The formation of thermodynamically stable structures enables lncRNAs to interact with various biomolecules. These interactions involve RNA, DNA, and proteins, allowing lncRNAs to exert regulatory control over gene expression at multiple levels, including pre-transcriptional, transcriptional, post-transcriptional, translational, and post-translational processes. LncRNAs exert regulatory control through several molecular mechanisms, which can be categorized into distinct archetype (Zhang P. et al., 2019; Nandwani et al., 2021). Below are described some of them.

I) **Decoy** lncRNAs sequester specific regulatory factors, including transcription factors, RNA-binding proteins (RBPs), and catalytic proteins. LncRNAs acting as miRNA sponges are also included in this group.

TABLE 1 The top 5 cancer types worldwide, considering estimated cases and deaths for both men and women.

Cancer site	Incidence		Mortality		PubMed
	No. of cases	% of all sites	No. of deaths	% of all sites	No. of publications[a]
Female Breast	2,261,419	11.7	684,996	6.9	3,277
Lung	2,206,771	11.4	1,796,144	18.0	4,169
Prostate	1,414,259	7.3	375,304	3.8	1,303
Colon	1,148,515	6.0	576,858	5.8	3,238
Stomach	1,089,103	5.6	768,793	7.7	2,456

[a]Publications related to the role of lncRNAs in various types of cancer.
Source: Adapted of Sung et al., 2021.

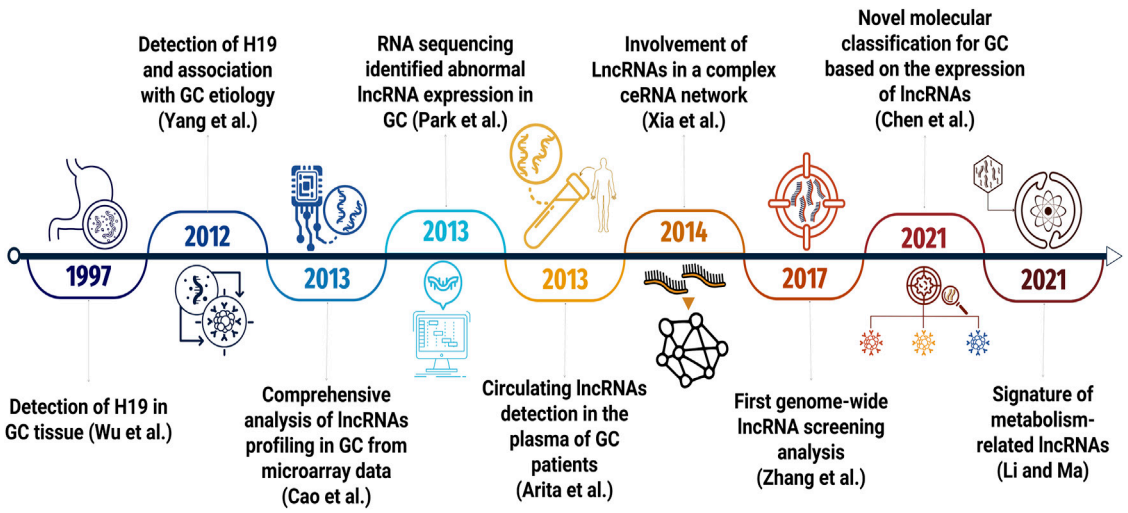

FIGURE 1
Timeline highlighting the key milestones in lncRNA research related to GC.

II) **Scaffolds** lncRNAs serve as building blocks of ribonucleoprotein complexes (RNP) complexes that regulate gene expression through epigenetic and transcriptional control.
III) **Signals** lncRNAs are expressed at specific time points and subcellular regions, where they act as molecular signaling. Their role involves interacting with chromatin-modifying enzymes, such as histone methyltransferases, in order to silence target genes or block their transcription via chromatin remodeling.
IV) **Guide** lncRNAs recruit transcription factors, RNAPII, and RNPs to specific loci, with the targeting being dependent on the biological context.

Detecting lncRNAs in human circulation further enhances their potential as targets for clinical applications. Extensive research has revealed the presence of lncRNAs in body fluids, including peripheral blood, gastric juice, and saliva (Anfossi et al., 2018). Notably, Shao et al. (2016) have demonstrated that the levels of lncRNAs in plasma are unchanged for up to 8 freeze-thaw cycles under different incubation temperatures (4°C and 20°C). The stability can be explained by their packing in extracellular vesicles such as apoptotic bodies, microvesicles, and exosomes. Circulating exosomal lncRNAs have emerged as promising biomarkers for GC (Zhang et al., 2021; Badowski et al., 2022; Sun et al., 2023).

3 A historical perspective on the role of lncRNAs in GC

The investigation of lncRNAs in GC is a relatively recent field of research, originating from early studies published in the late 1990s. However, for over a decade, the scientific community primarily directed its attention towards investigating lncRNAs in other cancer types. It was not until 2012 that substantial interest emerged in unraveling the involvement of these regulatory elements in the progression of gastric tumors. Figure 1 illustrates a timeline of major GC-related lncRNAs research milestones.

The first paper to investigate the expression profiles of lncRNAs in GC was published in 1997. Wu et al. (1997) evaluated the H19 lncRNA and *IGF2* gene in a group of 70 patients diagnosed with GC, focusing on transcriptional expression, loss of imprinting, and heterozygosity. Out of the patients assessed, 28 individuals showed heterozygosity for the H19, but no significant associations with clinicopathological features were detected.

Following this study, research involving lncRNA and GC remained stagnant for a long time. It was not until 2012 that interest in investigating these regulatory elements in this type of tumor resumed. In that particular year, Yang et al. conducted a comparative analysis of H19 expression in GC tissues and adjacent tissues. They discovered that the overexpression of H19 is associated with increased cell proliferation, whereas the suppression of these lncRNA induces apoptosis in GC cell lines (Yang et al., 2012).

The first comprehensive analysis of global expression profiles of lncRNAs in GC was published in 2013. From microarray mining data in Gene Expression Omnibus, Cao et al. identified 88 differentially expressed lncRNAs between tumor and adjacent non-tumour tissue. Among the most relevant lncRNAs in the research, they found LINC00152 and PVT1. These two lncRNAs were some of the most dysregulated in the GC. Furthermore, in a validation dataset, these results were 59% similar, providing substantial evidence for the functional significance of this class of transcripts in the context of GC (Cao et al., 2013).

During the same period, parallel investigations utilizing high-throughput RNA sequencing (RNA-seq) were underway. Park et al. (2013), in a pioneering work, identified 31 intergenic lncRNAs differentially expressed in GC, findings coincident with previous work using microarray data (Park et al., 2013).

The increased levels of lncRNAs in GC tissues has prompted investigations into their presence in the bloodstream of individuals. In 2013, Arita et al. conducted a study to assess the expression of H19, HOTAIR, and MALAT1 in plasma samples obtained from GC

patients and healthy controls. Only H19 showed higher levels in GC patients when compared to control, and a reduction in levels was also observed in postoperative plasma (Arita et al., 2013).

Another potential avenue in the field of investigating lncRNAs is a competing endogenous RNAs (ceRNA) hypothesis. From bioinformatics analyses, Xia et al. (2014) identified that lncRNAs may harbor microRNA response elements (MREs) and participate in a complex ceRNA network. Understanding these regulatory networks may be an alternative to developing new therapeutic approaches (Xia et al., 2014).

In the following years, extensive clinical and *in vitro* studies were conducted to elucidate the role of lncRNA in GC. Promising results showed that aberrant expression of lncRNAs is associated with the regulation of cell proliferation, invasion, apoptosis, response to treatment, tumor metastasis, and poor prognosis (Wang et al., 2014; Song et al., 2016; Zhang et al., 2017; Qin et al., 2018; Chen et al., 2020; Sun et al., 2021).

Only in 2017, the first genome-wide lncRNA screening analysis was published. The study was divided into four phases: discovery, training, validation, and external, and it brought together a total of 321 individuals. Microarray analyses revealed several differentially expressed lncRNAs; among them, five novel lncRNAs, TINCR, CCAT2, AOC4P, BANCR, and LINC00857, were detected in tumor tissue samples and pre and post-operative plasma. This signature made it possible to distinguish with high precision and sensitivity between GC patients, precancerous lesions, gastrointestinal, stromal tumors, and healthy controls. Additionally, this study demonstrated how lncRNA profiles could be highly dynamic, providing a less invasive alternative for GC monitoring and detection (Zhang et al., 2017).

Another significant advance in GC research was the development of a molecular classification based on the expression of 1,235 tumor-specific lncRNAs. Three clinically relevant molecular subtypes were identified: L1, L2, and L3 confirmed by microarray data analysis. The L3 subtype showed a worse prognosis, potentially due to the abundance of oncogenic lncRNAs, such as DUXAP8 and H19, associated with tumor progression. These results emphasize the dynamic nature of lncRNA expression and their utility as reliable prognostic markers for GC (Chen Y. et al., 2021).

More recently, lncRNAs have also been associated with GC metabolism. The metabolic profiles of individual tumors are highly heterogeneous, and the molecular action of lncRNAs strongly influences metabolic pathways. In a study published by Li and Ma (2021), a signature of 1,539 metabolism-related lncRNAs was identified, which allowed the classification of GC into two subtypes with different drug sensitivities (Li and Ma, 2021).

4 Exploring the role of LncRNAs in the regulation of GC development and progression

Dysregulated expression of lncRNAs is a common occurrence in cancers (Qian et al., 2020). In GC, these transcripts play a crucial role in promoting carcinogenesis through the modulation of cellular mechanisms, such as proliferation, stemness, tumor immune escape, invasion, angiogenesis, and drug resistance of tumor cells (Zhang X.-Z. et al., 2020; Gui et al., 2021; Jiang et al., 2021; Razavi et al., 2021; Sun et al., 2023).

4.1 Emerging role of lncRNAs in GC invasion and migration

Migration and invasion are essential mechanisms for cancer progression (Friedl and Wolf, 2003). Recent studies have shed light on the role of lncRNAs in regulating these processes by influencing cytoskeleton reorganization (Tang et al., 2018; Zhang G. et al., 2019; García-Padilla et al., 2022; Raei et al., 2022).

HOXA11-AS is a lncRNA implicated in the progression and metastasis of GC cells and tissues. It exerts its effects by modulating the miR-124-3-ITGB3 axis (You et al., 2021). ITGB3, a member of the integrin family, is positively regulated in GC and plays a critical role in focal contacts during cell migration by binding to extracellular matrix (ECM) ligands (Zhu et al., 2019).

Another lncRNA, DANCR, has been associated with cell migration and invasion in GC tissues. Its expression is positively regulated through the interaction between Enhancer Of Zeste 2 Polycomb Repressive Complex 2 Subunit (EZH2) and histone deacetylase 4 (HDAC4) (Mao et al., 2017). This interaction leads to the epigenetic suppression of lncRNA-LET transcription. EZH2 overexpression in GC cells contributes to the modulation of PTEN and Akt phosphorylation, promoting epithelial-mesenchymal transition. EZH2 also regulates the expression of metalloproteinases, such as MMP-9, associated with aggressive tumors in GC (Gan et al., 2018). Although no studies in GC demonstrate the dynamics between lncRNA-EZH2-MMP, this axis is a plausible candidate for future investigations aimed at better elucidating the migration and invasion process.

The lncRNA XIST is linked to multiple carcinogenesis aspects (Yang et al., 2021). Notably, in GC cell lines, XIST has been found to promote invasion and migration via its role as a molecular sponge for miR-337, which regulates the expression of *JAK2* (Zheng W. et al., 2020). The JAK-STAT3 signaling pathway has been previously implicated in regulating cellular motility, invasion, and migration (Teng et al., 2014). Thus, the involvement of XIST in this pathway further underscores its potential oncogenic role in GC.

Recent investigation has linked the lncRNA AK025387 to promoting cancer cell migration and invasion through the MAPK signaling pathway. This study revealed a positive correlation between AK025387 expression and the genes Raf-1, MEK2, and ERK (Sun et al., 2020). The MEK/ERK pathway has been activated in various types of tumors, including GC. Moreover, the proteins involved in the MAPK pathway contribute to regulating and activating MMPs and FAK, two essential proteins involved in focal adhesion and extracellular matrix degradation (Yang and Huang, 2015).

DSCR8, another lncRNA, promotes tumor cell progression in GC patients by acting as a miR-137 sponge and positively regulating Cdc42 expression. The reorganization of the cytoskeleton during tumor cell migration and invasion is typically dependent on Cdc42-mediated stimulation. DSCR8 is also closely associated with various clinicopathological features of GC, including tumor size, metastasis, and tumor-node-metastasis (TNM) stage (Chen Z. et al., 2021).

Moreover, LINC00152 is an onco-lncRNA overexpressed in GC tissues, particularly in patients with advanced GC, and associated with poor patient outcomes. Knockdown of LINC00152 has been shown to reduce the proliferative, migratory, and invasive capacity of cell lines and the size of the xenograft tumor by regulating the miR-193b-3p/ETS1 axis (Wang et al., 2019). These findings suggest that lncRNAs are crucial in cancer development and progression and may be potential therapeutic targets.

Functional studies conducted *in vitro* and *in vivo* have provided valuable insights into the oncogenic properties of another lncRNA, LINC00355. Specifically, LINC00355 has been identified as a promoter of crucial cancer-related processes, including proliferation, migration, and invasion, while inhibiting apoptosis in GC cells. At molecular level, LINC00355 interacts with histone deacetylase 3 (HDAC3) to suppress the transcriptional activity of tumor protein-induced nuclear protein 1 (TP53INP1), a stress-responsive protein with tumor-suppressor function. This interaction triggers the epithelial-mesenchymal transition process, which is closely associated with increased metastatic potential and disease progression in cancer (Zhao et al., 2023).

These findings underscore the critical role of lncRNAs in cancer development and progression. Further research in this field will contribute to a deeper understanding of the complex mechanisms involved in lncRNA-mediated regulation of migration and invasion, resulting in improved treatment options for GC patients.

4.2 LncRNAs-mediated modulation of drug response in GC

The management of GC necessitates a comprehensive multimodal approach, encompassing surgical resection, adjuvant and/or neoadjuvant chemotherapy, radiation, and targeted therapy as appropriate for specific cases. Chemotherapeutic regimens commonly incorporate a variety of pharmacological compounds, including platinum agents, taxanes, and antimetabolites (Yamashita et al., 2021). Nevertheless, the frequent development of therapy resistance poses a substantial barrier to improving survival outcomes. Recently, lncRNAs have emerged as crucial drug sensitivity and resistance mechanism regulators (Ahmad et al., 2021; Liu et al., 2022).

Platinum-based agents, including cisplatin and oxaliplatin (OXA), are classified as alkylating compounds that form bonds with DNA molecules, leading to errors in pairing DNA bases. Consequently, they prevent strand separation during DNA synthesis (Bukowski et al., 2020). According to a study by Zhang et al. (2020), high levels of MALAT1 are correlated with resistance to OXA. However, when MALAT1 was silenced, cell proliferation in resistant cell lines decreased, leading to apoptosis and increased sensitivity to OXA (Zhang Z. et al., 2020).

Similarly, the lncRNA EIF3J-DT has also been implicated in chemoresistance to OXA. Functionally, EIF3J-DT modulates the expression of *ATG14*, a gene encoding a protein essential for autophagosome assembly, through two distinct mechanisms. Firstly, it directly interacts with the mRNA of ATG14, increasing its stability and expression. Secondly, it sequesters the miRNA MIR188-3p, which prevents the degradation of *ATG14*. The EIF3J-DT-MIR188-3p-ATG14 axis has been identified as a crucial pathway involved in the activation of autophagy and chemotherapy resistance in GC cells (Menon and Dhamija, 2018; Luo et al., 2021).

Another lncRNA, PCAT-1, is overexpressed in both GC tumor tissues and cisplatin-resistant cell lines, and its increased expression has been associated with chemotherapy resistance, attributed to the epigenetic repression of the *PTEN* gene. PCAT-1 achieves this repression by recruiting EZH2 and promoting enhanced trimethylation of lysine 27 on histone 3 (H3K27me3) (Li et al., 2020). Moreover, PCAT-1 functions as a ceRNA for miR-128, thereby regulating the expression of its downstream target gene, *ZEB1* (Guo et al., 2019). These findings highlight the multifaceted regulatory roles of PCAT-1 in GC pathogenesis, encompassing epigenetic modification and ceRNA-mediated gene regulation.

The lncRNA LINC00942 has also been identified as contributing to GC cisplatin resistance. Microarray analysis revealed significant upregulation of LINC00942 in chemoresistant cells, and its knockdown resulted in increased apoptosis rates. LINC00942 localizes in the cytoplasm, allowing interactions with RBPs to modulate gene expression. Notably, LINC00942 specifically interacts with Musashi2 (MSI2), an RBP known for its tumorigenic properties and involvement in key signaling pathways like NOTCH and Ras/MAPK. By inhibiting β-Trcp-mediated degradation of MSI2, LINC00942 influences the expression of c-Myc mRNA. These results emphasize the importance of the LINC00942/MSI2/c-Myc axis in regulating chemotherapy sensitivity and its potential as a target for therapeutic intervention (Zhu et al., 2022).

Furthermore, the lncRNA UCA1 plays a role in modulating sensitivity to adriamycin in GC cells. UCA1 overexpression has been demonstrated to decrease cell apoptosis through its ability to regulate miR-27b negatively (Fang et al., 2016). These findings point to the pivotal role of lncRNAs in drug resistance mechanisms, thereby highlighting their potential as therapeutic targets for GC management.

4.3 The emerging role of lncRNAs in GC immune responses

The immune system possesses remarkable self-renewal and cell differentiation capabilities, crucial for developing various lymphocyte lineages, such as natural killer (NK), B, and T cells. Emerging evidence highlights the pivotal role of lncRNAs in orchestrating these intricate processes with their dynamic and cell-specific expression patterns (Chen et al., 2017; Bocchetti et al., 2021).

In the context of cancer, the dysregulation of lncRNAs has been implicated in immune evasion mechanisms, impacting patient survival (Denaro et al., 2019). For instance, studies have elucidated the influence of LncRNA HCG18 in GC-exosomal cells, which promotes the polarization of M2 macrophages through the upregulation of *KLF4* and downregulation of miR-875-3p. These molecular alterations have been associated with shorter patient survival times and increased malignancy, highlighting the prognostic value of such expression profiles as well as their potential as therapeutic targets (Gambardella et al., 2020; Piao et al., 2020; Hu et al., 2021).

Moreover, the dysregulation of lncRNAs contributes to the modulation of the tumor immune microenvironment (TIME), creating a conducive milieu for cancer growth and progression. These lncRNAs impact fundamental immune response mechanisms, such as antigen presentation, regulation of T cells, and modulation of programmed death-ligand 1 (PD-L1) (Mofed et al., 2022; Pi et al., 2021).

Exploring this field, researchers have developed and tested prognostic signatures utilizing the expression patterns of specific lncRNAs. A strong association was observed between immune infiltrating status and risk scores. Patients with higher immunophenoscores have better survival rates. This score is a measure of tumor immunogenicity. These findings propose that patients can be categorized into various prognostic groups based on their lncRNA signatures. Thus, it becomes possible to explore the development of potential immune checkpoint inhibitors (Ding et al., 2021).

Several studies have explored the role of lncRNAs in regulating the Programmed Cell Death 1 (PD-1)/PD-L1 pathway. For instance, the lncRNA SNHG15 has been found to correlate positively with *PD-L1* expression in GC cell lines. Functionally, SNHG15 acts as an endogenous competitor of miR-141, leading to increased PD-L1 expression and promoting immune resistance in GC (Dang et al., 2020).

Furthermore, the lncRNA NUTM2A-AS1 has been implicated in promoting tumorigenesis and drug resistance through its modulation of PD-L1. Acting as a ceRNA for miR-376a, NUTM2A-AS1 targets the expression of *TET1* and *HIF-1A*. Moreover, the study has shown that *TET1* interacts with HIF-1A to regulate the expression of *PD-L1*. These findings suggest that lncRNAs, through the lncRNA/miRNA/mRNA axis, play a role in immune evasion by modulating *PD-L1* expression (Wang et al., 2020).

LINC00152 has also emerged as a critical regulator in tumor cell growth by modulating the infiltration of $CD8^+$ T cells. LINC00152 recruits EZH2 to the promoters of chemokines *CXCL9* and *CXCL10/CXCR3*, leading to their repression. Conversely, silencing LINC00152 promotes the expression of these chemokines, resulting in increased infiltration of $CD8^+$ T cells. This influx of $CD8^+$ T cells into the tumor microenvironment and the expression of CXCL9 and CXCL10 may potentiate the therapeutic effects of immune checkpoint blockade, such as anti-PD-1 therapy. Hence, LINC00152's involvement in triggering antitumor T-cell immunity underscores as a potential target for immunotherapeutic interventions (Ou et al., 2021).

In summary, lncRNAs play intricate roles in immune regulation and tumor progression. Their dysregulation impacts immune evasion, immune cell infiltration, and modulation of crucial immune checkpoint molecules. Understanding the mechanisms underlying these interactions holds promise for developing novel therapeutic strategies targeting lncRNAs in cancer immunotherapy.

5 LncRNAs as potential biomarkers in GC

Biomarkers are essential indicators of specific conditions, encompassing normal biological processes, pathogenic processes, or pharmacological responses (Dancey et al., 2010). In clinical practice, protein- or peptide-based biomolecules are tumor markers. However, their sensitivity and specificity are limited, and traditional markers such as carcinoembryonic antigen (CEA) and cancer antigen 19–9 (CA19-9) have demonstrated ineffectiveness in the early detection of GC (Nakamura et al., 2019). Furthermore, despite being included in GC treatment guidelines, HER2-targeted therapies have not yielded satisfactory clinical outcomes (Hecht et al., 2016; Tabernero et al., 2018). Therefore, developing precise and reliable biomarkers is crucial for effective GC management.

Fortunately, high-throughput technologies have enabled the identification of more effective biomarkers, including lncRNAs. These molecules possess notable features such as high stability, abundance in body fluids, tissue-specific expression, versatile interactions with biomolecules, and diverse roles in gene expression regulation. Consequently, lncRNAs promise improved diagnosis, prognosis, and treatment of GC (Guimarães et al., 2018; Li et al., 2021; Liu et al., 2022; Hosseini et al., 2023).

Numerous studies have underscored the potential of lncRNAs in effectively distinguishing GC patients from healthy individuals with high sensitivity and specificity. Moreover, the clinical relevance of lncRNA expression in GC has been extensively investigated. To provide an overview of the most recent research in this field, Table 2 summarizes studies published within the last 4 years.

For instance, overexpression of LINC00152 has been consistently observed in GC (Mao et al., 2019; Ou et al., 2021; Li et al., 2022). In the serum and tissue, LINC00152 expression levels distinguished GC patients from healthy control and played a role as a robust prognostic indicator. Specifically, overexpression of LINC00152 exhibited a positive correlation with advanced TNM stage, lymph node metastasis, tumor invasion depth, and poorer overall survival, indicating a more aggressive disease phenotype (Ou et al., 2021).

In 2022, a newly identified lncRNA TCLlnc1 has shown higher expression levels in tissues and plasma samples from GC patients than in healthy controls. TCLlnc1 levels demonstrated significant distinguished early-stage and advanced-stage GC patients from healthy individuals, with respective area under the curve (AUC) values of 0.71 and 0.97, respectively. Furthermore, their overexpression was correlated with distant metastasis. These findings indicate that TCLlnc1 holds promise as a potential diagnostic and prognostic biomarker for GC (Hu et al., 2022).

Extracellular vesicles (EVs), secreted by viable cells, have been verified to show specific information from their cells of origin, specially lncRNAs levels. Xiao et al. (2021) observed CCAT1 levels significantly higher in the serum EVs from GC patients compared with healthy controls, patients with chronic gastritis, and atypical hyperplasia. EVs CCAT1 produced an AUC value of 0.890 with a sensitivity and specificity of 79.6% and 92.6%, respectively. These researchers determined CCAT1 as a lncRNA stable in serum EVs and a potential prognostic biomarker for GC (Xiao et al., 2021).

Recently, Zhao et al. found that LINC00355 exhibits significantly higher expression levels in exosomes derived from the plasma of GC patients than in healthy controls. Moreover, its expression is markedly elevated in GC tumor tissues compared to adjacent non-tumor tissues, with a positive correlation observed between

TABLE 2 LncRNAs as potential biomarkers of Gastric Cancer.

LncRNA	Expression	Potential biomarker	AUC	Sample type	Clinical implication	References
CCAT1	Up	Diagnosis and prognosis	0.89	Tissue and serum	Poor survival outcomes	Xiao et al. (2021)
TCLlnc1	Up	Diagnosis and prognosis	0.97	Tissue and plasma	Tumor distant metastasis and poor survival outcomes	Hu et al. (2022)
DRAIR	Down	Diagnosis and prognosis	0.89	Tissue and plasma	Poor survival outcomes	Jin (2021)
NR038975	Up	Diagnosis	0.71	Tissue and plasma	Advanced TNM stage	Wei et al. (2021)
lncRNA-GC1	Up	Diagnosis	0.90	Tissue and serum	Advanced TNM stage	Guo et al. (2020)
	Up	Prognosis and predictive	0.70	Plasma	Poor survival outcomes and worse response to chemotherapy	Song et al. (2022)
LINC00152/CYTOR	Up	Prognosis	—	Serum	Advanced TNM stage and poor survival outcomes	Ou et al. (2021)
PTCS3	Down	Diagnosis and prognosis	0.92	Plasma	Poor survival outcomes	Zhang et al. (2020)
LINC00355	Up	Prognosis	—	Tissue and plasma	Advanced TNM stage, distant metastasis, and poor survival outcomes	Zhao et al., 2023, Zhao et al., 2020
lnc-SLC2A12-10:1	Up	Diagnosis	0.77	Tissue and plasma	Advanced TNM stage	Zheng et al. (2020)
LINC01614	Up	Prognosis	—	Tissue	Poor survival outcomes	Chen et al. (2021)
LINC00941	Up	Prognosis	—	Tissue	Poor survival outcomes	Liu et al. (2019)
CEBPA-AS1	Up	Diagnosis and prognosis	0.82	Tissue and plasma	Tumor size, Bormann type, and TNM stage	Piao et al. (2020)
H19	Up	Diagnosis	0.85	Serum	Advanced TNM Stage	Zhou et al. (2020)
HOXA11-AS	Up	Diagnosis and prognosis	0.92	Tissue and serum	Poor survival outcomes and TNM stage	Liu et al. (2019)
B3ALT5-AS1	Up	Diagnosis and prognosis	0.81	Serum	Poor survival outcomes, LNM and TNM stage	Feng et al. (2020)
SSTR5-AS1	Up	Prognosis	—	Tissue	Poor survival outcomes and distant metastasis	Cheng et al. (2020)
MIAT	Up	Diagnosis	0.89	Serum	Poor survival outcomes and TNM stage	Xu et al. (2020)
DIRC1	Up	Diagnosis and prognosis	0.77	Tissue	Poor survival outcomes	Lin et al. (2022)
HCP5	Up	Diagnosis	0.82	Serum	Differentiation, lymph node metastasis, and nerve invasion	Qin et al. (2021)
p4516	Up	Prognosis	—	Tissue	Poor differentiation, advanced TNM stage and poor survival outcomes	Nie et al. (2019)
PANDAR, FOXD2-AS1, and SMARCC2	Up	Diagnosis	0.84	Plasma	Poor differentiation and advanced TNM	Yang et al. (2019)
FAM49B-AS, GUSBP11, and CTDHUT	Up	Diagnosis	0.82	Plasma	—	Zheng et al. (2019)

LINC00355 expression, the depth of invasion and TNM stage. Significantly, LINC00355 overexpression is associated with poorer overall survival outcomes in GC patients. Taken togheter, these findings indicate the oncogenic role of LINC00355 in GC and its potential as a diagnostic and prognostic biomarker (Zhao et al., 2023).

A comprehensive series of multi-phase studies have highlighted the potential clinical significance of lncRNA-GC1 as a valuable biomarker for various aspects of GC. The first investigation, published in 2020, encompassed 826 participants, including 522 individuals diagnosed with GC, 85 subjects with gastric precancerous lesions, and 219 healthy donors. The

findings demonstrated that elevated levels of exosomal lncRNA-GC1 exhibited accuracy in effectively distinguishing between GC patients and healthy donors, as evidenced by an AUC value of 0.903 (Guo et al., 2020).

Interestingly, lncRNA-GC1 levels were significantly higher in patients with early-stage GC, intestinal metaplasia, chronic atrophic gastritis, and positive *H. pylori* infection. This suggests that lncRNA-GC1 may serve as a reliable biomarker for early GC progression detection and monitoring. In addition, lncRNA-GC1 expression demonstrated a gradual increase in correlation with the progression of TNM stage, further supporting its potential as a prognostic indicator. An essential aspect of these studies was the simultaneous evaluation of commonly used clinical markers such as CEA, CA72-4, and CA19-9. The results indicated that lncRNA-GC1 outperformed these markers in terms of diagnostic efficiency. Notably, lncRNA-GC1 expression remained consistent after treatment with RNase and exposure to multiple freeze/thaw cycles, demonstrating its robustness and stability (Guo et al., 2020).

In 2022, a retrospective study conducted across multiple medical revealed that the levels of circulating exosomal lncRNA-GC1 could effectively distinguish patients who would benefit from fluorouracil-based adjuvant chemotherapy. GC patients with lower levels of lncRNA-GC1 exhibited better responses to chemotherapy and improved survival outcomes. The consistent results across different studies and the robustness of lncRNA-GC1 expression make it an attractive candidate for further clinical validation and potential integration into routine clinical practice (Song et al., 2022).

In addition to their potential as biomarkers, lncRNAs hold promise as novel therapeutic targets. The diverse and intricate functional roles of lncRNAs provide opportunities for various therapeutic interventions. These include the modulation of lncRNA genomic loci to induce transcriptional repression, hindrance of secondary structure formation to prevent interactions with biomolecules, the introduction of synthetic lncRNAs, and modifications of expression patterns. Despite the therapeutic potential of lncRNAs, it is essential to note that no lncRNA-based therapies have yet progressed to phase II or III clinical development (Winkle et al., 2021).

The findings presented in this study highlight the promising potential of lncRNAs as valuable tools in clinical practice. However, it is crucial to acknowledge that the translation of lncRNAs into clinical applications is still in its early stages, with limited success thus far. Currently, only one lncRNA, PCA3, has been successfully translated into an FDA-approved molecular diagnostic test, namely, PCA3 ProgensaTM (Gen-Probe Inc., San Diego, CA, USA), which is primarily recommended for patients who have previously had a negative biopsy for prostate cancer (Cui et al., 2016).

5.1 Challenges of incorporating lncRNAs into clinical practice

Several vital aspects must be addressed to overcome the challenges of implementing lncRNAs in clinical practice. A primary challenge in lncRNA research is the limited sample size often encountered in studies. Many investigations have a relatively small number of participants, which can compromise the statistical power and precision of the results. Additionally, including healthy individuals as controls is crucial for validating the specificity and sensibility of lncRNA biomarkers. Some studies' absence of appropriate control groups can introduce biases and limit the accurate evaluation of biomarker efficacy (Zheng, 2018).

Another significant challenge is the prevailing focus on specific regions or ethnicities in lncRNA research. This geographic bias may hinder the generalizability and reproducibility of findings in broader populations. To ensure the clinical relevance and applicability of lncRNA biomarkers, including diverse populations and considering the potential influence of genetic and environmental factors is imperative (Zheng, 2018).

The lack of standardization in pre-analytical and experimental procedures represents another challenge in the field. The absence of well-established protocols for sample collection, processing, and analysis may impede the comparability and reliability of results across different studies. The harmonization and standardization of these procedures are critical to facilitate robust comparisons between studies and enhance the overall quality (Anfossi et al., 2018).

Furthermore, retrospective study designs are prevalent in lncRNA research, which can introduce inherent biases. Prospective studies are essential to validate lncRNA biomarkers' predictive and prognostic value. Long-term follow-up is necessary to assess the performance of these biomarkers in predicting treatment response, disease progression, and patient outcomes, thereby providing valuable insights for clinical decision-making (Anfossi et al., 2018).

In addition to technical challenges, the field of lncRNA research faces inherent obstacles related to the nature of lncRNAs. For example, the poor conservation of lncRNAs across different species poses difficulties in evaluating their functions and effects in animal models. The lack of conservation hinders the translation of findings from model organisms to humans and limits our understanding of the broader biological implications of lncRNAs (Winkle et al., 2021).

Moreover, lncRNAs are often expressed at low levels, which presents challenges in their accurate measurement and detection (Mattick et al., 2023). The quantification of lncRNAs requires sensitive and specific techniques that can reliably distinguish them from background noise and accurately determine their expression levels. Detecting specific lncRNAs in physiological processes can be challenging due to their transient or cell-type-specific expression patterns. To fully harness the potential of lncRNAs in clinical applications, further research efforts are needed to unravel their functional significance (Anfossi et al., 2018; Fathi Dizaji, 2020; Statello et al., 2021; Winkle et al., 2021).

Understanding the precise roles of lncRNAs in gene regulation, cellular processes, and GC pathogenesis is crucial for developing targeted interventions. GC can be anatomically classified into two main subtypes, cardial and non-cardial GC, each with distinct epidemiological profiles and mechanisms of carcinogenesis. However, currently, there are no available studies characterizing the expression patterns of lncRNAs based on the anatomical subtypes. Performing an exploratory investigation to identify specific lncRNA expression patterns in these subtypes is crucial

for implementing a more effective screening strategy (Cao et al., 2013; Thrift et al., 2023).

6 Discussion

GC poses a significant public health challenge due to its high incidence and mortality rates. Therefore, the identification of precise biomarkers and novel therapeutic targets is crucial for improved management. Abnormal expression of lncRNAs plays a crucial role in the development and progression of GC. By acting as master regulators of gene expression, lncRNAs exert substantial influence on cancer hallmarks, including cell proliferation, evasion of cell death, immune evasion, and metabolic alterations.

The discovery of lncRNAs has revolutionized the field of molecular biology since the publication of the first paper in 1997. Substantial scientific progress has been made in elucidating the involvement of lncRNAs in gastric carcinogenesis.

Current studies contribute to a more comprehensive understanding of the mechanisms by which lncRNAs exert their actions in GC. LncRNAs interact with biomolecules, acting as miRNA sponges, interacting with RNA-binding proteins, and modulating the expression of critical genes within pro-tumorigenic pathways.

Notably, most lncRNAs' expression is highly specific to tissues and cell types. Moreover, the widespread and stable presence of lncRNAs in body fluids, including blood, saliva, urine, and gastric juice, makes them promising candidates for clinical applications, including diagnostic biomarkers, prognostic indicators, predictors of therapeutic response, and potential targets for the development of personalized cancer treatment strategies.

Extensive research is currently dedicated to developing therapeutic strategies targeting lncRNAs. Multiple approaches are being explored, including antisense oligonucleotides (ASO), CRISPR/Cas9 technology, RNA interference (RNAi) using viral vectors, and nanotechnology-based delivery systems.

ASOs are single-stranded deoxyribonucleotides with complementary sequences to RNA targets. In the context of lncRNAs, the ASOs can bind to the desired lncRNA and induce degradation. Remarkably, ASOs targeting natural antisense transcripts (NATs) have demonstrated promising preclinical results in gene reactivation within the central nervous system. Similar to this approach, RNAi technology utilizes small interfering RNAs (siRNAs) or short hairpin RNAs (shRNAs) to target and silence specific lncRNAs. Viral vectors, such as lentiviruses or adenoviruses, can deliver these siRNAs or shRNAs into cells, enabling efficient knockdown of the target lncRNAs. Both ASOs and siRNAs can enhance their delivery to specific cells or tissues through nanotechnology-based systems. These nanocarriers can improve therapeutic molecules' stability, bioavailability, and cellular uptake and enhance the efficacy of therapeutic targets. Alternatively, the CRISPR/Cas9 technology inhibits or alters the expression of lncRNAs by introducing specific modifications to the DNA sequences that transcribe lncRNAs.

Currently, no lncRNA-targeted therapeutic intervention has progressed to clinical development. Nevertheless, lncRNAs are actively investigated as potential biomarkers. The FDA has approved the first lncRNA-based diagnostic test, PCA3 ProgensaTM, for prostate cancer. Moreover, ongoing research endeavors explore the clinical relevance of lncRNAs in a diverse array of complex diseases, extending beyond cancer to neurological conditions.

Despite the potential of lncRNAs as valuable tools in GC management, much remains to be explored and understood. Challenges such as small sample sizes, incorporating adequate healthy controls, mitigating geographic bias, establishing standardized protocols, tolerability issues, inefficient intracellular delivery, and overcoming the reliance on retrospective study designs must be addressed for successful translation into routine diagnostic tests and to ensure the safety and efficacy of therapeutic interventions in clinical settings.

Continued research, integration of multi-omics approaches, a multidisciplinary team, and large-scale multicenter studies are essential to advance our understanding of lncRNAs' role in tumorigenesis. This comprehensive approach may establish lncRNAs as robust biomarkers, thus propelling personalized management of GC.

Author contributions

JS, DC, and PA were involved in the conception and design of the study. JS and ET participated in writing the manuscript. FM, RF, and RM contributed to the review process. 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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The pivotal role of EMT-related noncoding RNAs regulatory axes in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) remains a major health problem worldwide, being the leading cause of cancer-related deaths, with limited treatment options, especially in its advanced stages. Tumor resistance is closely associated with the activation of the EMT phenomenon and its reversal, being modulated by different molecules, including noncoding RNAs (ncRNAs). Noncoding RNAs have the potential to function as both tumor suppressors and oncogenic molecules, controlling the malignant potential of HCC cells. Basically, these molecules circulate in the tumor microenvironment, encapsulated in exosomes. Their impact on cell biology is more significant than originally expected, which makes related research rather complex. The temporal and spatial expression patterns, precise roles and mechanisms of specific ncRNAs encapsulated in exosomes remain primarily unknown in different stages of the disease. This review aims to highlight the recent advances in ncRNAs related to EMT and classifies the described mechanism as direct and indirect, for a better summarization. Moreover, we provide an overview of current research on the role of ncRNAs in several drug resistance-related pathways, including the emergence of resistance to sorafenib, doxorubicin, cisplatin and paclitaxel therapy. Nevertheless, we comprehensively discuss the underlying regulatory mechanisms of exosomal ncRNAs in EMT-HCC via intercellular communication pathways.

KEYWORDS

hepatocellular carcinoma, noncoding RNA, epithelial-mesenchymal transition, chemoresistance, exosomes

1 Introduction

Hepatocellular carcinoma (HCC) is a common lethal malignancy among patients with chronic liver disease, with approximately 800,000 deaths annually, according to the GLOBOCAN 2020 report (Sung et al., 2021). Several treatment options are available for therapeutic purposes, such as trans-arterial chemoembolization (TACE) with anthracyclines, cisplatin, and multikinase inhibitor, sorafenib (Pratama et al., 2019). However, these treatments become challenging to manage, due to the appearance of invasion, metastasis and recurrence, whose key molecular sign is EMT (Yan et al., 2018).

EMT (epithelial-mesenchymal transition) is a morphogenetic process in which epithelial cells get a mesenchymal phenotype. In early EMT, transcriptional factors (TFs) are activated to repress epithelial genes and activate the mesenchymal ones. These transcriptional changes trigger the following key events: cell-cell junction dissociations, apical-basal polarity loss,

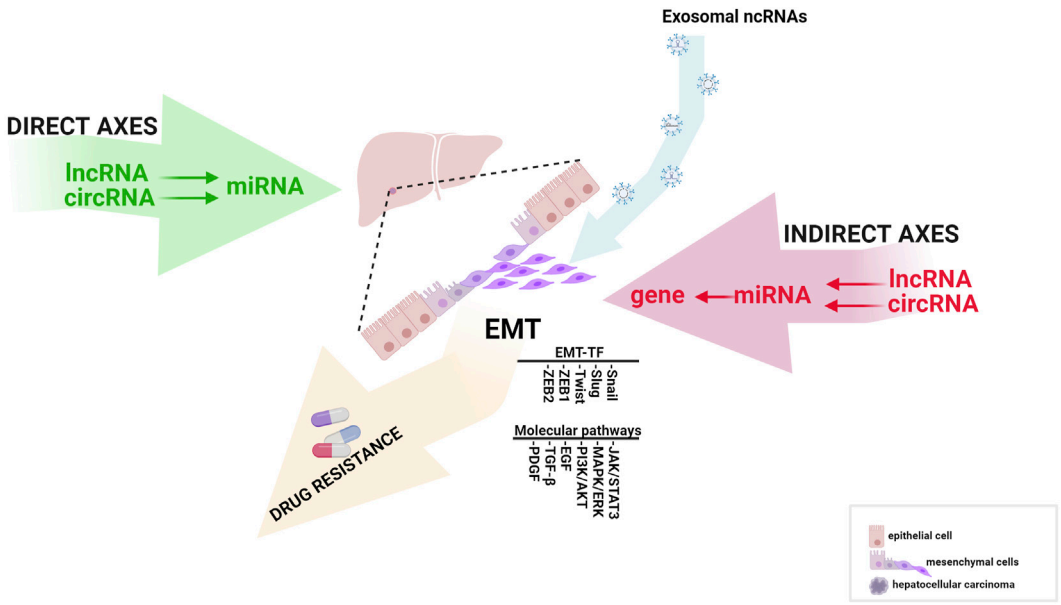

FIGURE 1
The influence of direct and indirect EMT-related ncRNA axes in HCC progression (created with biorender.com accessed on July 2023).

cytoskeleton architecture reorganization, the production of extracellular matrix (ECM) degradation enzymes, and cellular shape transformation. The activation of cellular pathways associates this process with proliferation, invasion, metastasis, and chemotherapy resistance (Yan et al., 2018; Dudas et al., 2020; Yang et al., 2020; Huang et al., 2022). Among these transformations, EMT is associated with numerous signaling pathways involved in inflammation, oncogenic and metabolic stress, hypoxia or apoptosis (Huang et al., 2022).

Moreover, many studies suggest that noncoding RNAs (ncRNAs), such as microRNAs (miRNAs), long-noncoding RNAs (lncRNAs) and circular RNAs (circRNAs), have been linked to both the EMT process activation and inhibition. Indeed, these types of RNAs have multiple roles in cancerous cells because one ncRNA transcript could target many molecules involved in different signaling pathways (Toden et al., 2021; Khanbabaei et al., 2022).

This review highlights ncRNAs' significant direct and indirect signaling pathways in the EMT process and how these mechanisms are involved in HCC progression and chemoresistance. Finally, we provide an update on developing exosome-based therapies against HCC and their molecular aspects in EMT (Figure 1).

2 EMT-related ncRNAs mechanisms of action

As mentioned above, noncoding RNAs (ncRNAs), including microRNAs, lncRNAs and circRNAs, have oncogenic and tumor suppressor roles and regulate essential processes involved in cancer progression.

MicroRNAs (miRNAs) are noncoding single-stranded RNAs of approximately 22 nucleotides transcribed in pri-miRNA by RNA Pol II (Bartel, 2004). As described in the canonical pathway, Ribonuclease III and double-stranded-RNA-binding protein, DGCR8, recognize this structure in the nucleus, generating a pre-miRNA of ~65 nucleotides. Pre-miRNA is exported to the cytoplasm by an Exportin 5 and Ran-GTP complex and recognized by RNase III Dicer, which forms a miRNA duplex. This mature form is incorporated into an RNA-induced silencing complex (RISC), directing RISC to complementary mRNA targets (Cai et al., 2004). In brief, miRNAs function as negative regulators of genes when binding to RNA 3′-untranslated region (3′-UTR) (Ha and Kim, 2014). Besides that, the interaction with coding sequences, gene promoters, and 5′-UTR has been proved (O'Brien et al., 2018). Because each miRNA can regulate multiple targets containing specific miRNA response elements (MREs) (Bassett et al., 2014) and play a crucial role in a variety of molecular processes, they have been studied in all cancer types (Esquela-Kerscher and Slack, 2006; Volinia et al., 2006; Nicoloso et al., 2009). In HCC, miRNAs modulate cell cycle, proliferation, apoptosis, epithelial-mesenchymal transition and metastasis (Sidhu et al., 2015). Furthermore, our previous studies have shown that miRNAs are an important tool in the prognostic and diagnostic HCC (Mjelle et al., 2019; Sorop et al., 2020).

Long noncoding RNAs (lncRNAs) are transcripts of approximately 200 nucleotides, which usually RNA Pol II transcribes, but so do RNA Pol I and RNA Pol III (Statello et al., 2021; Mattick et al., 2023). Moreover, they have a wide diversity, with an average of 100,000 human lncRNAs (Mattick et al., 2023). At first, lncRNAs were defined as transcriptional "junk" or "noise." Still, in the past few years, more studies have shown the involvement of lncRNAs in different molecular pathways (Sun et al., 2017), indicating their interaction with DNA, RNA, or protein. The interaction mechanism could be: scaffold, decoy, guide, signal, or SINEUPs. Scaffold lncRNAs could act as archetype RNAs and are involved in the assembly of transcriptional regulators. The decoy

TABLE 1 Summary of ncRNAs direct signaling pathways and their action on HCC tumor cell processes.

ncRNA	Expression	Target	Axis pathway	ncRNA involvement in cellular process	References
miR-509-3p	↓	TWIST	miR-509-3p/TWIST/EMT	(−) EMT, (−) proliferation, (−) metastasis	Zhang et al. (2021)
miR-361-5p	↓	TWIST1	miR-361-5p/TWIST1/EMT	(−) EMT, (−) proliferation, (−) migration, (−) invasion	Yin et al. (2020)
miR-370-3p	↓	TWIST1, SNAIL	IL-8/STAT3/miR-370-3p/TWIST1, SNAIL/EMT	(−) EMT, (−) metastasis	Peng et al. (2022)
LINC00992	↑	miR-361-5p	LINC00992/miR-361-5p/TWIST1	(+) EMT, (+) proliferation, (+) metastasis, (+) invasiveness	Li et al. (2022)
LINC01133	↑	miR-199a-5p	LINC01133/miR-199a-5p/SNAIL; LINC01133/ANXA2/STAT3/cyclin D1	(+) EMT, (+) proliferation, (+) migration, (+) invasion	Yin et al. (2021)
UCID	↑	miR-122, miR-203, miR-30b, miR-34a, miR-153	lnc-UCID/miR/SNAI1	(+) EMT, (+) metastasis, (+) migration, (+) invasion	Yuan et al. (2021)
circHIPK3	↑	miR-338-3p	circHIPK3/miR-338-3p/ZEB2	(+) EMT, (+) migration, (+) invasion, (+) metastases	Li et al. (2021)
circPTK2	↓	miR-92a	circPTK2/miR-92a/E-cadherin	(−) EMT, (−) proliferation, (−) invasion	Gong et al. (2020)

Note: downregulated expression (↓), upregulated expression (↑), inhibition of cellular process (−), enhance of cellular process (+).

mechanism implies acting as a competing endogenous RNA (ceRNA) or sponge of miRNAs, transcriptional factors, or RNA-binding proteins. In contrast, the guide mechanism involves the formation of a ribonucleoprotein complex, which targets a promoter or genomic loci (Rinn and Chang, 2012). Furthermore, lncRNAs could act as regulatory molecules (Nadhan et al., 2022) or SINEUPs containing SINE elements which enhance mRNAs translation (Toki et al., 2020).

Circular RNAs (circRNAs) are single-stranded RNAs with closed-loop structures and resistance to RNase R and exonucleases. They are generated from precursor RNA (pre-RNA) through back-splicing (Chen, 2016). This mechanism involves connecting a downstream donor site of a flanking downstream intron to an upstream acceptor site (Kristensen et al., 2019). Increasing research has revealed that circRNAs can sponge miRNAs, interact with proteins, interfere with transcription or splicing, or encode peptides (Zhang and Wang, 2021).

EMT plays a pivotal role in the early stage of metastasis (Bakir et al., 2020); thus, many studies have been conducted to determine the function of ncRNAs in this highly dynamic phenomenon. Therefore, this review underlines two types of mechanisms: direct and indirect.

2.1 Direct EMT-related ncRNAs' mechanism of action

Direct mechanism involves direct interaction between miRNA and EMT-regulatory factors, such as twist family bHLH transcription factor 1 (TWIST), snail family transcriptional repressor 1 (SNAIL), or zinc finger E-box binding homeobox 1/2 (ZEB1/2) (Skovierova et al., 2018). We defined this mechanism by three crucial axes: miRNA/EMT, lncRNA/miRNA/EMT, and circRNA/miRNA/EMT.

Several miRNAs, such as miR-509-3p (Zhang et al., 2021), miR-361-5p (Yin et al., 2020), and miR-370-3p (Peng et al., 2022), have been found to inhibit TWIST1 expression via targeting its 3′UTR and to abate the EMT process. Li et al. (2022) observe that LINC00992 downregulates miR-361-5p and upregulates TWIST1, thus promoting cell proliferation, migration, and invasion. In addition, miR-370-3p decreases TWIST1 and SNAIL, affecting interleukin 8 (IL-8) expression and restraining the metastasis capacity in HCC cells (Peng et al., 2022). In contrast, LINC01133 (Yin et al., 2021) and lnc-UCID (Yuan et al., 2021) increase EMT by acting as a sponge of miRNAs, increasing SNAIL expression. Furthermore, circHIPK3 promotes metastases and ZEB2 expression via inhibiting miR-338-3p (Li et al., 2021). In contrast, circPTK2 and E-cadherin compete for binding miR-92a that, aggravates proliferation and invasion, while circPTK2 suppresses miRNA's effect in HCC cells (Gong et al., 2020), as summarized in the direct mechanism part from Table 1.

2.2 Indirect EMT-related ncRNAs' mechanism of action

The indirect mechanism involves miRNA/mRNA, lncRNA/miR/mRNA, and circRNA/miRNA/mRNA regulatory axes that modulate an EMT molecule.

2.2.1 miRNA/mRNA axes

Numerous miRNA/mRNA axes have been found to be involved in the EMT process (Table 2).

For instance, Shen et al. (2021) have found that miRNA-10a-5p is downregulated in HCC tissues and decreases EMT in HCC cells by targeting spindle and kinetochore-associated complex subunit 1 (SKA1). SKA1 is upregulated in tumors, promoting cancer progression, and has a prognostic value in HCC (Chen et al.,

TABLE 2 Summary of miRNAs signaling pathways and their action on HCC tumor cell processes.

miRNA	Expression	Target	Axis pathway	miRNA involvement in cellular process	References
miR-10a-5p	↓	SKA1	miR-10a-5p/SKA1	(−) EMT, (−) migration, (−) invasion, (−) tumor formation *in vivo*	Shen et al. (2021)
miR-143-3p	↓	FGF1	miR-143-3p/FGF1/EMT	(−) EMT, (−) proliferation, (−) invasion	Peng et al. (2021)
miR-139-5p	↓	WTAP	miR-139-5p/WTAP/EMT	(−) EMT, (−) invasion, (−) proliferation	Liu et al. (2021)
miR-181 a/b/c/d	↑	CDX2, GATA6, NLK1	miR-181/CDX2, GATA6, NLK1	(+) stemness	Ji et al. (2009)
miR-181b	↑	TIMP3	miR-181b/TIMP3/TGF- β	(+) migration, (+) invasion, (+) tumor formation *ex vivo*	Wang et al. (2010)
miR-181a	↑	BIM	mir-181a/TGF- β/EMT	(+) EMT	Brockhausen et al. (2015)
miR-181ab1	↑	CBX7	mir-181/TGF- β/EMT	(+) EMT, (+) proliferation	Chen et al. (2022)
miR-23b-3p	↓	c-MET	miR-23b-3p/c-MET/TGF-β1/EMT	(−) EMT, (−) migration, (−) invasion	Park et al. (2022)
miR-4521	↓	FAM129A	miR-4521/FAM129A/EMT	(−) EMT, (−) migration, (−) proliferation, (+) apoptosis	Ayesha et al. (2022)
miR-7	↓	BCL2L1	miR-7/BCL2L1/P53/EMT	(−) EMT, (−) proliferation, (−) metastasis	Zhang et al. (2023)
miR-22-3p	↓	SPRY2	miR-22-3p/CBL/SPRY2/ERK/EMT	(−) EMT, (−) migration, (−) invasion, (−) Cancer stem cell features	Zeng et al. (2020); Cui et al. (2023)
miR-383	↓	RBM3	miR-383/RBM3/STAT3/EMT	(−) EMT	Zhang et al. (2022)

Note: downregulated expression (↓), upregulated expression (↑), inhibition of cellular process (−), enhance of cellular process (+).

2018; Song et al., 2022). Other oncosuppressors are miR-143-3p and miR-139-5p, which repress fibroblast growth factor 1 (FGF1) and Wilms' tumor 1-associating protein (WTAP). Those proteins increase EMT, proliferation and invasion of HCC cells (Liu et al., 2021; Peng et al., 2021). Moreover, Zhu et al. (2021) declare that miR-139-5p is regulated by lncRNA TTN antisense RNA 1 (TTN-AS1) and inhibits Sparc/osteonectin, cwcv, and kazal-like domains proteoglycan 1 (SPOCK1), an oncogenic proteoglycan involved in EMT (Vancza et al., 2022).

Growing studies have supported the importance of transforming growth factor beta (TGF-β) in HCC via SMAD/non-SMAD-dependent signaling pathways, which induce EMT-TFs (Hao et al., 2019). Several studies have shown that the miR-181 family positively correlates with TGF-β pathways, thus increasing EMT, tumor progression and stemness (Ji et al., 2009; Wang et al., 2010; Brockhausen et al., 2015; Chen et al., 2022). In contrast, miR-23b-3p has been proven to inhibit TGF- β1-induced EMT and block invasion and migration (Park et al., 2022).

Apoptosis or programmed cell death is a complex mechanism that involves death receptors (extrinsec pathway) and mitochondria (intrinsic pathway), by which it maintains cell homeostasis (Schattenberg et al., 2011). As discussed above, EMT confers resistance to apoptosis (Valdes et al., 2002). Interestingly, miR-4521 acts as an oncosuppressor in HCC cells by modulating mechanisms involved in proliferation and apoptosis. On the one hand, miR-4521 activates two apoptosis pathways (p-FAK/p-Akt/MDM2/P53 and FAK/p-Akt/BCL-2/BAX/Cytochrome-C/Caspase-3/Caspase-9) by decreasing the expression of family with sequence similarity 129 member A (FAM129A); on the other hand, it thereby attenuates invasivity by blocking TIMP-1/MMP9/MMP2, p-FAK/p-Akt and EMT pathways (Ayesha et al., 2022).

Moreover, the miR-7/BCL2L1/P53 and miR-22-3p/CBL/SPRY2/ERK axes decrease EMT, invasion, proliferation and migration (Cui et al., 2023; Zhang et al., 2023). Another EMT inhibitor is miR-383, which negatively regulates the multi-functional RNA-binding protein (RBM3) expression. As reported, RBM3 upregulates signal transducer and activator of transcription 3 (STAT3) expression via binding to its mRNA (Zhang et al., 2022). In addition, STAT3 targets the TWIST promoter and positively regulates its transcriptional activity in HCC cells, thus inducing EMT (Zhang et al., 2015).

Moreover, many studies highlight indirect mechanisms that imply lncRNA/miRNA/mRNA and circRNA/miRNA/mRNA axes.

2.2.2 lncRNA/miRNA/mRNA axes

Table 3 shows the lncRNA/miRNA/mRNA axes related to EMT in HCC. According to their oncological role, lncRNAs could be classified into two groups: onco-suppressor and oncotargets. Therefore, within the last 3 years, five lncRNAs, TMEM220-AS1 (Cao et al., 2021), lncRNA miR503HG (Song and Qiu, 2021), LINC02362 (Li et al., 2022), LINC02027 (Wang et al., 2023) and SATB2-AS1 (Huang et al., 2023), have been documented to function as miRNA sponge, to decrease a gene that promotes the EMT process. For instance, Huang et al. (2023) show that SATB2-AS1 is observably reduced in HCC tissues compared to adjacent tissues and its overexpression hampers tumor growth and metastasis *in vitro*. Besides, SATB2-AS1 also acts as a ceRNA for miR-3678-3p. This miRNA accelerates cell proliferation and suppresses cell apoptosis by blocking GRIM-19 (gene associated with retinoic-interferon-induced mortality 19), a negative STAT3/HIF-1α pathway regulator (Huang et al., 2023).

TABLE 3 Summary of lncRNAs signaling pathways and their influence in HCC tumor cells processes.

lncRNA	Expression	Target	Axis pathway	lncRNA involvement in cellular process	References
TMEM220-AS1	↓	miR-484	lnc-TMEM220-AS1/miR-484/MAGI1	(−) EMT, (−) proliferation, (−) invasion, (−) metastasis, (−) tumor growth, (+) apoptosis	Cao et al. (2021)
miR503HG	↓	miR-15b	lncRNA miR503HG/miR-15b/PDCD4	(−) EMT, (−) angiogenesis, (−) migration, (−) invasion	Song and Qiu (2021)
LINC02362	↓	miR-516b-5p	LINC02362/miR-516b-5p/SOCS2	(−) EMT, (−) proliferation, (−) migration, (−) invasion, (+) apoptosis	Li et al. (2022)
LINC02027	↓	miR-625-3p	LINC02027/miR-625-3p/PDLIM5	(−) EMT, (−) proliferation, (−) migration, (−) invasion	Wang et al. (2023)
SATB2-AS1	↓	miR-3678-3p	lnc-SATB2-AS/miR-3678-3p/GRIM-19/STAT3/HIF-1α	(−) EMT, (−) proliferation, (−) invasion, (−) migration, (−) metastasis, (−) tumor growth, (+) apoptosis	Huang et al. (2023)
LINC00668	↑	miR-532-5p	LINC00668/miR-532-5p/YY1	(+) EMT, (+) proliferation, (+) migration, (+) invasion	Xuan et al. (2020)
LINC00922	↑	miR-424-5p	LINC00922/miR-424-5p/ARK5	(+) EMT, (+) proliferation, (+) migration, (+) invasion	Ye et al. (2021)
UNC5B-AS1	↑	miR-4306	UNC5B-AS1/miR-4306/KDM2A	(+) EMT, (+) proliferation, (+) migration	Huang et al. (2021)
BACE1-AS	↑	miR-377-3p	lnc-BACE1-AS/miR-377-3p/CELF1	(+) EMT, (+) invasion, (+) migration, (+) metastasis	Liu et al. (2021)
DUXAP8	↑	miR-9-3p	lnc-DUXAP8/miR-9-3p/IGF1R	(+) EMT, (+) proliferation, (+) migration, (+) invasion	Guan et al. (2021)
LOC554202	↑	miR-485-5p	LOC554202/miR-485-5p/BSG	(+) EMT, (+) proliferation, (+) migration, (+) invasion	Yang et al. (2022)
SNHG1	↑	miRNA-376a	lnc-SNHG1/miR-376a/FOXK1/SNAIL	(+) EMT, (+) proliferation, (+) migration, (+) invasion, (−) apoptosis	Meng et al. (2021)
HAGLROS	↑	miR-26b-5p	lnc-HAGLROS/miR-26b-5p/KPNA2/p53	(+) EMT, (+) proliferation, (+) migration, (+) invasion, (−) apoptosis	Tang et al. (2022)
DARS-AS1	↑	miR-3200-5p	lnc-DARS-AS1/miR- 3200-5p/CKAP2/FAK/ERK	(+) EMT, (+) proliferation, (+) migration, (+) invasion, (+) cell growth, (+) metastasis, (−) apoptosis	Feng et al. (2021)
SNHG12	↑	miR-516a-5p	lnc-SNHG12/miR-516a-5p/HEG1	(+) EMT, (+) proliferation, (+) migration, (+) invasion, (−) apoptosis	Chen et al. (2021)
PRR34-AS1	↑	miR-296-5p	lnc-PRR34-AS1/miR-296-5p/E2F2/SOX12/Wnt/beta-catenin	(+) EMT, (+) proliferation, (+) migration, (+) invasion, (+) tumor growth	Qin et al. (2021)
NUTM2A-AS1	↑	miR-186-5p	lnc-NUTM2A-AS1/mIR-186-5p/KLF7/Wnt/beta-catenin	(+) EMT, (+) invasion, (+) cell growth, (+) stemness, (−) apoptosis	Long et al. (2023)
LINC01278	↑	miR-1258	β-catenin/TCF-4/LINC01278/miR-1258/SMAD2/3	(+) EMT, (+) invasion, (+) migration, (+) metastasis	Huang et al. (2020)
CRNDE	↑	miR-539-5p	lnc-CRNDE/miR-539-5p/POU2F1/AKT/NF-kB	(+) EMT, (+) proliferation, (+) migration, (+) invasion	Li et al. (2020)
HCP5	↑	miR-29b-3p	lnc-HCP5/miR-29b-3p/DNMT3A/AKT	(+) EMT, (+) invasion, (+) cell growth, (+) metastasis, (−) apoptosis	Zhou et al. (2021)
KDM4A-AS1	↑	miR-411-5p	lnc-KDM4A-AS1/miR-411-5p/KPNA2/AKT/HIF-1α	(+) EMT, (+) proliferation, (+) migration, (+) invasion, (+) metastasis, (+) tumor growth	Chen et al. (2021)
MAPKAPK5-AS1	↑	miR-154-5p	lnc-MAPKAPK5-AS1/miR-154-5p/PLAGL2/EGRT/AKT/HIF-1α	(−) EMT, (−) proliferation, (+) apoptosis, (−) metastasis	Wang et al. (2021)
TTN-AS1	↑	miR-139-5p	lnc-TTN-AS1/miR-139-5p/SPOCK1	(+) EMT, (+) proliferation, (+) migration, (+) invasion, (+) metastasis, (+) tumor growth, (−) apoptosis	Zhu et al. (2021)

Note: downregulated expression (↓), upregulated expression (↑), inhibition of cellular process (−), enhance of cellular process (+).

On the other hand, several lncRNAs increase EMT by sponging miRNAs that target oncogenes. LncRNAs such as LINC00668 (Xuan et al., 2020), LINC00922 (Ye et al., 2021), UNC5B-AS1 (Huang et al., 2021), BACE1-AS (Liu et al., 2021), DUXAP8 (Guan et al., 2021) and LOC554202 (Yang et al., 2022) were upregulated in HCC to contribute to specific lncRNA/miR/mRNA axes induced EMT. SNHG1 is another lncRNA with high expression levels in HCC; it is negatively correlated to a poor patient prognosis.

TABLE 4 Summary of circRNAs signaling pathways and their influence in HCC tumor cells processes.

circRNA	Expression	Target	Axis pathway	circRNA involvement in cellular process	References
circFGGY	↓	miR-545-3p	circFGGY/miR-545-3p/SMAD7	(−) EMT, (−) invasion, (−) migration, (−) cell growth	Feng et al. (2022)
circ_0000098	↓	miR-1204	circ_0000098/miR- 1204/ALX4	(−) EMT, (−) proliferation, (−) migration, (−) invasion	Li et al. (2021)
circEPB41L2	↓	miR-590-5p	circEPB41L2/miR-590-5p	(−) EMT, (−) proliferation, (−) migration, (−) invasion, (−) metastasis	Chen et al. (2021)
circ_0004913	↓	miR-184	circ_0004913/miR-184/HAMP	(−) EMT, (−) proliferation, (−) migration, (−) invasion, (−) tumor growth	Wu et al. (2020)
circ_0003998	↑	miR-143-3p	circ_0003998/miR-143 -3p/FOSL2; circ_0003998/miR-143 -3p/PCBP1/CD44v6	(+) EMT, (+) migration	Song et al. (2020)
circ_0101145	↑	miR-548c-3p	circ_0101145/miR-548c-3p/LAMC2	(+) EMT, (+) proliferation, (+) migration, (+) metastasis	Jin et al. (2020)
circBACH1	↑	miR-656-3p	circBACH1/miR-656-3p/SERB1	(+) EMT, (+) proliferation, (+) migration, (+) invasion, (+) tumor growth, (−) apoptosis	Li et al. (2021)
circPUM1	↑	miR-1208	circPUM1/miR-1208/MAP3K2	(+) EMT, (+) migration, (+) invasion	Zhang et al. (2021)
circ_0051040	↑	miR-569	circ_0051040/miR-569/ITGAV	(+) EMT, (+) proliferation, (+) migration, (+) invasion, (+) tumor growth, (+) metastasis	Ju et al. (2022)
circ_0001459	↑	miR-6165	circ_0001459/miR-6165/IGF1R	(+) EMT, (+) proliferation, (+) migration, (+) invasion, (+) tumor growth, (+) metastasis	Shen et al. (2022)
circSEC24A	↑	miR-421	circSEC24A/miR-421/MMP3	(+) EMT, (+) proliferation, (+) invasion, (+) migration, (+) cell growth	Zhang and Zhou (2022)
		miR-455-3p	circSEC24A/miR-455-3p/PPM1F	(+) EMT, (+) proliferation, (+) invasion, (+) metastasis, (+) tumor growth, (−) apoptosis	Liao et al. (2021)
circ_0003288	↑	miR-145	circ_0003288/miR-145/PD-L1	(+) EMT, (+) migration, (+) invasion	Xu et al. (2021)
circ_0091579	↑	miR-136- 5p	circ_0091579/miR-136-5p/TRIM27	(+) EMT, (+) proliferation, (+) migration, (+) invasion, (+) cell cycle progression	Mao et al. (2022)
circTOLLIP	↑	miR-516a-5p	circTOLLIP/miR-516a-5p/PBX3/EMT	(+) EMT, (+) proliferation, (+) metastasis	Liu et al. (2022)
circCDR1as	↑	miR-1287	circCDR1as/miR-1287/Raf1 and MEK/ERK	(+) EMT, (+) proliferation, (+) metastasis	Zhang et al. (2020)
circ-TLK1	↑	miR-138-5p	circTLK1/miR-138-5p	(+) EMT, (+) proliferation, (+) migration, (+) invasion	Lu et al. (2022)
circFoxo3	↑	miR-199a-5p	circFoxo3/miR-199a-5p/ABCC1	(+) EMT, (+) invasion, (+) tumor growth	Huang et al. (2020)

Note: downregulated expression (↓), upregulated expression (↑), inhibition of cellular process (−), enhance of cellular process (+).

SNHG1 regulates cell proliferation and invasion via EMT through miR-376a binding to elevate forkhead box protein K1 (FOXK1) expression, a molecule that binds and upregulates SNAIL (Meng et al., 2021). HAGLROS knockdown impaired HCC tumorigenesis *in vitro* and *in vivo*. HALGROS increases the karyopherin α2 (KPNA2) level and suppresses p53 signaling to abate apoptosis by acting as a miR-26b-5p sponge (Tang et al., 2022). DARS-AS1 induces EMT via interacting with miR-3200-5p, further promoting Cytoskeleton associated protein 2 (CKAP2) expression and FAK/ERK pathway activation (Feng et al., 2021).

SNHG12 (Chen et al., 2021), PRR34-AS1 (Qin et al., 2021) and NUTM2A-AS1 (Long et al., 2023) axes induce EMT via Wnt/β-catenin signaling. Furthermore, Huang et al. (2020) point out that miR-1258 is downregulated in HCC patients. *In vivo,* experiments showed that the miR-1258 overexpression in nude mice impeded metastatic lung nodule formation. At the molecular level, LINC01278 acts as a sponge of miR-1258 and upregulates SMAD2/3, thus suppressing E-cadherin and enhancing vimentin expression. Moreover, transcription factor 4 (TCF-4) binds to the promoter site of LINC01278 and increases β-catenin expression, TGF-β and Wnt/β-catenin pathways, thereby activating the LINC01278/miR-1258/Samd2/Smad3 axis (Huang et al., 2020).

CRNDE and HCP5 induce Akt pathway activation by sponging miR-539-5p andmiR-29b-3p, respectively, to promote the EMT and the progression of HCC (Li et al., 2020; Zhou et al., 2021). Furthermore, two others oncogenic lncRNAs, KDM4A-AS1 and MAPKAPK5-AS1, activated by hypoxia-inducible factor 1-alpha (HIF1α), have also been found to increase protein kinase B (Akt) (Chen et al., 2021; Wang et al., 2021); their corresponding axes being listed in Table 3.

2.2.3 circRNA/miRNA/mRNA axes

In the Table 4 there are highlighted critical pathways that involve circRNAs. In HCC, the levels of circFGGY (circ_0006633) (Feng et al., 2022), circ_0000098 (Li et al., 2021), and circEPB41L2 (Chen et al., 2021) are downregulated in tumor tissues and inhibit EMT, proliferation, migration, and invasion. In summary, authors highlight circFGGY/miR-545-3p/Smad7 (Feng et al., 2022), circ_0000098/miR-1204/ALX4 (Li et al., 2021) and circEPB41L2/miR-590-5p (Chen et al., 2021) axes as being important in HCC. Furthermore, Wu et al. (2020) revealed that circ_0004913 was downregulated in HCC tissues and that the overexpression of circ_0004913 constrained proliferation, EMT and metastasis by acting as a sponge of miR-184 and promoting hepcidin antimicrobial peptide (HAMP) expression. In brief, the circ_0004913/miR-184/HAMP axis regulates JAK2/STAT3/Akt signaling in HCC cells (Wu et al., 2020).

In contrast, six circRNAs, circ_0003998 (Song et al., 2020), circ_0101145 (Jin et al., 2020), circBACH1 (Li et al., 2021), circPUM1 (Zhang et al., 2021), circ_0051040 (Ju et al., 2022) and circ_0001459 (Shen et al., 2022), have been observed to manipulate various miR/mRNA axes to induce EMT. Besides, elevated level of circSEC24A leads to the expression of protein phosphatase, Mg2+/Mn2+ dependent 1F (PPM1F) and matrix metalloproteinase 3 (MMP3) by sponging miR-455-3p and miR-421, respectively (Liao et al., 2021; Zhang and Zhou, 2022). MMPs are a class of enzymes that degrade extracellular matrix (ECM) proteins (Klein and Bischoff, 2011). In HCC, it was reported that MMP3 promotes EMT and metastasis (Scheau et al., 2019).

Circ_0003288 is an oncogenic RNA that enhances EMT by increasing programmed death-ligand 1 (PD-L1) and Akt pathways via miR-145 sponging (Xu et al., 2021). Circ_0091579 has been demonstrated to pin HCC patients and its downregulation inhibits EMT and promotes apoptosis *in vitro*. Also, miR-136-5p is a direct target of circ_0091579 and its overexpression suppresses the malignant potential of HCC cells via regulating tripartite motif containing 27 (TRIM27) expression (Mao et al., 2022).

Moreover, the Toll interacting protein (TOLLIP)-derived circRNA (circTOLLIP) is also found to be involved in the EMT of HCC. CircTOLLIP is upregulated in HCC via eukaryotic translation initiation factor 4A3 (EIF4A3), an RNA-binding protein. This circRNA acts as a ceRNA for miR-516a-5p, thus upregulating PBX3 and exhibiting pro-tumor roles *in vitro* and *in vivo* (Liu et al., 2022).

CircRNA CDR1as is highly expressed in some cancers (Jiang et al., 2020). Specifically, circRNA CDR1as is overexpressed in HCC tissues and its expression positively regulates EMT, proliferation and metastasis in HCC cells via the miR-1287 sponge. This circRNA enhances Raf-1 proto-oncogene, serine/threonine kinase (RAF1) expression, a crucial molecule in the RAS/RAF/MEK/ERK pathway (Zhang et al., 2020).

3 The role of ncRNA/mRNA axes in HCC drug resistance

As discussed above, EMT is associated with chemotherapy resistance by avoiding cell death mechanisms (De Las Rivas et al., 2021). Therefore, a growing number of studies have supported the importance of EMT-related ncRNAs in molecular pathways of different therapies (He et al., 2022).

Sorafenib is the first-line FDA-approved treatment for HCC (Niu et al., 2021) and an oral multikinase inhibitor that targets vascular endothelial growth factor receptor 2 (VEGFR2), platelet-derived growth factor receptor (PDGFR), hepatocyte factor receptor (KIT), or other molecules to decrease angiogenesis. HCC cells acquire resistance to sorafenib by different molecular pathways, including EMT (Marisi et al., 2018; Tang et al., 2020). In this context, lncH19 knockdown has been reported to inhibit EMT in HCC cells by enhancing miR-675 expression, which is involved in sorafenib sensitivity. In brief, H19 promoted sorafenib resistance (Xu et al., 2020). LncRNA-POIR also has an oncogenic effect and suppresses miR-182-5p expression, inhibiting the EMT process and triggering sorafenib sensitivity (Chen et al., 2021). Additionally, small nucleolar RNA host gene 3 (SNHG3) induces EMT and CD151 expression by functioning as a ceRNA for miR-128. LncRNA-SNHG3 can induce sorafenib resistance and promote invasion *in vitro* (Zhang et al., 2019). In contrast, lncLIMT (LINC01089), which reppresses miR-665 expression and EMT, decreases sorafenib resistance. In addition, LIMT inhibits tumor growth *in vivo* in tumor nude mouse models (Sun et al., 2022). MiR-125b-5p is upregulated in sorafenib-resistant HCC cell lines and its overexpression induces EMT by repressing ataxin 1 (ATXN1) expression. Thus, it was reported that miR-125b-5p enhances sorafenib resistance *in vivo* (Hirao et al., 2021).

Besides Sorafenib, TACE with doxorubicin and cisplatin is used in HCC advanced patients (Lu et al., 2017; Couri and Pillai, 2019).

Doxorubicin (Adriamycin, DOX) is an anthracycline drug used as an antineoplastic agent. The most known mechanism of action involves the interaction with topoisomerase IIα (TOP2A) (Tewey et al., 1984) and the activation of apoptosis (Roos and Kaina, 2013). Anthracycline drug resistance is caused by the incapability of DOX to accumulate in the nucleus (Cox and Weinman, 2016). For instance, Zhang et al. (2021) reported that overexpression of linc-ROR (long intergenic non-protein coding RNA (linc)-regulator of reprogramming) increases DOX resistance in HCC cell lines by TWIST upregulation. Also, circFoxo3 has higher expression in adriamycin-resistant patients. It has been shown that circFoxo3 via miR-199a enhances ABCC1 expression, a known protein involved in drug resistance. Moreover, the downregulation of miR-199a promoted EMT signaling in HCC cells and reversed circFoxo3 inhibition effects (Huang et al., 2020).

Li et al. (2020) identified that circ_0003998 downregulation facilitated DOX-sensitivity by E2F Transcription Factor 3 (E2F3) regulation. They further identified circ_0003998 as a sponge of miR-218-5p and Eukaryotic initiation factor 5A2 (EIF5A2) as a direct target of miR (Li et al., 2020). Moreover, EIF5A2 is involved in genistein resistance, an essential anti-tumoral phytoestrogen that promotes apoptosis (Sarkar and Li, 2002) and inhibits EMT and stemness. MiR-1275 is a tumor suppressor that can bind 3′-UTR EIF5A2 as a protein that upregulated PI3K/Akt and EMT pathways. MiR-1275 was expressed at a higher level by genistein treatment (Yang et al., 2022). Furthermore, it has been shown that miR-140-5p is involved in drug resistance in HCC cells. In brief, miR-140-5p improves DOX sensitivity through PIN1 depletion (Gao et al., 2021) and catalpol sensitivity through EMT suppression (Wu et al., 2021).

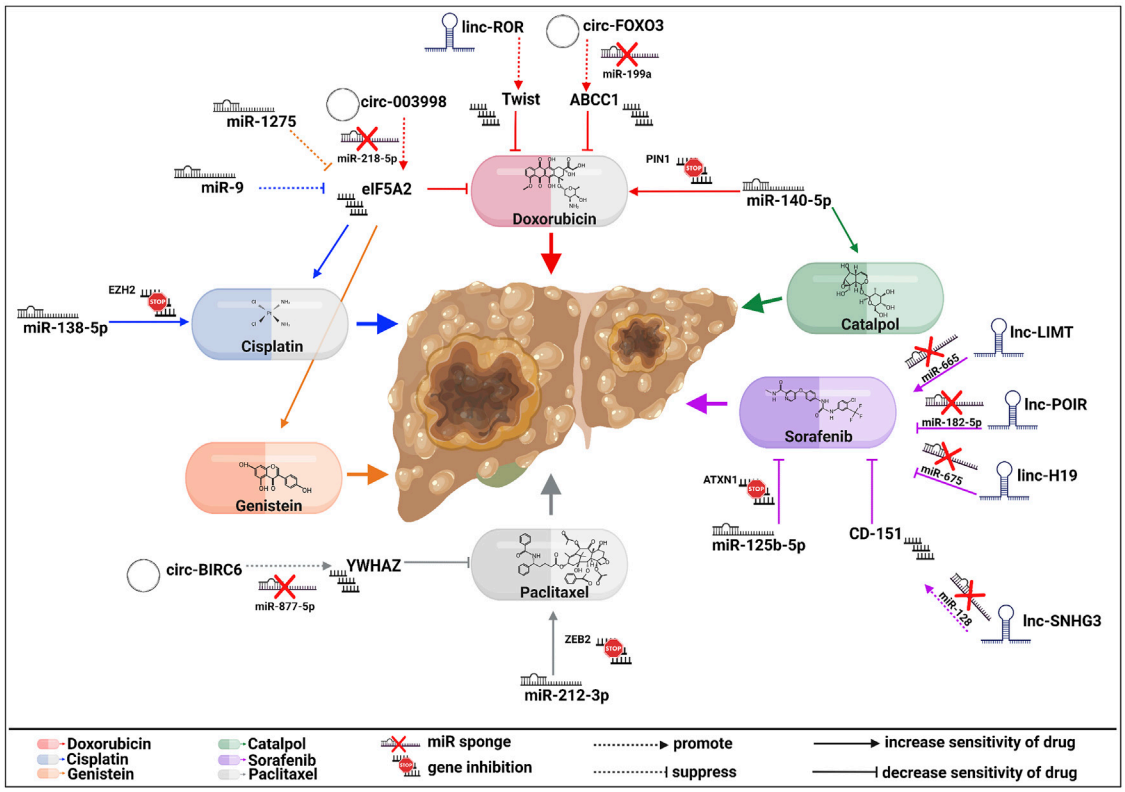

FIGURE 2
Molecular actions of EMT-related ncRNA axes in HCC drug resistance. Multiple regulatory components either increase or decrease sensitivity to sorafenib, paclitaxel, genistein, cisplatin, doxorubicin, or catalpol, affecting HCC progression. The signaling pathways of every drug are represented by different colors, as seen above (created with biorender.com accessed on July 2023).

Cisplatin is a chemotherapeutic that inhibits transcription and replication, inducing apoptosis and necrosis in HCC cells (Ishikawa, 2009). It has been shown that miR-9 increases cisplatin sensitivity *in vitro* and *in vivo* by targeting EIF5A2 and EMT process. Besides that, EIF5A2 depletion decreases vimentin expression and increases E-cadherin in HCC cell lines (Bao et al., 2020). Another ncRNA involved in cisplatin sensitivity is miR-138 by its direct target, enhancer of zeste homolog 2 (EZH2). This miRNA upregulates EMT markers; therefore, the miR-138/EZH2/EMT axis could regulate cisplatin resistance (Zeng et al., 2021), also involved in radiosensitivity. Bai et al. (2022) show that miR-138 is downregulated in HCC tissue and its expression is indirectly correlated with EZH2 expression, which is a direct target of miR-138-5p. By RNA-seq, they observed that miR-138-5p upregulation inhibits HIF-1α and EMT (Bai et al., 2022). Moreover, Lu et al. (2022) reported that miR-138-5p is negatively regulated by circ-TLK1.

Paclitaxel—a microtubule-stabilizing molecule, induces cell death (Weaver, 2014). As mentioned above, paclitaxel (PTX) is another drug whose resistance could be caused by different signaling pathways, including ncRNAs and EMT (Ashrafizadeh et al., 2021). Liu et al. (2020) pointed out circ-BIRC6 (circRNA baculoviral IAP repeat-containing 6) as an inhibitor of PTX sensitivity by sponging miR-8 77-5p to enhance tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta (YWHAZ) expression. Its role in drug resistance has been reported in ovarian cancer (Hong et al., 2018), bladder cancer (Yu et al., 2019), and gastric cancer (Zhao et al., 2021). Furthermore, miR-212-3p is decreased in PTX-resistant cells. This miRNA can bind to 3′UTR ZEB2, thus mediating chemoresistance in HCC cells. Transfection of miR-212-3p in resistant cells inhibited ZEB2 expression, reversing EMT (Yang et al., 2020). Figure 2 summarizes the ncRNAs axes involved in HCC drug resistance.

These investigations show the complex and dual role of ncRNAs in EMT. The exact mechanism by which every ncRNA is involved in the HCC will be difficult to decode because of its functions in many hepatocellular processes. One way to start is by classifying the miRNAs based on their direct or indirect impact on the EMT process. Undoubtedly, future studies are necessary to report new miRNAs associated with HCC-EMT and to map their function in this process, which can lead to the development of novel therapies.

Therefore, to translate ncRNAs in a therapeutic situation, tools must be developed to analyze these ncRNA axes functionally and to devise therapy strategies, so as to overcome off-target and toxicity consequences.

4 EMT-associated exosomal ncRNAs in HCC

Exosomes can be found in all human body fluids (blood, urine, saliva, ascites, cerebrospinal and synovial fluids) (Jiang

et al., 2022). They are extracellular 30–100 nm vesicles (EVs) having a lipid bilayer; they are generated from the luminal membranes of multivesicular bodies (MVBs) and released into the extracellular matrix after MVBs fusion with the cell membrane (Kim et al., 2020). The primary physiological role of exosomes is to mediate cell-cell communication by transferring bioactive molecules, such as proteins or nucleic acids (Chen et al., 2021), thus being one of the most studied tools for the interchange of substances between tumor cells and the tumor microenvironment (Jiang et al., 2022).

In the last decade, more studies have highlighted the regulatory effects of different bioactive molecules delivered by exosomes, such as ncRNAs, in the EMT process in various types of cancers, including HCC. Interestingly, they can promote or suppress the EMT phenomena in HCC cells.

According to RNAseq investigation, exosomal miR-92a-3p expression level increases in two established high-metastatic HCC cell lines (97 hm and Huhm). Besides, treatment with high-metastatic HCC-derived exosomal miR-92a-3p facilitates the aggressiveness of HCC cells via PTEN inhibition and Akt/Snail signaling activation, promoting EMT (Yang et al., 2020). Similarly, high levels of miR-4800-3p were found in Huh7 cell-derived exosomes. Thus, Lin et al. (2022) demonstrated that exosomal miR-4800-3p heightened the progression of HCC by regulating the Hippo signaling pathway and targeting STK25 in both *in vitro* and *in vivo* experiments. Moreover, the treatment of low metastatic HCC cells with exosomal miR-4800-3p downregulates the expression of E-cadherin and ZO-1 and increases the expression of N-cadherin, activating the EMT process (Lin et al., 2022).

Interestingly, M2 macrophages can influence tumor development by secreting various cytokines and exosomes that can be loaded with specific miRNAs. For instance, miR-660-5p-loaded M2 exosomes augmented EMT and enhanced the tumorigenic ability in HCC cells through downregulating Kruppel-like factor 3 (KLF3) expression (Tian et al., 2021).

Human umbilical cord mesenchymal stem cells (hucMSCs) have low immunogenicity and high proliferation and differentiation potential. Additionally, the treatment of HCC cells with hucMSC-Exo upregulates miR-451a. This miRNA inhibits a disintegrin and metalloprotease 10 (ADAM10), thus reducing EMT and aggressive phenotypes of HCC (Xu et al., 2021).

Several studies showed that TGF-β treatment induces EMT (Miyazono, 2009; Lin et al., 2020; Kim et al., 2021) and treatment with exosomes derived from these cells increases proliferation and metastasis in HCC cells (Lin et al., 2020) through intercellular communication. Lin et al. (2020) reported that 119 miRNAs are upregulated, such as miR-125b-5p, 374a-5p, miR-24-3p, miR-200b-3p, and miR-21-5p, and 186 are downregulated in EMT-Hep3B-derived exosomes (EMT-Hep3B exo), as compared to Hep3B exo. Moreover, treatment with EMT-Hep3B exo with miR-374a-5p interference inhibits hepatocellular metastasis by upregulation of growth arrest and DNA damage 45-alpha (GADD45A), a cell growth suppressor (Lin et al., 2020). In contrast, Huh7 cell-derived exosomes loaded with miR-125b (Exo-125b) blocks EMT and suppresses metastatic potential via inhibiting TGF-β1/SMAD pathways (Kim et al., 2021). Similarly, miR-374c-5p was found to be downregulated in the EMT model and transferred by exosomes derived from bone marrow mesenchymal stem cells (BMSC) suppresses EMT via targeting LIM domain kinase 1 (LIMK1) and inhibiting Wnt/β-catenin and TGF-β1 axes in HCC cells (Ding et al., 2023).

Yao et al. (2022) identified that lncRNA THEMIS2-211 is upregulated in plasma-derived exosomes from HCC patients. Knockdown of THEMIS2-211 increases E-cadherin and decreases N-cadherin and vimentin in HCC cells. Mechanistically, they showed that THEMIS2-211 is an oncogene that promotes proliferation, migration, invasion, and EMT by sponging miR-940 and increasing SPOCK1 expression (Yao et al., 2022).

Circ-0004277 and lncRNA PRR34-AS1 transfer via exosomes to human hepatic cells increases the malignant phenotype (Zhu et al., 2020; Zhang et al., 2022). Thus, PRR34-AS1 enhanced Rab27a expression to increase the exosome secretion of VEGF and TGF-β in HCC cells and transmitted them into the human liver epithelial (THLE-3) cells (Zhang et al., 2022).

In summary, these studies prove that exosomes act as ncRNAs cargo for tumor cells and have distinct regulatory effects on the EMT process in HCC and various underlying processes. Although exosomes are promising therapy in cancer, improvement of their purification, and additional studies on the interaction and mechanisms with other types of cells remain the main problems to be solved in their uses.

5 Conclusion and future perspectives

The development of transcriptomics approaches in the last decade has highlighted the essential roles of ncRNAs in cancer (Slack and Chinnaiyan, 2019; Winkle et al., 2021). The formation of ncRNA axes starts to become an essential tool in various cellular mechanisms, and its role in the progression of HCC is decisive (Wong et al., 2018). Furthermore, it will be crucial to comprehend how ncRNA axes regulate migration, proliferation, and EMT in HCC cells, so as to generate cutting-edge therapeutic medications based on ncRNAs, to prevent and manage HCC.

Taking together these observations, we find that defining ncRNA pathways in direct and indirect mechanisms could map a precise road to a therapeutic target as close to a clinical necessity as possible. The EMT-related miRNAs' direct mechanism of action could be a promissive path in developing new therapies against metastasis. However, more research is needed to understand how these miRNA axes work and to determine which transcripts are valuable targets. Undoubtedly, since a single miRNA could have several targets and can affect more therapeutic drugs, its use as a new therapy in cancer requires an in-depth study of the mechanisms involved.

Author contributions

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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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Identification of novel lactate metabolism-related lncRNAs with prognostic value for bladder cancer

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Background: Bladder cancer (BCA) has high recurrence and metastasis rates, and current treatment options show limited efficacy and significant adverse effects. It is crucial to find diagnostic markers and therapeutic targets with clinical value. This study aimed to identify lactate metabolism-related lncRNAs (LM_lncRNAs) to establish a model for evaluating bladder cancer prognosis.

Method: A risk model consisting of lactate metabolism-related lncRNAs was developed to forecast bladder cancer patient prognosis using The Cancer Genome Atlas (TCGA) database. *Kaplan–Meier* survival analysis, receiver operating characteristic curve (ROC) analysis and decision curve analysis (DCA) were used to evaluate the reliability of risk grouping for predictive analysis of bladder cancer patients. The results were also validated in the validation set. Chemotherapeutic agents sensitive to lactate metabolism were assessed using the Genomics of Drug Sensitivity in Cancer (GDSC) database.

Results: As an independent prognostic factor for patients, lactate metabolism-related lncRNAs can be used as a nomogram chart that predicts overall survival time (OS). There were significant differences in survival rates between the high-risk and low-risk groups based on the *Kaplan–Meier* survival curve. decision curve analysis and receiver operating characteristic curve analysis confirmed its good predictive capacity. As a result, 22 chemotherapeutic agents were predicted to positively affect the high-risk group.

Conclusion: An lactate metabolism-related lncRNA prediction model was proposed to predict the prognosis for patients with bladder cancer and chemotherapeutic drug sensitivity in high-risk groups, which provided a new idea for the prognostic evaluation of the clinical treatment of bladder cancer.

KEYWORDS

bladder cancer, lncRNAs, molecular subtype, prognostic model, lactate metabolism

1 Introduction

Globally, bladder cancer (BCA) is the most common urological malignancy and requires lifelong monitoring after diagnosis (Dobruch and Oszczudłowski, 2021). Twenty to thirty percent of BCA patients have progressed to muscle-invasive BCA (MIBCA) when diagnosed (Fletcher et al., 2011). Nearly 50% of MIBCA patients develop tumor metastasis after radical cystectomy (RC). BCA is estimated to cause 356,000 new cases and 145,000 deaths yearly (Antoni et al., 2017). Consequently, the guidelines recommend treating MIBC with neoadjuvant chemotherapy (NAC) and RC (Milowsky et al., 2016). Approximately 50% of MIBCA patients cannot tolerate suppressive adverse events resulting from chemotherapy, leading to treatment delays in nonresponders (Hanna et al., 2018). To improve cancer patients' clinical efficacy and prognosis, clarifying BCA pathogenesis and determining targets for diagnosis and treatment are imperative.

Urothelial BCA (UBCA) is one of the earliest cancers considered to have immunogenicity. With the FDA's approval of immune checkpoint inhibitors (ICIs) and pan-FGFR inhibitors, PD-1/PD-L1 therapy has shown an impressive lasting response in UBCA patients. However, the response rate has been low (Eckstein et al., 2019). To maintain uncontrolled growth and proliferation, BCA may use aerobic glycolysis-dependent metabolism (the Warburg effect) as the primary energy source (Yang et al., 2011). High lactic acid levels and subsequent acidification caused by glycolytic metabolic transformation may promote carcinogenesis and contribute to invasion, immune escape, metastasis, and chemoradiotherapy resistance (Wang et al., 2020). In addition, the Warburg effect is a feature of MIBCA and nonmuscle invasive BCA (NMIBC) (Burns et al., 2021). A significant portion of the glucose storage is converted into lactic acid by lactate dehydrogenase-A (LDH-A), resulting in glucose being used to promote growth, regardless of oxygen levels (Kim et al., 2006). *In vitro*, overexpression of LDH-A promoted BCA proliferation, invasion, and migration by stimulating epithelial-mesenchymal transformation (EMT) (Jiang et al., 2016). The metabolic state of tumor cells influences their interactions with the tumor microenvironment (TME), which is crucial for antitumor immunity (Bader et al., 2020). As lactic acid levels increase in the TME, tumor-associated macrophages differentiate into M2 subtypes, while activated macrophages promote tumor invasion through the CCL17/CCR-4/Mtorc1 signaling axis (Zhang et al., 2021). Lactic acid derived from tumor cells induces GPR81 expression in dendritic cells through a paracrine mechanism, inhibiting immune cell antigen presentation (Brown et al., 2020). These reports suggest that an in-depth understanding of lactate metabolism in BCA will provide new opportunities to predict the disease life cycle and find targets for tumor immunotherapy.

A long noncoding RNA (lncRNA) is an RNA transcribed over 200 nucleotides without the capability to code for proteins. Various cancers, including BCA, can be initiated and progress at different levels, including epigenetic, transcriptional, and posttranscriptional regulation (Iyer et al., 2015). In BCA patients, overexpression of Aly/REF export factor (ALYREF) promotes cell proliferation through PKM2-mediated glycolysis and high expression of pyruvate kinase M2 (PKM2), and ALYREF predicts poor survival (Wang et al., 2021). The low expression of AlkB homolog 5 (ALKBH5) results in poor prognosis in BCA patients, inhibits progression in a m6A-dependent manner, and sensitizes BCA cells to cisplatin through the casein kinase 2 (CK2)α-mediated glycolytic pathway (Yu et al., 2020). Due to their regulatory influences on BCA metabolism, lncRNAs are considered potential targets for drug screening and are a promising area of research.

In recent years, using high-throughput sequencing and data analysis in biomedical research has become increasingly important in identifying biomarkers, predicting prognosis, and monitoring recurrence and stratification (Zhang et al., 2019). Many studies have used a variety of biomarkers to establish clinical patient diagnosis or prognosis prediction models (Liu et al., 2021). Many studies have focused on hypoxia modulating tumor immune responses, while lactate has mainly been ignored in BCA metabolism.

Herein, LM_lncRNAs were analyzed using bioinformatics, a prognostic model for BCA was established, chemotherapy-targeted drugs were explored based on lactate metabolism groups, and a prediction model was developed for the prognosis of BCA. This study may benefit the innovation of customized precision diagnosis and treatment strategies for BCA.

2 Methods

2.1 Data acquisition

TCGA data are freely available to the public, and this study strictly follows access policies and publication guidelines. BCA RNA expression data were downloaded from TCGA GDC's official website (https://portal.gdc.cancer.gov/). A total of 408 BCA patients were evaluated for gene expression. This study included variables such as the age and sex of the participants, American Joint Committee on Cancer (AJCC) stage, histological grade, and survival rate. We excluded 11 samples of BCA patients with OS< 30 days and one sample without OS recorded. All remaining patients were included in our study. In this study, we included 397 patient samples and 19 paracancerous samples (Table 1). To select mRNAs with a p-value less than 0.05, fragments per kilobase million (FPKM) were converted into transcripts per million (TPM). The Molecular Signatures Database (MSigDB) contains a gene set related to lactate (Hallmark-lactate) (Liberzon et al., 2015).

2.2 Identification of differentially expressed LM_lncRNA

Our screening procedure used a $|\log_2 FC| > 1$ and a false discovery rate (FDR) < 0.05. The limma package was also used to identify all differentially expressed lncRNAs (Ritchie et al., 2015). It was determined whether there was a relationship between the LM_mRNAs in the sample and all lncRNAs differentially expressed data calculated by Pearson correlation. A correlation was demonstrated if $|R^2| > 0.3$ and $p < 0.001$.

TABLE 1 The clinical characteristics of patients in the TCGA dataset

Variable	Number of samples
Gender	
Male/Female	294/103
Age	
≤65/>65	159/238
Stage	
I/II/III/IV/NA	2/124/137/132/2
Grade	
High/Low/UN	376/18/3
T	
T0/T1/T2/T3/T4/UN	1/3/114/190/58/31
M	
M0/M1/MX/UN	187/10/198/2
N	
N0/N1/N2/N3/NX/UN	229/45/76/7/36/4

2.3 Development of the LM_lncRNA prognostic signature

Based on univariate Cox analysis, lncRNAs predict overall survival (OS) in BCA patients. Afterward, we selected lncRNAs with independent prognostic characteristics using multivariate Cox regression. In this study, we selected lncRNAs that are independent prognostic factors for patient survival using the survminer software package. The regression coefficient of the multivariate Cox regression model was multiplied by the linear combination of expression levels to generate a prognostic risk score based on LM_lncRNAs.

This model can be expressed as follows:

$$\begin{aligned}\text{Risk score} = {} & @\text{Expr of lncRNA (1)} \times \text{coefficient of lncRNA (1)}] \\ & + @\text{Expr of lncRNA (2)} \times \text{coefficient of lncRNA (2)}] + \ldots\ldots \\ & + @\text{Expr of lncRNA (n)} \times \text{coefficient of lncRNA (n)}]\end{aligned}$$

$$Riskscore = \sum_{i=0}^{N} (\text{Expi}^{*}\beta\text{i})$$

In this formula, Expi is the expression level of each prognostic lncRNA, and the coefficient is βi. Furthermore, patients were divided into high-risk and low-risk groups based on the median lactate-related risk scores calculated by the formula above. *Kaplan–Meier* survival curves, receiver operating characteristic curves (ROCs), and C-indices were used to predict patient outcomes and decision curve analysis (DCA).

2.4 Signature validation of LM_lncRNA

The TCGA dataset (dataset 1) contains 393 patients divided into two subgroups based on random selection. There were 197 patients in validation set 1 and 196 in validation set 2 (dataset 2). TCGA datasets were analyzed, prognostic features were identified, and the model's performance was validated in 2 datasets, validation sets 1 and 2. Having validated the prognostic value of lncRNA models based on the LM_lncRNA signature, we validated its impact on survival outcomes in BCA patients. The OS effects of prognostic factors were compared between high-risk and low-risk patients using log-rank tests and *Kaplan–Meier* survival curves. To evaluate the accuracy of the immune profile derived from the survival ROC software package, we calculated the area under the curve (AUC) using time-dependent ROC curves.

2.5 Coexpression network construction

Using Cytoscape, we constructed a correlation network between mRNAs and lncRNAs. With the help of the R software package ggalluvial, we analyzed the relationship between lncRNAs and risk.

2.6 Predictive nomograms and GSEA enrichment analysis

Separate gene expression analyses were conducted for high- and low-risk groups related to lactate metabolism (Subramanian et al., 2005). With an FDR q-value <0.25, the difference was considered statistically significant. To estimate the OS of patients at 1, 3, and 5 years, we constructed a Norman diagram and calibrated the statistics using the RMS package. After a calibration curve was developed, statistically significant values ($p < 0.05$) were calculated and compared with patient predictions at the 3- and 5-year marks.

2.7 Immunity reaction and sensitivity to immunotherapies/chemotherapies

Infiltration of immune cells in tumors in the high-risk and low-risk groups was estimated using the ESTIMATE algorithm (Yoshihara et al., 2013). Identifying immune checkpoints and m6A modification enabled quantification of immune function in high- and low-risk populations.

Every patient with BCA can be predicted to respond to chemotherapy using the Genomics of Cancer Drug Sensitivity database (GDSC) (Yang et al., 2013). The GDSC database predicts chemosensitivity in patients with two types of BCA. A half-maximum inhibitory concentration (IC50) was predicted using ridge regression in the "pRRophetic" package (Geeleher et al., 2014). Ten cross-validations are conducted to calculate accuracy.

2.8 Statistical analysis

R software was used for all data analysis and visualization (version 4.1.2). If the distribution of the groups was not expected or the variance was unknown, Wilcoxon rank-sum

tests or Kruskal–Wallis tests were used to compare them. Cox regression analysis was conducted on both the univariate and multivariate data. Survival differences were assessed using log-rank tests. We assessed the sensitivity and specificity of BCA prognosis and other clinicopathological features by calculating ROC curves and C-indices. The statistics were considered significant if $p < 0.05$.

3 Results

In Figure 1, a flow chart describes this study in more detail.

3.1 Identification of significantly enriched LM_lncRNAs

Twelve GSEA gene sets were related to lactate metabolism in the MSigDB database, and all of the lncRNAs were extracted, totaling 330. A total of 306 lncRNAs were enriched for lactate metabolism-related pathways after intersection processing with the entire gene set of the sample. Based on the Pearson correlation between mRNAs and lncRNAs in BCA, we screened lncRNAs significantly associated with lactate metabolism. We obtained 780 candidate gene expression data of lncRNAs with the criteria of $|R^2| > 0.3$ and $p < 0.001$ (Supplementary Table S2, S3). Among them, 548 lncRNAs

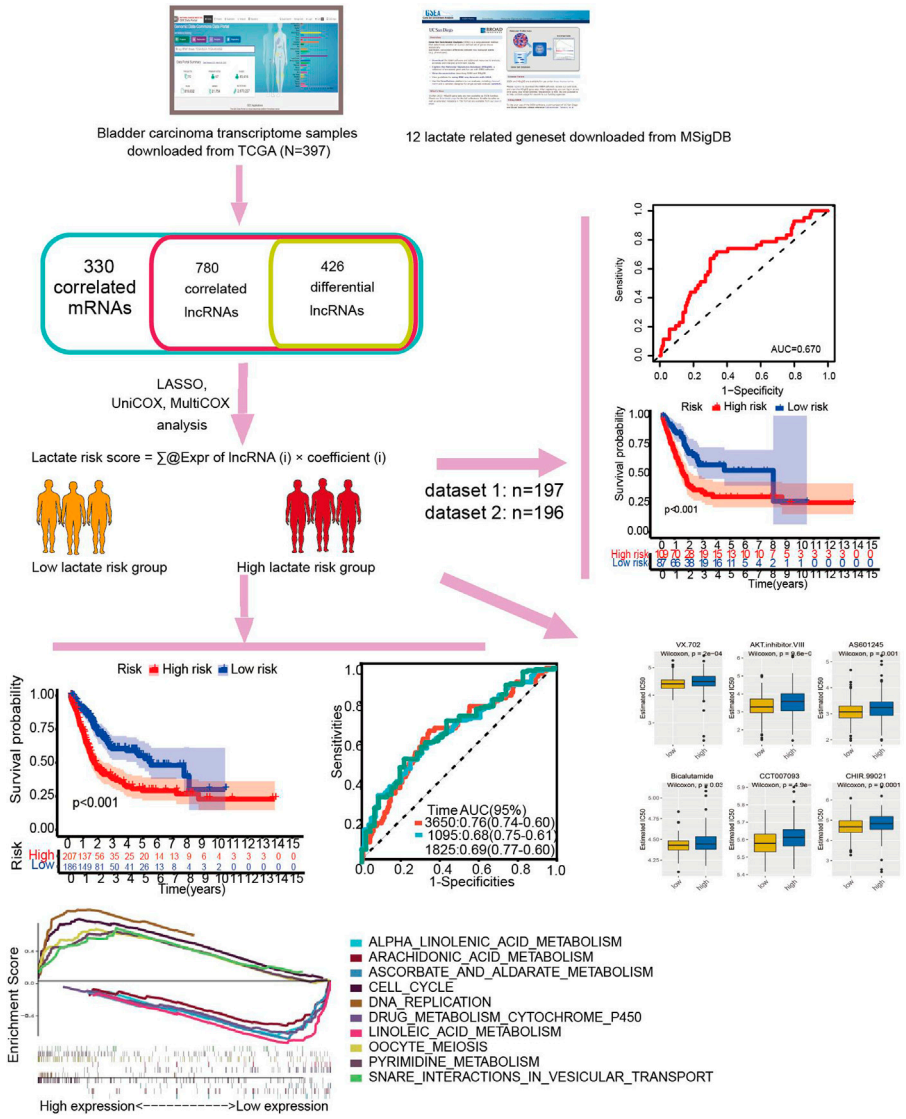

FIGURE 1
Study flowchart. Three hundred thirty lactate-related mRNAs and 780 related lncRNAs (LRLs) were obtained from the TCGA and MSigDB databases. Then, 426 lactate-related differentially expressed lncRNAs (LDELs) were identified according to their differential expression in the tumor and adjacent tumor. Next, univariate Cox, Lasso, and multivariate Cox analyses were applied to screen for prognostic LDELs. Based on this analysis, a 5-LDEL signature was constructed. Subsequently, GSEA analyses, immune-related analyses, m^{6}A-related analyses, and drug sensitivity assays were applied to identify the potential function of this signature. Finally, 2 internal validations were conducted to explore the expression and function of these LDELs.

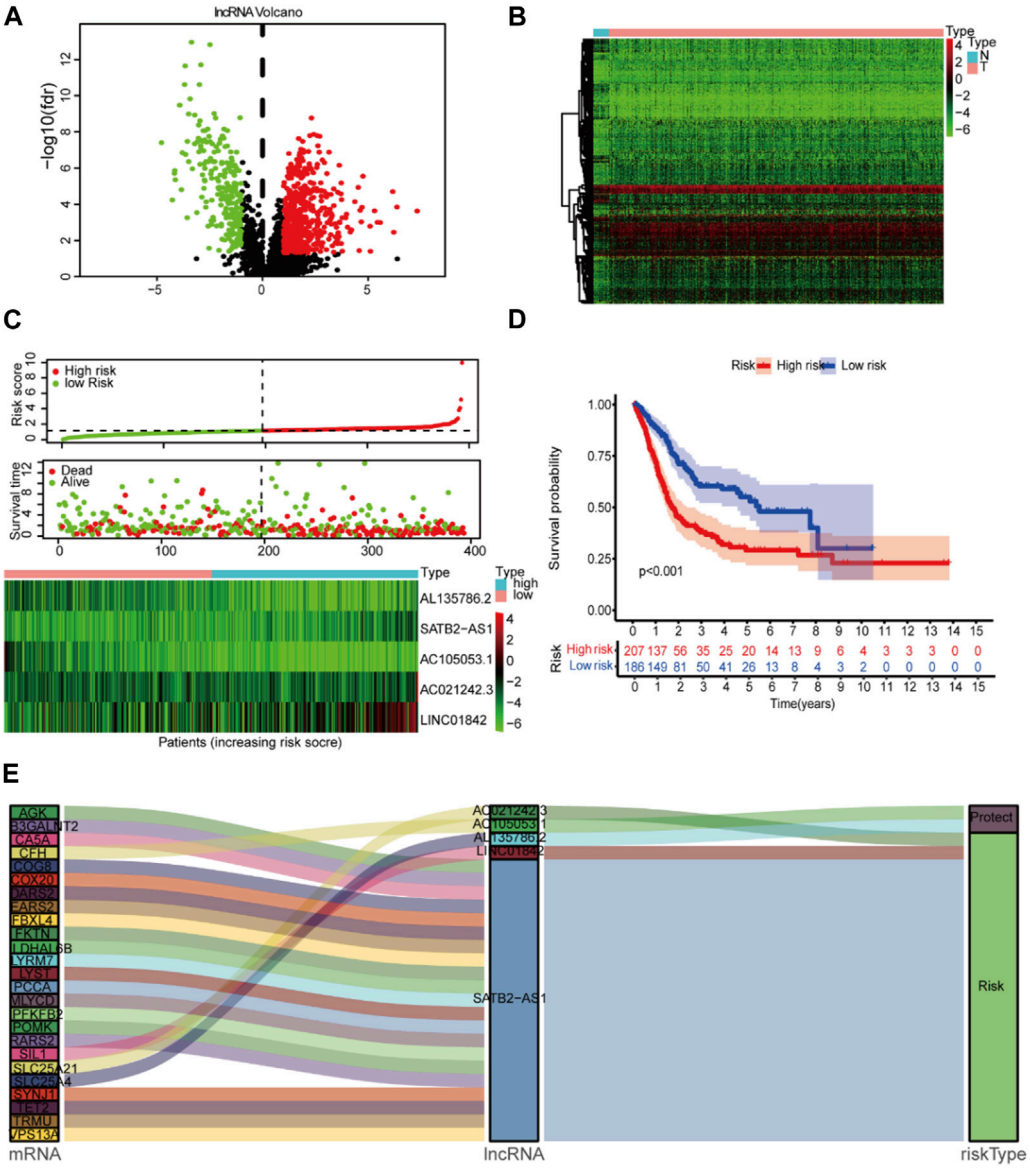

FIGURE 2
Lactate signature construction. **(A)** Volcano map for differentially expressed lncRNAs. **(B)** Heatmap for differentially expressed lncRNAs. **(C)** Risk score distribution and survival status of the two risk groups. **(D)** *Kaplan–Meier* curve analysis (*K-M* curve analysis) for the cohort. **(E)** The Sankey diagram presents the detailed connection between lactate-related lncRNAs and lactate-related genes.

were overexpressed, and 232 lncRNAs were downregulated. A total of 426 differential lncRNAs were identified with $p < 0.05$ and $|\log_2 FC| > 1$ criteria. The heatmap and volcano map of the different analyses are shown in Figures 2A,B.

3.2 Construction and multivariate evaluation of the prognostic significance of LM_lncRNA

This study included 397 BCA patients and 306 LM_lncRNAs in the TCGA cohort to determine prognostic risk models. The association between survival and LM_lncRNAs was determined by univariate Cox regression analysis. As a result, when the $p < 0.05$, we found seven lncRNAs significantly associated with OS in BCA patients. Figure 2C shows the prediction model constructed from five lncRNAs as the result of multivariate stepwise Cox regression analysis. A prognostic model based on LM_lncRNA was developed by dividing patients into two categories based on median risk scores. Compared to the low-risk group, the high-risk group had a shorter mortality and survival time (Figure 2D).

A prognostic risk score formula composed of these five lncRNAs is as follows:

$$\begin{aligned}\text{Risk score} = {} & (1.34455 \times \text{Expr of SATB2} - \text{AS1}) \\ & + (0.09399 \times \text{Expr of AC021242.3}) \\ & + (-6.07924 \times \text{Expr of AC105053.1}) \\ & + (-4.23667 \times \text{Expr of AL135786.2}) \\ & + (0.19263 \times \text{Expr of LINC01842})\end{aligned}$$

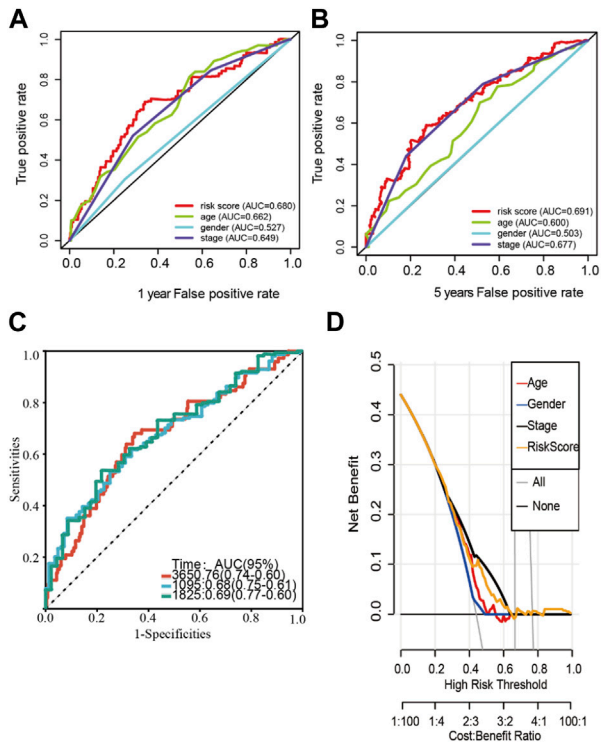

FIGURE 3
Stability verification of the lactate-related lncRNA signature model in the training cohort. **(A-B)** The 1-year AUC of this signature lncRNA was 0.681, and the 5-year AUC was 0.691. **(C)** The predicted 3-, 5-, and 10-year survival receiver operating characteristic (ROC) curves of the new lncRNA features were 0.67, 0.68, and 0.69, respectively. **(D)** The model's decision curve analysis (DCA) also shows that the model has good profitability.

In Cox regression analysis, three of these LM_lncRNAs, SATB2-AS1, AC021242.3, and LINC01842, showed positive coefficients, suggesting that their high expression is associated with poorer OS. While the coefficients of AC105053.1 and AL135786.2 were negative, the Sankey diagram indicated that this lncRNA was protective (Figure 2E).

An analysis of clinicopathological manifestations and LM_lncRNA prognostic features was conducted using a heatmap. Meanwhile, the 1-year AUC of this signature lncRNA was 0.681, and the 5-year AUC was 0.691, which was superior to standard clinicopathological features in predicting BCA prognosis (Figures 3A,B). Over the 3-, 5-, and 10-year periods, the survival ROCs were 0.67, 0.68, and 0.69, indicating that the predictive ability of the model was still good after 10 years (Figure 3C). As shown by DCA, the model had good profitability based on its C-index of 0.648 (Figure 3D).

3.3 Validation of the LM lncRNA signature

To validate the LM_lncRNA signature, its prognostic accuracy was further evaluated in an independent cohort. These two validation datasets were also downloaded from the TCGA database. Moreover, the data of "Dataset 2" and "Dataset 3" were randomly selected from the 397 patients obtained in the initial part of the present study. Two validation sets were randomly selected: validation set 1 (dataset 2: 197) and validation set 2 (dataset 3: 196). Low-risk patients had significantly longer survival, as evaluated by the ROC curve, with areas of 0.670 and 0.702 (Figures 4A,B) and the validation cohorts (Figures 4C,D), respectively.

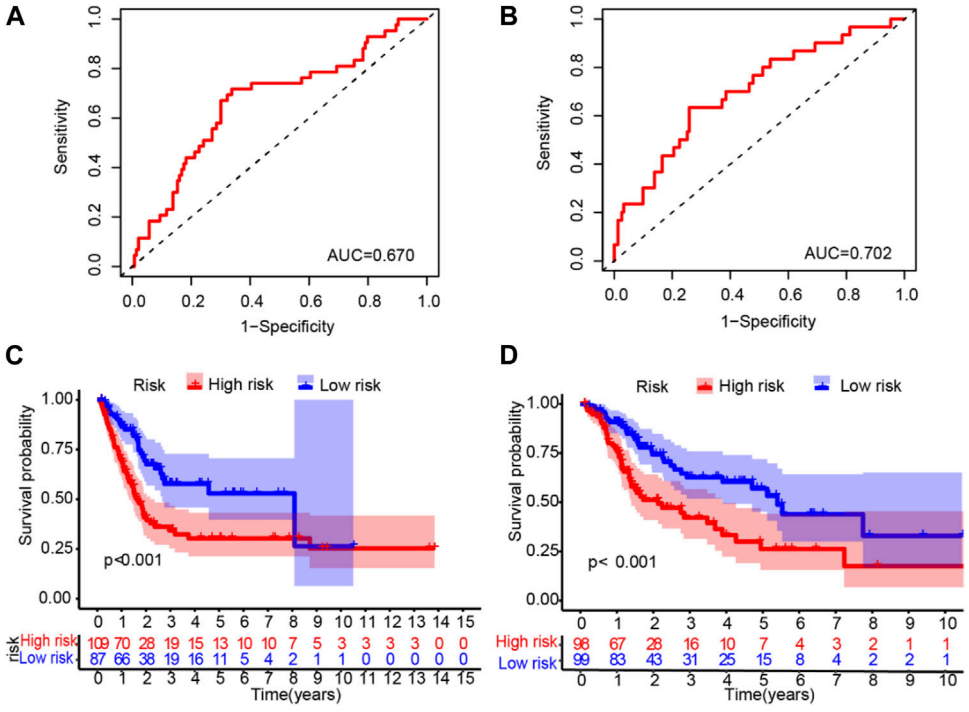

FIGURE 4
ROC validation and Kaplan–Meier curve analysis for the lactate-related lncRNA signature. **(A-B)** The ROC areas were 0.670 and 0.702 in validation sets 1 (dataset 2: n = 197) and 2 (dataset 3: 196), respectively. **(C–D)** Prolonged OS in low-risk *versus* high-risk patients in both validation cohorts (log-rank test, $p < 0.001$).

3.4 Construction of the nomogram in the TCGA cohort

According to univariate and multivariate regression analyses, BCA was an independent prognostic factor (Figures 5A,B). Figure 4C shows the nomogram derived from the five LM_lncRNAs. The mixed nomogram (Figure 5D) combining clinicopathological features and prognostic factors of LM_lncRNAs coupled with the 5-year calibration curve could be applied stably and accurately to treat BCA patients (Figure 5E).

In low- and high-risk individuals, GSEA identified pathways enriched with differentially expressed lncRNAs. According to these findings, LM_lncRNAs play a central role in cell cycle regulation, oocyte meiosis, pyrimidine metabolism, and DNA replication. Low-risk individuals showed higher steroid hormone biosynthesis, retinol metabolism, and linoleic acid oxidation (Figure 6A).

3.5 Subtype-specific genomic profiling and immune infiltration levels

Based on immune scores, no significant differences were found between the high- and low-risk groups. In the high-risk group, stromal scores were significantly different from those in the low-risk group; moreover, as shown in Figures 6B,C, immune infiltration of the matrix was significantly different in the high-risk group. As immune checkpoint inhibitors are a critical component of immunotherapy, we explored differences between groups in immune checkpoint expression. HNRNPA2B1, HNRNPC, IGF2BP2, IGF3, ALKBH5, and YTHDF2 were significantly different between the high-risk and low-risk groups in terms of m6A modification (Figure 7A). The two patient groups expressed significantly different levels of lncRNAs, such as TNFRSF18, TNFRSF14, TNFRSF9, TNFRSF8, TNFSF4, HAVCR2, LAG3, LGALS9, SIGLEC15, SIGLEC9, SIGLEC7, and LAIR1 (Figure 7B). According to the results of the Pearson correlation calculation in the previous section, with $|R^2| > 0.3$ and $p < 0.001$ as the correlation criteria, to identify independent prognostic factors for LM_mRNAs, a network diagram was drawn (Figure 7C).

3.6 Predicting chemotherapeutic response

We utilized the GDSC website to assess the outcome considering that chemotherapy resistance directly affects patient outcomes. Furthermore, we assessed the response of the two subgroups to

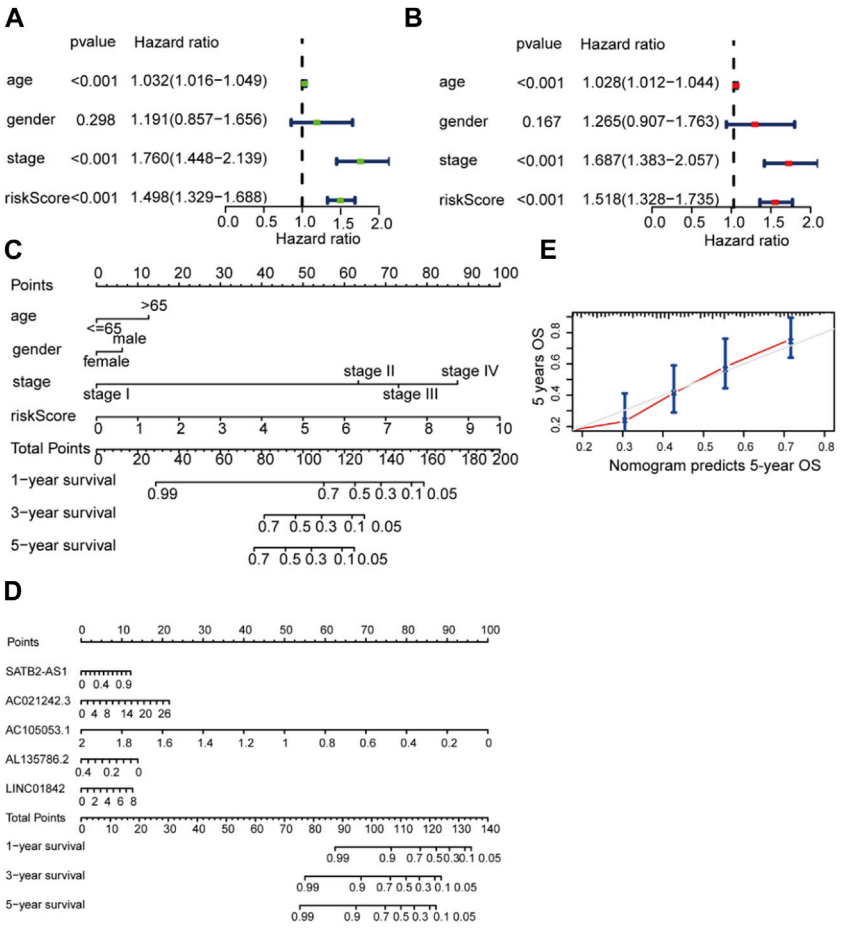

FIGURE 5
Independent prognostic value of the LDEL risk model. **(A, B)** Univariate **(A)** and multivariate Cox **(B)** analyses in the training cohort. **(C)** A nomogram for the lactate lncRNA signature. **(D)** A nomogram for both prognostic lactate lncRNAs and pathological factors. **(E)** C-index analysis of the nomogram.

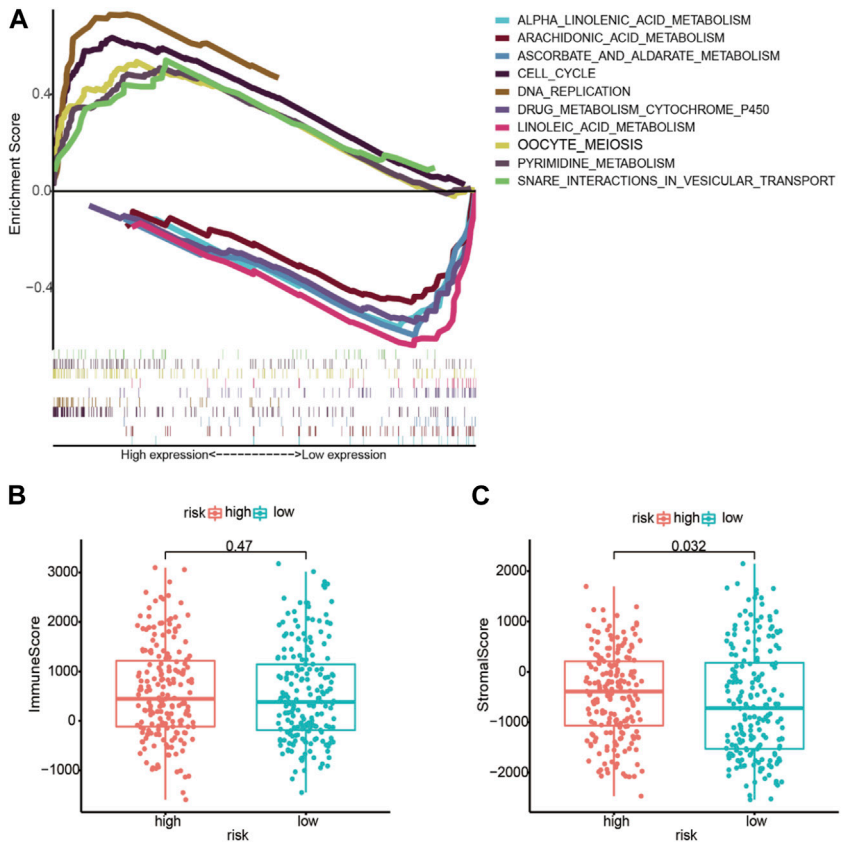

FIGURE 6
(A) Enrichment of genes in the representative pathways by GSEA function analysis. **(B)** Immune scores for the high-risk and low-risk groups. **(C)** The stromal score for the high-risk and low-risk groups.

chemotherapeutic agents using the GDSC cell line dataset (Figure 8). A total of 22 drugs were found to be more sensitive to high-risk subtypes, increasing a patient's prognosis when chemotherapy drugs were used on patients with high-risk subtypes Table 1. The above results can help to screen for more suitable chemotherapy drugs for precise treatment. Several specific targeted therapeutic drugs with the smallest IC50, such as AKT inhibitor VIII, AS601245, axitinib, FH535, MG.132, MS.275, and PD.0332991, have more significant potential to be developed into a high-risk group for the treatment of BCA.

4 Discussion

BCA has a poor prognosis partly because of the lack of an effective early diagnosis. The clinical diagnosis of BCA mainly relies on cystoscopy biopsy and urine cytology. Cystoscopic biopsy is invasive and expensive, and urine cytology is less sensitive for identifying early low-grade BCA (Lokeshwar et al., 2005). Moreover, due to the lack of sensitivity and specificity of diagnosis, a series of BCA-related biomarkers (such as nuclear matrix protein, bladder tumor antigen, and cytokeratin) have limited application value in the early detection of BCA (Chao et al., 2001). Therefore, developing new biomarkers with high sensitivity and specificity is critical for the early diagnosis and prognostic analysis of UBCA.

The study of this type of UBCA, its molecular mechanism, and the prognosis of MIBCA patients is essential for the prognosis of this type of UBCA. This study first used TCGA and MSigDB data to confirm the LM_mRNAs further screened by correlation analysis. Recent reports indicated that patient data in TCGA with follow-up times <30 days or OS < 30 days were excluded (Dai et al., 2021; Fang et al., 2021; Li et al., 2021). We performed the analysis in the present study according to such a modality.To identify differentially expressed lncRNAs, we conducted a differential analysis on the lncRNAs above. Univariate and multivariate analyses identified LM_lncRNAs that might be independent risk factors for UBCA. This study screened five differentially expressed lncRNAs: SATB2-AS1, AC021242.3, LINC01842, AC105053.1, and AL135786.2. SATB2-AS1, an inhibitor of microRNA155-3p, regulates the migration and proliferation of breast cancer cells (Liu et al., 2017). Studies of SATB2-AS1 in colon tumors demonstrated that it could regulate SATB2 to affect the colon tumor microenvironment (Xu et al., 2019). The lncRNA SATB2-AS1 regulates the proliferation of lung cancer cells by coordinating with other lncRNAs (Lu et al., 2021). In previous studies on lung cancer-related lncRNAs, LINC01842 was considered to regulate lung cancer cell proliferation in a ceRNA pattern with CASC8 and VPS9D1-AS1

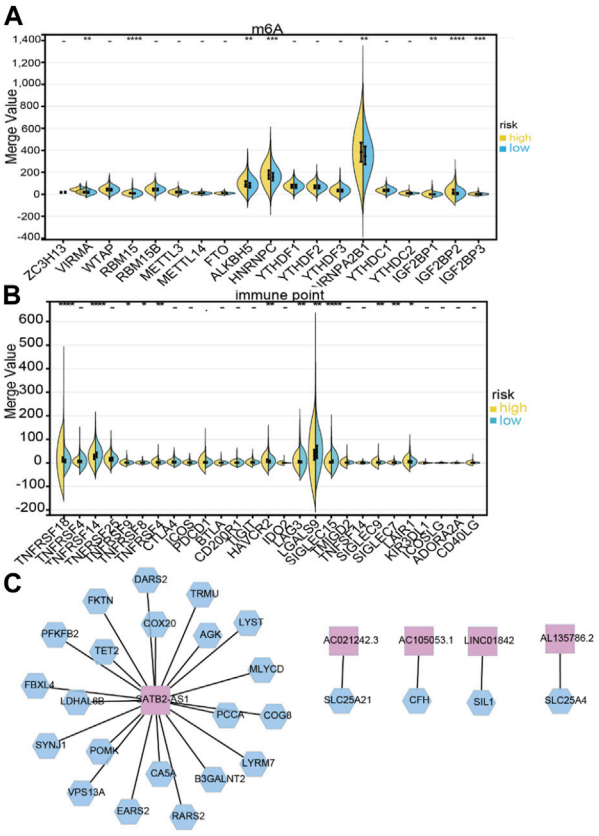

FIGURE 7
Correlation between LDELs and immunometabolic modification. **(A)** Expression of m^{6}A genes between high- and low-risk subgroups ($^{-}p \geq 0.1$, $\cdot p < 0.1$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$). **(B)** Distribution of immune checkpoints between the high- and low-risk subgroups. **(C)** Protein–protein interaction (PPI) network of 5 LDELs and lactate metabolism genes.

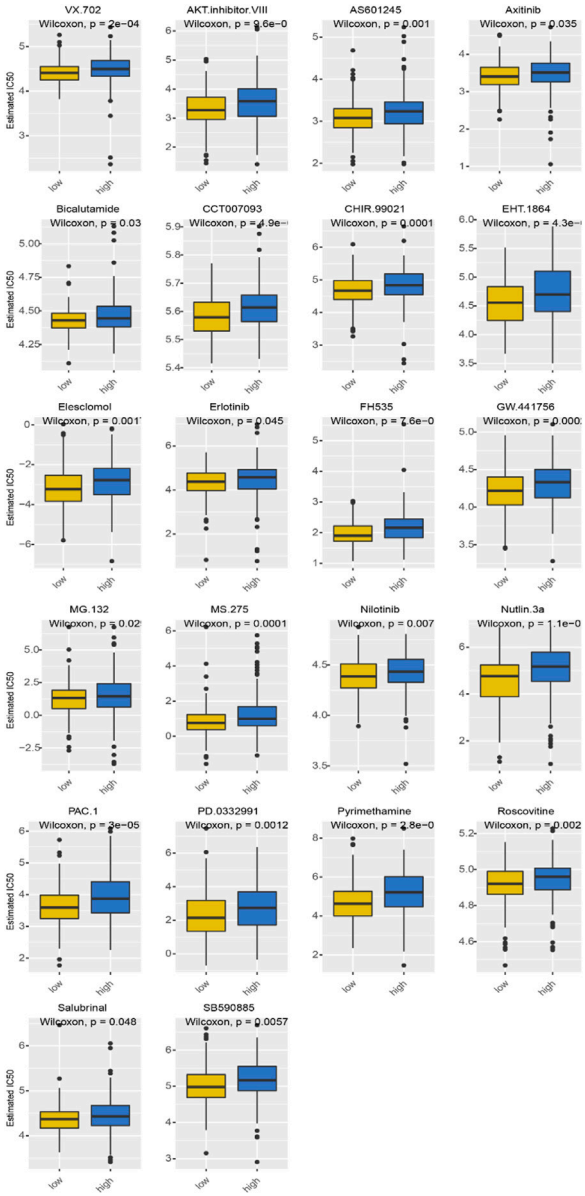

FIGURE 8
Comparative analysis of chemotherapy drugs with good efficacy in the high-risk group.

(Dai et al., 2020). However, there are no reports on the expression and function of AC105053.1, AC021242.3, and AL135786.2 in tumors. Research on tumor and immune regulation and the TME has gradually become a hotspot in recent years. However, most of the research on the immune regulation-related mechanism of UBCA is limited to animal experiments and direct sequencing data. There needs to be in-depth research on the mechanism, especially the mechanism of lactic acid in UBCA (Conde et al., 2015). The expression levels of immune checkpoints are predictive biomarkers of immunotherapy response, showing broad potential for precision therapeutics. In metastatic UBCA, immunotherapy targeting suppressive immune checkpoints has often been used as a second-line therapy, but only 30% of patients respond to ICI immunotherapy (Lopez-Beltran et al., 2021). Earlier studies related to immunotherapy and pan-cancer research demonstrated that methylation played a critical role in immune cell infiltration (Guo et al., 2021). The process of m6A modification was proven to be the key to methylation (Ma et al., 2019). ALKBH5 regulates target gene splicing, leading to changes in lactate in the tumor microenvironment (Li et al., 2020). METTL3-mediated RNA m6A modification regulates lactate metabolism in the TME (Xiong. et al., 2022). We hypothesize that those with high lactate risk scores may benefit more from immunotherapy. In comparison to low-risk groups, high-risk groups exhibited significantly elevated levels of m6A modification, as well as TNFRSF18, HAVCR2, and LAG3, suggesting that these m6A modification suppressive agents may be considered for patients with a high lactate risk.

There were 22 drugs identified in the GDSC cell line dataset that were highly specific to the high-risk lactate group, which provides new targets for treating UBCA more precisely. By controlling aerobic glycolysis, overactivated PTEN/PI3K/Akt/mTOR promotes cancer metabolic conversion and tumor cell proliferation. AKT inhibitor VIII has been proven to protect gastric cancer cells, clear cell renal cell carcinoma, and breast cancer cells. AS601245, an anti-inflammatory JNK inhibitor, and clofibrate induce cell responses and alter gene expression

profiles in Caco-2 colon cancer cells (Cerbone et al., 2012). In addition to inhibiting VEGFR1, VEGFR2, and VEGFR3, axitinib inhibits platelet-derived growth factor receptors and C-Kit. Treatment has been used for advanced renal cell carcinoma patients who have not responded to cytokines or tyrosine inhibitors.

Nevertheless, it is not used in the treatment of BCA. *In vitro* and *in vivo*, blocking the SDF-1/CXCR4/β-catenin axis inhibits the growth of BCA cells, but there are few related reports (Zhang et al., 2018). FH535, an inhibitor of the β-catenin pathway, inhibits the release of the proangiogenic cytokines vascular endothelial growth factor (VEGF), interleukin (IL)-6, IL-8, and TNF-α. It inhibits angiogenesis *in vitro* and *in vivo* (Liu et al., 2016). The proteasome inhibitor MG-132 inhibits mitochondrial-mediated intrinsic myocardial apoptosis and NF-κB-mediated inflammation, and less research has been done on cancer treatment. An investigation of MS-275, a potent cytotoxic HDACi selective for classes I/IV, in RMS xenograft models demonstrated modest antitumor activity alone and combined with standard chemotherapy (Cassandri et al., 2021). A selective CDK4/6 inhibitor, palbociclib, has shown outstanding results in phase II clinical trials in patients with estrogen receptor-positive HER2-negative breast cancer (Bollard et al., 2017).

5 Conclusion

Based on ROC analysis, DCA, and calibration curve analysis of the TCGA dataset, we identified a novel, efficient, and highly prognostic LM_lncRNA signature. LM_lncRNAs were found to act as independent predictors of OS in the TCGA database. Validation by random grouping within the dataset shows its effectiveness. In addition, 22 chemotherapeutic agents sensitive to the high-risk group were predicted, which could be used to treat tumors with tumor-related sensitive drugs. This study developed a new method for diagnosing and evaluating UBCA patients' survival prognoses based on lactate metabolism.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

XW and JP conceived and designed the study and analyzed the data, QG, NR, and PW implemented the methodology, and drafted the manuscript. MW and ZL are involved in the conceptualization and constructive discussions, resources, writing review/editing, and funding support. 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1215296/full#supplementary-material

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Comprehensive analysis of ceRNA network composed of circRNA, miRNA, and mRNA in septic acute kidney injury patients based on RNA-seq

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Background: Sepsis is a complex, life-threatening clinical syndrome that can cause other related diseases, such as acute kidney injury (AKI). Circular RNA (circRNA) is a type of non-coding RNA with a diverse range of functions, and it plays essential roles in miRNA sponge. CircRNA plays a huge part in the development of various diseases. CircRNA and the competing endogenous RNA (ceRNA) regulatory network are unknown factors in the onset and progression of septic AKI (SAKI). This study aimed to clarify the complex circRNA-associated regulatory mechanism of circRNAs in SAKI.

Methods: We collected 40 samples of whole blood of adults, including 20 cases of SAKI and 20 cases of healthy controls. Moreover, five cases were each analyzed by RNA sequencing, and we identified differentially expressed circRNA, miRNA, and mRNA (DEcircRNAs, DEmiRNAs, and DEmRNAs, respectively). All samples were from SAKI patients with intraperitoneal infection.

Results: As a result, we screened out 236 DEcircRNAs, 105 DEmiRNAs, and 4065 DEmRNAs. Then, we constructed two co-expression networks based on RNA–RNA interaction, including circRNA–miRNA and miRNA–mRNA co-expression networks. We finally created a circRNA–miRNA–mRNA regulation network by combining the two co-expression networks. Functional and pathway analyses indicated that DEmRNAs in ceRNA were mostly concentrated in T cell activation, neutrophils and their responses, and cytokines. The protein–protein interaction network was established to screen out the key genes participating in the regulatory network of SAKI. The hub genes identified as the top 10 nodes included the following: ZNF727, MDFIC, IFITM2, FOXD4L6, CIITA, KCNE1B, BAGE2, PPIAL4A, USP17L7, and PRSS2.

Conclusion: To our knowledge, this research is the first study to describe changes in the expression profiles of circRNAs, miRNAs, and mRNAs in patients with SAKI. These findings provide a new treatment target for SAKI treatment and novel ideas for its pathogenesis.

KEYWORDS

septic, acute kidney injury, transcriptome analysis, ceRNA, noncoding RNA

Introduction

Sepsis is highly susceptible to organ dysfunction because it causes dysregulation of the patient's response to infection (Singer et al., 2016). Sepsis without treatment or effective therapies can cause shock and multiorgan failure if not treated immediately. As part of sepsis, kidney is one of the most frequently impaired organs in patients with sepsis, that is, septic acute kidney injury (SAKI) (Koyner, 2019). Intensive care unit patients are most likely to suffer from acute kidney injury (AKI) due to sepsis, and around 60% of sepsis cases are complicated by AKI. Furthermore, AKI frequently occurs early in the course of sepsis (Uchino, 2005; Bagshaw et al., 2008). In addition, the increasing incidence of sepsis and AKI in critically ill patients represents a high risk of death (Parmar et al., 2009). In 2012, Kidney Disease: Improving Global Outcomes determined AKI occurrence by measuring urine output and serum creatinine, but these markers have a particular hysteresis (Khwaja, 2012; Wen and Parikh, 2021). The root cause is the unclear molecular mechanisms of SAKI (Wen et al., 2018). Thus, studying the mechanisms underlying SAKI pathogenesis and developing biomarkers for early diagnosis and treatment is essential.

Circular RNA (circRNA) was first reported in 1976. At that time, circRNA was assumed to be a plant viroid (Sanger et al., 1976). With the continuous progress of circRNA research, knowledge and awareness of circRNA have been refined. circRNA is a new non-coding RNA with a unique structure. CircRNA forms are derived from a 30–50 connection between the two ends of linear RNA molecules (Danan et al., 2011; Kristensen et al., 2019). Based on the components of parental genes, at least three different groups of circRNA, including ecircRNA, ciRNA, and eIciRNAs, exist in animal cells (Li et al., 2015; Zhang et al., 2018). Several studies confirmed that circRNA is present in human blood and other tissues and expressed during the development of numerous diseases. Gene expression can be influenced by miRNA sponges and other mechanisms. CircRNA also has excellent stability and sensitivity in biological fluids; thus, it can be a suitable potential biomarker of cancer or other diseases (Meng et al., 2017; Zhang et al., 2018). In 2011, Salmena recommended for the first time the competing endogenous RNA (ceRNA) hypothesis (Salmena et al., 2011). CeRNA is a research hotspot and the most popular mechanism for circRNA to regulate gene expression. CircRNA can competitively combine with miRNA response elements (MREs), regulate the expression of downstream mRNA, and is involved in the occurrences and progression of a number of diseases through the mechanism of ceRNA (Taulli et al., 2013; Tay et al., 2014; Zhang et al., 2018). Meanwhile, the function and mechanism of circRNA-associated ceRNA in SAKI are still being elucidated.

This study investigated the pathogenesis of SAKI and the potential treatment target. First, we utilized RNA sequencing (RNA-seq) to compare the expression profiles of circRNA, miRNA, and mRNA between SAKI patients and healthy controls. Differential expression of 10 selected circRNAs (four upregulated and six downregulated) confirmed the consistency with sequencing data by real-time quantitative polymerase chain reaction (QRT-PCR). Afterward, based on the sequencing data, co-expression networks were formed for circRNA–miRNA and miRNA–mRNA. By combining circRNA and miRNA pairs, a circRNA–miRNA–mRNA ceRNA network was established. Pathway and functional analyses were applied to elucidate the potential functional pathways of differentially expressed mRNAs (DEmRNAs). We then constructed a network of protein–protein interaction (PPI) and identified hub genes (Martino et al., 2021). New targets for the diagnosis and treatment of SAKI can be possibly screened out due to this study.

Methods

Patient sample collection

The clinical experimental specimens were obtained from the whole blood of SAKI patients and healthy individuals. Twenty samples were obtained from SAKI patients between June 2022 and November 2022 from the General Hospital of Ningxia Medical University. All samples were obtained from SAKI patients with intraperitoneal infection. We selected five cases for each of the two groups for RNA-seq, and the rest were used to identify the accuracy of RNA-seq results by qRT-PCR. In this study, the Human Research Ethics Committee at the General Hospital of Ningxia Medical University approved the research, and the experimental specimens were kept at −80°C until their extraction. Table 1 and Table 2 show basic information about the patients and healthy controls, respectively.

High-throughput sequencing

In accordance with the manufacturer's instructions, total RNA was isolated from clinical experimental specimens using TRizol reagent (Invitrogen RNA simple kit). We evaluated the integrity of RNA by electrophoresis in agarose gels using an Agilent 2100 bioanalyzer (Agilent Technologies, United States of America) of the extracted RNA for quality check (optical density (OD) 260/OD280: 1.8–2.2; OD260/OD230 > 2.0; RNA integrity number >= 7). After rRNA depletion, the remaining RNA was purified, fragmented, and readied for cDNA synthesis. Next, for the first step, fragmentation buffer and Invitrogen reverse transcriptase (SuperScript IV) were used to reverse transcribe the RNA fragments with randomly selected primers to synthesize first-strand cDNA. As a part of the next step, DNA polymerase I was used to synthesize the second-strand cDNA. RNase H was used for reverse transcription, and dNTP was used to replace dUTP (instead of dTTP), whereas a buffer was used for its preparation. As a part of the library construction process, the RNA-seq library chain was made specifically with a high-fidelity PCR polymerase, and double-stranded cDNAs were obtained. A single nucleotide of A was added to each end of the double-stranded cDNA to ensure the quality of the library. After the ligation of adapters and library fragment screening, PCR amplification was performed. Given that the dUTP on the second-strand cDNA hindered the amplification of high-fidelity polymerase, amplified libraries were only derived from the first-strand cDNA. Library quality of the PCR products was validated using an Agilent 2100 Bioanalyzer. In the end, 150 bp paired-end reads were obtained from the libraries using Illumina's Hiseq 2500 platform.

TABLE 1 Information of the SAKI patients and healthy people for RNA-seq.

Characteristics	Control group (n = 5)	SAKI group (n = 5)	p-value
Male gender	4(80.00)	4(80.00)	>0.999
Age, years	45.20 ± 1.985	48.40 ± 8.029	0.716
BMI, kg/m^2	22.22 ± 2.195	23.10 ± 1.886	0.519
Kidney disease	0 (100.00)	0 (100.00)	>0.999
Abdominal Infection	0(100.00)	5(100.00)	*(p < 0.005)
Serum creatinine	69.4 ± 3.99	359.18 ± 56.92	0.007
Urea concentration	5.18 ± 0.17	18.39 ± 2.27	0.004

Abbreviations: Data are presented as mean (SD) or number (percentage); The difference between the two groups was analyzed by independent-sample t-test and One-way ANOVA BMI, body mass index.

TABLE 2 Information of the SAKI patients and healthy people for RT-qPCR validation.

Characteristics	Control group (n = 15)	SAKI group (n = 15)	p-value
Male gender	10(66.67)	12(80.00)	0.427
Age, years	47.20 ± 1.619	51.67 ± 3.952	0.309
BMI, kg/m^2	21.89 ± 0.63	22.43 ± 0.57	0.535
Kidney disease	0 (100.00)	0 (100.00)	>0.999
Abdominal Infection	0 (100.00)	10 (66.67)	*(p < 0.005)
Serum creatinine	65.67 ± 2.75	314.60 ± 26.83	*(p < 0.005)
Urea concentration	5.27 ± 0.15	18.95 ± 0.80	*(p < 0.005)

Abbreviations: Data are presented as mean (SD) or number (percentage); The difference between the two groups was analyzed by independent-sample t-test and One-way ANOVA BMI, body mass index.

RNA-seq data analysis

Raw sequencing data were quality controlled by FastQC and R software (Ward et al., 2019; Sepulveda, 2020). To obtain high-quality clean reads, we further processed the raw sequencing reads by fastp. The main step was the removal of sequencing primers and low-quality reads. A reference genome (hg19) alignment was performed using STAR software (Dobin et al., 2012). Gene expression levels were represented by fragments per kilobase of exon per million mapped fragments values. For circRNA, a database of sequencing reads (count) was used to detect the expression of circRNA in different samples using CIRCexplorer2 (Luo et al., 2019). To sequence small RNAs, using miRDeep2, we compared the sequences of small RNAs of each sample with those of miRNA precursors and mature miRNAs of corresponding species in the miRBase database (https://www.mirbase.org) (Friedländer et al., 2011). In addition, the closely related known miRNAs were obtained by combining them with human miRNA sequences, and the expressions of known miRNAs in each sample were counted.

Differential expression analysis

We selected five healthy control samples and five SAKI samples for RNA-seq analysis. After obtaining clean data by methods described previously, we aligned them to the reference genome to obtain differentially expressed genes (DEGs). DEGs were analyzed using DESeq2 (Love et al., 2014). Differential gene screening was performed using the edgeR filter criteria (log2fold change >2, false discovery rate >0.05) (Robinson et al., 2009). Upregulated and downregulated DEGs were categorized by log2(Fold Change) > 1 and log2(Fold Change) < −1, respectively.

Enrichment of gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG)

For differentially expressed circRNAs (DEcircRNAs) and DEmRNAs, we used GO and KEGG analysis to predict their functions. GO is a standard for describing gene functions. After screening for differential genes, in accordance with the gene–function classification system of GO, biological processes (BPs) were used to categorize DEGs, molecular functions (MFs), and components of cellular metabolism (CC). Enrichment analysis can indicate the manifestation of gene function of sample differences from the perspective of biological pathways. KEGG pathway databases contain pathways that represent molecular interactions, reactions, and relationships. We also analyzed differentially expressed circRNA host genes. DEGs were enriched by GO and KEGG pathways using the clusterProfiler R package (Yu et al., 2012). You can find out more about GO at http://www.geneontology.org and KEGG at http://www.genome.jp/kegg.

Co-expression network analysis of circRNA, miRNA, and mRNA

In this study, we built networks of co-expression between circRNA and miRNA and between miRNA and mRNA using co-expression analysis. To determine the Pearson correlation coefficient, we used the R function "cor.test ()" (Zhang et al., 2020) Cytoscape (https://cytoscape.org) was used to visualize the two co-expressions networks.

Construction of circRNA–miRNA–mRNA network

CeRNA contains miRNA binding sites; circRNA can compete with miRNAs and inhibit mRNA-mediated gene regulation. circRNA binds to miRNAs competitively and acts as an endogenous miRNA sponge. When the expression of circRNA in cells decreases, more miRNAs bind to the mRNA. MiRNAs negatively regulate mRNAs due to their negative regulatory effects, and the expression of mRNA decreases. MiRanda was used to predict the circRNA's miRNA target (http://www.MiRNA.org/MicroRNA/home.do). Two bioinformatics tools (miRanda and RNAhybrid) predicted the miRNA target genes (mRNA). As a final result, the intersection of the two tools was obtained. Then, we calculated Pearson's correlation coefficient using the R function cor. We used it to denote the size of RNA–RNA interaction. Based on the ceRNA theory, an endogenous RNA network composed of circRNA, miRNA, and mRNA was constructed. Visualization was performed using Cytoscape software (https://cytoscape.org).

Gene set enrichment analysis (GSEA)

According to GSEA, to a certain extent, the random error introduced by the limit threshold was largely avoided. In addition, the proportion of upward and downregulated genes in the GO or pathway can be determined by calculating the enrichment score of a gene. Therefore, GSEA was necessary, and a GSEA software was used (version 4.1.0, https://www.gsea-msigdb.org).

Identifying hub genes for and building the PPI network

Known and predicted PPI are stored in the STRING database, and we used them to make a PPI network of DEmRNA. After the visualization was carried out with Cytoscape software (https://cytoscape.org), the 10 best genes were selected as hub genes.

QRT-PCR

Sequencing results were verified by qRT-PCR. In SAKI patients and healthy individuals, total RNA was extracted using TRIzol. Reverse transcription was performed following the manufacturer's instructions. QRT-PCR was also conducted. Table 3 provides a list of all primer sequences. We analyzed the data using the $2^{-\triangle\triangle CT}$ method.

Statistical analysis

In this study, we conducted statistical analyses using R and SPSS. Independent sample *t*-tests were used to determine statistical significance between groups. We performed all bioinformatics analyses with R packages of R software. Statistics were considered significant when $p > 0.05$, whereas $p > 0.05$ was not considered significant.

Result

Identifying circRNA, mRNA, and mRNA expression differences

We collected five whole blood samples from SAKI patients and another five samples from the healthy controls for high-throughput sequencing. As a result, 29856 circRNAs were detected. circBase and circatlas databases were used for gene annotation. In the circBase database, 10972 circRNAs with circBase IDs were annotated. Meanwhile, in the circatlas database, 24196 circRNAs were identified. The scatter plot in Figure 1 shows the visualized circRNAs with different expressions in SAKI samples and healthy controls. Principal component analysis demonstrated significant differences between the two groups (Figure 1). The heatmap_top50_sample_cluster exhibited the expression of DEcircRNAs (Figure 1). The number of up-and-down-regulated genes did not differ considerably from those in healthy controls. Among the total DEcircRNAs, 129 were significantly upregulated, and 107 were significantly downregulated. CircBase included 33 upregulated and 48 downregulated DEcircRNAs. The rest were observed for the first time. Volcano maps showed all DEcircRNAs (Figure 1). Similarly, differentially expressed miRNAs (DEmiRNAs) and DEmRNAs was detected in the two groups. A total of 76 DEmiRNAs were upregulated, and 29 were downregulated. A total of 2125 genes were upregulated by DEmRNA, and 1940 genes were downregulated. Figure 2 shows the volcano and heatmap_top50_sample_cluster of DEmiRNAs and DEmRNA.

Functional analysis of GO and KEGG

To explore the possible features of DEcircRNAs and DEmRNAs in SAKI, we analyzed their host genes using KEGG pathway and GO enrichment analyses. Figure 3 shows the gene enrichment analysis (GO) of DEcircRNAs. BP, CC, and MF enrichment analysis results indicated that the host genes of DEcircRNAs were primarily located in the "regulation of GTPase activity," "negative or positive regulation of catabolic process," "nuclear speck," "cytoplasmic ribonucleoprotein or ribonucleoprotein granule," "active transmembrane transporter activity," "transcription corepressor activity," "modification–dependent protein binding," and other processes. In accordance with KEGG pathway enrichment analyses, DEcircRNAs of host genes were mainly enriched in "Amyotrophic lateral sclerosis," "Nucleocytoplasmic transport," and "Th1 and Th2 cell differentiation"

TABLE 3 Primer sequences for quantitative real-time polymerase chain reaction analysis of differentially expressed circRNA levels.

Name	Forward primer sequence	Reverse primer sequence
chr12:66203711|66228370	TGTGGCAGTATATCAAGCAGAGA	CACCGATGGTCTTGTTTTTCTGT
chr1:113829592|113834439	TGTTCCACCCCATTCCAGTG	ACAGACACTGAAGACTCCTGG
chr21:33414888|33432871	GCCTGTTTCTTCCTGGTCCTG	TGGGAAAGAGGGTCTCTTCTATCT
chr15:64499293|64500166	GGGAACTAAACCGGAGCCAG	ACATGCCCAGTGGACAACATC
chr19:15397150|15404042	CAAGTTCCATATCCCTGCGGT	CAGCCTGCGACCTCTTCATT
chr19:47084345|47094608	CAACTCTCCATCTTCCCCAGGT	GGAAGCTACCGGAGTCGTGA
chr19:54142929|54153840	TGACGAGTTTGAGCAGCGTC	CTCACCTTGGAGTTTGCGCT
chr1:233198939|233236980	GCGAGCCACCATCAGTAACA	TGCAGTGAAGAGAGATGCAGG
chr9:96458379|96465778	AAAGATACCAGGCCAGAAGCG	CTCCGCTCAGCTCTTTCGAG

(Figure 3). In addition, we examined DEmRNA enrichment in ceRNA based on GO and KEGG. The GO analysis showed that numerous BPs correlated with T cell activation, neutrophil, and their responses, and cytokines were significantly enriched in cytoplasmic and vesicle lumen (Figure 3). According to KEGG analysis, DEmRNAs were mainly found in cytokine–cytokine receptor interactions and cell adhesion molecules (Figure 3).

GSEA

To predict DEmRNA-related pathways and BPs in SAKI with greater accuracy, we also performed GSEA for all DEmRNAs of RNA-seq data. GSEA (Figure 4) showed that DEmRNAs in SAKI were mainly enriched in "Cytokine–cytokine receptor interaction," "Cell adhesion molecules," "Th17 cell differentiation," and "Th1 and Th2 cell differentiation." In line with KEGG analysis, this result confirms the validity of our findings.

Co-expression network analysis of circRNA, miRNA, and mRNA

Correlations between DEcircRNAs, DEmiRNAs, and DEmRNAs were determined based on Pearson's correlation coefficient, and DEcirc/DElnc/DEmRNAs without significant interactions were excluded based on certain conditions. As shown in Figure 5, we used Cytoscape software to establish a circRNA–miRNA co-expression network containing 217 DEcircRNAs and 72 DEmiRNA. circRNA–miRNA and miRNA–mRNA co-expression networks containing 60 DEmiRNAs and 943 DEmRNAs, respectively (only interactions with correlation p-value less than 0.05 were plotted).

Construction of the circRNA–miRNA–mRNA network

As previously described, the ceRNA network consists of miRNAs negatively regulated by circRNAs and mRNAs. CircRNA, mRNA, and miRNA RNA-seq results were used to create a ceRNA network between these three molecules. The RNA used to build ceRNA has several requirements. First, significant differences exist between the three molecules. Second, miRNA has a targeted relationship with circRNA, as do the miRNA and mRNA, and they were negatively correlated (p-value <0.05, cor <= −0.8). Third, the same miRNA must have a targeting relationship with mRNA and circRNA. Fourth, circRNA and mRNA that have a targeted relationship with the same miRNA are significantly correlated (p-value <0.05). Figure 6 shows that 36 circRNA, 20 miRNA, and 56 mRNA were selected to create a circRNA-associated ceRNA regulatory network. These results provide new information about pathogenetic mechanisms and potential treatments for SAKI.

Gene identification and network construction of PPI

Network visualization was performed using the String database to uncover potential PPI networks in SAKI and identify hub genes for SAKI development. STRING contains known and predicted protein interactions. The physical interaction between two proteins and the functional interaction between two proteins are respectively termed direct and indirect PPIs. DEGs were extracted for the species directly included from the database. With the igraph package of the R language, we calculated the network modularity and module division using fast greedy optimization (Baciu et al., 2017; Sepulveda, 2020). This package divides the blocks into blocks and drawings. The plots are presented in Figure 7. After clustering, modules with more than 10 significantly different genes were analyzed again for GO and KEGG enrichment. Hub genes identified as the top 10 best genes included the following: ZNF727, MDFIC, IFITM2, FOXD4L6, CIITA, KCNE1B, BAGE2, PPIAL4A, USP17L7, and PRSS2.

QRT-PCR

QRT-PCR was used to confirm the consistency of gene expression and RNA-seq data. We randomly selected 9 circRNAs (4 upregulated and 5 downregulated) for validation. As shown in Figure 8, the results of qRT-PCR and sequencing data were consistent.

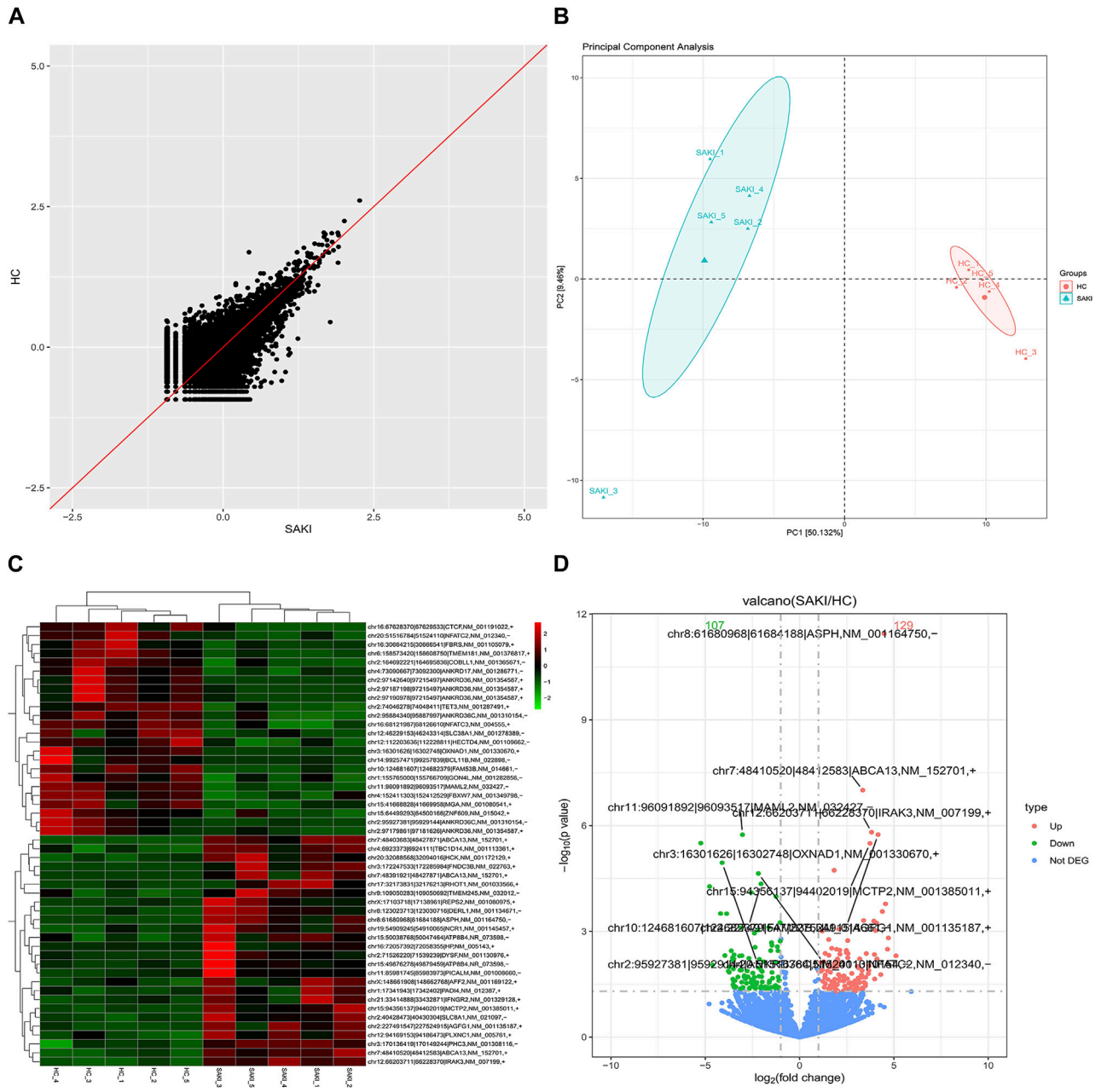

FIGURE 1
Differentially circular RNAs analysis in SAKI. Abbreviations: **(A)** Scatter plot showed the visualizing circRNAs different expression in SAKI samples and healthy control. **(B)** Principal component analysis (PCA) of SAKI samples and healthy control. **(C)** Heatmap and cluster analysis of top 50 genes of DEcircRNAs. Each column represents a sample and each row a different gene. The color represents the expression levels of the gene. Red indicates that the gene has a higher expression level; green indicates that the gene has a lower expression. **(D)** DEcircRNAs in volcano plot, significantly top 5 marks are depicted. The blue dots indicate no significant difference circRNAs; The red dots indicate upregulated circRNAs; The green dots mean downregulated circRNAs.

Discussion

Sepsis can cause SAKI, which is one of its most severe complications. No consensus has been reached regarding the mechanisms underlying sepsis-induced AKIs. Based on available research, the root cause of the exact timing of kidney damage in sepsis is uncertain. When patients show signs and symptoms of sepsis, we appropriate empiric antibiotics passively. Patients may receive hemodialysis treatment when the infection seriously endangers the kidneys or other organs (Bottari et al., 2021; Patel et al., 2022; Roggeveen et al., 2022). A variety of potential markers may be useful in early detection of SAKI and targeting of its therapeutic targets. Most studies involved noncoding RNAs. Noncoding RNAs are a class of substances that may play a role at the gene level. A broad involvement has been observed in a number of diseases, including SAKI (Rong et al., 2017). A great deal

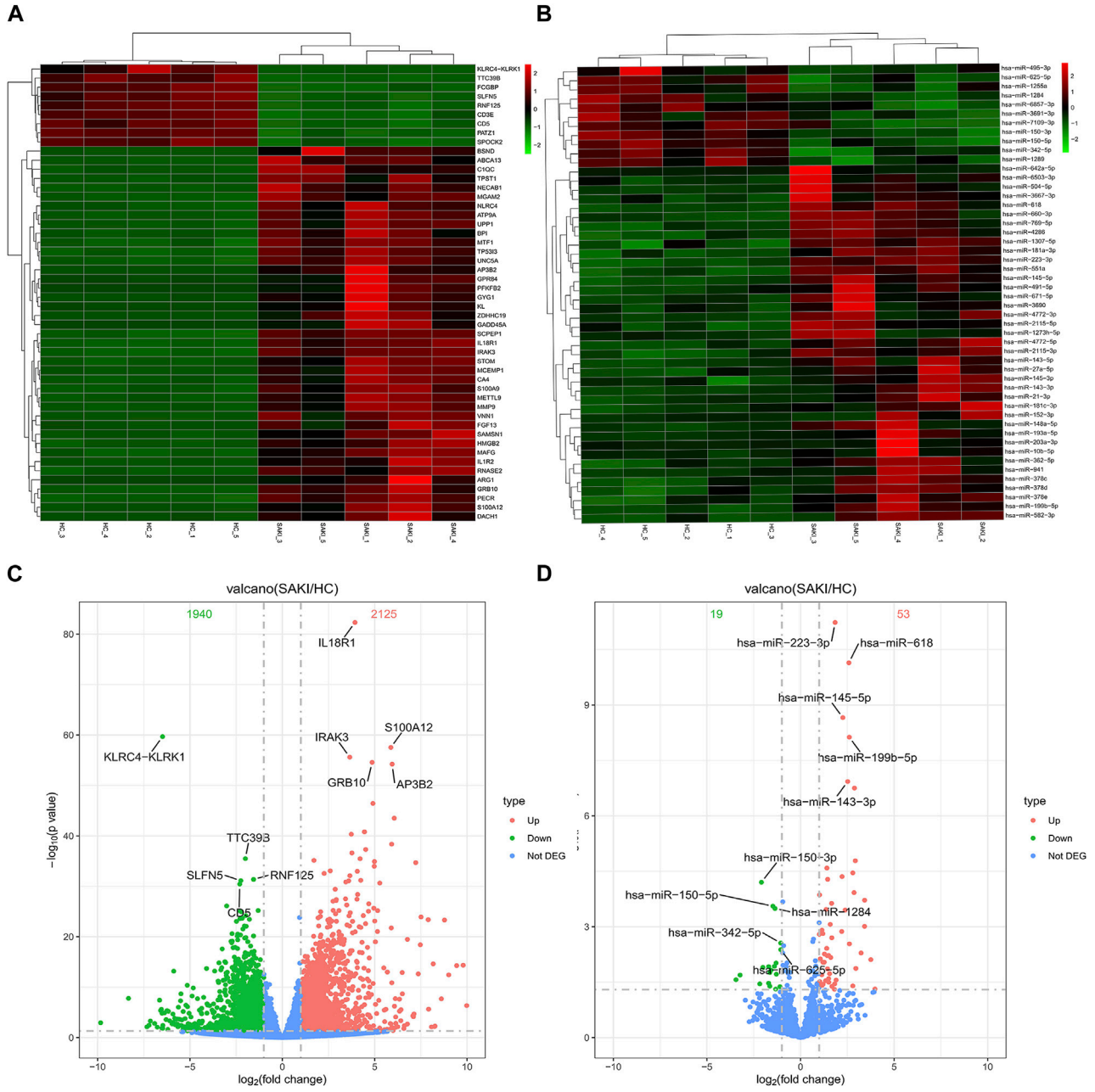

FIGURE 2
Differentially miRNAs and mRNAs analysis in SAKI. Abbreviations: **(A)** Heatmap and cluster analysis of top50 genes of DEmRNAs. **(B)** Heatmap and cluster analysis of top 50 genes of DEmiRNAs. **(C)** DEmRNAs in volcano plot, significantly top 5 marks are depicted. **(D)** DEmiRNAs in volcano plot, significantly top 5 marks are depicted.

of research has been conducted on miRNAs in SAKI. Numerous miRNAs, such as MiR-107, MiR-210, and MiR-150-5p, affect the growth and advancement of SAKI (Wang et al., 2017; Lin et al., 2019; Shi et al., 2021). However, SAKI is still relatively understudied regarding circRNA profiles and ceRNA networks associated with circRNAs. CircRNAs, miRNAs, and mRNAs were sequenced in SAKI using high-throughput sequencing. We have identified 236 DEcircRNAs, 105 DEmiRNAs, and 4065 DEmRNAs. We studied their mutual interactions initially and constructed a circRNA-associated-related network. In light of this finding, several significant dysregulated RNAs may be used as potential biomarkers of SAKI.

CircRNA has a particular structure that can make it more stable than other RNA (Rong et al., 2017). It has been an important topic of numerous research. Circ_0114428, Circ_0091702, and CircRNA TLK1 are involved in the process of SAKI according to related literature. For prediction of circRNA functions, differentially expressed host genes in the presence of circRNAs were analyzed, and GO and KEGG analyses were conducted. Ten randomly selected CircRNAs were analyzed with qRT-PCR to validate the RNA-seq

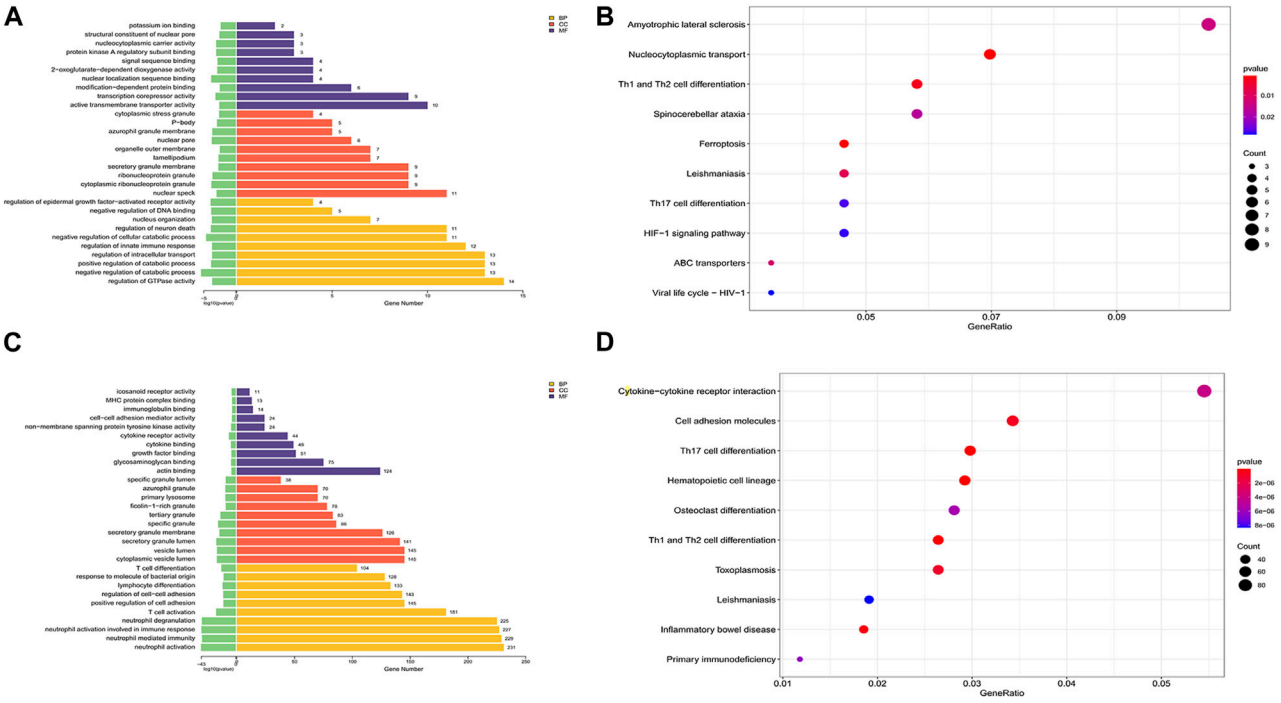

FIGURE 3
GO and KEGG analysis of DEmRNAs and the host genes of DEmiRNAs. Abbreviations: **(A)** GO analysis of the host genes of DEcircRNAs under the theme of BP, CC and MF. **(B)** KEGG analysis of the host genes of DEcircRNAs. **(C)** GO analysis of DEmRNAs under the theme of BP, CC and MF. **(D)** KEGG analysis of DEmRNAs.

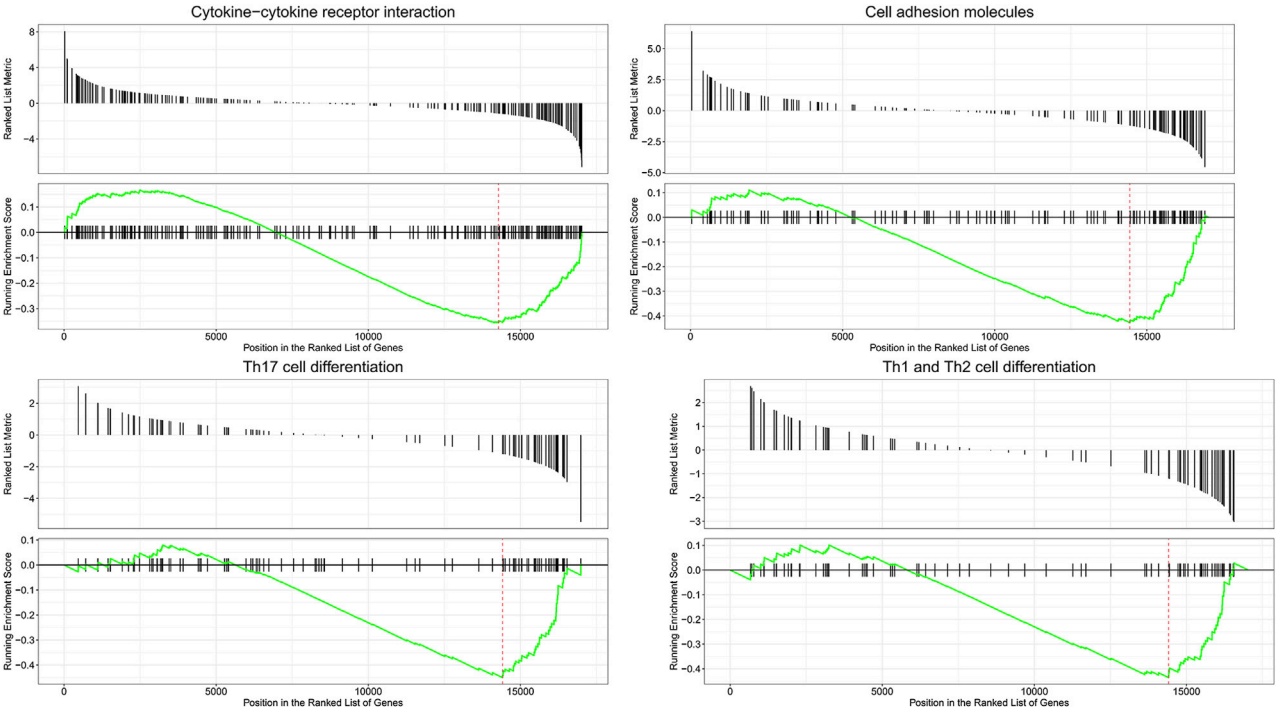

FIGURE 4
Enrichment plot of four KEGG among RNA processes. Abbreviations: The title represents a description of the gene. The abscissa represents the score of gene set members in the target gene list. The ordinate represents the enrichment score of the run. Ranked list metric represents the position of each member in the target gene set.

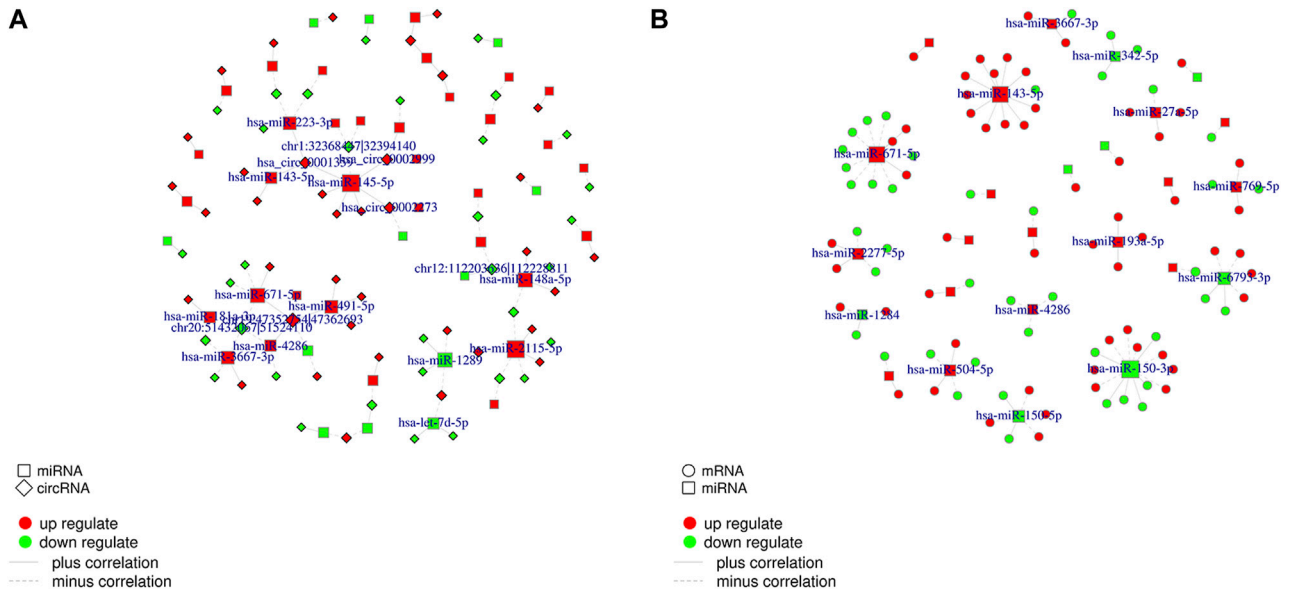

FIGURE 5
CircRNA-miRNA co-expression network and miRNA-mRNA co-expression network. Abbreviations: **(A)** Networks of circRNA-miRNA. Only RNA-RNA interactions with a correlation *p*-value less than 0.05 are plotted. The red color line indicates that the expression of this RNA is positively correlated, green indicates a negative correlation for the expression of RNA. **(B)** Networks of miRNA-mRNA.

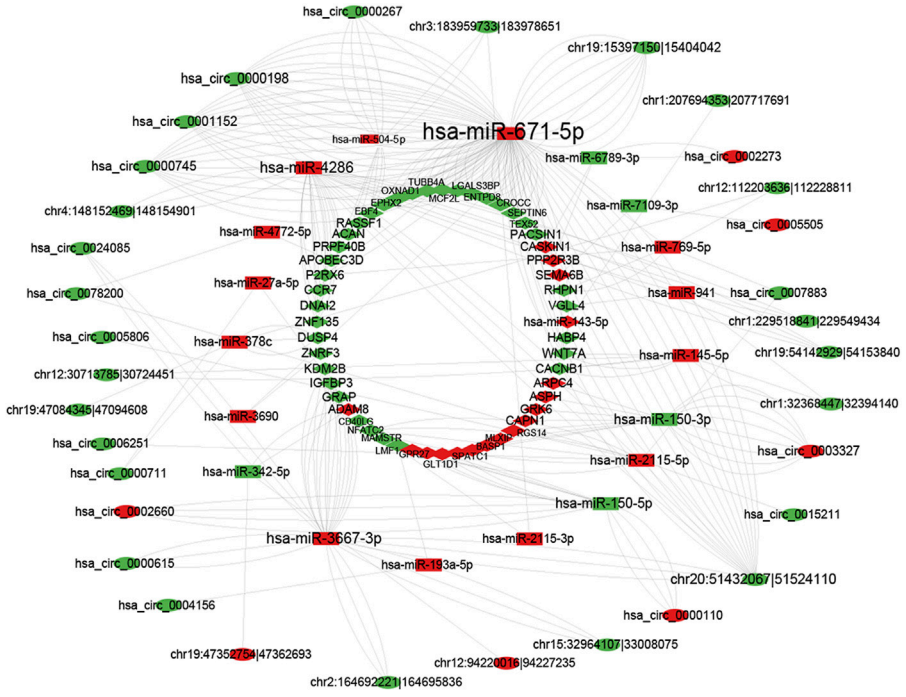

FIGURE 6
CircRNA–miRNA–mRNA network. The red color line indicates that the expression of this RNA is positively correlated, green indicates a negative correlation for the expression of RNA.

results (four upregulated and five downregulated). They coincided with the results of RNA-seq. According to GO enrichment analysis, six GO terms (GO:0043087, GO:0009895, GO:0009896, GO:0032386, GO:0016607, and GO:0022804) were significantly enriched. We also observed that the KEGG pathway enrichment analysis enriched the top 10 KEGG terms, including amyotrophic lateral sclerosis, nucleocytoplasmic transport, Th1 and Th2 cell differentiation, spinocerebellar ataxia, ferroptosis, leishmaniasis,

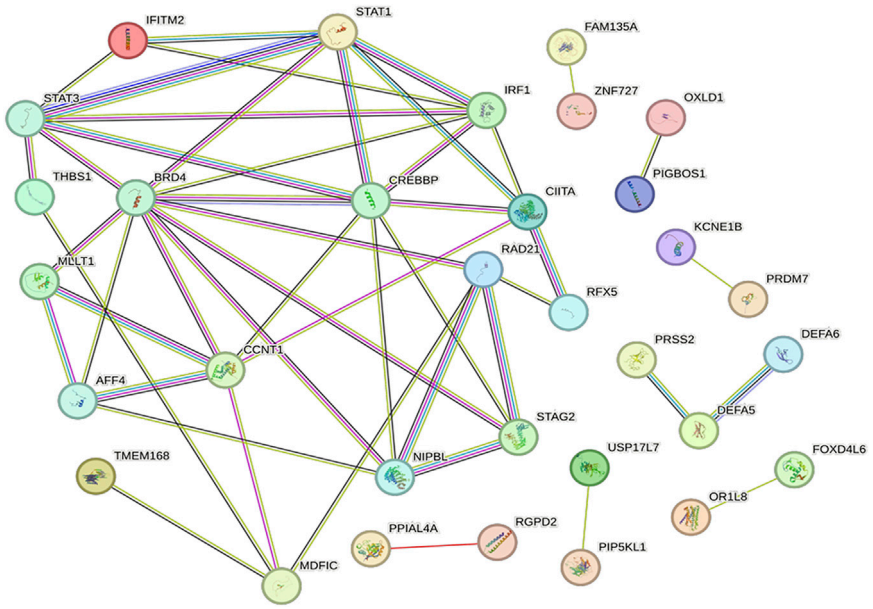

FIGURE 7
PPI network and hub genes. Proteins are represented as nodes and functional relationships by edges.

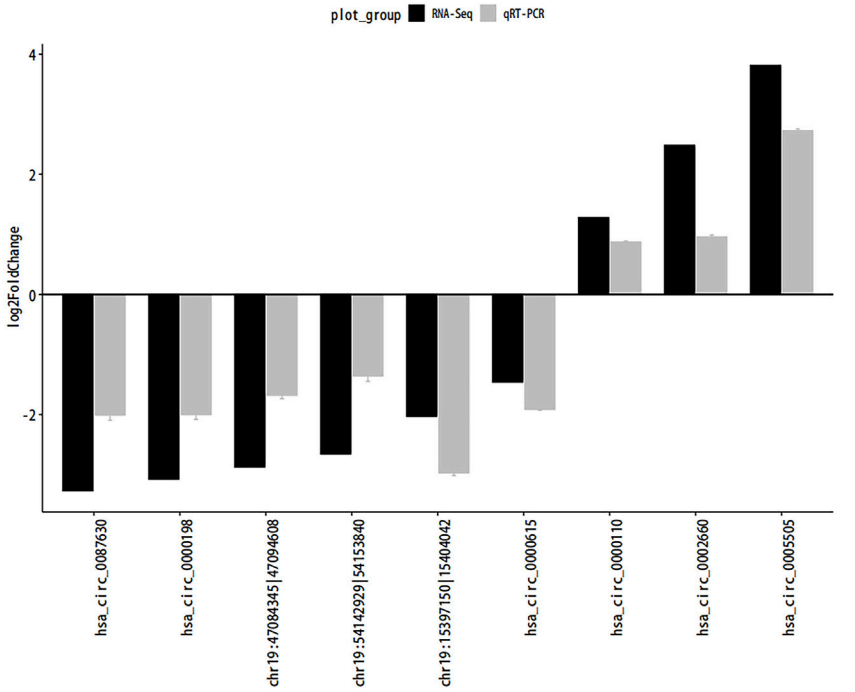

FIGURE 8
QRT-PCR. Abbreviations: The quantitative Real-time PCR (qRT-PCR) verification for the expression patterns of circRNAs. Gray and black legends represent qRT-PCR and RNA-seq results, respectively.

Th17 cell differentiation, HIF-1 signaling pathway, ABC transporters, and viral life cycle (HIV-1).

CircRNA is regulated by ceRNA as described previously. mRNA expression is regulated by CircRNA, which acts as a miRNA sponge and competes with MREs. As a result of RNA-seq, we identified 36 circRNAs, 20 miRNAs, and 52 mRNAs of differential expression. Based on RNA-RNA interactions, we constructed two co-expression networks of circRNA–miRNA

and miRNA–mRNA. By combining the co-expression networks of miRNA–mRNA and circRNA–mRNA, we finally established a circRNA–miRNA–mRNA regulation network to understand SAKI mechanisms. We analyzed mRNAs in ceRNA in terms of GO enrichment and KEGG pathways to identify and explore possible biological functions. GO enrichment analysis showed that T cell activation, neutrophil and their responses, and cytokines may be related to the pathological process of SAKI. Significant enrichment of GO–CC was found in genes involved in cytoplasm and vesicle lumen. According to functional enrichment analysis of KEGG, cytokine, cell adhesion molecules, and T-helper cells may participate in the initiation and progression of SAKI. Although the immune system depends heavily on neutrophils, its activation is harmful in sepsis. It can induce an immune reaction and lead to thrombosis. This condition may be the reason for SAKI (Stiel et al., 2018). In Mi Han et al. (2017) stated that delta neutrophil index as a serum marker can be used to judge SAKI patients' condition (Han et al., 2017). Subsets of CD4+T-cell, Th1, Th17, and regulatory T (Treg) cells were observed. By analyzing the Th17/Treg ratio, one can determine the severity and prognosis of sepsis patients. Septic patients are characterized by persistently high Th2/Th1 levels in the peripheral blood. Moreover, T cell activation profiles can cause the identification of sepsis early. Numerous T helper cells are strongly correlated to cytokines. Th1 cells are strongly correlated to interferon-γ((IFN-γ), as do Th17 cells and tumor necrosis factor-α(TNF-α) or interleukin-17(IL-17). This result suggests that T cells and their related cytokines are important materials involved in sepsis and cause inflammation. In addition, AKI can confer an altered cytokine profile (Gupta et al., 2016; Coakley et al., 2020; Chaturvedi et al., 2021; Liu et al., 2021). A number of cell adhesion molecules are found in cells, including intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). They play an important role in defense against infections. VCAM-1 and ICAM-1 are markers of vascular endothelial damage in sepsis and can be used to monitor the development of organ dysfunction, such as kidney injury (Amalakuhan et al., 2016). Martijn van Griensven observed that ICAM-1 had a strong pathogenic effect on sepsis. ICAM-1 j/j mice had a low mortality in sepsis. This result was caused by the decreased cytokine level (Griensven et al., 2006). To avoid random errors introduced by limiting thresholds, we also examined the potential functions of DEmRNAs in the ceRNA network by performing GSEA. Our results matched those above. "Cytokine-cytokine receptor interaction" was reported in the result of GSEA analysis of DEmRNAs of SAKI in 2020 (Yang et al., 2020).

A PPI network was established to screen out the key genes involved in the regulatory network of SAKI. Hub genes identified as the top 10 nodes comprised the following: ZNF727, MDFIC, IFITM2, FOXD4L6, IGBOS1, CIITA, KCNE1B, BAGE2, PPIAL4A, USP17L7, and PRSS2.

Limitations

Our research encountered several flaws and limitations. First, we lacked sequence samples and genes to verify. As a result, our study may lack reliability. Second, we did not use kidneys from SAKI patients for RNA-seq because these samples are lacking in clinical practices. Third, the ceRNA network is a hypothesis. More experiments are needed to understand its mechanism deeply.

Conclusion

To our best knowledge, this research is the first report that examined changes in circRNA, miRNA, and mRNA expression in patients with SAKI. We analyzed whole blood samples from patients and healthy individuals for RNA sequencing,A circRNA–miRNA–mRNA regulation network was constructed using the differentially expressed genes obtained from the sequencing Functional and pathway analyses indicated that DEmRNAs in ceRNA were mostly concentrated in T cell activation, neutrophils and their responses, and cytokines. A PPI network was also established to screen out the key genes participating in the regulatory network of SAKI. The hub genes identified as the top 10 nodes included the following: ZNF727, MDFIC, IFITM2, FOXD4L6, CIITA, KCNE1B, BAGE2, PPIAL4A, USP17L7, and PRSS2. These findings provide a new treatment target for SAKI treatment and novel ideas for its pathogenesis. We will conduct more detailed studies on sepsis and septic acute kidney injury in the future. This will include RNA sequencing of whole blood samples from septic patients along with animal and cellular experiments. The aim is to identify therapeutic targets for the disease, more accurately.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/geo/subs/?view=series, GSE232404 and GSE242059. GSE232404 contains data for circRNAs and mRNAs, and GSE242059 contains data for miRNAs.

Ethics statement

The studies involving humans were approved by the Medical Research Ethics Review Committee, General Hospital of Ningxia Medical University, Ningxia Medical University, China (2020-642). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

S-RM and QM drafted the manuscript and searched the literature to identify eligible trials. S-RM and Y-NM analyzed the data. W-JZ received the funding for this study. 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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Insights into the involvement of long non-coding RNAs in doxorubicin resistance of cancer

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Doxorubicin is one of the most classical chemotherapeutic drugs for the treatment of cancer. However, resistance to the cytotoxic effects of doxorubicin in tumor cells remains a major obstacle. Aberrant expression of long non-coding RNAs (lncRNAs) has been associated with tumorigenesis and development via regulation of chromatin remodeling, transcription, and post-transcriptional processing. Emerging studies have also revealed that dysregulation of lncRNAs mediates the development of drug resistance through multiple molecules and pathways. In this review, we focus on the role and mechanism of lncRNAs in the progress of doxorubicin resistance in various cancers, which mainly include cellular drug transport, cell cycle disorder, anti-apoptosis, epithelial-mesenchymal transition, cancer stem cells, autophagy, tumor microenvironment, metabolic reprogramming and signaling pathways. This review is aimed to provide potential therapeutic targets for future cancer therapy, especially for the reversal of chemoresistance.

KEYWORDS

long non-coding RNA, doxorubicin, drug resistance, cancer, molecular mechanisms

Introduction

Cancer has become one of the most common diseases and a leading cause of death. It is estimated that 19.3 million new cancer cases occurred worldwide in 2020 with almost 10.0 million cancer deaths (Sung et al., 2021). In 2040, the global cancer burden is even expected to reach 28.4 million cases, representing a 47% rise compared to 2020 (Sung et al., 2021). Research on cancer has drawn extensive attention and great progress has been made regarding to cancer screening, early diagnosis and effective treatment. For example, the mortality rate of breast cancer (BRCA) is shown to fall steadily, with an about 35% decline over the past three decades (Malvezzi et al., 2019). Chemotherapy alongside surgery and radiotherapy, usually constitutes the standard regimen of cancer therapy (Mariette et al., 2007). However, when cancer is advanced or patients cannot suffer surgery, chemotherapy then becomes the last strategy.

Doxorubicin (DOX) is an anthracycline antibiotic which was isolated from the pigment-producing *Streptomyces* peucetius early in the 1960s (A et al., 2000). It is one of the most widely employed chemotherapeutic agents for the treatment of both hematological and solid tumors, including breast cancer, ovarian cancer (OC), bladder cancer (BLCA), lung cancer

(LC), and acute myeloblastic leukemia (AML) (Blum and Carter, 1974; Young et al., 1981; Hulst et al., 2022). Doxorubicin stabilizes a reaction intermediate in which DNA strands are cut and covalently linked to tyrosine residues of topoisomerase II (Top2), eventually blocking DNA relegation (Minotti et al., 2004; Pommier et al., 2010). In addition, doxorubicin generates free radicals, leading to DNA damage or lipid peroxidation; interferes with DNA unwinding or DNA strand separation and helicase activity; induces apoptosis in response to Top2 inhibition (Gewirtz, 1999; Minotti et al., 2004; Pommier et al., 2010). DOX can also induce histone eviction from open chromatin, which attenuates the DNA damage response, triggers epigenetic alterations and induces apoptosis (Pang et al., 2013).

Since its discovery, DOX has brought a substantial improvement in cancer therapy. The introduction of DOX into the adjuvant therapy of BRCA demonstrated definite benefit in disease-free survival and overall survival (Hortobágyi and Buzdar, 1993). Gastric cancer (GC) was ever considered refractory to chemotherapy, whereas the addition of DOX produced encouraging response rates over 40% and increased median overall survival (Wadler et al., 1985). Along with the wide application, intrinsic and acquired resistance to DOX remains a major clinical problem. Some studies revealed resistance to DOX due to increase of drug efflux and reduction in drug accumulation, mediated by members of the ATP-binding cassette (ABC) superfamily (Grant et al., 1994; Velamakanni et al., 2007; Broxterman et al., 2009). The members of ABC transporters regulate the absorption, distribution, and clearance of pharmacological agents (Vasiliou et al., 2009). However, though many investigations are devoted to the development of transporter inhibitors for reversal of resistance, it has not been successful in improving the clinical response to chemotherapy, causing our consideration on the real nature of chemoresistance (Abraham et al., 2009). Undoubtedly, it will be of key importance for clinical studies to define the exact mechanisms mediating doxorubicin resistance.

Long non-coding RNAs (lncRNAs) are a kind of transcriptional products with a length longer than 200 nucleotides and no or low protein-encoding ability (Alexander et al., 2010; Uchida and Dimmeler, 2015; Yang L et al., 2014). Similar to coding genes, lncRNAs are usually transcribed by RNA polymerase II and have a poly-A tail (Gibb et al., 2011), but their sequence are less conserved than that of mRNAs (Pang et al., 2006). LncRNA has only been regarded as the "transcriptional noise" of the genome, rather than having biological functions, for a long time after its discovery (Ponjavic et al., 2007; Struhl, 2007). In recent years, more and more studies have shown that lncRNAs are widely involved in the regulation of gene expression at epigenetic, transcriptional and posttranscriptional levels (Yang Get al., 2014), playing an important role in cell differentiation, organogenesis, tissue homeostasis and other critical life activities (Mercer et al., 2009; Hung and Chang, 2010; Schmitz et al., 2016). In addition, the abnormal expression of lncRNAs is also closely related to the occurrence and development of cancer and chemoresistance (Batista and Chang, 2013; Evans et al., 2016). Insights into the role of lncRNAs in DOX resistance will help to deepen our understanding of chemoresistance formation and provide potentially targetable or predictive biomarkers of chemotherapy, which is the point of our review.

lncRNA and doxorubicin resistance

LncRNAs have received extensive attention for its modulation in cancer progress as well as therapeutic response. Aiming to uncover the role and mechanism of lncRNAs in DOX resistance, databases were searched for published reports focused on "lncRNA and doxorubicin resistance". The qualified articles were further straightened out and categorized in this section with the main mechanisms and implicated lncRNAs summarized in Figure 1.

ABC transporters

The ATP-binding cassette (ABC) transporter family is a big family regulating cellular levels of hormones, lipids, ions, xenobiotics and other small molecules (Robey et al., 2018). Altered membrane transport and enhanced drug efflux mediated by over-expression of ABC superfamily, including ABCB1 and ABCC1, is one of the main and most studied mechanisms of doxorubicin resistance (Lehne, 2000). Known LncRNAs mediating the regulation of transporter expression were summarized in Figure 2.

H19 was the first lncRNA found to be implicated in ABCB1 regulation in human hepatocellular carcinoma (HCC). Reduced expression of H19 could suppress ABCB1 expression, which led to the increase of cellular DOX concentration and DOX sensitivity (Tsang and Kwok, 2007). Mechanistically, ABCB1 gene promoter was hypomethylated in resistant HCC cells, while H19 silencing induced a marked increase in ABCB1 promoter methylation and decrease in ABCB1 expression (Tsang and Kwok, 2007). There was also evidence that lncRNA MRUL could contribute to DOX resistance by playing an enhancer-like role in promoting ABCB1 expression in GC (Wang et al., 2014). MRUL knockdown led to increased drug accumulation and apoptosis in DOX-resistant SGC7901 cell (Wang et al., 2014). LncRNA microarray revealed that the expression levels of over 3,000 lncRNAs were altered in the DOX-resistant osteosarcoma (OSA) cell line MG63/DXR compared with the parental MG63 cell and ODRUL was the most upregulated one (Zhang et al., 2016). ODRUL might participate in DOX resistance by targeting ABCB1. In addition, the clinical results showed that high expression of ODRUL was correlated with poor chemotherapy response and prognosis (Zhang et al., 2016). Recently, Zhang et al. found that lncRNA FOXC2-AS1 expression was significantly higher in DOX-resistant OSA cell lines and tissues, and correlated with poor prognosis (Zhang C. L. et al., 2017). Functional studies revealed that silencing of FOXC2-AS1 abolished the growth of DOX-resistant OSA cell and improved the sensitivity to DOX *in vitro* and *in vivo*. Further mechanistic studies demonstrated that FOXC2-AS1 promoted the expression of transcription factor FOXC2 at both the transcription and post-transcription levels, further stimulating the expression of downstream ABCB1. CASC9, a lncRNA upregulated in doxorubicin-resistant BRCA cell, might regulate the expression of ABCB1 through EZH2. EZH2 was demonstrated to be a binding protein of CASC9. Meanwhile, EZH2 depletion resulted in suppressed ABCB1 expression (Jiang et al., 2018). Linc00518 and ABCC1 expression were both upregulated in DOX-resistant BRCA cell. Linc00518 could act as

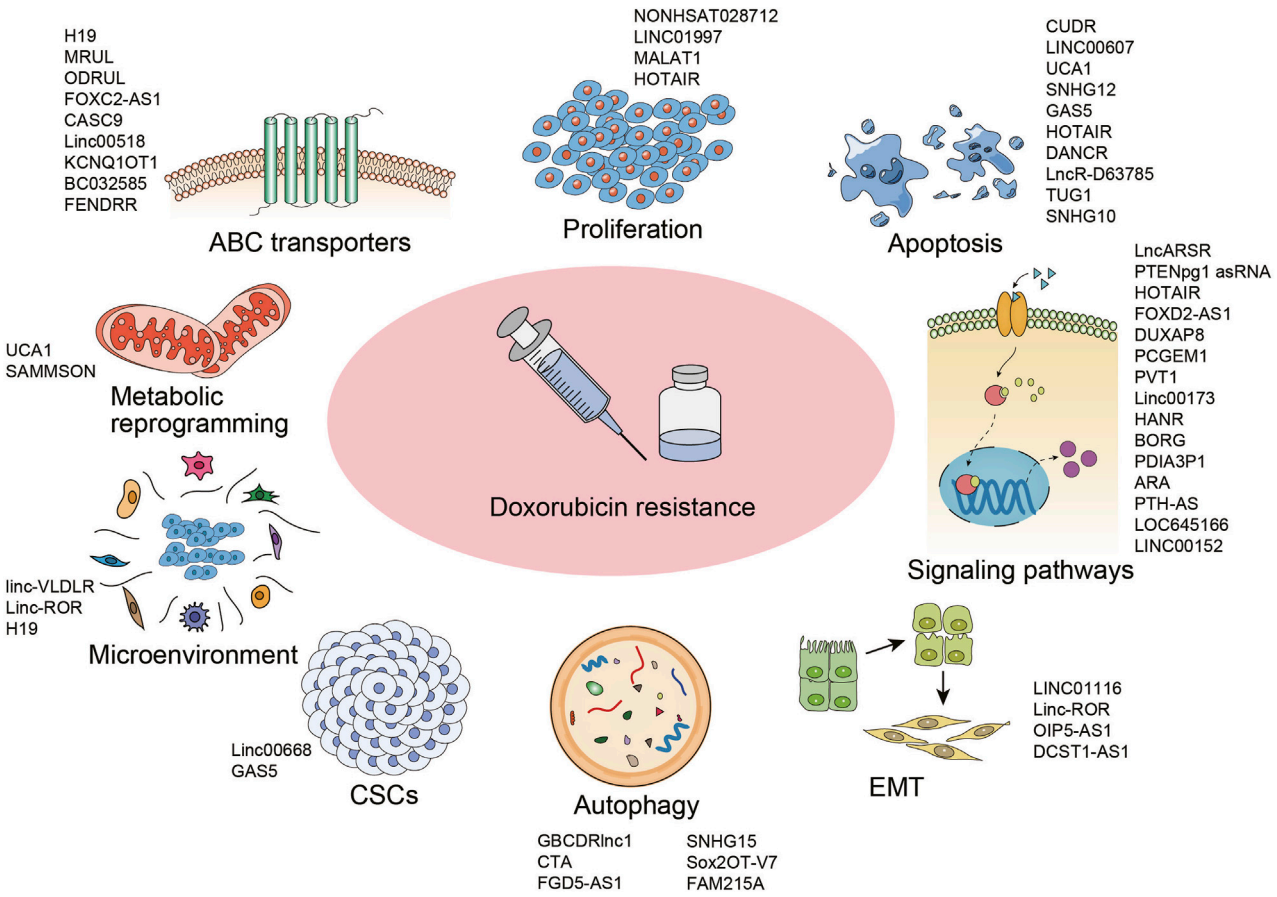

FIGURE 1
Mechanisms underlying doxorubicin resistance in cancer and the implicated lncRNAs. Abbreviations: ABC, ATP-binding cassette; EMT, epithelial to mesenchymal transition; CSCs, cancer stem cells.

a molecular sponge of miR-199a to upregulate ABCC1 expression, thus conferring chemoresistance to DOX (Chang et al., 2018). LncRNA KCNQ1OT1 was upregulated in DOX resistant AML samples and cells. Through adsorbing miR-193a-3p, KCNQ1OT1 induced the expression of Tspan3. Unfortunately, the underlying mechanism of Tspan3 in chemoresistance was not revealed by the authors or reported elsewhere. However, the expression of ABCC1 and ABCB1 was found to be strictly regulated by Tspan3 (Sun et al., 2020).

Using the lncRNA expression profiling of BRCA patients from Gene Expression Omnibus datasets, our group screened out three lncRNAs (AK291479, U79293, and BC032585) to be significantly associated with anthracycline-based chemotherapeutic response (Zeng et al., 2019). BC032585 was further chosen to figure out its molecular function *in vitro*. It was observed that knockdown of BC032585 resulted in a stronger resistance to DOX as accessed by cell viability and this function was at least partly mediated by the upregulation of ABCB1. Collectively, this study had opened out a new approach for the identification of clinically useful lncRNA markers.

LncRNA could also negatively regulated ABCB1 expression and acted as a chemosensitivity mediator. LncRNA microarray found that FENDRR was the most downregulated lncRNA with a 22-fold change in the paired DOX-resistant and sensitive human OSA cell lines (Kun-Peng et al., 2017a). Functional studies revealed that FENDRR suppressed cell cycle, promoted apoptosis and increased DOX sensitivity of OSA cells *in vitro*. Moreover, further studies demonstrated that FENDRR inhibited DOX resistance through negatively affecting posttranscriptional expression of ABCB1 and ABCC1 (Kun-Peng et al., 2017b). FENDRR was also downregulated in resistant chronic myeloid leukaemia (CML) cells. The overexpression of FENDRR attenuated DOX resistance, as shown by increased DOX accumulation and enhanced cell apoptosis *in vitro* and *in vivo*. Both FENDRR and ABCB1 mRNA contained several AU-rich elements and competitively bound to the RNA-binding protein HuR (Zhang et al., 2019). Previous studies indicated that this interaction with RNA-binding protein was beneficial to keeping the mRNA stabilization and/or regulating the translation (Bish and Vogel, 2014). As a result, aberrations in FENDRR expression led to the opposite change of ABCB1 level.

Apoptosis

In addition to targeting the multidrug transporter proteins, a part of lncRNAs involved in DOX resistance have been shown to

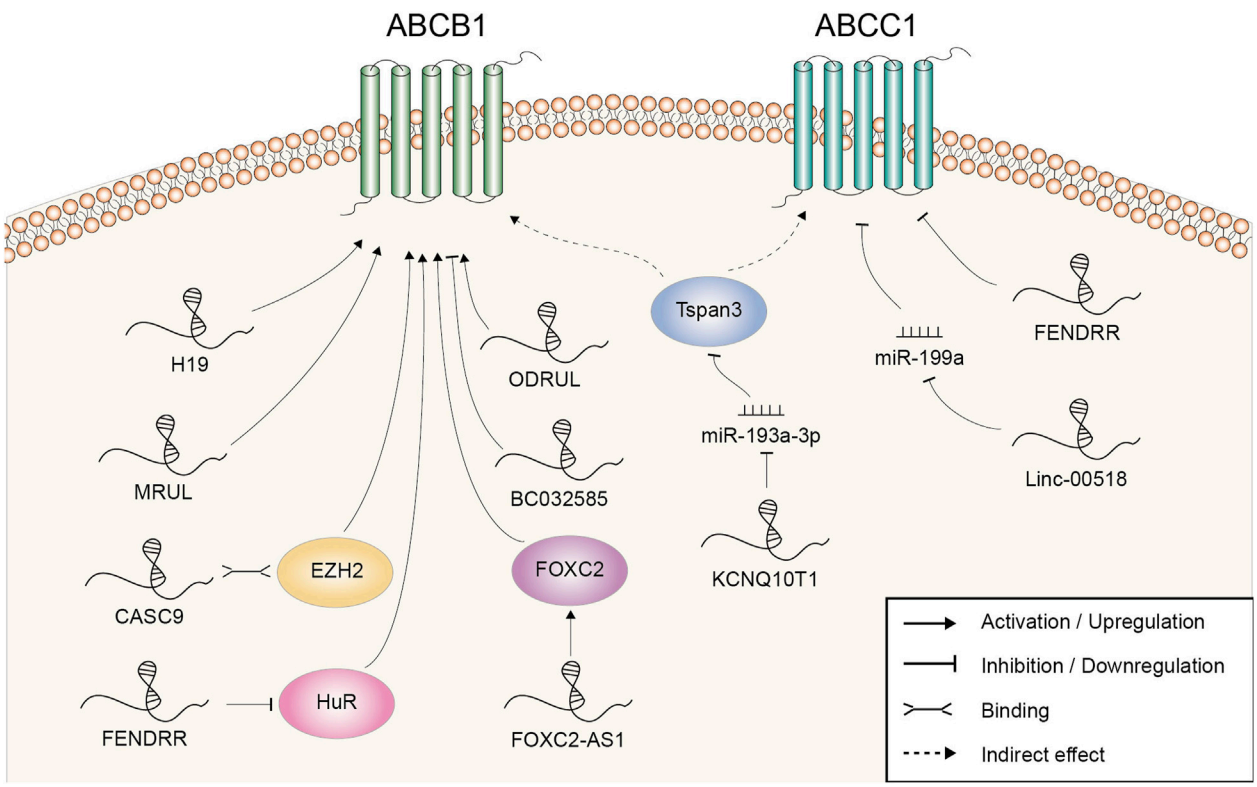

FIGURE 2
Doxorubicin resistance-related lncRNAs involved in the regulation of ABCB1 and ABCC1 and the underlying mechanisms.

regulate apoptosis-related genes (Figure 3). It is unsurprising because triggering apoptosis induction to eliminate malignant cells is exactly the way how most chemotherapeutic drugs work (Mohammad et al., 2015).

Caspases are evolutionarily conserved cysteine proteases with a well-defined role in apoptosis. Mammalian apoptotic caspases are generally divided into the initiators (caspase 2, 8, 9, and 10) and the effectors (caspase 3, 6, and 7), all of which must undergo proteolytic activation to execute their function (Shi, 2002; Van Opdenbosch and Lamkanfi, 2019). CUDR was a novel gene found to be overexpressed in A10A cell, a DOX-resistant subline of human squamous carcinoma (HSC) A431 cell (Tsang et al., 2007). Since the CUDR cDNA sequence contained no distinct open reading frames, it was inferred that CUDR possibly exerted its function as a long non-coding RNA. Further study indicated that the CUDR-inhibited apoptosis was at least dependent on downregulation of caspase 3 (Tsang et al., 2007). LINC00607 was upregulated in DOX-resistant thyroid cancer (TC) cell. It decreased caspase 9 expression by promoting the methylation of caspase 9 promoter, thereby inhibiting the apoptosis induction and augmenting DOX resistance (Li L. et al., 2021).

Above-mentioned initiator caspases activation can be mediated by anti-apoptosis protein Bcl-2-regulated pathway under cytotoxic drugs-induced cellular stress (Shalini et al., 2015). Shang et al. found that UCA1 silencing advanced cell apoptosis induced by DOX in GC cell through promoting cleaved PARP protein expression and depressing the expression of Bcl-2, indicating a promoting role in resistance development (Shang et al., 2016). Another study also demonstrated that UCA1 increased chemoresistance of GC cell via negatively regulating miR-27b. Mechanistically, UCA1 knockdown or miR-27b overexpression increased DOX-induced cell apoptosis by decreasing Bcl-2 protein expression and increasing cleaved caspase 3 (Fang Q. et al., 2016). High expression of SNHG12 was correlated with chemoresistance to DOX and a poor overall survival in OSA. In addition, a higher expression of SNHG12 was revealed in DOX-resistant cells compared to parental sensitive cells. SNHG12 mainly targeted miR-320a to upregulate MCL1, which has been reported to be a Bcl-2 family apoptosis regulator and exhibit a crucial function in suppressing cell apoptosis (Zhao et al., 2017; Zhou B. et al., 2018). Notably, lncRNA GAS5 was reported to inhibit rather than promote chemoresistance in bladder transitional cell carcinoma (BTCC). Overexpression of GAS5 promoted the induction of apoptosis by DOX and depressed Bcl-2 expression, whereas upregulated Bcl-2 largely reversed GAS5-induced sensitivity to DOX. Clinically, BTCC patients with lower level of GAS5 had a significantly worse disease free survival (Zhang H. et al., 2017). Altogether, these data confirmed that lncRNAs could affect the response of cancer to DOX according to their regulation pattern in Bcl-2 expression.

HOTAIR was upregulated in the DOX-resistant small cell lung cancer cell (SCLC). Depletion of HOTAIR increased drug sensitivity by enhancing cell apoptosis and decelerating cell cycle progression. Moreover, HOTAIR knockdown reduced HOXA1 methylation by decreasing DNMT1 and DNMT3b expression. Summarily, HOTAIR modulated chemotherapy resistance in SCLC by regulating HOXA1 methylation (Fang S. et al., 2016). DANCR was found to be suppressed by DOX in a high throughput screening in colorectal cancer (CC) cell. Via interacting with the

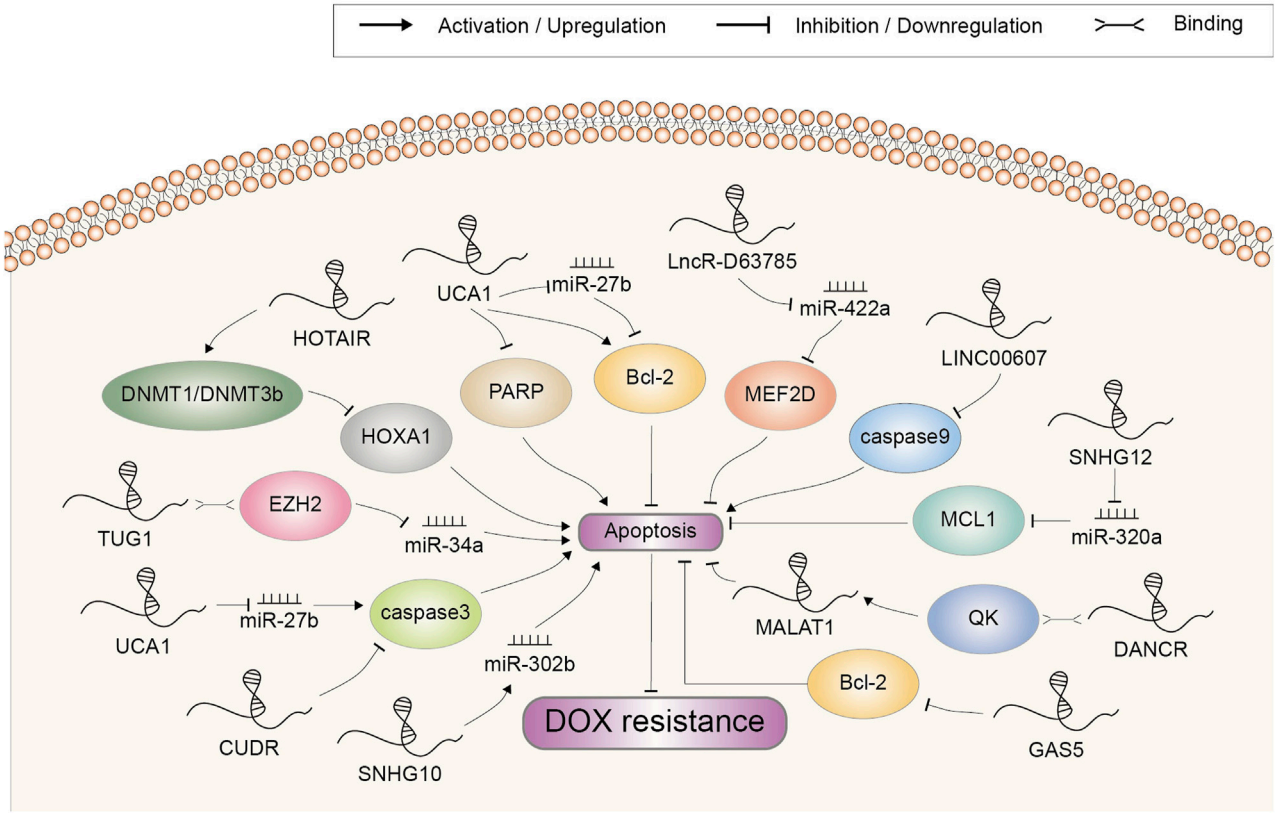

FIGURE 3
Doxorubicin resistance-related lncRNAs involved in the regulation of cell apoptosis and the underlying mechanisms. Abbreviations: DOX, doxorubicin.

RNA-binding protein QK, DANCR enhanced the RNA stability of MALAT1, which further mediate the suppressive function of DANCR on DOX-induced apoptosis (Xiong et al., 2021). This study established DANCR as an important repressor of apoptosis in CC. LncR-D63785 was highly expressed in GC tissues and cells. Knockdown of lncR-D63785 fostered the apoptosis of GC cells treated with DOX. It functioned as a sponge of miR-422a and promoted chemoresistance by blocking miR-422-dependent suppression of MEF2D (Zhou Z. et al., 2018). TUG1, a lncRNA upregulated in DOX-resistant AML tissues and cells, could epigenetically suppress miR-34a expression via recruiting EZH2 to its promoter. Either TUG1 knockdown or miR-34a overexpression remarkably facilitated cell chemosensitivity by enhancing DOX-induced apoptosis (Li Q. et al., 2019). LncRNA SNHG10 was downregulated in triple negative breast cancer (TNBC) cells after DOX treatment, and overexpression of SNHG10 significantly promoted DOX-induced apoptosis. Mechanism research showed that SNHG10 could inhibit the development of resistance to DOX by upregulating miR-302b through methylation modulation (Aini et al., 2022).

Cell proliferation

Uncontrolled proliferation is a hallmark of cancer, typically utilized by cancer cells to resist chemotherapeutic agent-induced growth suppression (Zheng et al., 2019). LncRNAs were also found to participate in aberrant cell proliferation and DOX resistance (Figure 4). Microarray analysis revealed that NONHSAT028712 was significantly increased in DOX-resistant BRCA cells. Further study indicated that NONHSAT028712 mediated the development of chemoresistance through cis-regulating nearby CDK2 gene, which was required for the transition of cell cycle from G1 to S phase (He et al., 2016). LINC01977 could significantly promote BRCA cell proliferation and chemoresistance to DOX in *vitro* assays (Li Z. et al., 2021). It sponged miR-212-3p to prevent miRNA-mediated repression of GOLM1, which was reported to function as a key promoter of cell proliferation in several cancer types (Chen et al., 2015; Xu et al., 2017). MALAT1 was reported to be highly expressed in DOX-resistant BRCA cells. It could promote cell proliferation and colony formation to increase DOX resistance, mechanistically through recruiting E2F1 and activating downstream AGR2 expression (Li S. et al., 2022). MiR-570-3p was another target of MALAT1, which could inhibit the proliferation of BRCA cells and mediate the regulatory role of MALAT1 on DOX resistance (Yue et al., 2021). In HCC, elevation of MALAT1 also mediated tumor growth and DOX resistance via a MALAT1/miR-3129-5p/Nova1 axis (Cao et al., 2021). Other lncRNAs enhancing DOX resistance through increasing cell proliferation included HOTAIR (Wang H. et al., 2018). In GC cells, HOTAIR mainly counteracted with miR-217 to inhibit its suppressing effect in DOX resistance (Wang H. et al., 2018).

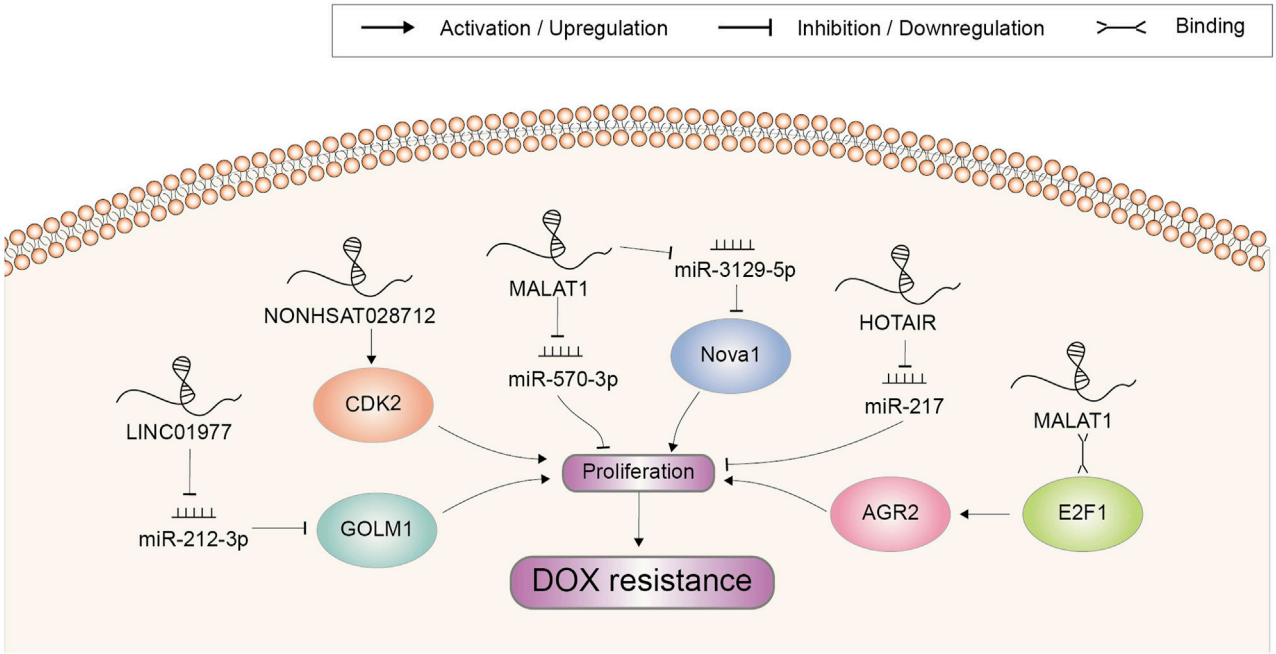

FIGURE 4
Doxorubicin resistance-related lncRNAs involved in the regulation of cell proliferation and the underlying mechanisms. Abbreviations: DOX, doxorubicin.

Signaling pathways

A fine-tuned regulation of signal transduction pathways is crucial for maintaining cellular and tissue homeostasis (SMA, 2020). Aberrant activation of oncogenic signaling pathways often lead to the transformation of normal cells to cancer cells with the acquirement of malignant phenotype (Palla et al., 2022). Many drugs with the ability of blocking dysregulated signaling pathways have been developed for cancer treatment. However, due to the crosstalk inside signaling network, awakening of alternative survival signaling pathways have become one dominating mechanism of chemoresistance (Dent et al., 2009). Similarly, any survival signaling pathway activated in response to toxic stress might also help cancer cells to escape DOX-based chemotherapy (Table 1).

PI3K signaling pathway

PTEN tumor suppressor is a negative regulator of the PI3K/Akt pathway and is epigenetically silenced in multiple cancers (Álvarez-G et al., 2019). LncARSR overexpression inhibited DOX-induced cell apoptosis and enhanced DOX resistance in HCC while knockdown of lncARSR showed the opposite effects (Li et al., 2017). LncARSR decreased PTEN expression and activated the PI3K/Akt pathway. Furthermore, the effects of lncARSR on DOX resistance could be reversed by PTEN depletion or PI3K/Akt pathway inhibitors. Taken together, upregulated lncARSR promoted DOX resistance through activating the PTEN/PI3K pathway (Li et al., 2017). Subsequent study by Li et al. revealed that lncARSR further activated NF-κB in a PI3K pathway-dependent manner. NF-κB transactivated lncARSR through direct binding and activation of lncARSR promoter, forming a positive feedback regulatory loop among lncARSR, Akt and NF-κB. And this regulatory loop together promoted DOX resistance (Li Y. et al., 2022). LncRNA PTENpg1 regulated PTEN expression through sequestering numerous PTEN-targeting miRNAs. Moreover, two antisense RNA (asRNA) transcripts isoforms (α and β) were encoded from the *PTENpg1* locus (Johnsson et al., 2013). The α isoform epigenetically regulated PTEN transcription via localizing to the *PTEN* promoter and catalyzing the formation of H3K27me3, while the β isoform interacted with PTENpg1 as an RNA:RNA pairing and post transcriptionally affected PTEN production. Suppression of this asRNA isoforms-regulated network led to a clear induction of PTEN protein level and a concomitant downregulation of pAKT. As a result, the OSA cells were significantly sensitized to DOX (Johnsson et al., 2013). Unlike PTENpg1 asRNA transcripts, HOTAIR was reported to modulate PTEN expression by increasing the hypermethylation of its promoter locus, thus suppressing PTEN expression and conferring DOX resistance in AML (Zhou et al., 2021). HOTAIR was also reported to reinforce DOX resistance by promoting the phosphorylation of AKT and activating AKT/mTOR signaling pathway in BC (Li Z. et al., 2019). Other lncRNAs implicated in PI3K signaling pathway and DOX resistance included FOXD2-AS1 in BC and DUXAP8 in B-cell acute lymphoblastic leukemia (B-ALL), further uncovering the central role of PI3K pathway in cancer DOX resistance (Nong et al., 2021; Zhang et al., 2022).

P53 signaling pathway

LncRNA PCGEM1 was specifically expressed in prostate tissue, and associated with prostate cancer (PC). The overexpression of PCGEM1 attenuated DOX-induced apoptosis in LNCaP cells

TABLE 1 LncRNAs implicated in doxorubicin resistance of cancer through multiple signaling pathways.

LncRNA	Cancer type	Molecular mechanism	Role in DOX response	References
PI3K signaling pathway				
LncARSR	HCC	decreasing PTEN expression, activating PI3K pathway and NF-κB	Resistance	Li et al. (2017), Li et al. (2022b)
PTENpg1 asRNA	OSA	Promoting PTEN transcription and PTEN mRNA stability	Resistance	Johnsson et al. (2013)
HOTAIR	AML	Increasing the hypermethylation of *PTEN* promoter	Resistance	Zhou et al. (2021)
HOTAIR	BC	Increasing PI3K, AKT and mTOR phosphorylation	Resistance	Li et al. (2019b)
FOXD2-AS1	BC	Increasing PI3K and AKT phosphorylation	Resistance	Nong et al. (2021)
DUXAP8	B-ALL	Increasing PIK3CA expression through sponging miR-29a	Resistance	Zhang et al. (2022)
P53 signaling pathway				
PCGEM1	PC	Inhibiting the expression of p53 and $p21^{Waf1/Cip1}$	Resistance	Fu et al. (2006)
PVT1	BLCA	Promoting p53 ubiquitination through MDM2/AURKB cascade	Resistance	Jiang et al. (2022)
Wnt/β-catenin signaling pathway				
Linc00173	SCLC	Sponging miR-218 to upregulate the expression of Etk, NDRG1 and GSKIP	Resistance	Zeng et al. (2020)
HANR	HCC	Binding to GSKIP for regulating the phosphorylation level of GSK3β	Resistance	Duffy et al. (2014), Xiao et al. (2017)
NF-κB signaling pathway				
BORG	TNBC	Binding to and activating RPA1	Resistance	Gooding et al. (2019)
PDIA3P1	HCC	Binding to miR-125a/b and miR-124 to upregulate TRAF6	Resistance	Xie et al. (2020)
MAPK signaling pathway				
ARA	HCC	Unkown	Resistance	Jiang et al. (2014a)
JAK-STAT signaling pathway				
PTH-AS	BC	Upregulating the expression level of STAT1	Resistance	Akimoto et al. (2022)
LOC645166	BC	Binding to and recruiting NF-κB to promote GATA3 transcription	Resistance	Zheng et al. (2020)
Hippo signaling pathway				
LINC00152	RB	Sponging miR-613 to positively regulate YAP1	Resistance	Lu et al. (2010)
Keap1/Nrf2/ARE signaling pathway				
PVT1	TNBC	Promoting the protein stability of Nrf2 by inhibiting the binding of Keap1 to Nrf2	Resistance	Luo et al. (2020)

Abbreviations: DOX, doxorubicin; HCC, hepatocellular carcinoma; OSA, osteosarcoma; AML, acute myeloblastic leukemia; BC, breast cancer; B-ALL, B-cell acute lymphoblastic leukemia; PC, prostate cancer; BLCA, bladder cancer; SCLC, small cell lung cancer cell; TNBC, triple negative breast cancer; RB, retinoblastoma.

(Fu et al., 2006). Moreover, the induction of p53 and $p21^{Waf1/Cip1}$ due to DOX treatment was attenuated by PCGEM1 overexpression, as well as the protein levels of cleaved caspase 7 and cleaved PARP. These implied that PCGEM1 induced DOX resistance by inhibiting the function of p53-dependent apoptotic machinery (Fu et al., 2006). In BLCA cells, lncRNA PVT1 could interact with MDM2, promoting its expression and cascaded MDM2/AURKB-mediated p53 ubiquitination. Thus, p53 pathway-mediated tumor suppressor genes were suppressed, leading to elevated proliferation, invasion, and DOX resistance. Furthermore, addition of the MDM2 inhibitor Nutlin-3 could offset the increased DOX resistance induced by PVT1 overexpression, while overexpression of MDM2 or AURKB reversed PVT1 knockdown-induced sensitivity to DOX (Jiang et al., 2022).

Wnt/β-catenin signaling pathway

Linc00173 was first shown to be associated with the clinical stages and chemotherapeutic responses in SCLC. Elevated Linc00173 enhanced chemoresistance and cancer progression by sponging miR-218 to upregulate Etk expression. NDRG1 and GSKIP were positively regulated by Etk, which further induced the accumulation of β-catenin in the nucleus and activated Wnt/β-catenin pathway (Zeng et al., 2020). LncRNA HANR was demonstrated to be upregulated in HCC patients and predict a poor survival. Knockdown of HANR markedly enhanced the chemosensitivity of HCC cell lines to DOX, while overexpression of HANR showed the opposite effects. It was found that HANR

bound to GSKIP for regulating the phosphorylation level and activity of GSK3β (Xiao et al., 2017). As a downstream target of GSK3β, Wnt/β-catenin pathway was thought to correspondingly perform its oncogenic function and impair the therapeutic outcome of DOX (Duffy et al., 2014).

NF-κB signaling pathway

NF-κB signaling pathway could be provoked by genotoxic agents-induced DNA damage, augmenting the transactivation of varieties of anti-apoptosis genes and subsequent chemoresistance of cancer cells (Taniguchi and Karin, 2018). LncRNA BORG was greatly induced within TNBC cells when subjected to chemotherapeutic stresses. It fostered the cell survival and rendered them resistant to the cytotoxic effects of DOX both *in vitro* and *in vivo*. This chemoresistant activity of BORG was contingent upon its binding to RPA1, as well as the concomitant stimulation of NF-κB signaling. Interestingly, the activation of NF-κB amplifies BORG expression, which further enhances NF-κB activation, forming a novel feed-forward NF-κB signaling loop (Gooding et al., 2019). LncRNA PDIA3P1 was upregulated in human HCC and associated with poorer recurrence-free survival. DOX treatment could also upregulate PDIA3P1 level by disrupting the binding of hMTR4 to PDIA3P1 and abrogating the subsequent hMTR4-mediated degradation. TRAF6 was ordinarily suppressed by miR-125a/b and miR-124, while upregulated PDIA3P1 could bind to miR-125a/b and miR-124 to relieve their repression on TRAF6, leading to the activation of NF-κB pathway and reduced DOX-triggered apoptosis (Xie et al., 2020).

MAPK signaling pathway

Jiang et al. discovered a new upregulated lncRNA named ARA in DOX-resistant BC cells, the expression of which was further found to be significantly associated with DOX sensitivity in a panel of BC cells as well as HCC cells. Knockdown of ARA inhibited cell proliferation and migration, induced G2/M cell cycle arrest and cell death, which together contributed to DOX resistance reverse. To investigate the functional role of ARA, microarray transcriptomic analysis was performed and genes regulated by ARA were enriched in multiple KEGG pathways, among which MAPK signaling pathway was the most outstanding (Jiang M. et al., 2014).

JAK-STAT signaling pathway

Emerging data indicate that JAK-STAT pathway confers cellular resistance to antitumor treatment with DNA-damaging agents, including DOX (Khodarev et al., 2012). Akimoto et al. reported that ectopic expression of lncRNA PTH-AS in BC cell T47D markedly upregulated the level of STAT1 and its downstream interferon-related DNA damage resistance signature (IRDS) genes (Akimoto et al., 2022). As expected, when treated with DOX at a relatively high concentration, T47D cells with forced PTH-AS expression exhibited a significant resistance to drug-induced inhibition. LncRNA LOC645166 was identified to be upregulated in DOX-resistant BC cells as well as tissues of nonresponsive patients. It strengthened the tolerance of breast cancer to DOX via binding and recruiting NF-κB to promote GATA3 transcription, further leading to the activation of STAT3 (Zheng et al., 2020). The NF-κB/GATA3/STAT3 signaling pathway provided a promising target for overcoming DOX resistance in breast cancer.

Hippo signaling pathway

In human retinoblastoma (RB), LINC00152 was reported to boost DOX resistance by sponging miR-613 to positively regulate YAP1 (Wan et al., 2020). YAP1 is a downstream effector of the Hippo signaling pathway, which is widely recognized as an important regulator in both organ size control and tumorigenesis (Lu et al., 2010). In previous studies, YAP1 had also been reported to have an effect on cell sensitivity to 5-fluorouracil and docetaxel in esophageal cancer (Song et al., 2015). Thus, Hippo signaling pathway might be another molecular cascade responsible for lncRNA-mediated chemoresistance in cancer cells.

Keap1/Nrf2/ARE signaling pathway

Keap1/Nrf2 signaling pathway plays an important role in maintaining cellular redox balance (Buti et al., 2013). Aberrant activation of this pathway has frequently been detected in human cancers and is also related to resistance to chemotherapies in established cancers (Ge et al., 2017). It was revealed that PVT1 promoted the protein stability of Nrf2 by inhibiting the binding of Keap1 to Nrf2, which potentiated the resistance of TNBC cells to DOX (Luo et al., 2020).

Epithelial to mesenchymal transition (EMT)

EMT is a biological process in which epithelial cells transform into mesenchymal cells acquiring a motile phenotype (Pastushenko and Blanpain, 2019). Researches have revealed that EMT is not only closely related to tumor metastasis but also affects chemotherapy resistance. DOX resistance-related lncRNAs implicated in EMT regulation were summarized in Figure 5. In OSA, inhibition of LINC01116 suppressed cell viability, migration, and invasion, along with upregulated E-cadherin and downregulated vimentin. Accordingly, DOX resistance was attenuated. Further investigations indicated that LINC01116 regulated HMGA2 expression via EZH2-associated silencing of miR-424–5p and induced EMT (Li R. et al., 2021). Long intergenic non-protein coding RNA (linc)-regulator of reprogramming (ROR) was reported to promote invasion and metastasis in HCC. Knockdown of it notably suppressed EMT by downregulating TWIST1, increasing sensitivity of HCx`x`C cell to DOX (Zhang et al., 2021). FN1, is a glycoprotein present at the cell surface and in extracellular matrix tightly related to cellular adhesion and migration (Topalovski and Brekken, 2016). It was found to be

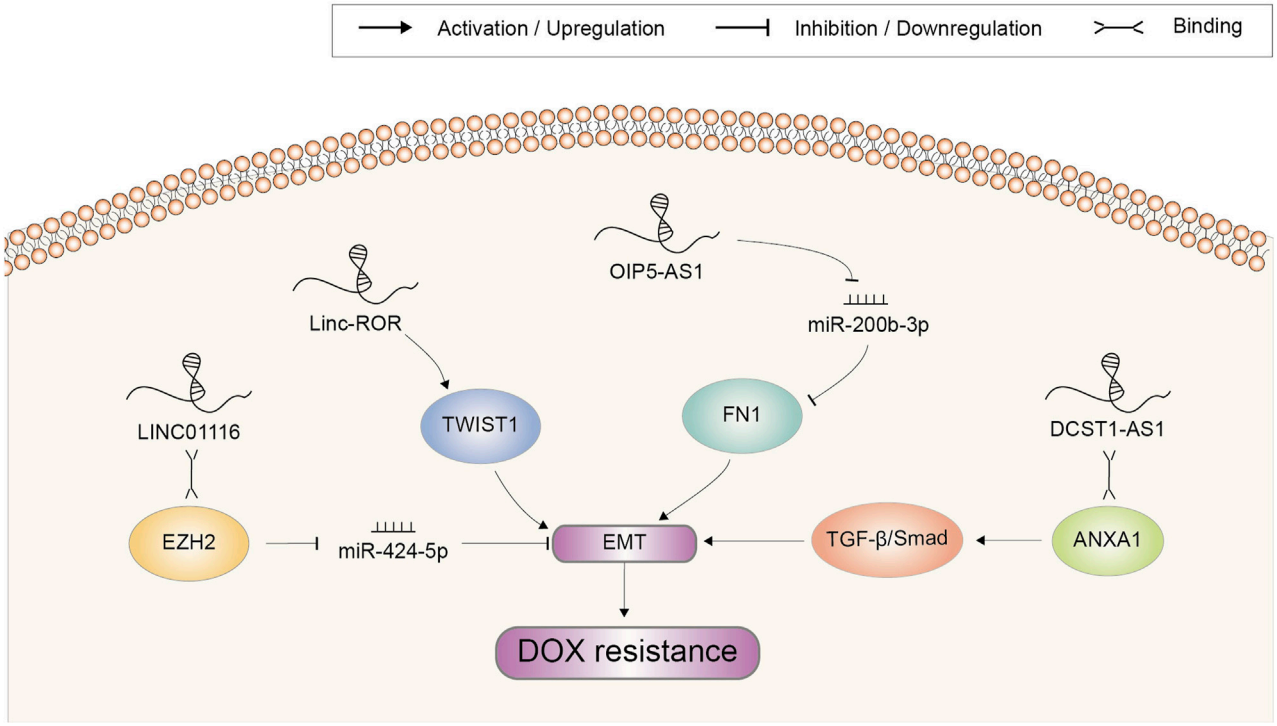

FIGURE 5
Doxorubicin resistance-related lncRNAs involved in the regulation of epithelial to mesenchymal transition and the underlying mechanisms. Abbreviations: DOX, doxorubicin; EMT, epithelial to mesenchymal transition.

significantly upregulated in the chemoresistant OSA cell lines and tissues and was related to unfavourable prognosis. LncRNA OIP5-AS1 acted as an upstream regulator of FN1 through sponging miR-200b-3p. Therefore, OIP5-AS1/miR-200b-3p/FN1 axis might be a promising target in treatment of OSA resistance to DOX (Kun-Peng et al., 2019). LncRNA DCST1-AS1 enhanced TGF-β/Smad signaling in TNBC cells through binding to ANXA1 and increasing its expression. Subsequently, the expression or secretion of proteins such as E-cadherin, SNAI1 and vimentin were coordinated to promote EMT and chemoresistance to DOX. Therefore, DCST1-AS1 represented a potentially promising therapy target for metastatic breast cancer (Tang et al., 2020).

Autophagy

Accumulating evidence supported the cytoprotective role of autophagy in drug resistance of cancer. When under cytotoxic effects of chemotherapeutic drugs, autophagy could contribute to maintaining the intracellular homeostasis and prolonging the survival of cancer cells through autophagosomes (Carew et al., 2007). Recent researches have suggested that dysregulated lncRNA play a role in the development of chemoresistance via autophagy (Figure 6). For example, lncRNA GBCDRlnc1 served as a critical regulator of the autophagic activity and DOX-resistant property of gallbladder cancer. Through direct molecular interaction, GBCDRlnc1 prevented the ubiquitination of PGK1, leading to the upregulation of PGK1 protein level. The ATG5-ATG12 conjugate, an essential complex for autophagy initiation, might be a downstream target of the GBCDRlnc1/PGK1 axis. Knockdown of GBCDRlnc1 dramatically downregulated PGK1, ATG5 and ATG12, suppressed autophagy and improved the sensitivity of gallbladder cancer cells to DOX (Cai et al., 2019).

Wang et al. found that lncRNA CTA could be activated by DOX but was downregulated in DOX-resistant OSA cells. Overexpression of CTA could inhibit autophagy to overcome DOX resistance and promote apoptosis by competitively binding miR-210 (Wang et al., 2017). On the contrary, lncRNA FGD5-AS1 was upregulated in DOX-resistant OSA cells. It was reported to regulate the miR-154-5p/WNT5A axis by sponging miR-154-5p and thus potentiate autophagy-associated DOX resistance (Fei et al., 2022). Small nucleolar RNA host gene 15 (SNHG15) was also upregulated in DOX-resistant OSA cell lines. It elevated autophagy via targeting the miR-381-3p/GFRA1 axis to enhance DOX resistance (Zhang et al., 2020). Sox2OT-V7, another lncRNA involved in DOX resistance of OSA cells, could modulate autophagy through directly targeting miR-142/miR-22 (Zhu et al., 2020).

Lysosomes could sequester macromolecules generated from autophagy for degradation and recycling, and mediate multiple drug resistance in cancer (Piao and Amaravadi, 2016). In HCC, FAM215A was overexpressed and increased the resistance of cells to DOX-induced inhibition. This cell protective effect was proved to be achieved by stabilizing LAMP2, which constitutively contributed to lysosome formation and the maintenance of lysosomal content (Huang et al., 2020).

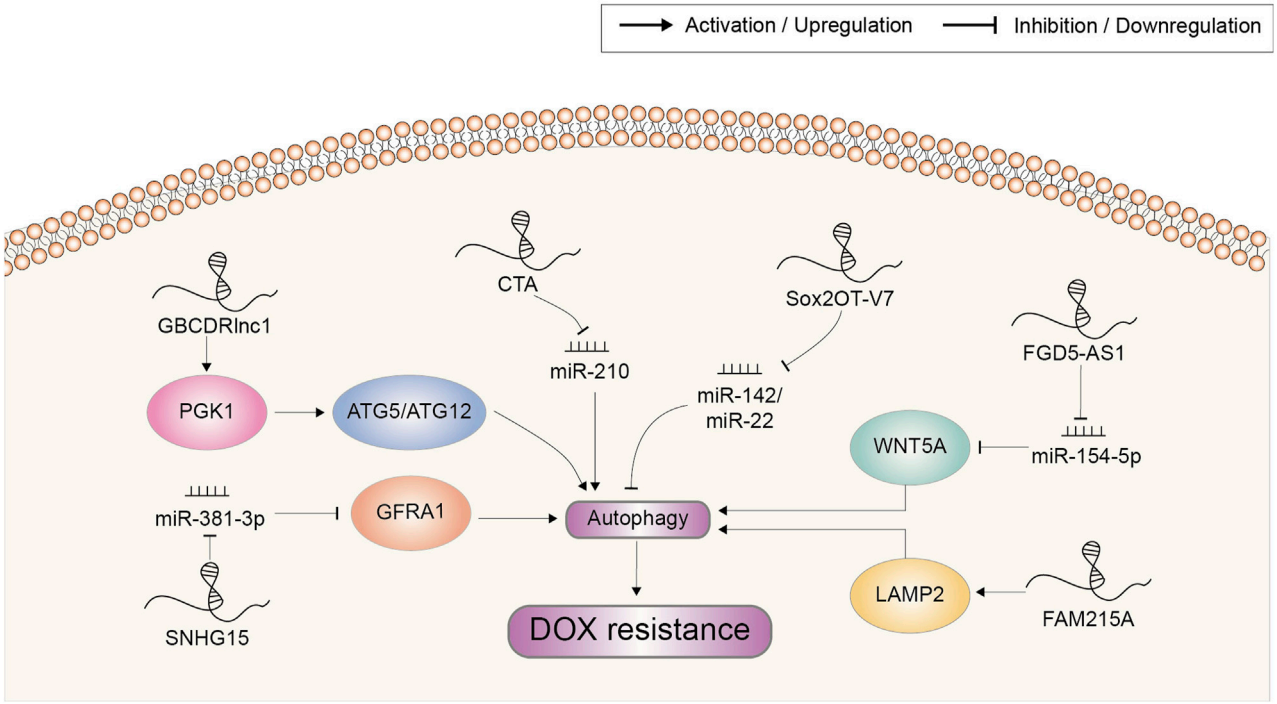

FIGURE 6
Doxorubicin resistance-related lncRNAs involved in the regulation of autophagy and the underlying mechanisms. Abbreviations: DOX, doxorubicin.

Cancer stem cells (CSCs)

CSCs represent a small fraction of cells in the tumor featured by their potential of self-renewal and initiating tumors. Studies demonstrated that CSCs were responsible for chemoresistance and tumor recurrence following chemotherapy (Wicha et al., 2006).

Linc00668 was observed to be increased in BC compared to normal tissues. Forced expression of Linc00668 enhanced self-renewal capacity of BC cells as well as DOX resistance. Mechanistically, Linc00668 interacted with SND1 to augment its transcriptional activity and the expression of target genes, including Nanog, Sox2, and Oct4, which were critical regulators of stem cell-like properties (Qian et al., 2020). GAS5 was reported to function in maintaining stemness in human CC cell line HCT116-derived CSCs. GAS5 knockdown suppressed the self-renewal capacity of CSCs and sensitized them to DOX by inducing apoptosis. Moreover, inhibition of Nodal growth differentiation factor (NODAL) signaling presented the similar results. Therefore, it was hypothesized that GAS5 exerted protective effects in CSCs under DOX treatment in a NODAL signaling-dependent manner (Zhou and Xiao, 2020).

Tumor microenvironment

Extracellular vesicles (EVs), mainly comprised of exosomes and microvesicles, are an important component of the tumor microenvironment (van Niel et al., 2018). EVs are a group of membrane-derived structures released by donor cell into the interstitial fluid. These EVs carry biological macromolecules such as protein, lipids and RNA, and can be taken up by recipient cells to achieve intercellular communication (Tkach and Théry, 2016). EVs are now considered as an additional mechanism for modulation of multiple physiological and pathological processes including chemoresistance (Mashouri et al., 2019).

Takahashi et al. (Takahashi et al., 2014a) identified a subset of lncRNAs in HCC that could be detected in EVs with at least 2-fold enrichment compared to donor cells. Among these lncRNAs, linc-VLDLR was also found to be significantly upregulated in malignant hepatocytes compared to non-malignant hepatocytes. Exposure of HCC cells to DOX increased linc-VLDLR expression within both cells and released EVs, and incubation with such EVs could reduce DOX-induced cell death in recipient cells. Further studies revealed that knockdown of linc-VLDLR suppressed cell viability, blocked cell-cycle progression and reduced the expression of ABCG2, leading to increased sensitivity of HCC cells to DOX (Takahashi et al., 2014a). Linc-ROR was another EV-transferred lncRNA in HCC (Takahashi et al., 2014b). Incubation with EVs originating from HCC cells increased linc-ROR expression and reduced DOX-induced cell death in recipient cells, whereas knockdown of linc-ROR augmented DOX-induced cytotoxicity. Besides, linc-ROR might mediate TGFβ-dependent chemoresistance in HCC, as TGFβ-increased expression of CD133+ tumor-initiating cells and colony growth were attenuated by linc-ROR knockdown. These findings all suggested an important role for linc-ROR in chemotherapeutic

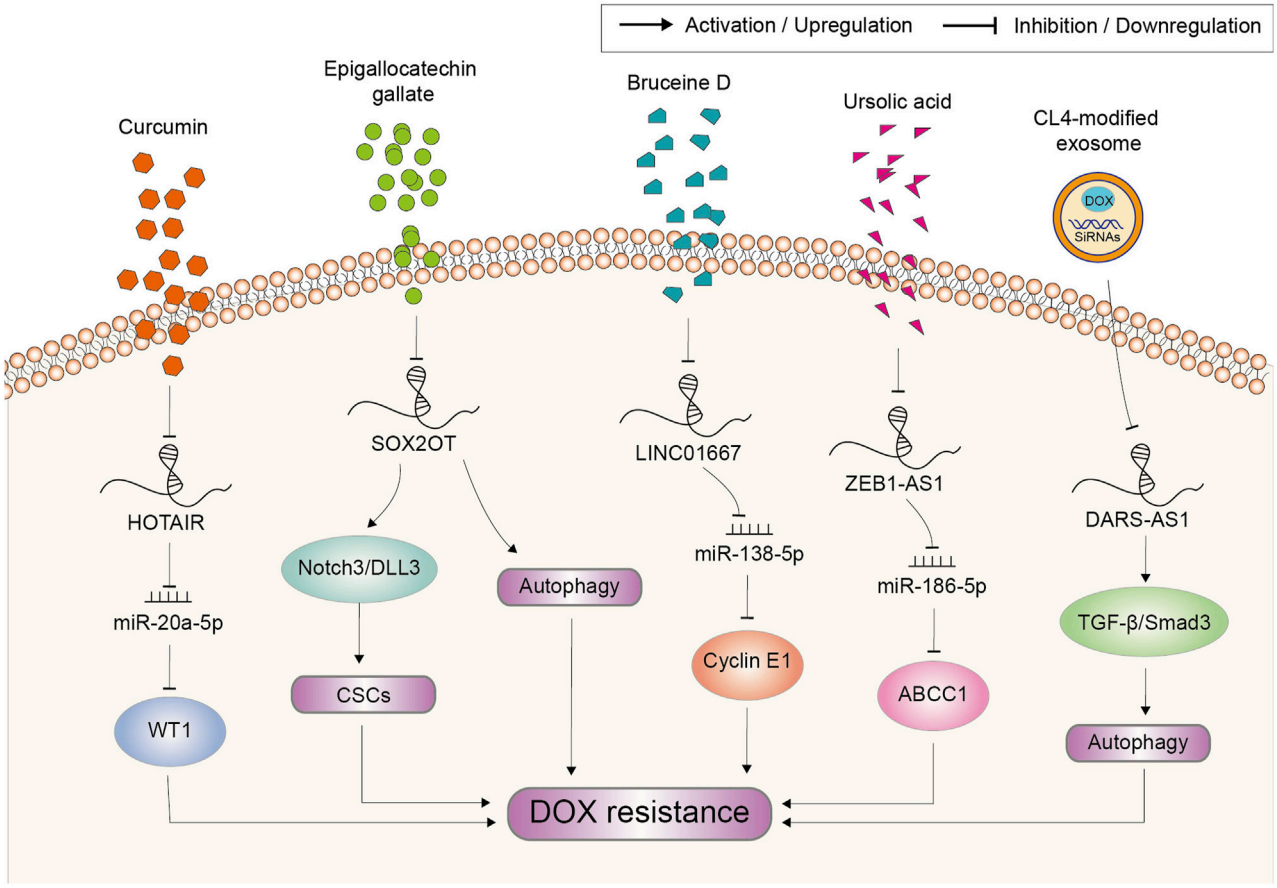

FIGURE 7
Potential strategies based on natural compounds and genetic tools to target lncRNAs for the reverse of doxorubicin resistance in cancer. Abbreviations: DOX, doxorubicin; CSCs, cancer stem cells.

response of HCC (Takahashi et al., 2014b). LncRNA H19 had been proved to mediate the resistance of BC cells to DOX. Moreover, extracellular H19 could be incorporated into exosomes and delivered to sensitive cells, leading to the dissemination of DOX resistance. Therefore, exosomal H19 might be a potential target to reduce DOX resistance in BC (Wang et al., 2020).

Metabolic reprogramming

Cancers have been shown to evade chemotherapy by switching to alternate metabolism. Aerobic glycolysis is recognized as an emerging hallmark of malignant tumors. Normal cells process glucose through mitochondrial oxidative phosphorylation, whereas glycolysis is preferred in most cancer cells for energy production, even under aerobic conditions (Hanahan and Weinberg, 2011). Moreover, emerging evidence has revealed that augmented glycolysis might also contribute to the development of acquired chemoresistance (Tamada et al., 2012). For example, the effect of UCA1 on DOX resistance in AML cell centered around its regulation of HIF-1α-dependent glycolysis. Ectopic expression of UCA1 exhibited a remarkable increase of glucose consumption and effectively enhanced HIF-1α level (Zhang et al., 2018). As a pivotal transcription factor, HIF-1α has been documented to play a critical role in metabolic reprogramming and chemoresistance in various tumor cells (Warfel and El-Deiry, 2014). LncRNA SAMMSON was overexpressed in DOX-resistant BC cell (Orre et al., 2021). Silencing of SAMMSON revealed a decreased glycolytic metabolism and an increased oxidative metabolism. Concomitantly, less ROS were produced from the mitochondrial respiratory chain, while mitochondrial replication, transcription and translation were enhanced. These results highlighted the role of SAMMSON in the metabolic rewiring and development of chemoresistance in BC.

Targeting lncRNAs for reversing doxorubicin resistance

Natural compounds including DOX occupy an important position in cancer therapy because of their diversity in structure and biological activity (Newman and Cragg, 2012). Owe to the multi-targeting capability, lncRNA might also be one of the targets of natural compounds. The recent reports regarding lncRNA-targeting natural compounds involved in DOX resistance are shown in Figure 7. Curcumin is a main active

flavonoid component existing in Chinese herb *Curcuma longa* with the anti-tumor property (Moradi-Marjaneh et al., 2018). It had also been proved to suppress the resistance to DOX in acute myeloid leukemia. Mechanism study showed that lncRNA HOTAIR was inhibited by curcumin, which further mediated the sensitization effect of curcumin through the miR-20a-5p/WT1 axis (Liu et al., 2021). Epigallocatechin gallate is the highest content of polyphenol in green tea, which was reported to exert significant inhibitory effect on osteosarcoma cells including induce apoptosis, inhibit cell proliferation and invasion (Jiang L. et al., 2014). Moreover, Wang et al. reported that epigallocatechin gallate could produce synergistic effects with DOX on osteosarcoma cells by targeting lncRNA SOX2OT variant 7. On the one hand, epigallocatechin gallate decreased SOX2OT variant 7 to reduce DOX-induced autophagy, which played a pro-survival role in protecting cells from the growth inhibition of DOX. On the other hand, epigallocatechin gallate targeting SOX2OT variant 7 could partially inactivate the Notch3/DLL3 signaling cascade to reduce cell stemness then abate DOX resistance (Wang W. et al., 2018). Bruceine D (BD) is a quassinoid extracted from *Brucea javanica* which has an anti-tumor activity in various cancers (Lau et al., 2009). BD treatment in GC cells significantly downregulated the expression of LINC01667, further inhibiting the expression of Cyclin E1 by releasing miR-138-5p from LINC01667 sponge (Li et al., 2020). Thus, BD could inhibit the growth of GC cells and enhance the chemosensitivity of GC cells to DOX. Ursolic acid (UA), a pentacyclic triterpenoid compound, was reported to reverse DOX resistance in TNBC. It could inhibit the expression of ZEB1-AS1, which sponged miR-186-5p to upregulate ABCC1. Hence, UA treatment led to the decrease in ABCC1 expression (Lu et al., 2022). Together, combined therapy of above natural compounds with DOX might serve as an effective strategy to reduce the occurrence of chemoresistance and improve the curative effect in certain cancer, which needs further verification in the clinical practice.

Cancer occurs as a result of loss function of suppressor genes and activation of oncogenes (Caspi et al., 2021; Megyesfalvi et al., 2023). However, the conventional therapeutic using natural compounds or their analogs always lacks specific targets and induces serious side effects. Consequently, much attention has been directed towards the application of genetic tools in anticancer therapy. Small interfering RNA (siRNA) is the most extensively used tool applied in cancer therapy in the *in vitro* and *in vivo* study because of its potential in suppressing oncogenes (Mirzaei et al., 2021). As such tumor-promoting factors account for chemoresistance, targeting them through siRNA also provides an important strategy to reverse DOX resistance. However, the translational application of siRNA is still at its initial stage. There exist multiple limitations that challenge its efficacy, mainly including instability in blood circulation and incapability to enter cells (Ashrafizadeh et al., 2020). To overcome these difficulties, a variety of platforms have been developed for siRNAs delivery, which consist of lipid nanoparticles, liposomal nanoparticles, polymeric nanoparticles, silicon dioxide nanoparticles, carbon nanotubes, gold nanoparticles, iron oxide nanoparticles, aptamers and so on (Acharya et al., 2017). Nowadays, attempts based on these delivery platforms to target lncRNAs is still quite rare. A most recent study employed aptamer CL4-modified exosomes for the targeted delivery of DARS-AS1 siRNA and DOX to TNBC cells (Liu et al., 2023). The tumor growth was synergistically suppressed *in vivo*, while the delivery system did not induce any observed safety issues in mice. Meanwhile, *in vitro* experiments revealed that silencing DARS-AS1 decreased DOX resistance by suppressing autophagy via inhibition of the TGF-β/Smad3 signaling pathway (Figure 7). This study shows the outstanding application potential of genetic tool represented by siRNA in anti-cancer therapy and chemoresistance reverse.

Conclusion and perspectives

Resistance to therapeutic drugs represented by DOX is a major burden for successful cancer treatments. However, the underlying mechanisms of chemoresistance are not yet fully elucidated. Multiple reasons for DOX resistance have been summarized and listed here, mainly including cellular drug transport, cell cycle disorders, anti-apoptosis, epithelial-mesenchymal transition, cancer stem cells, autophagy, tumor microenvironment, metabolic reprogramming and oncogenic signaling pathways. It should be noticed that cancer might develop resistance to DOX through more than one mechanism. What's more, recently found hallmarks, such as altered metabolic reprogramming and tumor microenvironment (Hanahan and Weinberg, 2011), have not only affected the development of new means to treat human cancer, but also enriched the connotation of chemoresistance. Future study on the nature of cancer is still in urgent need and will undoubtedly provide direction for deepening our understanding of how chemoresistance develops.

At present, most of the reported lncRNAs associated with DOX resistance were identified from laboratory-based results, which were far away from clinical status. This might explain why the clinical translation of chemoresistance reversal is difficult. For example, after the discovery of ABCB1, a number of inhibitors were identified and added to chemotherapy regimens. However, they all failed in clinical trials due to the inefficiency or unbearable toxicity (Gottesman et al., 2002; Binkhathlan and Lavasanifar, 2013). Therefore, future work should be focused on identifying target lncRNAs through well-designed clinical approaches. Obtainment of matched pre- and post-progression tumor biopsies from patients with acquired DOX resistance would be of great importance.

Although the study of lncRNAs on chemoresistance is in its infancy, growing evidence suggests that lncRNAs may serve as potential molecular targets for cancer therapy as well as reversal of chemoresistance. Still, the method to target lncRNAs *in vivo* remains an unsolved problem. Compounds such as curcumin and epigallocatechin gallate can regulate lncRNA expression, but they are lack of specificity. To take lncRNAs as novel therapy targets, there is still a long way to go. Nevertheless, studies over the last decades have established a solid foundation to warrant further investigation of lncRNAs on reversing chemoresistance.

Author contributions

Conceptualization, H-BZ, YH, and YZ; resources, H-BZ and YZ; writing—original draft prepa-ration, H-BZ and YH; writing—review and editing, H-BZ, YH, and J-LD; visualization, J-LD; supervision, G-YF and YZ; project administration, G-YF and YZ. 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.

Publisher's note

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The construction of a prognostic model of cervical cancer based on four immune-related LncRNAs and an exploration of the correlations between the model and oxidative stress

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Introduction: The immune-related lncRNAs (IRLs) are critical for the development of cervical cancer (CC), but it is still unclear how exactly ILRs contribute to CC. In this study, we aimed to examine the relationship between IRL and CC in detail.

Methods: First, the RNAseq data and clinical data of CC patients were collected from The Cancer Genome Atlas (TCGA) database, along with the immune genes from the Import database. We used univariate cox and least absolute shrinkage and selection operator (lasso) to obtain IRLs for prediction after screening the variables. According to the expression levels and risk coefficients of IRLs, the riskscore were calculated. We analyzed the relationship between the model and oxidative stress. We stratified the risk model into two as the high and low-risk groups. We also evaluated the survival differences, immune cell differences, immunotherapeutic response differences, and drug sensitivity differences between the risk groups. Finally, the genes in the model were experimentally validated.

Results: Based on the above analyses, we further selected four IRLs (TFAP2A.AS1, AP000911.1, AL133215.2, and LINC02078) to construct the risk model. The model was associated with oxidative-stress-related genes, especially SOD2 and OGG1. Patients in the high-risk group had a lower overall survival than those in the low-risk group. Riskscore was positively correlated with resting mast cells, neutrophils, and CD8+ T-cells. Patients in the low-risk group showed a greater sensitivity to immunosuppression therapy. In addition, we found that patients with the PIK3CA mutation were more sensitive to chemotherapeutic agents such as dasatinib,

Abbreviations: CTRP2.0, Cancer Therapeutics Response Portal 2.0; CC, cervical cancer; CCLE, Cancer Cell Line Encyclopedia; CCK-8, Cell counting kit-8; CNV, copy number variation; DCA, decision curve analysis; GSVA, Gene set variation analysis; GSEA, Gene set enrichment analysis; HRD, homologous recombination deficiency; HPV, human papillomavirus; IRLs, immune-related lncRNAs; lncRNAs, long-stranded non-coding RNAs; MSI, microsatellite instability; PRISM, Profiling Relative Inhibition Simultaneously in Mixtures; ROC, receiver operating characteristic; SNP, single nucleotide polymorphism; TCGA, The Cancer Genome Atlas, lasso, least absolute shrinkage and selection operator; TPM, transcripts per kilobase million; TMB, Tumor mutational burden.

afatinib, dinaciclib and pelitinib. The function of AL133215.2 was verified, which was consistent with previous findings, and AL133215.2 exerted a pro-tumorigenic effect. We also found that AL133215.2 was closely associated with oxidative-stress-related pathways.

Discussion: The results suggested that risk modeling might be useful for prognosticating patients with CC and opening up new routes for immunotherapy.

KEYWORDS

cervical cancer, immune-related lncRNAs, prognosis, immunotherapy, oxidative stress

Introduction

Globally, CC is the fourth leading cause of cancer-related deaths among women (Sawaya et al., 2019; Wen et al., 2020). Although the screening for CC and human papillomavirus (HPV) vaccination programs has been developed, the number of newly-diagnosed CC cases is on the rise, implying that CC remains a major public health concern (Arbyn et al., 2020). Surgery, radiotherapy and chemotherapy are the three common treatments for patients with CC, however, its 5-year survival rate remains unsatisfactory, owing to recurrence, metastasis and drug resistance (Sol et al., 2009; Kumar et al., 2018; Marquina et al., 2018). The progression and treatment of CC are influenced by the immune system (Chen et al., 2019); hence, immunotherapy is an effective treatment option for patients with CC. With the use of immune checkpoint inhibitors for cancers, great progress has been made in immune-targeted therapies for CC. Immunotherapies comprising anti-CTLA4 and anti-PD1 drugs are effective against CC(Drews et al., 2019). However, the consistently-low positive immunotherapeutic response limits the development and application of immunotherapies for patients with CC (Chung et al., 2019). It is therefore crucial to identify new therapeutic targets and biomarkers for the early diagnosis and prognosis of CC. Non-coding RNAs with more than 200 nucleotides are called long-stranded non-coding RNAs (lncRNAs), which can be involved in post-transcriptional modifications (Kung et al., 2013)and play a key role in processes such as antigen presentation, cancer immunity as well as immune cell infiltration (Denaro et al., 2019; Zhang L. et al., 2020). The lncRNA CamK-A, for example, is highly expressed in several human cancer types and can regulate the Ca2+-signaling-mediated remodeling of the tumor microenvironment (Sang et al., 2018). In addition, the overexpression of HLA-F-AS1 in colorectal cancer cells suppresses miR-375 and promotes the expression of PFN1, thereby exacerbating tumorigenesis (Zhang et al., 2021). LncRNAs can influence the response of patients with cancers to immunotherapies and the tumor microenvironment (Zhang Y. et al., 2020). However, little has been reported about the action mechanism of IRLs in patients with CC. Oxidative stress is involved in the development and progression of many diseases, including cancers (Valko et al., 2007), which is mainly because it can cause inflammation and thus affect cancer development (Reuter et al., 2010). Oxidative stress also plays an important role in CC. It has been shown that triflavin can induce apoptosis by regulating oxidative stress, thereby inhibiting cervical carcinogenesis (Zhu et al., 2021). In addition, oxidative stress is critical in lipid peroxidation, which has a positive effect on the elimination of HPV-related cancers (Cruz-Gregorio et al., 2021). Therefore, it is necessary to discover a new IRL as a potential marker of CC and explore its associations with oxidative stress.

Using the TCGA database and RNA sequencing data, we identified IRLs and established a 4-IRL risk model through the Lasso method. We also explored the potential links between the risk model and oxidative stress. Subsequently, we examined several clinical characteristics of patients with CC that were associated with the model. Additionally, the correlations of the IPS with single nucleotide polymorphism (SNP) mutations, copy number variations (CNVs) and immune cell infiltration were also analyzed. An analysis of drug sensitivity was conducted to improve drug treatment. Overall, these findings may provide a strategy for the prognostic prediction of patients with CC, along with the identification and development of immune-related treatment targets.

Materials and methods

The acquisition of data and the screening of immune-related lncRNAs

The transcriptomic and clinical data (detailed information about the demographic characteristics of the patients in Supplementary Table S3) on CC (normal = 3,tumor = 306) was obtained from the TCGA database (https://tcga-data.nci.nih.gov/), and the immune-related genes were accessed from the Import database (https://www.immport.org/). Count values of raw data were converted to transcripts per kilobase million (TPM) values for subsequent analyses; count values were used only to identify the differential genes. To identify differentially-expressed lncRNAs, we compared different gene expressions between normal and tumor samples with a threshold of |log2 FC (Log2 Fold Change)| > 2 and FDR (false discovery rate) < 0.01. IRLs were obtained based on the relationship between the expression of lncRNAs and immune genes using the Person correlation test (correlation coefficients >0.6). By taking the intersections of DElncRNAs and IRLs, the relevant IRLs were obtained.

The construction and validation of risk model

Machine learning is widely used in applications such as nearest neighbour search in large-scale data (Yan et al., 2021), dimensionality reduction of features, etc., We filtered the significant prognostic lncRNAs with $p < 0.05$ through univariate Cox analysis and

identified the final lncRNAs using lasso regression analysis. In order to create a prognostic risk model, we used the coefficients obtained from the lasso to calculate the riskscore of each patient with CC. The calculation was as follows:

$$riskScore = \sum_{i=1}^{n} Coef\,i * xi$$

The coefficient is Coefi, while xi is the count value of each DElncRNA. Based on the median riskscore (The advantages of the median are that it makes full use of all data information to reflect the centralized trend of a group of data, is not affected by extreme data, and is easy to find. It can clarify the middle level and is less affected by extreme data. Disadvantages: It is easily affected by extreme values), patients were divided into two groups, namely, the high-risk and the low-risk group. Furthermore, based on the survival duration of patients, a Kaplan-Meier analysis was performed to evaluate their prognostic value. Model-based receiver operating characteristic (ROC) curves were plotted for the first, third and fifth year; based on the survival time of patients, Kaplan-Meier analyses were performed, and survival curves were used to display the risk model results.

The acquisition of oxidative-stress-related genes

We collected several common oxidative-stress-related genes from published studies, including SOD1, SOD2 (Zelko et al., 2002), PON1(Teranishi et al., 2012), NOS3(Katkam et al., 2018), UCP2(Hu et al., 2019), GSR (Couto et al., 2016), GPX1 (Teranishi et al., 2012) and GSTM1(Cadoni et al., 2006). 8-hydroxy-2 deoxyguanosine (8-OHDG) is known as a key marker of oxidative stress (Reuter et al., 2010) and we collected 8-OHDG-related genes from GeneCards (https://www.genecards.org/) and obtained gene enrichment pathways using ClueGO. A protein-protein interaction network was then obtained through the String website (https://cn.string-db.org/). Four methods based on the naximal clique centrality (MCC), the density of maximum neighborhood component (DMNC), the maximum neighborhood component (MNC) and degree of cytoHubba were used to screen key genes, the top 10 of which were crossed. Correlations between the risk model and oxidative-stress-related genes were analyzed using the Spearman test.

The correlation between the risk model and clinical characteristics

The correlations between the model and the age, grade, clinical stage as well as TNM stage of patients with CC were assessed using the Chi-square test.

The correlation between targeted therapeutic markers and the risk model

Microsatellite instability (MSI), tumor mutational burden (TMB) and homologous recombination deficiency (HRD) are common molecular characteristics of genomic instability, which are validated biomarkers for targeted therapies (26). We performed a Kaplan-Meier analysis for TMB, MSI and HRD to assess their prognostic values. A Chi-square test was also used to evaluate their associations with the risk model.

Gene mutations and copy number variants

We downloaded the data on SNPs and CNVs of patients with CC from TCGA and UCSC databases. The SNPs and CNVs were visualized using circos (http://circos.ca) and R. The focus was on the demonstration of their locations on the chromosomes where the genes were present in the model. Significantly-mutated genes ($p < 0.05$) and gene mutation interactions between the high- and low-risk group were analyzed using the MAFTOOLS software. In both analyses, only the genes mutating more than 10 times in at least one group were considered, whose expression was probed using GEPIA. A statistical test for significant mutation rates was performed using a one-sided z-test. Copy number alterations among patients with CC were analyzed with GISTIC 2.0 (Mermel et al., 2011). The copy number gistic score, together with the percentage of patients in both risk groups, was also analyzed.

The infiltration of immune cells

We obtained most of our immune cell data from XCELL (Aran et al., 2017), EPIC(Racle et al., 2017) and CIBERSORT (Newman et al., 2015). Next, the immune cell infiltration was quantified using ssGSEA for subsequent analyses (Barbie et al., 2009; Bindea et al., 2013). Further analyses were conducted on the correlations between immune cell types and immune cell content of the risk groups. Based on a Pearson correlation analysis, we analyzed how immune cells and IRLs interacted.

The prediction of immunotherapeutic response

We used unsupervised subclass mapping methods (https://cloud.genepattern.org/gp) to predict the responses of different risk groups to immunotherapies (Lu et al., 2019).

Drugs with differential sensitivities in high- and low-risk groups

PIK3CA mutations are more common in CC (Xiang et al., 2015). As a result, mutations in the PIK3CA gene can be used as a target biomarker for patients with CC. We segregated the patients with CC carrying PIK3CA mutations. After downloading data on drug sensitivity AUC value from the Cancer Therapeutics Response Portal (CTRP2.0), Profiling Relative Inhibition Simultaneously in Mixtures (PRISM) repurposing dataset and the Cancer Cell Line Encyclopedia (CCLE) expression profile, we performed a differential drug

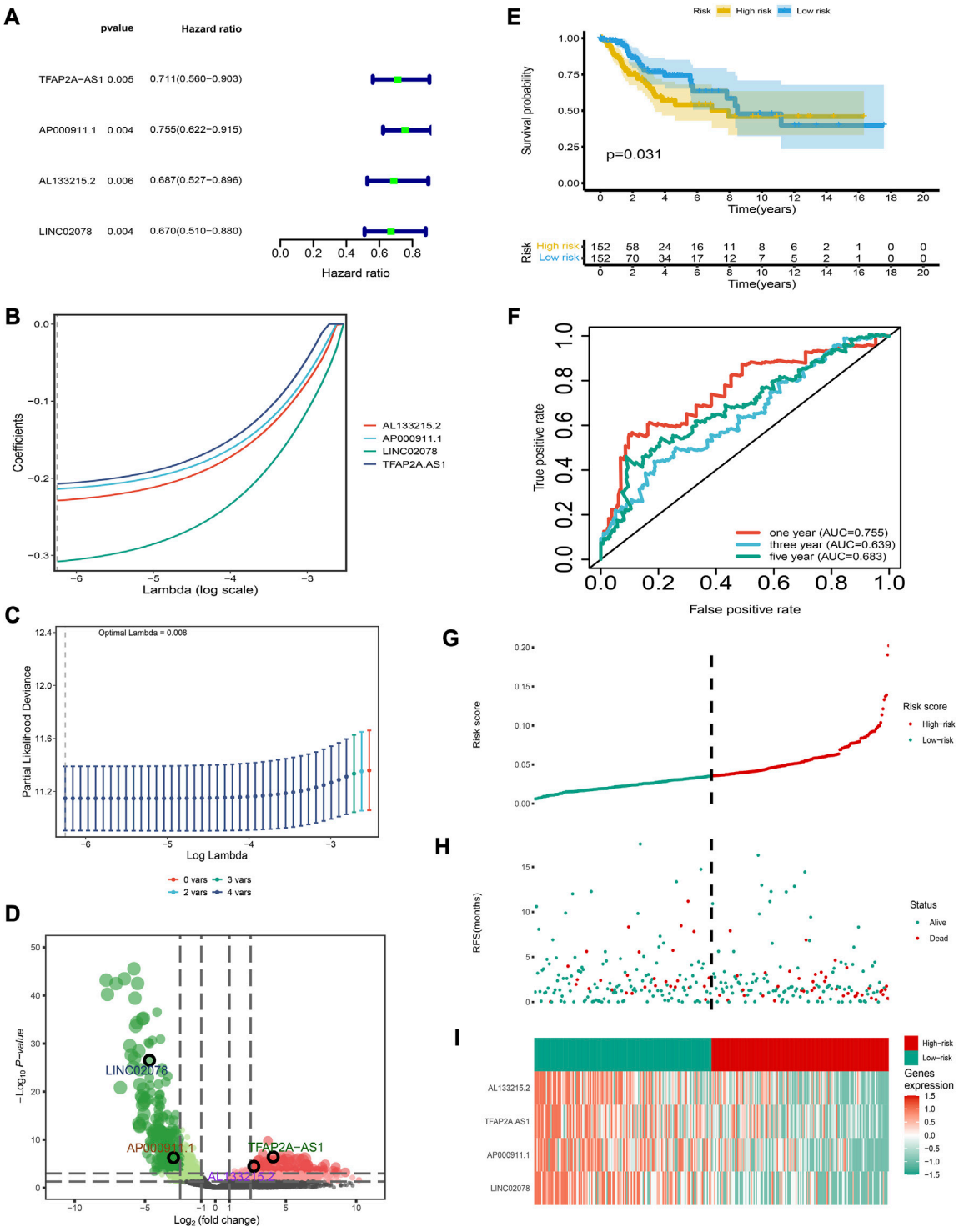

FIGURE 1
Establishment and verification of the risk model **(A)** Forest map for univariate Cox analysis. **(B)** LASSO coefficient distributions for four lncRNAs **(C)** Partial likelihood deviation of the LASSO coefficient distribution. Vertical dashed lines indicate lambda values. **(D)** Volcanic map of DEirlncRNAs. **(E)** The 3- and 5-year ROC curves for the risk model. **(F)** Patients in the low-risk group show longer survival as indicated by the Kaplan-Meier test. **(G–I)** Distribution of risk score, survival status, and molecular expression.

response analysis on patients in both risk groups. Spearman correlation analysis ($r < -0.30$ for CTRP; $r < -0.35$ for PRISM) was used to screen compounds with negative correlation coefficients (Yang et al., 2021).

Experimental verification

A total of 22 pairs of cancerous and non-tumorous tissues were collected from patients with CC at the First Affiliated

Hospital of Zhengzhou University. This study was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (Ethics Number: 2022-KY-0093-002), and an informed consent was obtained from patients. qRT-PCR was used to determine the expression of lncRNAs through the risk model. Primers for TFAP2A-AS1, AP000911.1, AL133215.2 and LINC02078 were designed using primer 5.0 (Supplementary Table S1). Total RNA was extracted with a trizol (CWBIO, China), and the first strand of cDNA was synthesized using a reverse transcription kit (Takara, Kyoto, Japan). Finally, cDNA was quantified through qRT-PCR using SYBR green master mix (Vazyme, China). GAPDH was used as an internal reference for calibration. The $2^{-\Delta\Delta CT}$ method was chosen to calculate the relative expression of lncRNAs. Cellular functional assays were performed for AL133215.2, which was knocked down in the HeLa and SiHa cell line via transfection. Cells transfected with siRNA and controls were stained with an Annexin V-FITC apoptosis detection kit (Beyotime, Shanghai, China). The stained cells were then analyzed through flow cytometry. Relative cell viability was monitored 24, 48, 72, and 96 h after transfection using cell counting kit-8 (CCK-8, Beyotime, Shanghai, China).

Gene set variation analysis (GSVA) and gene set enrichment analysis (GSEA)

We used GSVA to analyze the 50 hallmark pathways described in the molecular signature database (Subramanian et al., 2005; Hänzelmann et al., 2013). Next, we used a limma package to obtain pathways that differed significantly between patients in the high- and low-risk group. A GSEA (Subramanian et al., 2005) was conducted for both risk groups, and we selected significantly-enriched pathways based on *p*-values and FDR q-values that were below 0.05 and 0.25 respectively. We obtained the previously-reported gene sets related to immunotherapy from Hu et al. (2021). In addition to the immune-related gene sets we collected, gene ontology (GO) pathways associated with oxidative stress were also enriched using GSVA. Finally, we examined the associations between genes in the model and the enrichment scores.

Developing a predictive nomogram

The nomogram-integrated factors including the risk score, T, N, MSI. calibration curves and ROC were used to evaluate the accuracy and predictive ability of the nomogram; decision curve analysis (DCA) was used to evaluate the clinical effectiveness of the nomogram.

Statistical methods

R version 4.4.1 was used to perform all statistical tests in this manuscript. The $\chi 2$ test was used for appropriate categorical data, and the two-sample Wilcoxon test was used for continuous data. Survival analyses were performed using the R package "survival". Correlation analysis was performed using the Pearson correlation test. Statistical significance was defined as a *p*-value of less than 0.05 for all statistical analyses.

Results

The construction and validation of the risk assessment model

We obtained a total of 493 differentially-expressed lncRNAs; among them, 96 were immune-related lncRNAs (Supplementary Table S2). A total of four IRLs were identified and selected for risk modeling through univariate cox analysis (Figure 1A) and lasso analysis (Figures 1B, C); among them, TFAP2A-AS1 and AL133215.2 showed a significantly high expression, while LINC02078 and AP000911.1 had a significantly low expression in cancer tissues (Figure 1D). The risk score of each patient was computed as follows:

$$\begin{aligned}\text{Riskscore} = {} & \text{TFAP2A} - \text{AS1}^{*}(-0.207) + \text{AP000911.1}^{*}(-0.214) \\ & + \text{AL133215.2}^{*}(-0.229) + \text{LINC02078}^{*}(-0.308)\end{aligned}$$

The risk model had a good clinical predictive power, with a ROC value of 0.763, 0.645 and 0.678 for 1-, 3- and 5-year survival respectively (Figure 1E). The C-index and IBS of the risk model were 0.918 and 0.035, respectively, which also show that the risk model had a good predictive performance (details of the calculation process were in Supplementary Material S1). Patients in the low-risk group had a higher overall survival rate (Figure 1F). Furthermore, we determined the distribution of risk scores, the survival statistics of patients in different risk categories and the expression characteristics of the four IRLs (Figures 1G–I). As is shown in the graph, the low-risk patients showed an overexpression of these four protective lncRNAs.

The relationship between the risk model and oxidative stress-related genes

Among the eight common oxidative stress-related genes, the risk model was positively correlated with SOD2 while negatively correlated with UCP2 (Figure 2A). One of the four lncRNAs, AL133215.2, correlated significantly positively with SOD2 while negatively with UCP2; another was TFAP2A-AS1, which correlated significantly positively with UCP2. (Figure 2B). There was a significant positive correlation between SOD1 and GPX1 among the eight genes related to oxidative stress (Figure 2C). We obtained 62 8-OHDG-related genes from GeneCards, and the protein interaction network showed more interactions among TP53, OGG1, SOD2, CAT and other proteins (Figure 2D). The pathway enrichment results showed that 8-OHDG-related genes were significantly enriched in the negative regulation of oxidative stress-induced intrinsic apoptotic signaling pathways, responding to oxidative stress and other pathways (Figure 2E). Five key 8-OHDG-related genes were obtained through MCC, DMNC, MNC and Degree, namely, SOD2, OGG1, TP53, NFE2L2 and CAT (Figure 2F). The risk model and OGG1 were significantly negatively correlated (Figure 2G), and AL133215.2, TFAP2A-AS1 as well as OGG1 were significantly positively correlated (Figure 2H).

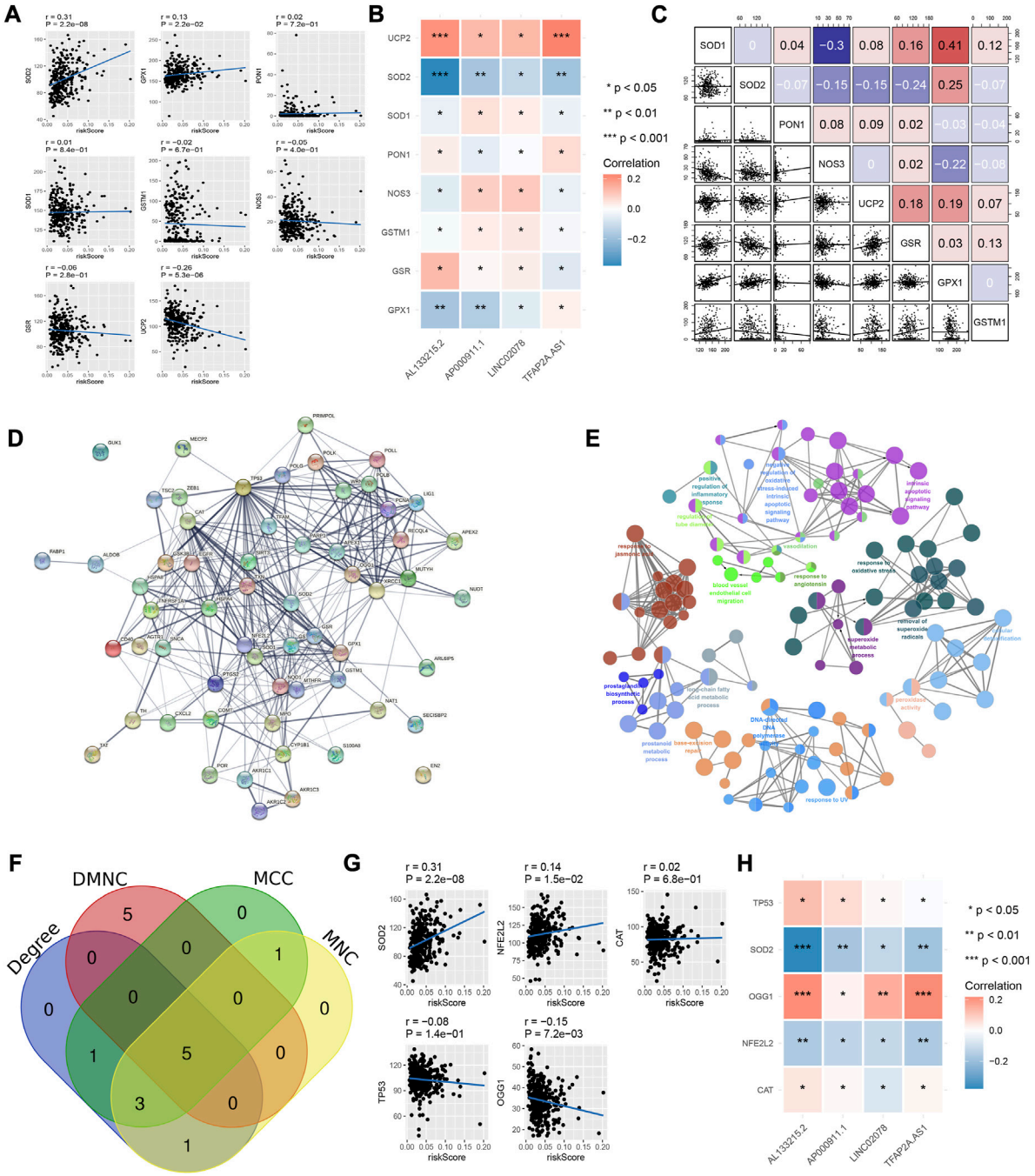

FIGURE 2
Relationship between risk model and oxidative stress-related genes. **(A)** Correlation of risk models and oxidative stress-related genes. **(B)** Correlation between the four IRLs and oxidative stress-related genes. **(C)** Correlation between oxidative stress-related genes. **(D)** Protein-protein interaction network of 8- OHDG-related genes. **(E)** Enrichment pathways of 8- OHDG-related genes. **(F)** Intersection genes of MCC, DMNC, MNC, and Degree. **(G)** Correlation of risk model and key genes **(H)** Correlation of 4 IRLs and key genes.

Differences in clinical characteristics among risk groups

Among all clinical characteristics, N and T stage were significantly associated with the risk model (Figure 3). More patients in the low-risk group were in Stage N0, more of whom in the high-risk group were in Stage T3 and T4. Perhaps this is one of the reasons for the shorter overall survival time of patients in the high-risk group.

The correlation between targeted therapeutic markers and the model

Of the three markers, MSI and the risk groups were significantly correlated (Figure 4A). The high-risk group had a higher MSI value (Figure 4B). However, there was no significant difference between the two groups for TMB (Figure 4C) or HRD (Figure 4D). In a combined analysis on MSI and riskscore, highMSI-highRisk patients

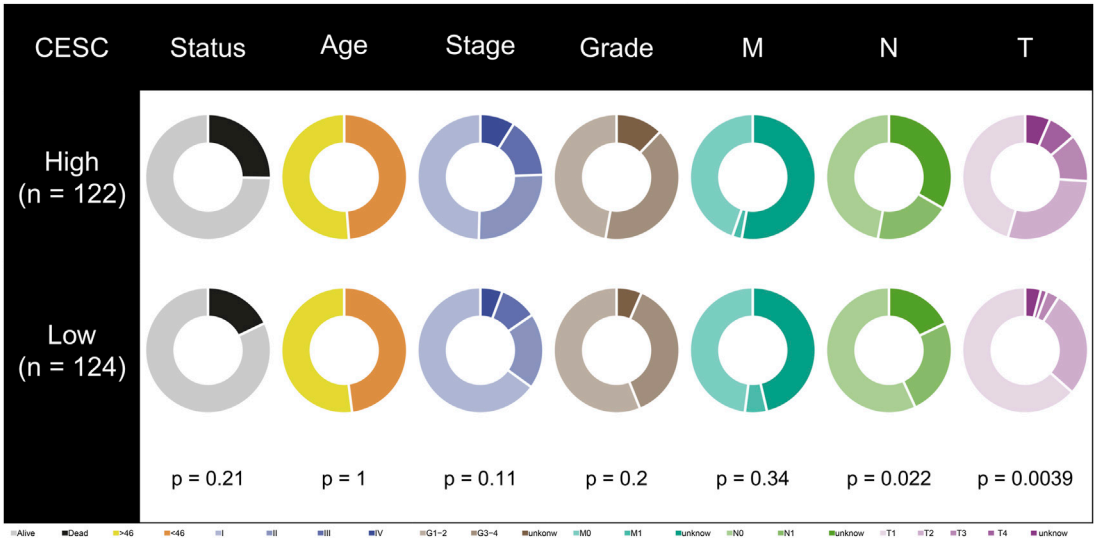

FIGURE 3
Correlation between risk model and clinical characteristics. T-stage and N-stage are different in high- and low-risk groups.

had a significantly shorter survival duration than lowMSI-lowRisk ones (Figure 4E). These results indicated that high-risk patients tended to have a higher MSI value, which contributed to a poorer prognosis.

The mutation status of groups at a high and low risk

From the analysis, TTN, PIK3CA and KMT2C had a high mutation frequency in both high- and low-risk group (Figure 5A, B). There were two genes with a high frequency of mutations in high-risk patients, namely, DNAH2 and AHNAK. There were 12 genes with a high mutation frequency in low-risk patients, namely, DNAH2 and AHNAK (Figure 5C). These genes exhibited a significant co-occurrence (Figure 5D). AHNKA, DMD, CACAN1H, KINA1109 and BRCA1 were differentially expressed between the CC and normal group (Figures 5E–I). CNV results from patients with CC showed a greater significant increase in gene copy number on chromosomes 1 and 3 (Figure 5J). The CNV of chromosomes 6, 10, 11, and 17, where the four lncRNAs TFAP2A.AS1, AL133215.2, AP000911.1, and LINC02078 were located respectively, are shown in Figure 5K. As is shown in the figure, there was a higher gene copy number loss in these chromosomes. A significant similarity in the chromosomal aberrations of patients in the high- and low-risk group was also observed (Figure 5L).

A comparison of the immune landscapes of high-risk and low-risk patients

Among the immune cell types, assessed by several methods, a significant positive correlation was shown between neutrophils and riskscore (Figure 6A). When ssGSEA was used to quantify immune cells, most of them showed a strong positive relationship with each other (Figure 6B). Neutrophils, NK cells and pDCs were significantly different between the high- and low-risk group (Figure 6C). Among the four lncRNAs in the risk model, the expression of TFAP2A-AS1 and AL133215.2 was negatively correlated with that of the majority of immune cells, while LINC02078 and AP000911.1 showed opposite trends (Figure 6D). We used the submap analysis to compare the gene expression profiles of the defined high- and low-risk group with another dataset containing 47 melanoma patients who showed immunotherapeutic responses (Roh et al., 2017). Anti-PD-1 therapies were more likely to be effective for low-risk patients (Bonferroni corrected $p = 0.015$) (Figure 6E). We screened several drugs that showed sensitivity among low-risk patients, including bleomycin A2, dasatinib and afatinib in CTRP2.0 (Figure 6F) as well as NVP-AUY922, dinaciclib, pelitinib, obatoclax, echinomycin, dasatinib and dacomitinib in PRISM (Figure 6G). Based on these studies, we could develop individualized treatment plans for patients in different risk groups.

The experimental validation of molecules in the model

The qRT-PCR assay indicated that TFAP2A-AS1 and AL133215.2 showed a significantly high expression in cancer tissues, while LINC02078 and AP000911.1 had a significantly low expression, which were consistent with our previous predictions (Figures 7A–D). Given the above analyses, we found a close association between AL133215.2 and oxidative-stress-related genes, so AL133215.2 was selected for experimental validation. CCK-8 assay showed that the proliferation of CC cells could be significantly inhibited by knocking down AL133215.2 (Figures 7E, F). We analyzed CC cells through flow cytometry and showed that apoptosis was significantly accelerated in

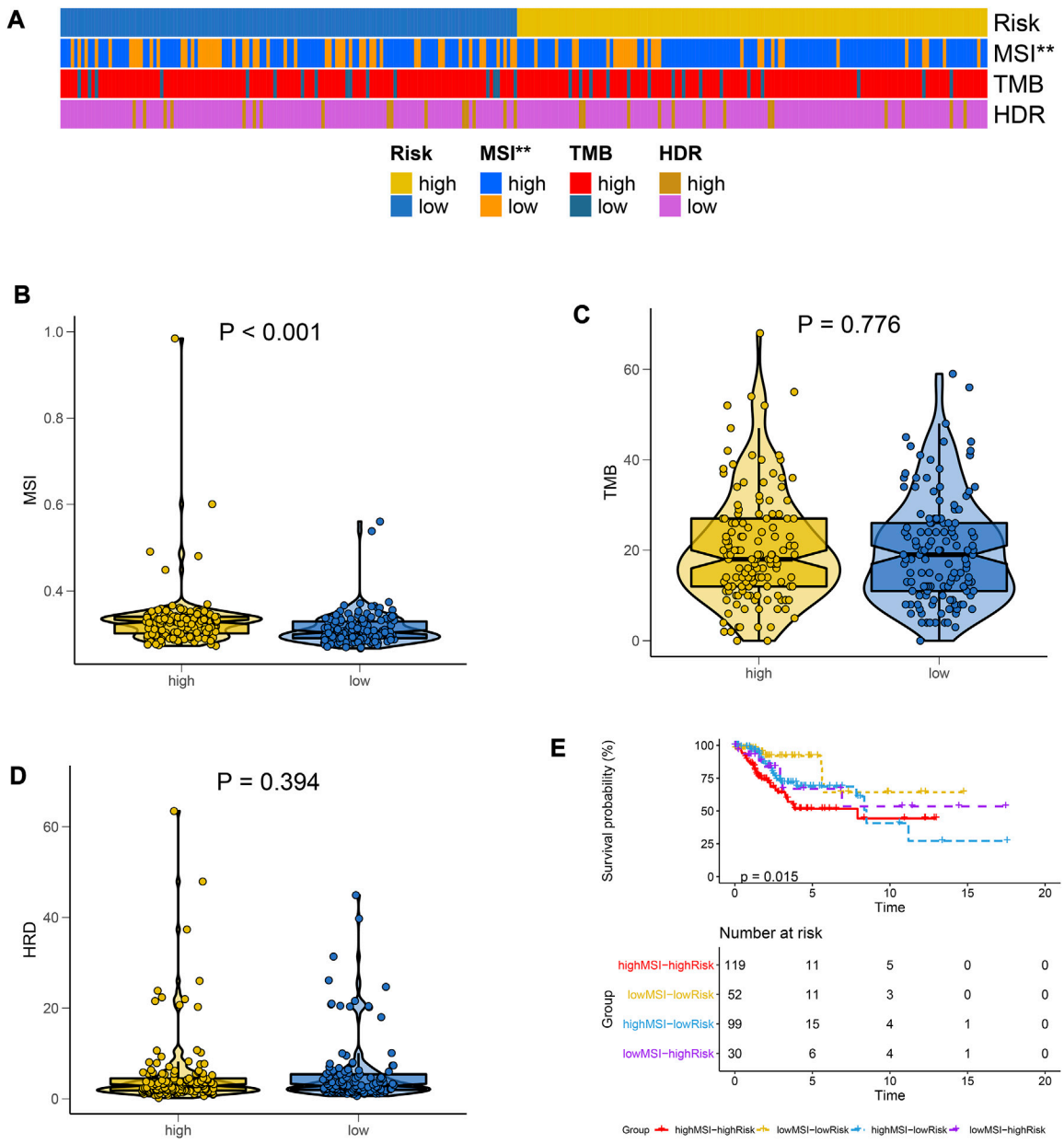

FIGURE 4
Differences in immunotherapy markers between the risk groups. **(A)** Heat maps for the distributions of MSI, TMB, and HRD in high- and low-risk groups. **(B–D)** The Violin plot for differences in expressions of MSI, TMB, and HRD between high- and low-risk groups. **(E)** MSI combined risk model was used to plot the survival curve.

both Siha and Hela cells after AL133215.2 knockdown (Figures 7G–I). These experimental results demonstrated the ability of AL133215.2 to promote the progression of CC.

Pathway enrichment analysis

GSVA was performed on each patient to compare pathways between high- and low-risk group. Among high-risk patients, inflammatory response and oxidative phosphorylation were significantly enriched (Figure 8A). According to GSEA results of the HALLMARK and KEGG gene set, inflammatory response and oxidative phosphorylation were significantly enriched in high-risk individuals (Figures 8B–D). A positive correlation was found between AL133215.2 and most immunotherapeutic predictive pathways, while a negative one was seen in all immune precursor pathways (Figure 8E). The AL133215.2 low-expression group had a higher enrichment score in terms of the oxidative stress pathways (Figure 8F). This was consistent with our previous study, where the high-risk group corresponded to a lower expression of AL133215.2 and a more pronounced oxidative stress.

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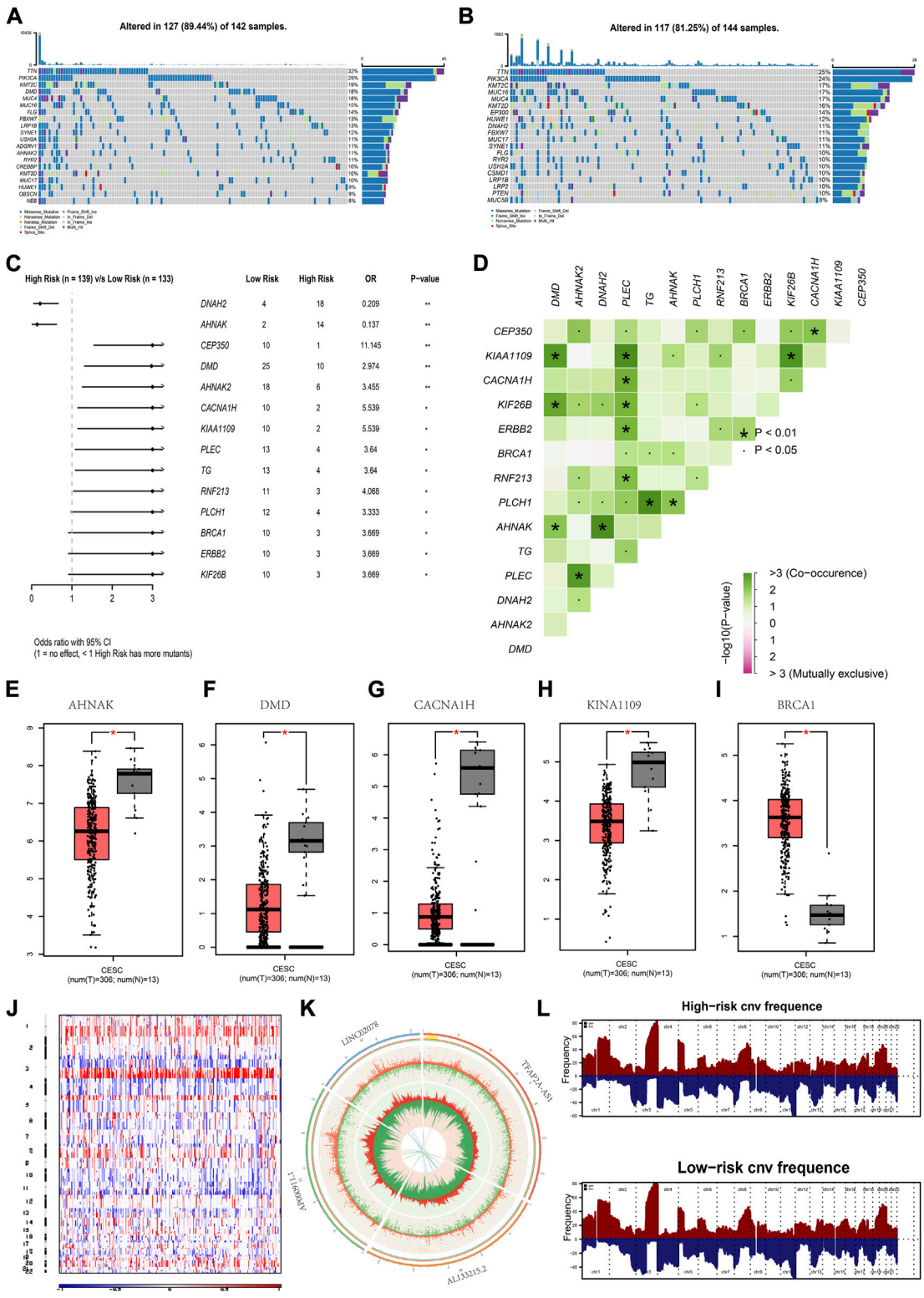

FIGURE 5
Mutations in high- and low-risk groups **(A,B)** Gene mutation waterfall map for low-risk group and high-risk group. **(C)** Forest map of the differentially mutated genes in the low-risk group and high-risk group. **(D)** Interaction of differentially mutated genes between the low-risk group and high-risk group. **(E–I)** Mutant genes showing expression differences between normal and CC patients. **(J)** Heat map of CNVs **(K)** Circle map of CNVs for the chromosomal location of genes of the risk model. **(L)** The chromosomal aberrations in high- and low-risk groups. *$p < 0.05$, **$p < 0.01$ indicated the statistical significance of data.

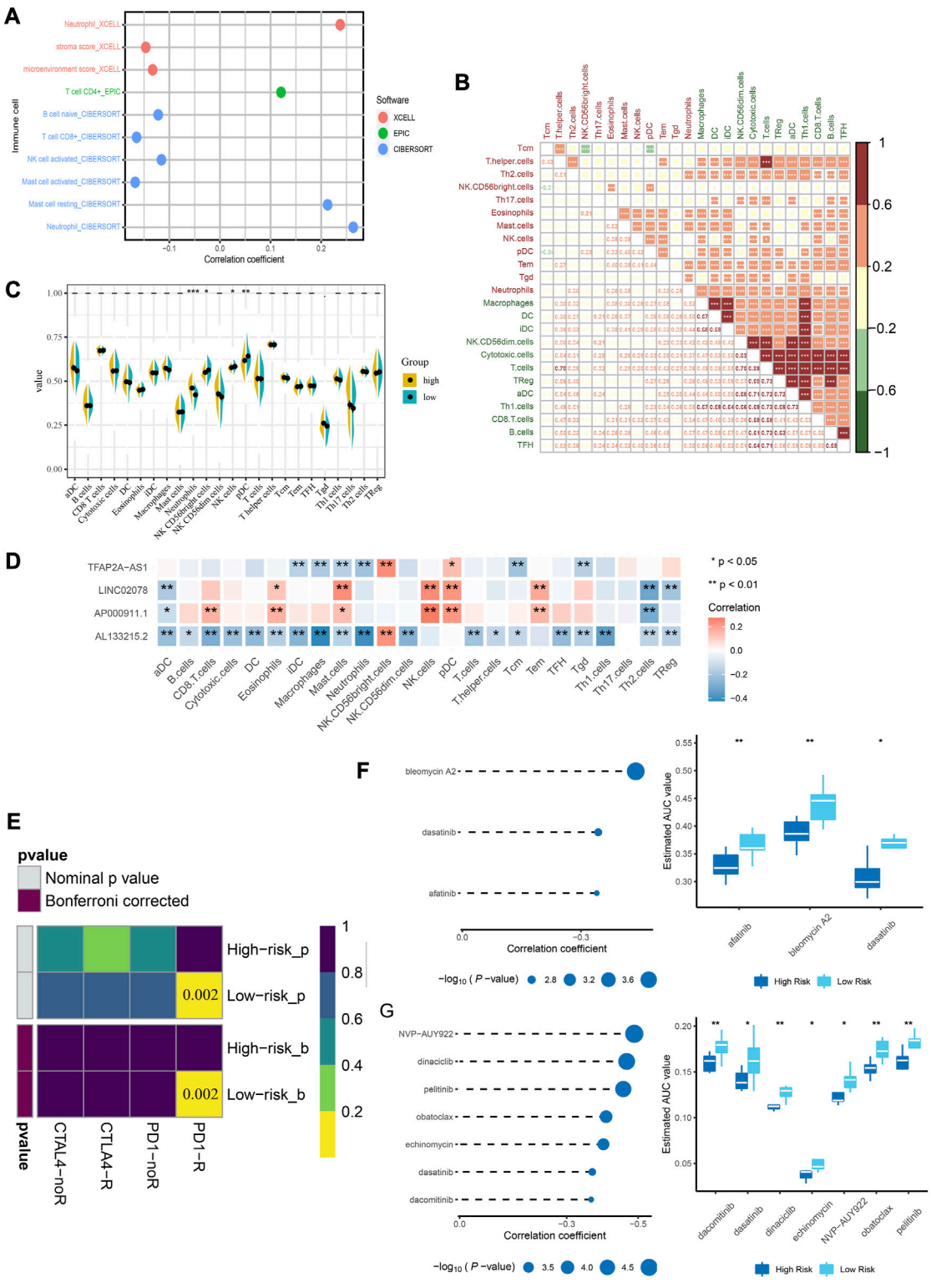

FIGURE 6
Immune infiltration landscape of CC and estimation of immunosuppressed genes using the risk model. **(A)** Correlation between immune cell types and riskscore. **(B)** Correlation matrix for the immune cells. **(C)** Comparison of the expressions of immune infiltrating cells in low- and high-risk groups. **(D)** Correlation between lncRNA and immune cells. **(E)** Submap analysis shows that patients in the low-risk group are more sensitive to PD-1 inhibitors. **(F)** Correlation analysis and drug response analysis for three differential drugs in CTRP. **(G)** Correlation analysis and drug response analysis for seven differential drugs in PRISM. *$p < 0.05$, **$p < 0.01$ indicated the statistical significance of data.

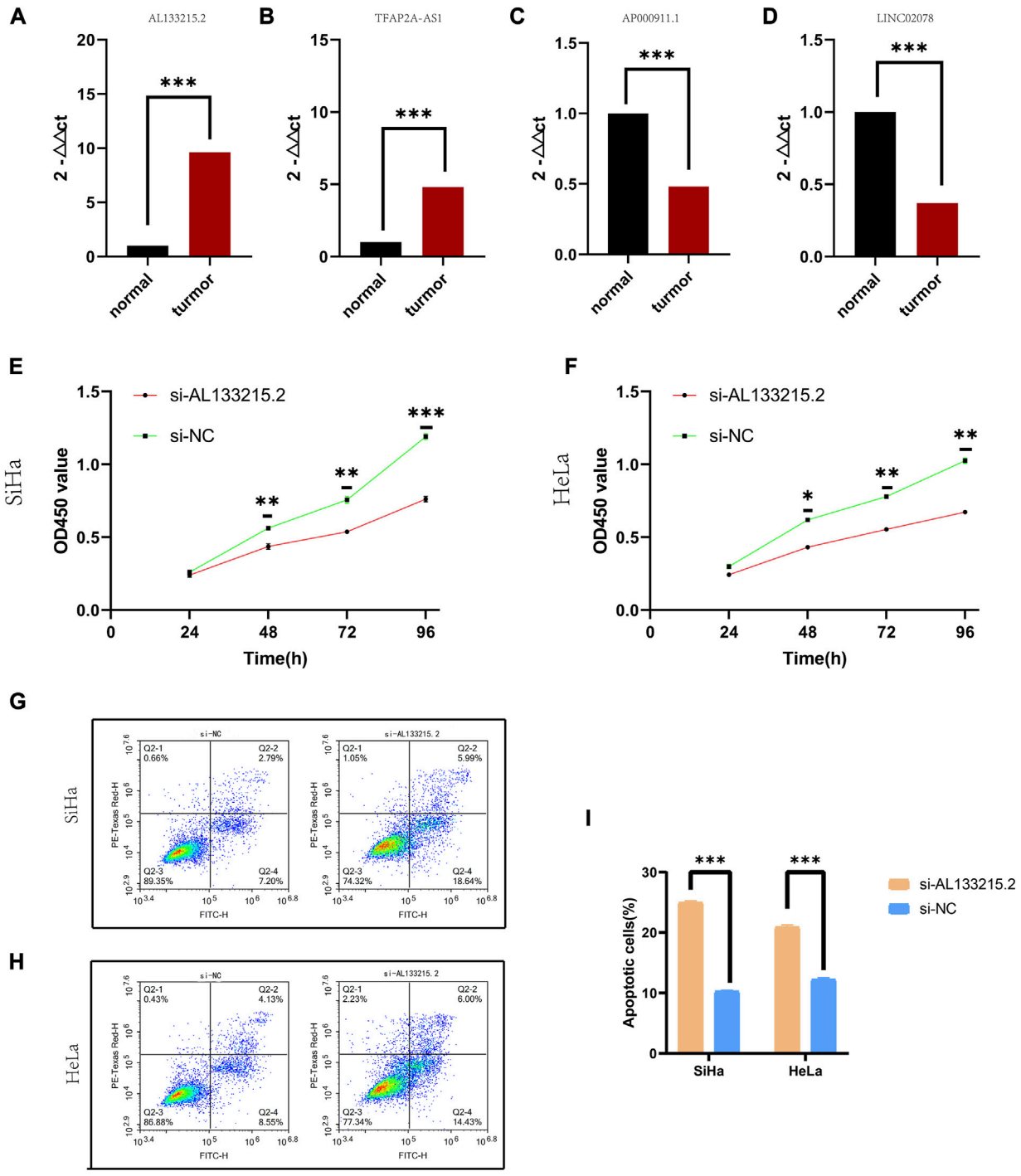

FIGURE 7
Experimental validation of genes in the risk model **(A–D)** qRT-PCR results for TFAP2A.AS1, AP000911.1, AL133215.2, and LINC02078. **(E)** CCK-8 results for knockdown of AL133215.2 in SiHa cell line. **(F)** CCK-8 results for knockdown of AL133215.2 in the HeLa cell line. **(G)** Apoptosis after knocking down AL133215.2 in SiHa cells **(H)** Apoptosis after knocking down AL133215.2 in HeLa cells **(I)** Histogram for apoptosis rates. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ indicated the statistical significance of data.

Nomogram construction and evaluation

A nomogram was constructed to predict survivals after 3 and 5 years (Figure 9A). The calibration curve validated that the nomogram had a good accuracy in predicting patient survivals (Figure 9B). ROC and DCA showed that the risk score had a better clinical efficacy compared to several other characteristics (Figure 9C, D).

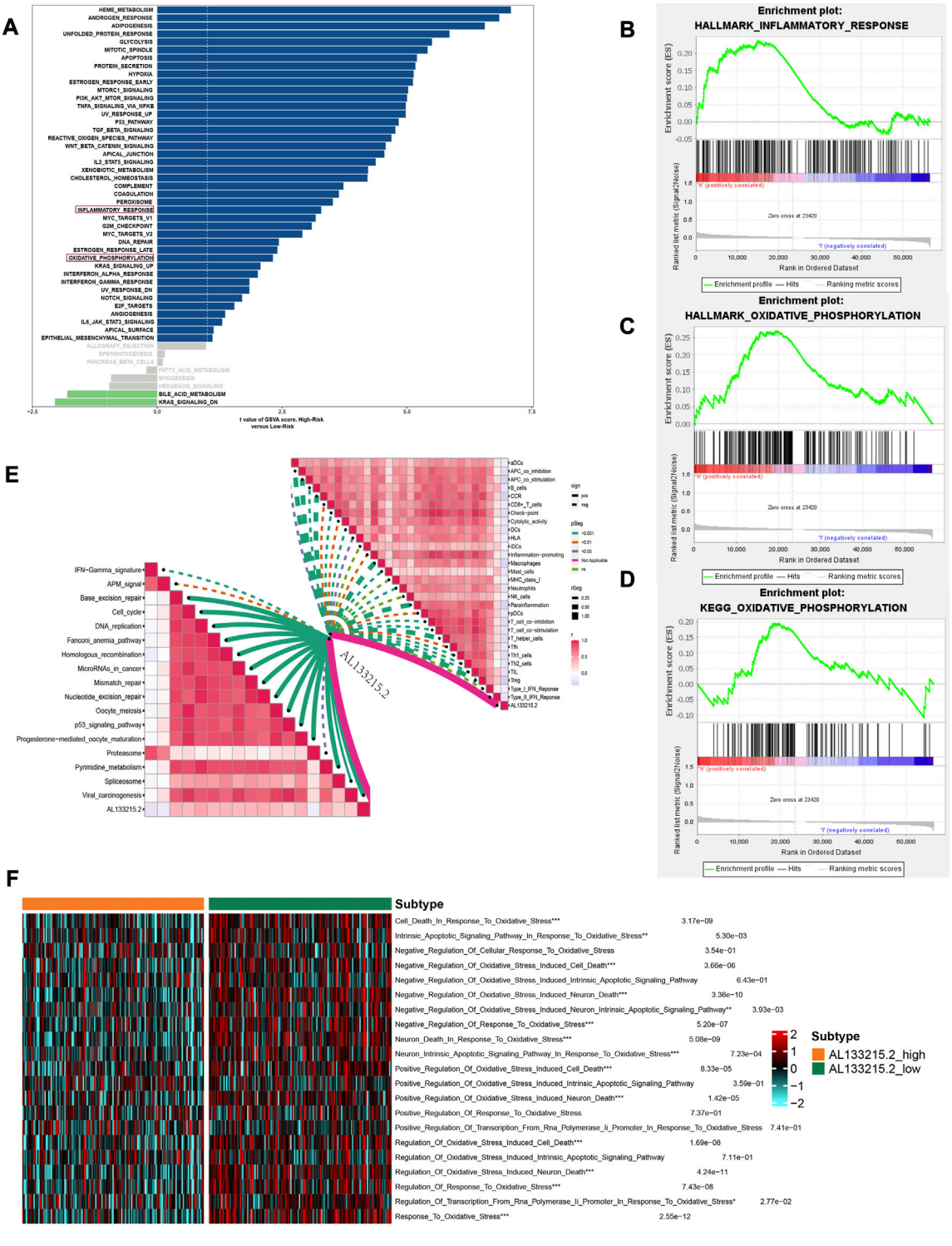

FIGURE 8
Gene set variation analysis and gene set enrichment analysis results. **(A)** Differential pathways between high- and low-risk groups. **(B)** Inflammatory_response in the HALLMARK gene set is significantly enriched in the high-risk group **(C)** Oxidative_phosphorylation in the HALLMARK gene set is significantly enriched in the high-risk group **(D)** Oxidative_phosphorylation in the KEGG gene set is significantly enriched in the high-risk group **(E)** Correlation of AL133215.2 with immune-related pathways **(F)** Differences in oxidative stress-related pathways in the high- and low-expression groups of AL133215.2. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ indicated the statistical significance of data.

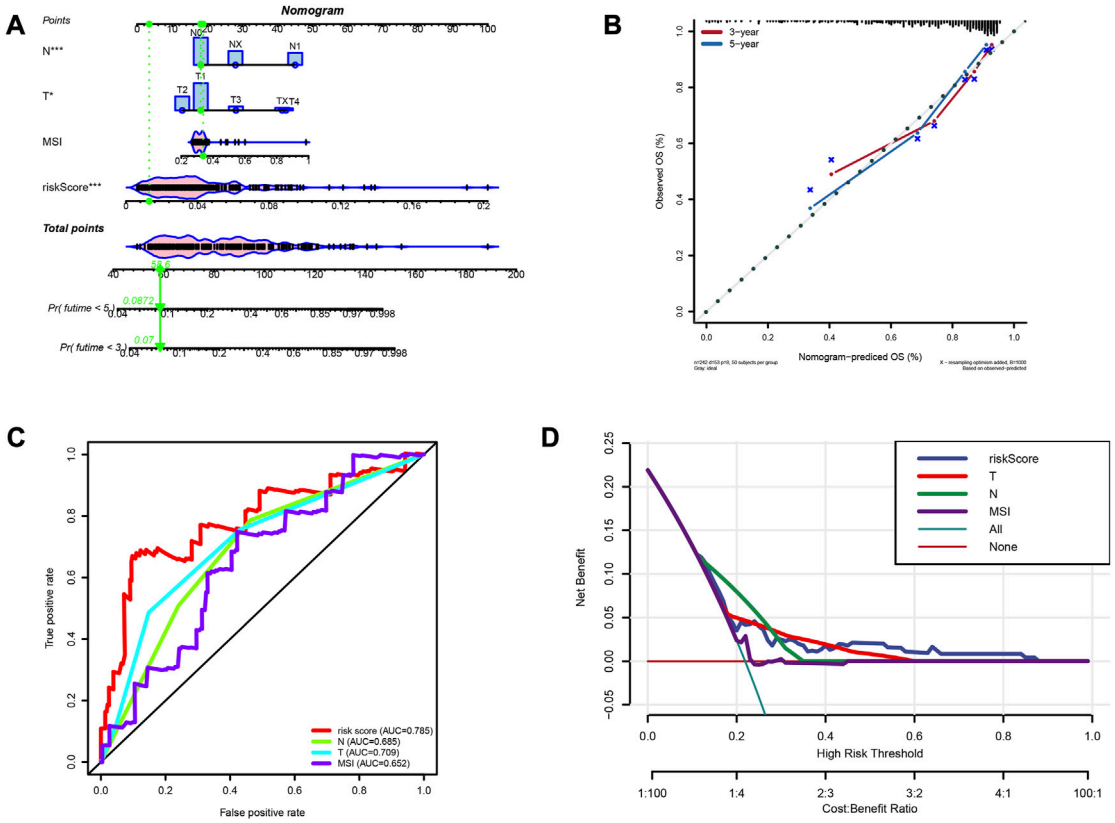

FIGURE 9
Construction of prognostic nomogram. **(A)** The nomogram predicts the probabilities of the 3- and 5-year outcome survival **(B)** The calibration plot for the nomogram predicts the probabilities of the 3- and 5-year outcome survival. **(C)** AUC values for factors in the nomogram. **(D)** The decision curve for the nomogram.

Discussion

In the immune system, lncRNAs regulate gene expression and play a key role in tumorigenesis as well as progression (Li et al., 2020). In addition to serving as prognostic markers, IRLs may also serve as therapeutic targets for cancers (De Felice et al., 2018). lnc-INSR suppresses the immune microenvironment by regulating the differentiation of Treg cells (Liu et al., 2020), thereby promoting tumor growth. In diffused B-cell lymphoma, ncRNASNHG14 promotes immune escape by regulating immune checkpoints (Zhao et al., 2019). Oxidative stress can lead to inflammatory pathways through which normal cells are converted into tumor cells, and studies have shown that oxidative stress plays an important role in the progression of CC (18, 19). However, the exact role of IRLs in the prognosis of CC and their associations with oxidative stress remain unclear. In this study, we identified four lncRNAs and construct an immune risk scoring system. TFAP2A-AS1 is a tumor suppressor of breast cancer as it competes for miR-933, thereby releasing SMAD2 (Zhou et al., 2019). AL133215.2 is identified and used to construct a prognostic model for CC (Chen et al., 2020). LINC02078 and AP000911.1 have not yet been reported in this context. Next, we will discuss the potential utility of risk modeling as a new immunotherapeutic tool and analyze the relationship between risk modeling and oxidative stress.

The risk model was stratified into two groups based on risk level: low-risk and high-risk model, which was associated with oxidative-stress-related genes SOD2 and OGG1. SOD2 plays an important role in vascular oxidative stress (Dikalova et al., 2017), and OGG1 acts as a DNA repair enzyme that can counteract DNA damage caused by oxidative stress (Li et al., 2018). Patients in the low-risk group had a longer outcome survival, as well as a higher MSI and immunogenicity, who were also more suitable for anti-PD-1 therapies. Additionally, we also screened for drugs that showed a great sensitivity among low-risk patients, including bleomycin A2, dasatinib, afatinib, dinaciclib, and pelitinib. Risk scores were compared to other clinical characteristics, which were found to be independent risk factors. In a ROC curve analysis, the AUC value of the risk model was significantly higher than that of other characteristics, suggesting that risks were a better predictor of patient prognosis. A nomogram was constructed to predict patient survival after 1, 3, and 5 years. Importantly, we validated the expression of lncRNAs identified using the model through qRT-PCR and functionally validated one of the genes. This, to some extent, demonstrated the prognostic value of the risk model.

To evaluate the efficiency of risks in immunotherapies, the immunogenicity of the tumor microenvironment needs to be investigated (Gasser et al., 2017). TMB is a biomarker of immunotherapeutic response (Hellmann et al., 2018; Samstein et al., 2019), the higher the TMB is, the greater the benefit of immunotherapies will be. MSI is a major predictor of

immunotherapeutic sensitivity; tumors with a high MSI (MSI-H) can be better treated with ICIs (Baretti and Le, 2018). HRD induces genomic instability and increases immunogenicity for patients with tumors, thereby leading to an increased response to ICIs (Dai et al., 2018). In this study, we found that risk grouping was significantly correlated with MSI grouping; the high-risk group had a higher MSI value. However, since microsatellite instability status does not effectively represent the potential benefit of immunotherapies (Camidge et al., 2019), other methods of evaluation need to be developed.

Mutations in certain genes are closely associated with immunotherapies, such as TP53, along with their associated co-mutations that can increase the expression of TMB and immune checkpoints, thereby affecting patients' response to immunotherapies (Assoun et al., 2019). Of the 14 genes identified that showed mutational differences between the high- and low-risk group, 12 showed higher mutations in low-risk patients. AHNAK2 and BRCA1 are involved in the regulation of the immune system (Ju et al., 2020; Xie et al., 2020). Mutations in BRCA1, a homologous repair gene, can affect the efficacy of immunotherapies (Fumet et al., 2020).

Infiltration of immune cells can be used to predict the response to cancer immunotherapies (Junttila and de Sauvage, 2013). We used XCELL, EPIC and CIBERSORT algorithms to estimate the relationship between risks and immune cells, and the correlations between neutrophils and risks were positive. Neutrophils are early infiltrative inflammatory cells that enable tumor cells to escape immune surveillance (Fetz et al., 2021). A comparison between immune cells of the high- and low-risk group was analyzed with ssGSEA, which showed that the high-risk group had a higher neutrophil infiltration, while the low-risk group had a higher infiltration of NK-CD56 bright cells, NK cells and pDCs. The activation of plasmacytoid dendritic cells (pDCs) can induce T-cell activation or tolerance; the NK CD56 bright cells can be used as antitumor effectors in cancer immunotherapies (Wagner et al., 2017). From the above results, the low-risk group seemed to have a better immunogenicity. Combined with the results of the comparison of immune datasets, we speculated that patients in the low-risk group might respond better to immunotherapies.

Dinaciclib is an effective anti-PD1 inhibitor that induces immunogenic cell deaths (Hossain et al., 2018). Dasatinib, combined with low-intensity chemotherapies, is effective in Philadelphia-positive acute lymphoblastic leukemia (Rousselot et al., 2016). Obatoclax improves the response of patients with bladder cancer to cisplatin chemotherapies and their treatment outcomes (Steele et al., 2019). Although the role of these drugs in CC is rarely reported, in our study, by analyzing their potential efficacy in patients carrying PIK3CA mutations, we speculated that several drugs, including dasatinib, dinaciclib, and obatoclax, might show better efficacies in the low-risk group of patients. This could provide targeted therapy options for low-risk patients.

Our study innovatively identified and validated four IRLs for CC, uncovered immune-associated risk models for predicting clinical outcomes, and established links to oxidative stress. The key features we selected may define a new therapeutic strategy that will serve as new immune biomarkers for future CC immunotherapy. Meanwhile, in the risk model, a significant increase of the inflammatory response and oxidative phosphorylation was observed in the high-risk group of patients, suggesting that both inflammation and oxidative stress could lead to increased risks. AL133215.2 was lowly expressed in high-risk patients, therefore, the oxidative-stress-related pathways were significantly enriched in the AL133215.2 low-expression group.

Conclusion

In conclusion, we developed a prognostic risk model by identifying IRLs and explored the associations between the risk model and oxidative stress. In actual clinical practice, we can perform transcriptome sequencing of 4 IRLs from patients, assess the risk scores and stratification of patients based on risk modeling formulas, and make comprehensive judgments on prognosis in conjunction with the clinical characteristics of patients. We can also propose the appropriate treatment plan according to the patient's risk stratification. However, there are limitations to our study, including insufficient sample size, limited generalisability due to lack of information on patient treatment and long-term follow-up, and lack of more in-depth mechanistic studies. These limitations may affect the interpretation of our findings, but they do not negate the reliability of our study.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the all participants signed an informed consent form approved by the ethics committee of the First Affiliated Hospital of Zhengzhou University (ethics number: 2022-KY-0093-002). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

XL, Conceptualization, Data curation, Formal analysis, Methodology, Validation, Visualization, Writing–original draft; YJ, Provided ethics committee and collected tissue samples, Funding acquisition; JL and SD, Data curation, Writing–review and editing; EY, Supervision, Project administration. The corresponding author had full access to all of the data and the final responsibility for the decision to submit this article for publication. 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1234181/full#supplementary-material

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A disulfidptosis-related lncRNA prognostic model to predict survival and response to immunotherapy in lung adenocarcinoma

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Background: Lung adenocarcinoma (LUAD) is the major subtype of lung cancer and has a poor prognosis. Disulfidptosis is a novel regulated cell death form characterized by aberrant disulfide stress and actin network collapse. This study aimed to identify disulfidptosis-related lncRNAs, and predict LUAD patients' prognosis and response to antitumor therapies by establishing a disulfidptosis-related lncRNA model.

Methods: Transcriptome and clinical data of LUAD patients were obtained from the TCGA database. Pearson correlation and Cox regression analysis was used to identify disulfidptosis-related lncRNAs associated with overall survival. LASSO regression analysis was adopted to construct the prognostic model. GO, KEGG and GSEA analysis was used to identify cellular pathways related to this model. Immune cell infiltration was investigated by ESTIMATE and CIBERSORT algorithms. Tumor mutational burden (TMB) and its association with model-derived risk score were analyzed using simple nucleotide variation data. Patients' response to immunotherapy and other antineoplastic drugs was predicted by the TIDE algorithm and GDSC tool, respectively.

Results: We identified 127 disulfidptosis-related lncRNAs, and a prognostic model that consists eight of them (KTN1-AS1, AL365181.3, MANCR, LINC01352, AC090559.1, AC093673.1, AP001094.3, and MHENCR) was established and verified. The prognostic model could stratify LUAD patients into two distinct risk-score groups. A high risk score was an independent prognosis factor indicating poor overall survival, and correlated with reduced immune cell infiltration, high TMB, and lower activity of tumor immune response. Immune checkpoint blockade might bring more survival benefits to the high-risk LUAD patients, whereas low-risk patients might be more responsive to targeted therapy and diverse kinase inhibitors.

Conclusion: We established a disulfidptosis-related lncRNA model that can be exploited to predict the prognosis, tumor mutational burden, immune cell infiltration landscape, and response to immunotherapy and targeted therapy in LUAD patients.

KEYWORDS
lung adenocarcinoma, disulfidptosis, lncRNA, prognosis, immunotherapy

Introduction

Lung cancer remains one of the leading causes of global cancer incidence and mortality (Sung et al., 2021; Zheng et al., 2023). Adenocarcinoma is the main histological type of non-small cell lung cancer (NSCLC) and accounts for around 40% of all lung cancers (Duma et al., 2019). Early detection and diagnosis are directly related to clinical outcome, and its failure often leads to miss of the optimal opportunity of clinical intervention. For patients with stage I or II disease, surgical resection is recommended. For patients at advanced stages, besides traditional radiotherapy and chemotherapy, systemic therapeutic strategies comprising targeted therapy and immunotherapy are optional for NSCLC treatment according to the gene mutation scenarios (e.g., *EGFR* mutation, *ALK* translocation) and expression of programmed cell death protein-ligand 1 (PD-L1) (Duma et al., 2019). Lung adenocarcinoma (LUAD) is molecularly and phenotypically diverse, and approximately 60% of LUAD have an oncogenic driver mutation that in many cases is associated with certain clinicopathologic features and predicts treatment response (Sholl, 2015; Tavernari et al., 2021). For example, *KRAS* and *KEAP1* are among the most frequently mutated genes in LUAD. *KEAP1* mutations confers shorter overall survival (OS) in *KARS* mutant LUADs in response to anti-PD-(L)1 immunotherapy (median OS (95%CI): 4.8 months (4.0–8.0) for *KEAP1* mutant versus 18.4 months (14.9–221.7) for *KEAP1* wild-type), but not in *KRAS* wild-type LUADs (Ricciuti et al., 2022). In another LUAD cohort treated with immune checkpoint inhibitors, *KEAP1* inactivation mutations due to somatic mutation and loss of heterozygosity are correlated with worse clinical outcomes and an immune-excluded phenotype (Scalera et al., 2023). The survival rate remains dismal despite of advances in genotype-based diagnosis and therapy modalities (Zhang et al., 2019). To improve LUAD management, a solid understanding of molecular events that correlate with LUAD malignant degree is necessary.

Resisting regulated cell death is a hallmark of cancer (Hanahan, 2022). Increasing evidence shows that different regulated cell death forms can affect cancer progression and response to therapy (Peng et al., 2022). For example, ferroptosis, characterized by iron-dependent lipid hydroperoxide accumulation, was found to be implicated in T cell immunity and contribute to immunotherapy efficacy (Wang. et al., 2019a). Disulfidptosis is a recently identified regulated cell death type induced by aberrant accumulation of intracellular disulfides in SLC7A11-overexpressing cells under a glucose starvation condition (Liu et al., 2023). Increased SLC7A11-mediated cystine uptake, in couple with glucose starvation, causes severe disulfide stress and facilitates aberrant disulfide bonding in actin cytoskeleton proteins, leading to actin filament contraction and detachment from the plasma membrane (Liu et al., 2023). A recent study by Chen et al. highlights that disulfidptosis plays a role in regulating bladder cancer progression and therapy efficacy (Chen et al., 2023). However, it remains unclear whether disulfidptosis is involved in LUAD progression and affects prognosis of LUAD patients.

Long non-coding RNAs (lncRNAs) are transcripts of more than 200 nucleotides that are not translated into proteins. LncRNA-encoding loci are among the most numerous regulatory and functional units in the non-coding regions of the genome (Uszczynska-Ratajczak et al., 2018). They play critical roles in regulating gene expression and protein function by interacting with DNA, RNA and proteins (Liu et al., 2015; Blank-Giwojna et al., 2019; Statello et al., 2021). The involvement of lncRNAs in gene expression regulation under pathological conditions suggests that they are related to a broad range of diseases. In terms of LUAD, a growing number of studies have demonstrated that lncRNAs promote disease progression (e.g., UPLA1 and LINC00628) and immune evasion (e.g., SChLAP1), and can serve as prognosis biomarkers and potential drug targets (Xu et al., 2019; Han et al., 2020; Du et al., 2021).

In this study, we aimed to identify disulfidptosis-related lncRNAs that affect prognosis of LUAD patients. We constructed and validated a prognostic model based on disulfidptosis-related lncRNAs, and this model exhibits high accuracy in predicting survival rate (area under the curve (AUC) for 1 year survival: 0.703). Moreover, the model-derived risk score can be used to evaluate tumor immune micro-environment landscape and sensitivity to immunotherapy and chemotherapy. Besides tumor stage, our prognostic model is an independent factor with potential to identify patients with high risk (hazard ratio (HR): 1.245, 95% CI: 1.167–1.328, $p < 0.001$). Our findings demonstrate key regulatory roles of disulfidptosis-related lncRNAs in LUAD progression and provide potential targets for precision treatment of LUAD.

Materials and methods

Data acquisition

The RNA-sequencing (RNA-seq)-based transcriptome profiling data, clinical information and somatic mutation data of over 500 LUAD patients were downloaded from The Cancer Genome Atlas (TCGA) database. Normal control samples were excluded for further analysis. LUAD cases with insufficient information about survival time, age, and tumor stage were also removed.

Screening for disulfidptosis-related lncRNAs

We obtained 25 disulfidptosis-related genes based on previous studies (Liu et al., 2023; Yang et al., 2023; Zhao et al., 2023). Pearson correlation analysis was performed to identify lncRNAs that exhibit co-expression patterns with the disulfidptosis-related genes, with the absolute value of correlation coefficient >0.4 and $p < 0.001$ as the screening threshold. These lncRNAs were defined as disulfidptosis-related lncRNAs.

Establishment and validation of a disulfidptosis-related lncRNA prognosis model

A total of 507 LUAD samples with survival information were randomly divided into two groups, one for model construction (the

training group, n = 254) and one for model validation (the test group, n = 253). In the training group, disulfidptosis-related lncRNAs that were associated with patients' overall survival were obtained by performing univariate Cox regression analysis. After LASSO regression analysis to determine lncRNAs with minimum deviation, a prognostic model based on eight disulfidptosis-related lncRNAs was established through multivariate Cox regression analysis. The risk score was the sum of products of the expression value of each of the eight lncRNAs and its regression coefficient, risk score = $\sum_{i=0}^{n} \beta i \, Exp \, i$ (Zhu et al., 2020). Based on the median risk score, patients were grouped into high- and low-risk subgroups, and survival analysis was carried out to evaluate the significance of the prognostic model. Samples in the test group were used to validate the reliability of this prognostic model. Multivariate Cox regression analysis was conducted to evaluate whether the risk score derived from the model is an independent prognostic factor of LUAD patients.

Functional enrichment analysis of differentially expressed genes

Differentially expressed genes (DEGs) were screened between the high and low risk groups, according to the screening criteria: |log2 fold change| > 1 and false discovery rate (FDR) < 0.05. After that, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were carried out to gain insights into possible molecular events that distinguish between high- and low-risk groups. GO terms or KEGG pathways were considered significantly enriched when FDR was less than 0.05. With a focus on Gene Ontology gene sets, gene set enrichment analysis (GSEA) was also performed based on gene expression profiles between the two groups. A gene set was considered enriched when *p*-value and FDR were less than 0.05 and 0.25, respectively.

Tumor infiltrating immune cells analysis

The ESTIMATE R package was used to analyze the abundances of infiltrating stromal and immune cells in LUAD tissues using gene expression data (Yoshihara et al., 2013). The ESTIMATE algorithm generates three scores based on single sample GSEA, including stromal, immune and estimate scores. Their differences between high and low risk LUAD groups were compared.

The CIBERSORT tool (Chen et al., 2018) was further employed to estimate the abundances of 22 immune cell types in each of the LUAD samples. In addition, single sample GSEA was performed using immune-related gene sets to evaluate multiple immune functions of each sample, and the activities of these immune functions were compared between two risk groups.

Tumor mutational burden analysis

According to the total number of somatic base substitutions, the tumor mutational burden (TMB) and mutation frequencies in each sample were calculated. Differences in TMB between the high- and low-risk groups of patients were analyzed. According to the median TMB score, LUAD patients were divided into two groups and survival analysis was performed to explore the influence of TMB on patients' overall survival. The combined effect of TMB and risk score on patient prognosis was also investigated.

Immunotherapy response and drug sensitivity prediction

We exploited the Tumor Immune Dysfunction and Exclusion (TIDE) platform (Jiang et al., 2018) to predict LUAD patient response to anti-PD-1 and anti-CTLA4 immunotherapy. TIDE prediction scores are negatively associated with immunotherapy response. Differences in response to immunotherapy between the high- and low-risk groups of patients were analyzed by comparing the TIDE scores.

The oncoPredict R package was used for predicting drug sensitivity in LUAD patients based on gene expression data. The required training sets were derived from the Genomics of Drug Sensitivity in Cancer database (GDSC) and downloaded from oncoPredict's Open Science Framework (https://osf.io/c6tfx/) (Maeser et al., 2021). We used the calcPhenotype function to obtain drug sensitivity scores of each patient. Differences in response to multiple drugs between the high- and low-risk groups were compared based on the drug sensitivity scores.

Statistical analysis

Data analysis was performed in R (4.2.2). Two-tailed Student's t-test was used to compare statistical differences between two groups. The Kaplan-Meier estimate and log-rank test were used for survival analysis. Unless otherwise indicated, differences were considered statistically significant when $p < 0.05$.

Results

Identification of disulfidptosis-related lncRNAs

RNA-seq data of LUAD patients were downloaded from TCGA. According to the annotation of gene type, protein coding mRNAs and lncRNAs were distinguished. To identify lncRNAs implicated in disulfidptosis, Pearson correlation analysis was conducted based on expression levels of lncRNAs and 25 disulfidptosis-related genes. Following stringent screening criteria (|Pearson R| > 0.4 and $p < 0.001$), 127 lncRNAs were screened out and their expression were correlated with 20 of the 25 disulfidptosis-related genes (Figure 1A; Supplementary Table S1).

Establishment of the disulfidptosis-related lncRNA prognostic model

We randomly divided 507 LUAD patients with survival information into two groups, the training group was for model

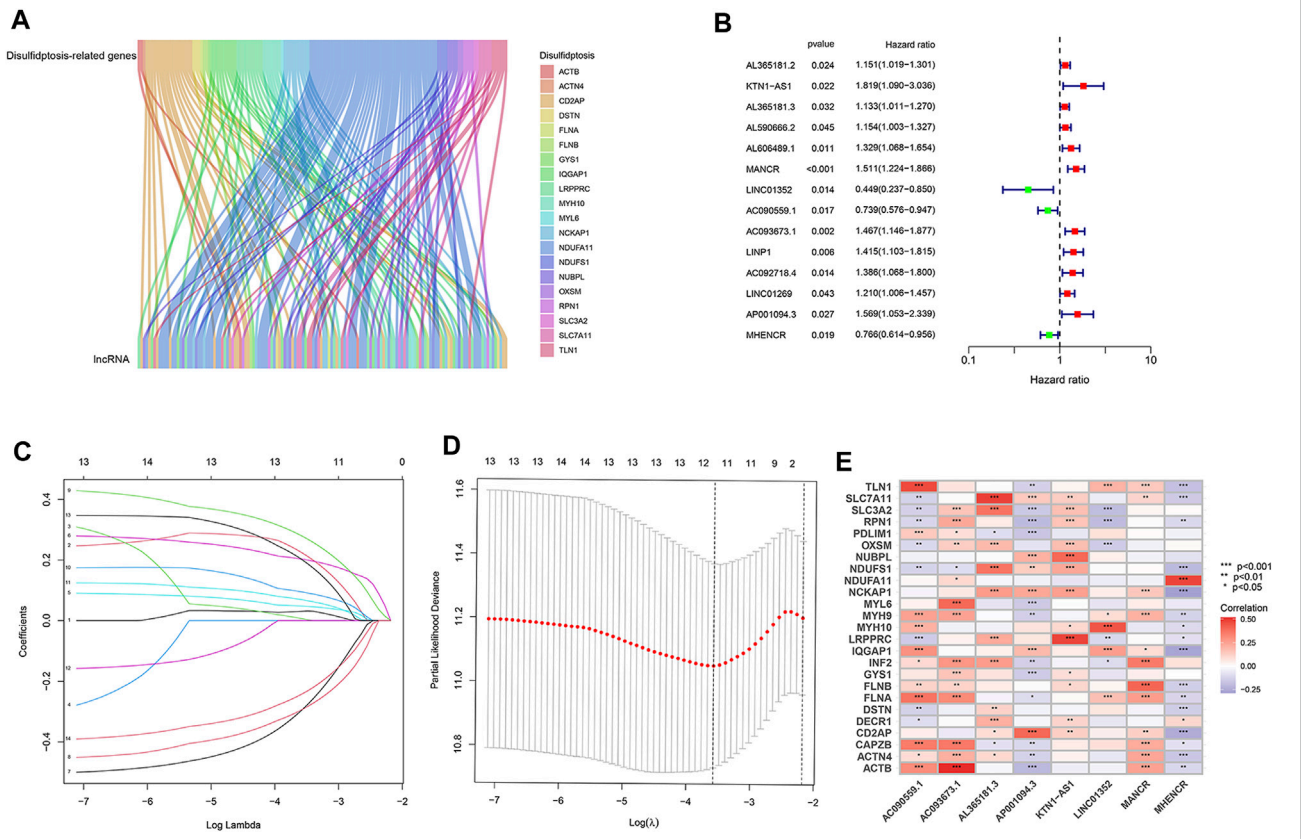


FIGURE 1
Identification of disulfidptosis-related lncRNAs and construction of prognostic model in LUAD. **(A)** The Sankey diagram showing significant expression correlations of disulfidptosis-related genes with 127 lncRNAs. **(B)** Disulfidptosis-related lncRNAs that affect the overall survival of LUAD patients according to univariate Cox regression analysis. **(C)** LASSO coefficients of the 14 lncRNAs that correlate with overall survival. **(D)** Cross-validation of LASSO regression, the dashed lines denote the optimal log(λ) value. **(E)** Heatmap showing the expression correlation between the eight lncRNAs used for model construction and disulfidptosis-related genes. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

construction and the test group for model validation. Among the 127 lncRNAs, only those associated with LUAD patient survival were considered to be enrolled in model construction. We performed univariate Cox regression analysis to exclude lncRNAs that showed no significant effect on survival, and 14 disulfidptosis-related lncRNAs were left. Of them, three were prognostically favorable lncRNAs and eleven were prognostically unfavorable ones (Figure 1B). Based on these 14 prognosis-associated disulfidptosis-related lncRNAs and using LASSO Cox regression analysis, a prognostic model comprised of 8 disulfidptosis-related lncRNAs was further established (Figures 1C, D). Then we assigned each patient a risk score per the formula of the prognostic model: risk score = (0.433 * KTN1-AS1 expression value (EV)) + (0.099 * AL365181.3 EV) + (0.274 * MANCR EV)—(0.604 * LINC01352 EV)—(0.404 * AC090559.1 EV) + (0.425 * AC093673.1 EV) + (0.374 * AP001094.3 EV)—(0.340 * MHENCR EV). The expression correlations between the 8 lncRNAs and 25 disulfidptosis-related genes were shown in Figure 1E, AC093673.1 and AL365181.3 showed positive associations while AP001094.3 and MHENCR showed negative correlations with most disulfidptosis-related genes. According to the median risk score, the training group was divided into high-risk and low-risk groups. As expected, patients in the high-risk group had shorter overall survival time, demonstrating the prognostic significance of the 8 lncRNAs-based model (Figure 2A). Similar survival analysis results were observed in the test group and after combination of the two groups (Figures 2B, C), which indicates that our prognostic model is reliable. Moreover, the 8 disulfidptosis-related lncRNAs exhibited consistent expression patterns per the risk scores between the training and test groups (Figure 2D).

We merged the training and test groups into one and divided it into two groups. Each patient's risk score and survival state were shown in Figures 2E, F a high risk score was positively correlated with an increased probability of death. In addition to predicting dismal overall survival, a high risk score also indicated poor progression-free survival (Figure 2G).

The disulfidptosis-related lncRNA model is an independent prognostic indicator

We next asked whether our prognostic model was interfered by other clinical factors. We enrolled four clinical features of LUAD patients, including age, gender, tumor stage and risk score, for Cox regression analysis. According to univariate analysis, tumor stage (HR: 1.639, 95% CI:1.426–1.884, $p < 0.001$) and the 8 disulfidptosis-

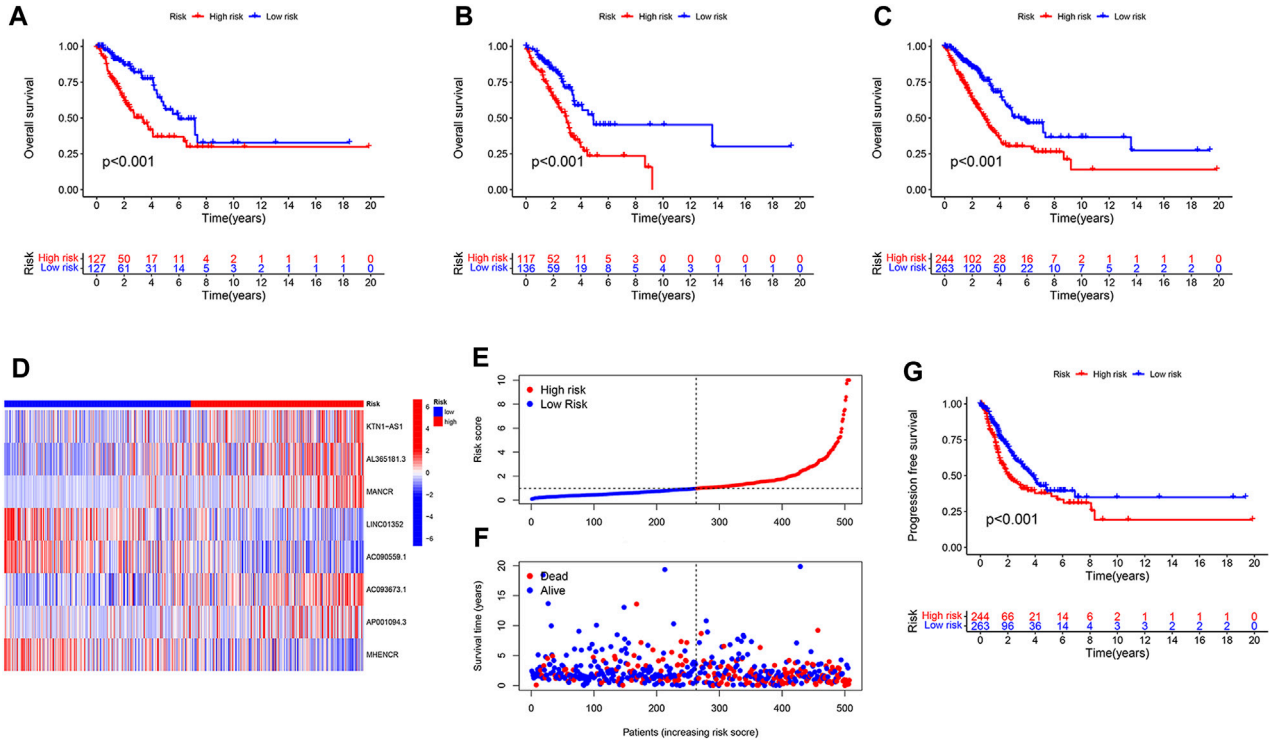


FIGURE 2
Evaluation and verification of the prognostic value of the disulfidptosis-related lncRNA model. **(A,B)** Kaplan-Meier curves showing the difference in overall survival between high- and low-risk LUAD patients in the training group **(A)** and in the test group **(B)**. **(C,D)** Kaplan-Meier curves showing the difference in overall survival **(C)** and progression free survival **(D)** in the combined LUAD cohort with high-risk and low-risk. **(E)** The risk score of each LUAD patient ordered from low to high is shown. **(F)** Survival status of LUAD patients that are ordered from low to high according to the risk score. **(G)** Heatmap showing expression of the eight disulfidptosis-related lncRNAs in LUAD patients with high-risk or low-risk.

related lncRNAs-based risk score (HR: 1.225, 95% CI: 1.155–1.299, $p < 0.001$) are two hazardous factors that affect prognosis (Figure 3A). Moreover, the multivariate Cox regression analysis suggested that risk score (HR: 1.245, 95% CI: 1.167–1.328, $p < 0.001$), together with tumor stage (HR: 1.647, 95% CI: 1.428–1.900, $p < 0.001$), are independent prognostic factors (Figure 3B). To further evaluate the predictive accuracy of the lncRNA-based prognostic model, ROC curve analysis was performed. The AUC values for 1-, 3-, and 5-year survival are 0.703, 0.673, and 0.654, respectively, indicating high accuracy of our prognostic model (Figure 3C). Of note, our prognostic model is almost as accurate as tumor stage in prognosis prediction, as reflected by similar AUC values (0.673 versus 0.687) (Figure 3D). These results suggest that our disulfidptosis-related lncRNAs-based model can serve as an independent and accurate prognostic indictor.

The practicability of the lncRNA-based model in prognosis prediction of LUAD patients with the same disease stage.

Since both our prognostic model and tumor stage are good survival predictors, we wondered what are the advantages of our model as compared with tumor stage in terms of prognosis prediction. To that end, LUAD patients were divided into early stage group (stage I or II) and advanced stage group (stage III or IV) according to the disease stage. It was found that in both groups, LUAD patients with high risk scores had a poorer overall survival rate than patients with low risk scores (Figures 3E, F). These results suggest that our prognostic model can distinguish between patients at high and low risk, even at the same disease stage.

Involvement of the disulfidptosis-related lncRNA model in immune regulation

To further gain insights into the biological differences between the high- and low-risk groups, we performed differentially expressed gene analysis and identified 643 DEGs between the two groups. GO enrichment analysis results indicated that these DEGs are associated with microtubule-based movement, humoral immune response, cilium movement, and other biological processes (Figure 4A). KEGG pathway analysis showed that the DEGs are involved in systemic lupus erythematosus and neutrophil extracellular trap formation (Figure 4B). Furthermore, GSEA that incorporates transcriptome data was carried out. Our analysis showed that nucleosome assembly, DNA packaging complex, nucleosome, protein DNA complex, and structural constituent of chromatin are the top five significantly enriched terms in the high-risk group, while in the low-risk group, B cell receptor signaling pathway, complement activation, immunoglobulin complex, T cell receptor complex, and immunoglobulin receptor binding are the top five significantly enriched cellular processes (Figures 4C, D).

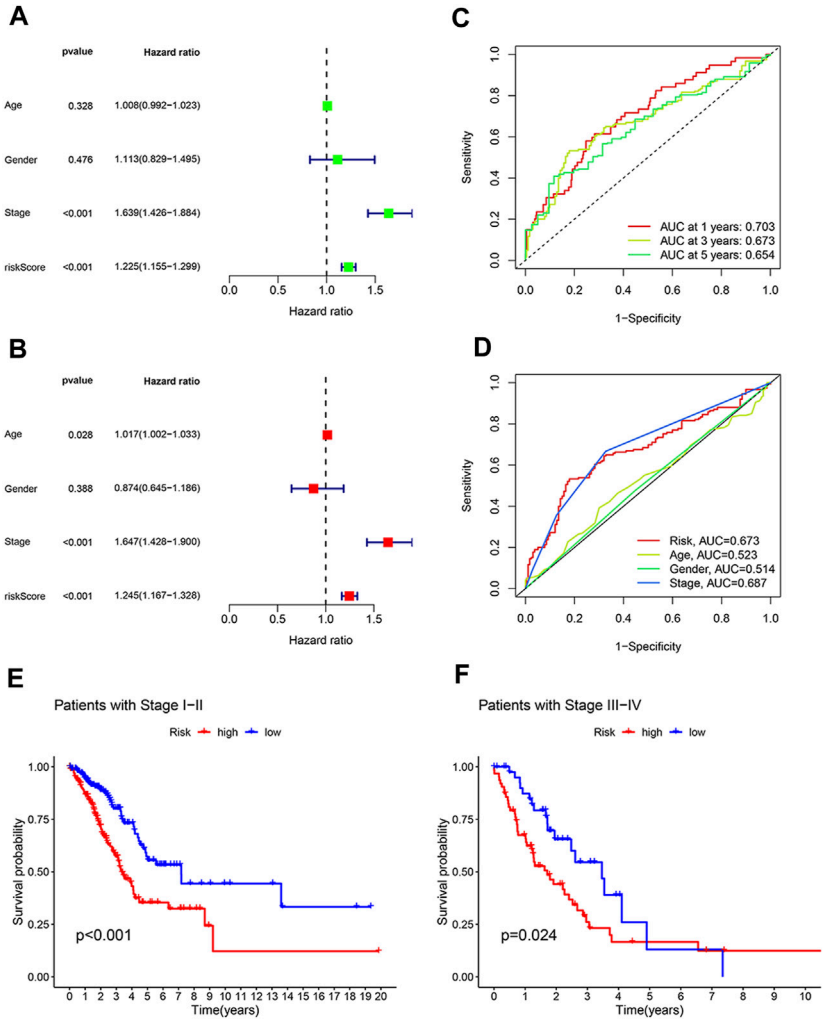
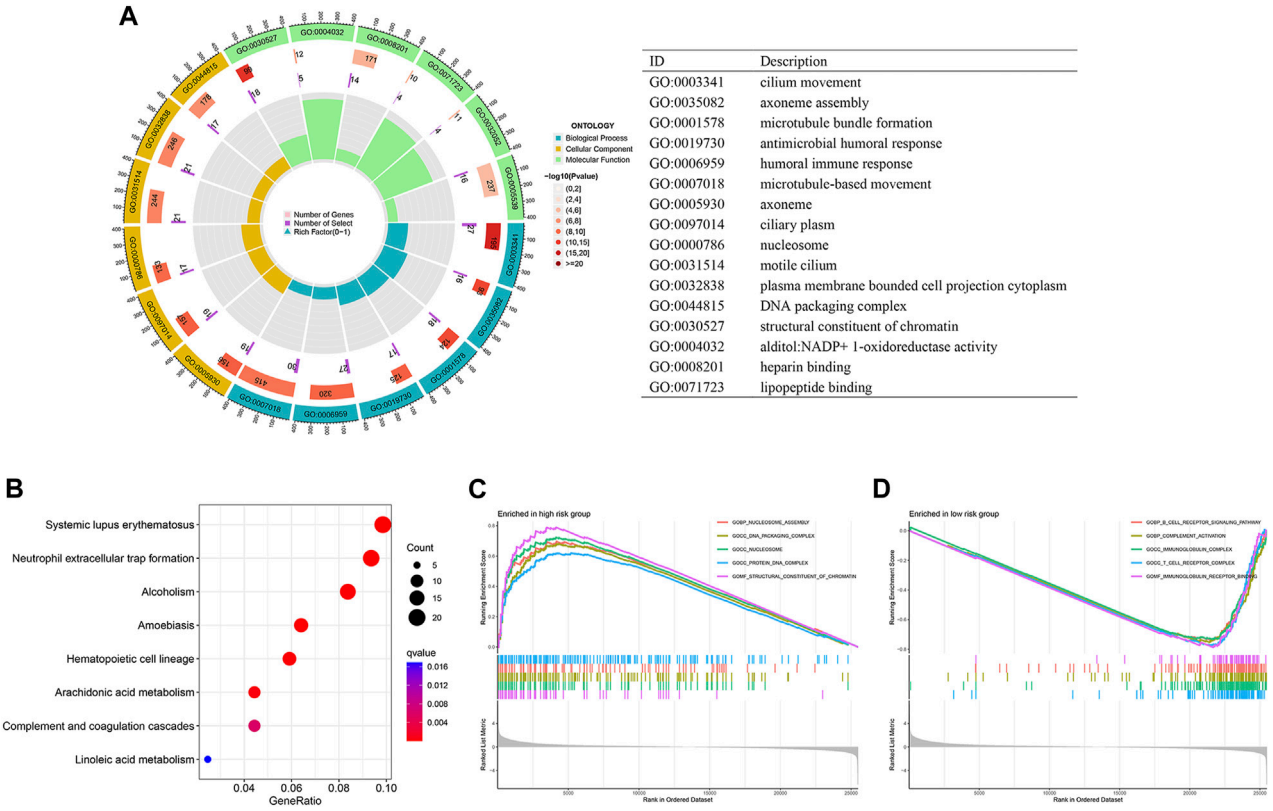


FIGURE 3
The model based on eight disulfidptosis-related lncRNAs is an independent prognosis indicator with high accuracy. **(A)** Forest plot showing prognostic value of age, gender, tumor stage and the model-derived risk score according to univariate regression analysis. **(B)** Forest plot showing tumor stage and our model-derive risk score are independent prognostic factors based on multivariate regression analysis. **(C)** The prognostic accuracy of our model-derived risk score for predicting 1-year, 3-year, and 5-year survival. **(D)** The accuracy of risk score, tumor stage, age and gender in predicting LUAD patients' survival. **(E,F)** Kaplan-Meier curves showing the difference in overall survival between high- and low-risk LUAD patients at early stages **(E)** and at advanced stages **(F)**.

Tumor immune microenvironment plays key roles in determining tumor progression. Considering the above GSEA result that showed enrichment of immune related processes in the low-risk group, we speculated that the tumor immune microenvironment is different between the high- and low-risk LUAD groups. According to the ESTIMATE algorithm, the immune scores are significantly lower in the high-risk group than in the low-risk group (Figure 5A), indicating less infiltration of immune cells in high-risk LUAD. We next used CIBERSORT approach to investigate the abundances of diverse immune cell types in the LUAD tissues. As shown in Figure 5B, high-risk LUADs have less infiltration of monocytes, resting Dendritic cells and resting mast cells, but increased infiltration of M0 macrophages. In addition, we analyzed multiple immune functions between the two LUAD groups. Strikingly, among the 29 kinds of immune functions, 25 showed lower function scores in high-risk LUADs than in low-risk LUADs, such as B cells, $CD8^+$ T cells, and cytolytic activity (Figure 5C). Together, these results suggest that high-risk LUADs, as classified by our disulfidptosis-related lncRNA model, may have compromised immune responses in their tumor microenvironment, resulting in tumor progression and worse overall survival.

Mutational landscape of the two LUAD groups classified by the disulfidptosis-related lncRNA model

TMB, the number of somatic mutations per megabase of genomic sequence, is a potential predictive biomarker in many solid tumors (Sha et al., 2020). We analyzed and compared gene mutation frequency and TMB between the two LUAD groups. High-risk LUADs exhibited significant higher TMB than low-risk LUADs



ID	Description
GO:0003341	cilium movement
GO:0035082	axoneme assembly
GO:0001578	microtubule bundle formation
GO:0019730	antimicrobial humoral response
GO:0006959	humoral immune response
GO:0007018	microtubule-based movement
GO:0005930	axoneme
GO:0097014	ciliary plasm
GO:0000786	nucleosome
GO:0031514	motile cilium
GO:0032838	plasma membrane bounded cell projection cytoplasm
GO:0044815	DNA packaging complex
GO:0030527	structural constituent of chromatin
GO:0004032	alditol:NADP+ 1-oxidoreductase activity
GO:0008201	heparin binding
GO:0071723	lipopeptide binding

FIGURE 4
Differential function enrichment between the high- and low-risk LUAD patients. **(A,B)** GO enrichment **(A)** and KEGG **(B)** analysis of differentially expressed genes in the two LUAD groups. **(C,D)** GSEA results showing cellular processes significantly enriched in high-risk LUADs **(C)** and low-risk LUADs **(D)**.

(Figure 6A). The TMB score of each LUAD sample and the top 20 most frequently mutated genes as well as their mutation types were shown in Figures 6B, 6C. Mutation frequencies of almost all of these genes were higher in high-risk LUADs than in low-risk LUADs. *TP53* and *TTN* were mutated in over half of the high-risk LUADs. High TMB can bring benefits for LUAD patients in their survival, and the overall survival rate is much higher in high TMB patients than in low TMB patients (Figure 6D). We next investigated the effects of risk score and TMB on LUAD patients' overall survival, patients were divided into four subgroups based on these two factors. We found that patients with high TMB and low risk scores exhibit the best prognosis, their 10-year survival rate is around 60%. In contrast, patients with low TMB and high risk scores have the poorest prognosis, the 5-year survival rate is merely about 25%. There are no significant differences in overall survival between high-risk patients with high TMB and low-risk patients with low TMB, and the survival rate of these patients is between the other two subgroups (Figure 6E).

Prediction of sensitivity to immunotherapy and other antitumor drugs

Drug resistance is a major cause of cancer relapse and cancer-related death. Immune checkpoint inhibitors (ICBs) have exhibited impressive therapeutic effects in certain cases of NSCLC. To explore the role of our disulfidptosis-related lncRNA model in predicting response to immunotherapy, we analyzed the correlations between LUAD risk score derived from the model and TIDE score. High-risk LUAD patients have significant lower TIDE scores (Figure 7A), suggesting that immune checkpoint inhibitors are more effective in these patients. Since our analysis showed that high-risk LUADs have high TMB (Figure 6A), our results are in line with previous finding that higher TMB was associated with clinical efficacy of anti-PD-1 therapy (Rizvi et al., 2015). In addition, the associations between LUAD risk score and sensitivity to other antitumor agents were also investigated. Drug sensitivity scores were generated by the calcPhenotype function in the oncoPredict package, based on gene expression data of LUAD patients and preprovided training datasets. As compared with patients in the low-risk group, patients in the high-risk group are less sensitive to diverse types of antineoplastic drugs, including EGFR tyrosine kinase inhibitors (gefitinib, erlotinib, and AZD3759) (Figure 7B), MEK and ERK inhibitors (Trametinib, PD0325901, Ulixertinib and ERK_6604) (Figure 7C), inhibitors of cell cycle-related kinases (AZD7762, BI-2536 and MK-1775) (Figure 7D), MET inhibitors (Savolitinib, Foretinib and Crizotinib) (Figure 7E), and drugs that disturb genome integrity (Talazoparib, AZD6738, VE821 and GDC0810) (Figure 7F). These results suggest that our disulfidptosis-related lncRNA model is a potential tool to predict response of LUAD patients to ICBs and other common antineoplastic drugs.

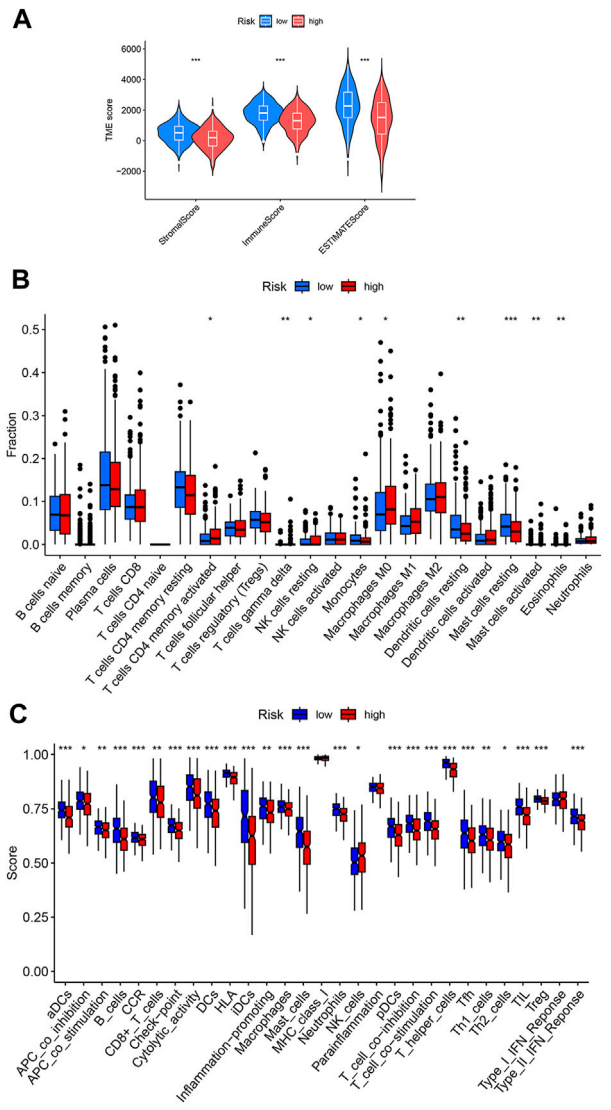


FIGURE 5
Different immune cell infiltration levels in the high- and low-risk LUADs. **(A)** Violin plots showing the differences of stromal and immune scores between the high-risk and low-risk groups. **(B)** Differences in infiltration degrees of 22 immune cell types in the tumor microenvironment of high- and low-risk LUADs. **(C)** Differences in diverse immune functions activities in the tumor microenvironment of high- and low-risk LUADs. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Discussion

Disulfidptosis is characterized by aberrant disulfide bonding among actin cytoskeleton proteins and subsequent actin network collapse due to cystine overload and NADPH shortage (Liu et al., 2023). Considering cancer cell vulnerability to disulfidptosis, targeting this newly identified cell death form is suggested as a potential therapeutic strategy in cancer treatment. Disulfidptosis-based signature can predict prognosis in various tumor types, including bladder cancer and hepatocellular carcinoma (Chen et al., 2023; Wang et al., 2023). LncRNAs play an important role in regulating malignant behaviors of tumor cells and have been demonstrated as potential biomarkers and targets for cancer diagnosis and treatment (Chi et al., 2019). Hitherto, lncRNAs related to disulfidptosis remain largely unknown and their prognostic significance in LUAD are also unclear. In this study, we identified lncRNAs that exhibit expression correlations with disulfidptosis-related genes, and established a prognostic model for LUAD patients comprised of eight disulfidptosis-related lncRNAs.

Our study identified 127 disulfidptosis-related lncRNAs, and those associated with LUAD patients' overall survival were screened out for model construction. A risk score model containing eight 8 disulfidptosis-related prognostic lncRNAs was established using LASSO regression analysis. Its predictive efficacy was evaluated in the training and test groups comprising over 500 LUAD patients. A high risk score derived from the model is an indicator for poor overall survival and progression free survival. Tumor stage reflects disease progression and severity. As expected, our analysis showed that tumor stage is an independent prognostic factor for LUAD. Similar to tumor stage, our lncRNA model-derived risk score was proved as a factor with independent prognostic value, and our model is as sensitive as tumor stage to predict three- and 5-year survival. Moreover, one advantage of our model, compared with tumor stage, is that it can distinguish between high- and low-risk patients that are at the same disease stage. Hence, this disulfidptosis-related lncRNA model is an accurate and reliable prognostic predictor for LUAD patients.

Crosstalk between immune cells and tumor cells within the tumor microenvironment have a profound influence on the fate of the later. The quantity and quality of tumor-infiltrating lymphocytes are key factors that forecast prognostic and therapeutic benefits in many types of cancer, such as oral squamous cell carcinoma, HER2-positive breast cancer, and epithelial ovarian cancer (Salgado et al., 2015; Hwang et al., 2019; Shaban et al., 2019; Paijens et al., 2021). In our study, we found that high- and low-risk LUAD patients have different immune activities and immune cell infiltration degrees. High risk scores had significant negative associations with T cell receptor complex, B cell receptor signaling pathway, and immunoglobulin complex. T cell receptors are required for effective antitumor immune responses through participating in tumor antigen recognition and T cell activation (Zhong et al., 2013). B cells mediate humoral immunity and can inhibit tumor growth by secreting immunoglobulins (Wang et al., 2019b). These results indicate a reduced antitumor immune activity in high-risk LUADs. Besides, high-risk LUAD patients had lower immune scores that represent reduced infiltration of immune cells within the tumor microenvironment, according to the ESTIMATE analysis results. Except NK cells that showed a higher function score in the high-risk LUADs, B cells, $CD8^+$ T cells, Dendritic cells and macrophages displayed significant lower function scores in these LUADs. Based on these findings, we reason that reduced immune cell infiltration and activity lead to poor prognosis of LUAD patients, and these patients can be distinguished by our disulfidptosis-related lncRNA model.

Despite that immune checkpoint inhibitors demonstrate remarkable survival benefit in NSCLC patients, only a minority of patients respond to them (Dong et al., 2017; Marinelli et al., 2020). Therefore, we wondered whether this

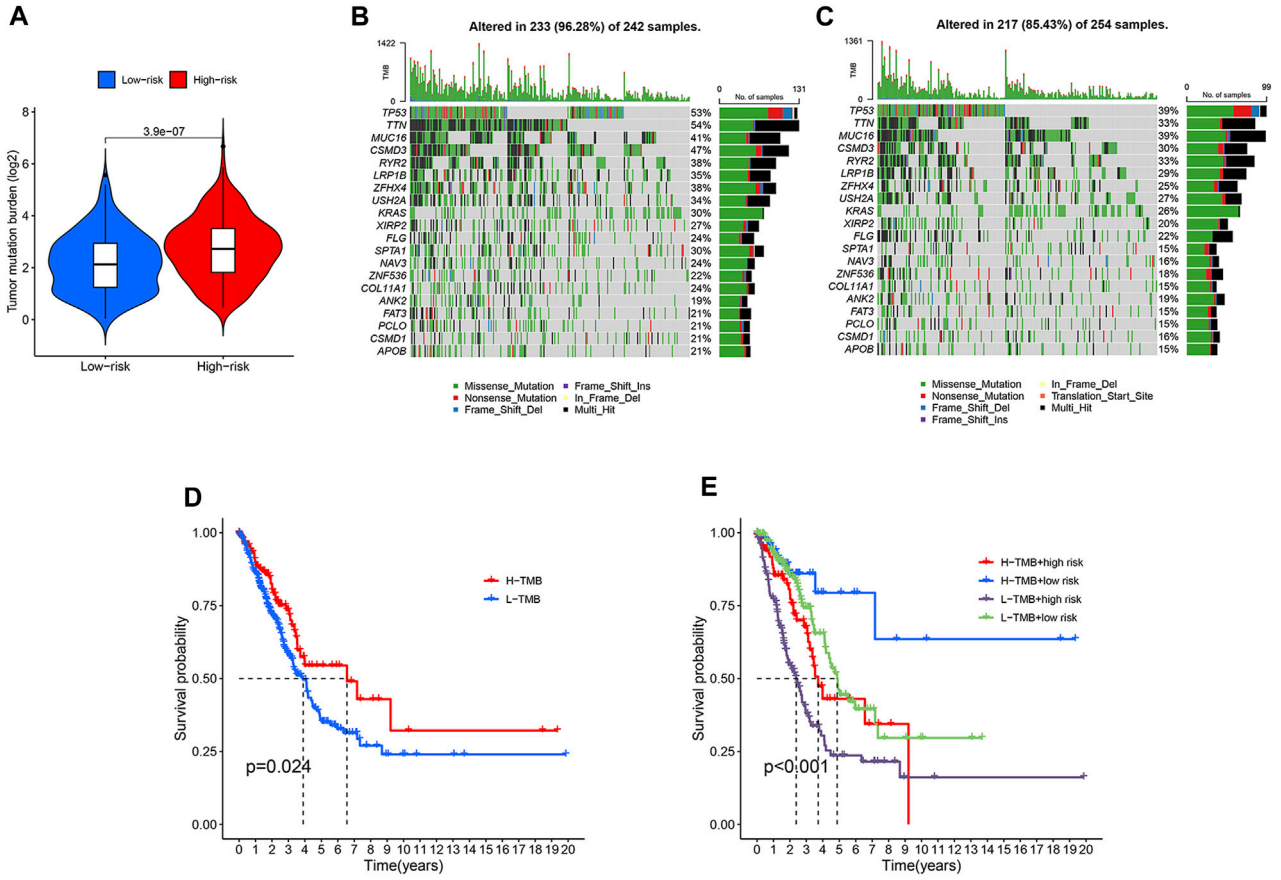


FIGURE 6
Differential tumor mutational burden and somatic mutation frequencies in the high- and low-risk LUADs. **(A)** Violin plot embedded with box plot showing the difference in the tumor mutational burden between the high-risk and low-risk LUADs. **(B,C)** Mutation frequencies of the top 20 most frequently mutated genes in the high-risk **(B)** and low-risk **(C)** LUADs were shown in the waterfall plots. The upper histograms of the plots represent tumor mutational burden of each sample. The mutation types of each gene are indicated with different colors and the right histograms show the sample number with a certain mutation type of the corresponding genes. **(D)** Kaplan-Meier curves showing the difference in overall survival between LUAD patients with high and low tumor mutational burden. **(E)** Kaplan-Meier curves of overall survival of the four subgroups that are classified based on different tumor mutational burden and different risk score derived from the lncRNA model.

disulfidptosis-related lncRNA model can be used as a predictive marker for clinical response to ICBs in LUAD patients. It turned out that high-risk patients had lower TIDE scores, suggesting that these patients are more likely to benefit from immune checkpoint blockade. This may be explained by higher TMB in high-risk patients, as a high TMB is considered as an indicator for better response to immunotherapy (Rizvi et al., 2015; Hellmann et al., 2018). In contrast, we found that high-risk patients are less sensitive to other antitumor therapies, such as EGFR tyrosine kinase inhibitors, MEK/ERK inhibitors, MET inhibitors, and drugs that disturb genome integrity and cell cycle progression.

Somatic driver mutation is a major cause of tumorigenesis and tumor progression. As higher TMB was found in high-risk LUADs, we further investigated genes with high mutation frequencies and compared their differences between the two LUAD groups. Of the 20 most frequently mutated genes, 19 had elevated mutation frequencies in the high-risk group. In the high-risk group there were 10 genes with a mutation frequency greater than or equal to 30%, while in the low-risk group there were only 5. With a mutation frequency of 54% in the high-risk LUADs, *TTN* was the most frequently mutated gene, which may account for the higher TMB in the high-risk group since *TTN* mutations represent high TMB (Oh et al., 2020). Mutation of the tumor suppressor *TP53* gene is among the most common genetic alterations in cancer, which were observed in 53% of patients in the high-risk group. We found higher mutation frequencies of oncogenes such as *MUC16* (Kanwal et al., 2018) and *KRAS* (Tomasini et al., 2016) in high-risk LUADs, which may be another cause, other than reduced immune cell infiltration and activity, of poor prognosis of high-risk LUAD patients.

In conclusion, we identified disulfidptosis-related lncRNAs, based on eight of which we established and validated a prognostic model that can predict independently overall survival of LUAD patients, reflect their immune activity within the tumor microenvironment, and forecast response to immunotherapy, targeted therapy and chemotherapy. This study provides preliminary insights into the association between disulfidptosis and tumor immune response. There are certain limitations in our

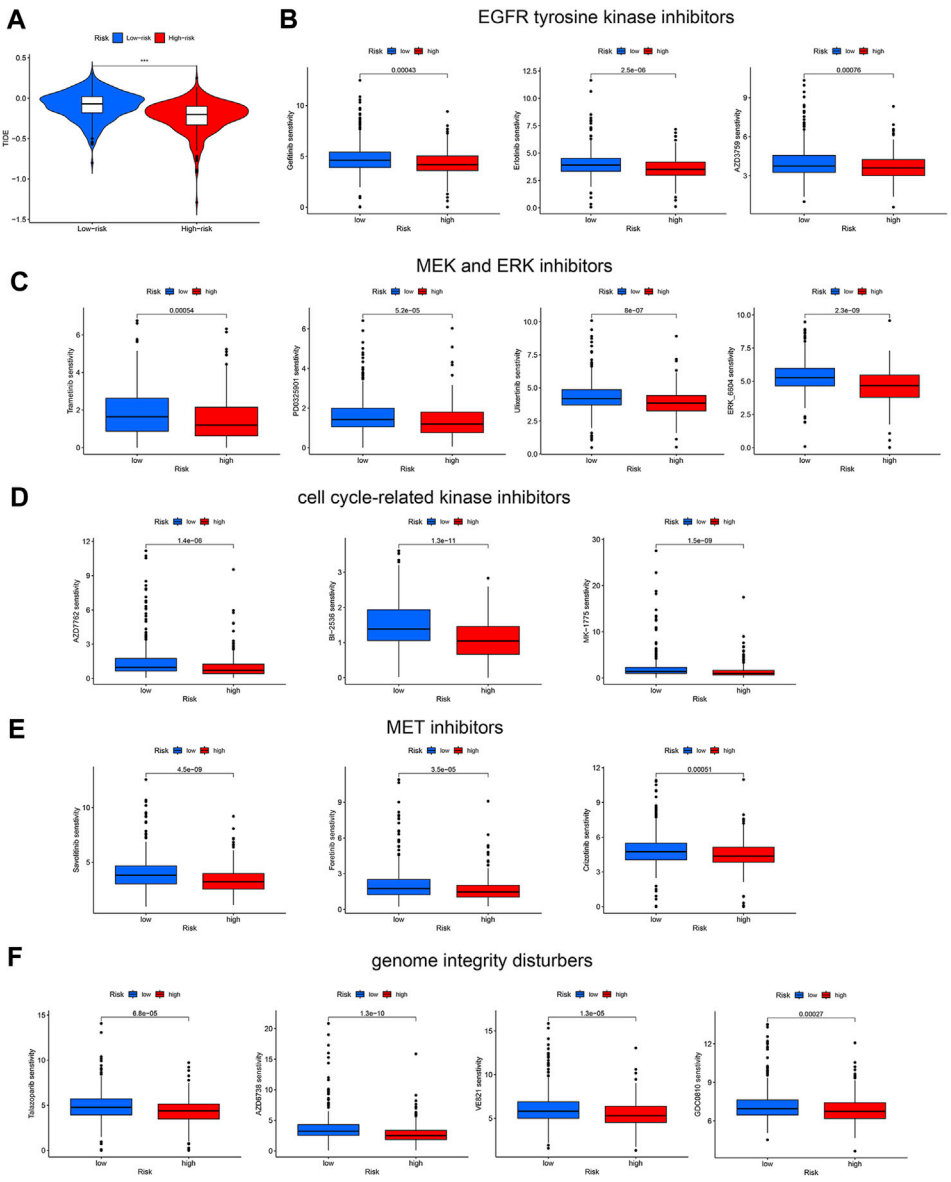


FIGURE 7
Prediction of response of the two LUAD patient groups to immune checkpoint blockade and other antitumor therapies. **(A)** Violin plots showing the difference in TIDE scores between the high-risk and low-risk LUAD groups. **(B–E)** Different sensitivities of the two LUAD groups to kinase inhibitors, including EGFR tyrosine kinase inhibitors **(B)**, MEK and ERK inhibitors **(C)**, cell cycle-related kinase inhibitors **(D)**, and MET inhibitors **(E)**. **(F)** Different sensitivities of the two LUAD groups to drugs disturbing genome integrity. ***, $p < 0.001$.

study. Although the prognostic model has been verified and its accuracy evaluated in over 500 LUAD samples, only TCGA RNA-seq data were used for analysis. Further verification of this model by transcriptome data of other independent LUAD cohorts is needed, and it also remains to be determined whether this model is appliable to data generated by other platforms. In addition, despite that the eight lncRNAs used for model construction show expression correlations with one or more disulfidptosis-related genes, their exact roles in regulating disulfidptosis need further research. Also, the specific molecular mechanisms of disulfidptosis-related lncRNAs in regulating the prognosis of LUAD patients and their response to antitumor therapies remains experimental exploration.

Data availability statement

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

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study

was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

H-BZ: Writing–review and editing, Data curation, Formal Analysis, Writing–original draft. J-YP: Writing–original draft, Writing–review and editing, Conceptualization. TZ: Conceptualization, Writing–review and editing, Funding acquisition, Supervision.

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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/fphar.2023.1254119/full#supplementary-material

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Unlocking the role of non-coding RNAs in prostate cancer progression: exploring the interplay with the Wnt signaling pathway

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Prostate cancer (PCa) is one of the most common cancers in males, exhibiting a wide spectrum of clinical manifestations that pose challenges in its diagnosis and treatment. The Wnt signaling pathway, a conserved and complex pathway, is crucial for embryonic development, tissue homeostasis, and various physiological processes. Apart from the classical Wnt/β-catenin signaling pathway, there exist multiple non-classical Wnt signaling pathways, including the Wnt/PCP and Wnt/Ca^{2+} pathways. Non-coding RNAs (ncRNAs) are involved in the occurrence and development of PCa and the response to PCa treatment. ncRNAs are known to execute diverse regulatory roles in cellular processes, despite their inability to encode proteins. Among them, microRNAs, long non-coding RNAs, and circular RNAs play key roles in the regulation of the Wnt signaling pathway in PCa. Aberrant expression of these ncRNAs and dysregulation of the Wnt signaling pathway are one of the causes of cell proliferation, apoptosis, invasion, migration, and angiogenesis in PCa. Moreover, these ncRNAs affect the characteristics of PCa cells and hold promise as diagnostic and prognostic biomarkers. Herein, we summarize the role of ncRNAs in the regulation of the Wnt signaling pathway during the development of PCa. Additionally, we present an overview of the current progress in research on the correlation between these molecules and clinical features of the disease to provide novel insights and strategies for the treatment of PCa.

KEYWORDS

non-coding RNA, miRNA, lncRNA, circRNA, Wnt signaling pathway, prostate cancer

1 Introduction

In males, prostate cancer (PCa) is one of the most common cancers and the fifth leading cause of cancer-related deaths (Sung et al., 2021). According to estimates for 2020, the worldwide incidence of PCa was 1.4 million new cases, with more than 375,000 males dying owing to the disease (Sandhu et al., 2021). PCa is a complex and heterogeneous disease, exhibiting a wide range of clinical manifestations, ranging from indolent to aggressive (He et al., 2022). Thus, investigating the mechanisms of the occurrence and development of PCa is crucial for its diagnosis and treatment. Although the molecular mechanisms underlying PCa progression remain unclear, genetic alterations and signaling pathways have been found to play key roles (Wang et al., 2022b).

The Wnt signaling pathway is a conserved pathway that plays crucial roles in embryonic development, tissue homeostasis, and stem cell maintenance (Zhou et al., 2022a). The Wnt

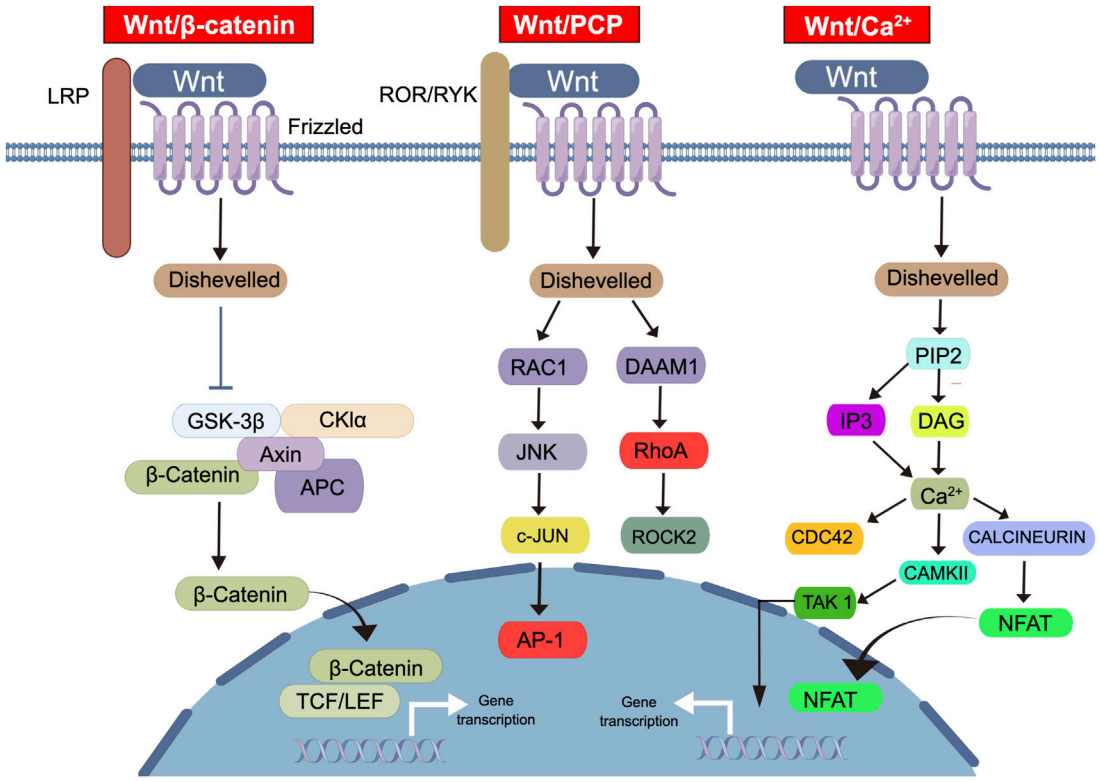

FIGURE 1
The diverse types and molecular mechanisms of the Wnt signaling pathway. This diagram illustrates the classification and members of the Wnt signaling pathway. The Wnt signaling pathway can be categorized into three classes: (1) Wnt/β-catenin signal transduction, (2) Wnt/PCP signal transduction, (3) Wnt/Ca^{2+} signal transduction. In the Wnt/β-catenin pathway, Wnt ligands bind to Frizzled receptors and LRP co-receptors, leading to the activation of the disheveled protein and subsequent inactivation of Axin protein. This results in the accumulation of β-catenin in the cytoplasm, its translocation into the nucleus, and binding to TCF/LEF transcription factors, ultimately inducing downstream gene expression. Wnt/PCP signaling pathway involved in cell polarity, tissue morphogenesis, cell adhesion, and directional migration through JNK and Rho GTPase signaling pathways. The Wnt/Ca^{2+} signaling pathway modulates gene expression and cell behavior by regulating intracellular Ca^{2+} levels. Abbreviations: Wnt, Wingless-related integration site; LRP, Low-density lipoprotein receptor-related protein; GSK3β, Glycogen Synthase Kinase 3 beta; CKIα, Casein kinase I alpha; Axin, Axis inhibitor; APC, Adenomatous Polyposis Coli; TCF, T-cell factor; LEF, Lymphoid enhancer-binding factor; RAC1, Ras-related C3 botulinum toxin substrate 1; DAMM1, Disheveled-associated activator of morphogenesis 1; JNK, c-Jun N-terminal kinase; RhoA, Ras homolog family member A; c-JUN, Cellular Jun oncogene; ROCK2, Rho-associated coiled-coil kinase 2; AP-1, Activator protein 1; PIP2, Phosphatidylinositol 4,5-bisphosphate; IP3, Inositol trisphosphate; DAG, Diacylglycerol; CDC42, Cell division control protein 42 homolog; TAK1, Transforming growth factor-beta-activated kinase 1; CAMKII, $Ca2^{+}$/calmodulim-dependent protein kinase II; NFAT, Nuclear factor of activated T-cells.

signaling pathway can be categorized into three classes: 1) Wnt/β-catenin signal transduction, 2) Wnt/PCP signal transduction, 3) Wnt/Ca^{2+} signal transduction (Asano et al., 2022). Dysregulation of the Wnt signaling pathway is associated with many diseases, including cancer (Yeh et al., 2019). In PCa, aberrant activation of the Wnt signaling pathway leads to dysregulation of cell proliferation, apoptosis, invasion, and angiogenesis (Koushyar et al., 2022). Understanding the mechanisms underlying Wnt signaling dysregulation can provide valuable insights into the pathogenesis of PCa and elucidate potential therapeutic targets.

Non-coding RNAs (ncRNAs) have been recognized as critical regulatory factors in gene expression and signal transduction in both normal physiology and disease pathogenesis, particularly in PCa (Ferri et al., 2022; Szaflik et al., 2022). ncRNAs are known to execute diverse regulatory roles in cellular processes, despite their inability to encode proteins (Wang et al., 2022a). Recent studies have elucidated several types of ncRNAs, such as microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), which play key roles in the regulation of the Wnt signaling pathway in PCa (Goodall and Wickramasinghe, 2021; Xue et al., 2022). The alterations in the expression patterns of these ncRNAs at distinct stages of PCa progression indicate their potential as diagnostic and prognostic biomarkers (He et al., 2020; Song et al., 2020). For example, the high expression of the lncRNA CCAT2 and SOX2-OT is associated with the diagnosis and prognosis of PCa, indicating their potential utility as biomarkers (He et al., 2020; Song et al., 2020). Moreover, these ncRNAs affect the characteristics of PCa cells such as proliferation, invasion, migration, and apoptosis; additionally, these ncRNAs can influence the therapeutic response of cancer cells by regulating the Wnt signaling pathway (He et al., 2020; Song et al., 2020; Wang et al., 2021). Therefore, Wnt signaling pathway-related ncRNAs are promising prospects as therapeutic targets for PCa.

In conclusion, our review presents a comprehensive elucidation of the intricate interplay between ncRNAs and the Wnt signaling pathway in the context of PCa occurrence, progression, and therapeutic approaches. Significantly, we underscore the profound implications of ncRNAs as promising diagnostic and prognostic biomarkers, accentuating their pivotal role in modulating PCa aggressiveness and

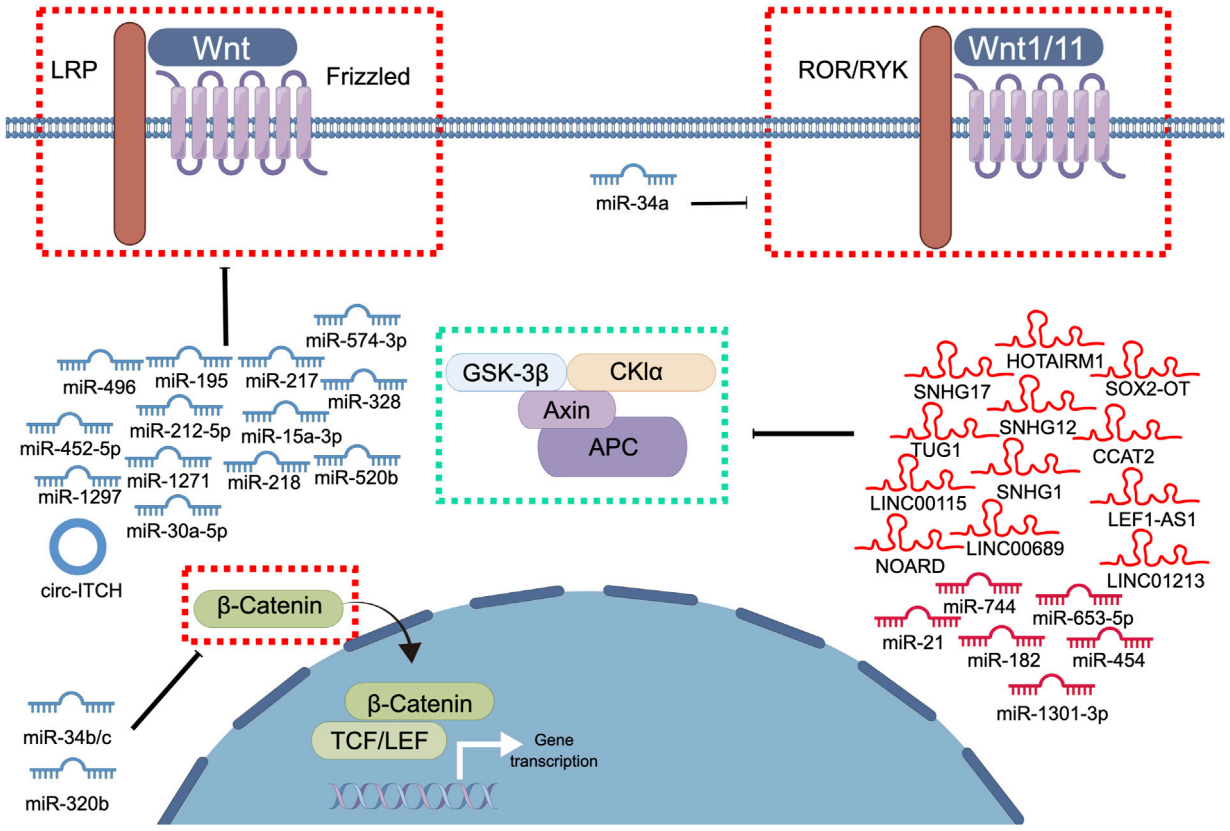

FIGURE 2
The involvement of ncRNAs in modulating the Wnt signaling pathway in prostate cancer. This diagram illustrates the involvement of ncRNAs in modulating the Wnt signaling pathway in PCa. Blue represents the role of suppressing the Wnt signaling pathway. Red represents roles that facilitate the Wnt signaling pathway.

therapeutic response. Moreover, we delve deep into the immense potential of targeting ncRNAs as therapeutic interventions for PCa, exploring a plethora of strategic avenues. Finally, we address the contemporary challenges encountered in this ever-evolving field.

2 Wnt signaling pathway and its role in PCa development

The Wnt signaling pathway is a complex intracellular signaling network that is involved in the regulation of cell fate, proliferation, and differentiation in many tissues (Figure 1) (Asano et al., 2022; Zhou et al., 2022a). Dysregulation of this pathway is associated with the occurrence and progression of many cancers, including PCa (Nusse and Clevers, 2017; Pisano et al., 2021). The Wnt signaling pathway can be categorized into the following two types: canonical and non-canonical (Rim et al., 2022). The canonical Wnt/β-catenin pathway operates through a mechanism whereby the binding of the Wnt protein to its receptor Frizzled triggers the activation of the Disheveled protein, leading to the inactivation of Axin protein and ultimately reducing the degradation of β-catenin (Rim et al., 2022). Consequently, β-catenin gradually accumulates in the cytoplasm and subsequently translocates into the nucleus, where it binds to TCF/LEF transcription factors to induce downstream gene expression (Rim et al., 2022). The non-canonical pathway includes the Wnt/PCP and Wnt/Ca^{2+} signaling pathways (Menck et al., 2021; Sarabia-Sanchez et al., 2023; VanderVorst et al., 2023). The Wnt/PCP signaling pathway is primarily involved in the regulation of cell polarity and tissue morphogenesis (VanderVorst et al., 2023). Additionally, it regulates cell adhesion and directional migration by activating the JNK and Rho GTPase signaling pathways (VanderVorst et al., 2023). The Wnt/Ca^{2+} signaling pathway primarily modulates gene expression and cell behavior by regulating intracellular Ca^{2+} levels (Sarabia-Sanchez et al., 2023). Moreover, it plays a critical role in embryonic nervous system development, cell polarity, and glial cell differentiation (Sarabia-Sanchez et al., 2023). The Wnt/Ror signaling pathway was recently discovered, and its function remains to be comprehensively elucidated (Menck et al., 2021). However, it has been shown to induce the formation and repair of synapses in neurons through certain Wnt ligands (Menck et al., 2021).

The Wnt signaling pathway is activated through the interaction of ligands and receptors on cell surfaces, leading to subsequent signal transduction through intracellular signaling molecules such as β-catenin and TCF/LEF transcription factors (Zhou et al., 2022b). In PCa, aberrant activation of the Wnt signaling pathway leads to dysregulation of cell proliferation, apoptosis, invasion, and angiogenesis (Khurana and Sikka, 2019; Lin et al., 2020). This dysregulation often results from mutations or alterations in key components of the Wnt signaling pathway, such as adenomatous polyposis coli (APC), Axin, and β-catenin, which promote cell cycle progression by activating cyclin D1 and c-myc and enhancing the induction effect exerted by TGF-β signaling (Reya and Clevers, 2005;

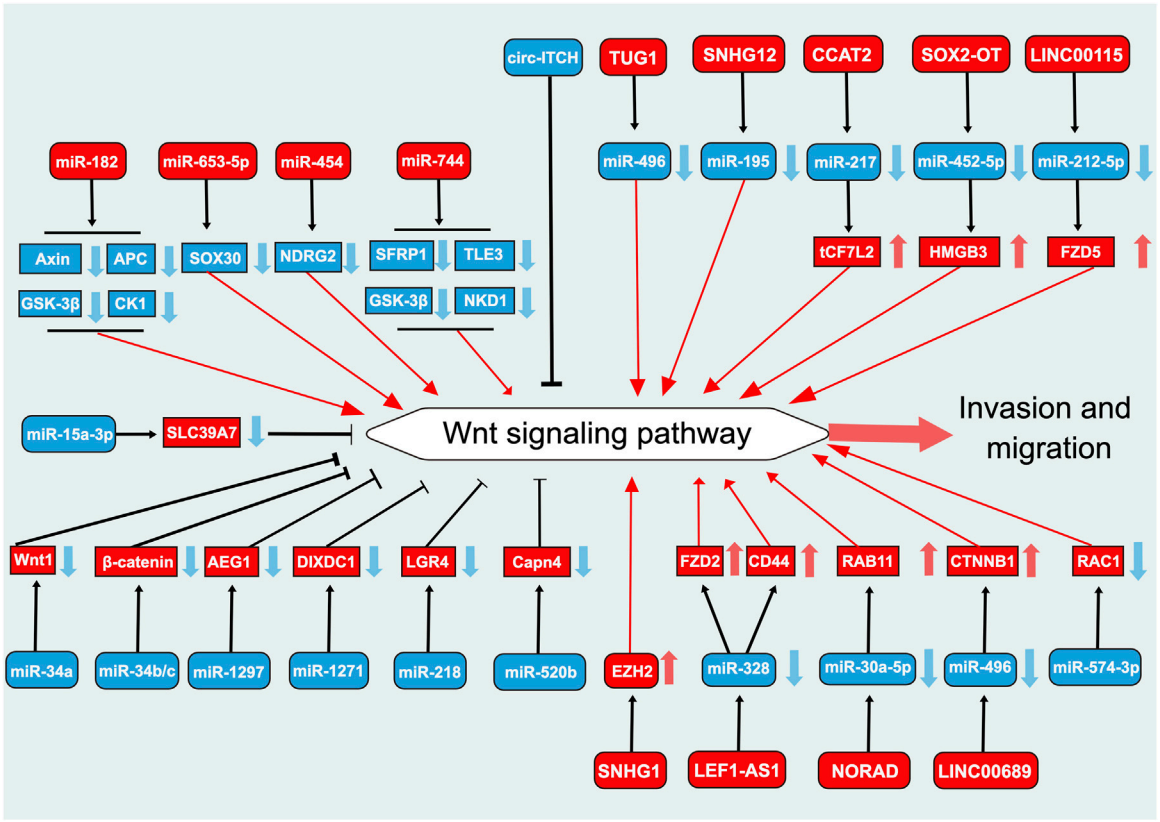

FIGURE 3
Role of Wnt signaling pathway-related ncRNAs in the regulation of invasion and migration in prostate cancer. This diagram illustrates the role of Wnt signaling pathway-related ncRNAs in the regulation of invasion and migration in PCa. Blue represents the role of suppressing the Wnt signaling pathway. Red represents roles that facilitate the Wnt signaling pathway.

Zhong et al., 2020; Yu et al., 2021). In addition, these components possibly promote neovascularization and tumor invasion by increasing the expression of VEGF, MMP-9, and IL-8 (Vallee and Lecarpentier, 2018).

Aberrant activation of the Wnt signaling pathway can serve as a biomarker for PCa (Yeh et al., 2019; Pisano et al., 2021). The expression of β-catenin protein in PCa tissue is significantly increased, and mutations and dysregulation of Wnt signaling pathway-related genes can also lead to the aberrant activation of this pathway (Yeh et al., 2019; Pisano et al., 2021). Distinct mutations in the Wnt signaling pathway genes are associated with specific subtypes of PCa. For example, APC mutations are frequently observed in early-stage and low-grade PCa, whereas β-catenin mutations are observed in advanced-stage and high-grade PCa (Desai et al., 2022; Mangolini et al., 2022). Furthermore, in metastatic PCa, an increased proportion of activation mutations in the Wnt/β-catenin signaling pathway-related genes is observed (Desai et al., 2022; Mangolini et al., 2022). The aberrant activation of the Wnt signaling pathway is also associated with the staging and metastasis of PCa (Yeh et al., 2019; Pisano et al., 2021). The classical Wnt/β-catenin signaling pathway is activated in late-stage PCa and bone metastatic PCa, facilitating cell proliferation and drug resistance (Yeh et al., 2019; Pisano et al., 2021).

Multiple components of the Wnt signaling pathway are potential targets in PCa therapeutic interventions (Koushyar et al., 2022). The inhibition of the Wnt signaling pathway in PCa may be crucial for its prevention and treatment. Few studies have investigated methods for inhibiting the Wnt/β-catenin signaling pathway (Brown, 2005; Park and Kim, 2023). For example, researchers have developed and evaluated the efficacy of Protac/molecular glue, antibody-drug conjugates, and anti-sense oligonucleotides in inhibiting the Wnt signaling pathway in preclinical models and clinical trials (Park and Kim, 2023). Additionally, RNA interference technology has shown promising results in preclinical models (Brown, 2005). However, the investigation of these methods is at its nascent stages, and further investigation is necessary to ascertain their efficacy in clinical treatment. Epigenetic alterations also regulate the activity of the Wnt signaling pathway in PCa (Xiong et al., 2009; Eismann et al., 2023). Alterations in DNA methylation and histone modification patterns frequently occur in PCa cells, leading to alterations in gene expression and the activity of the Wnt signaling pathway (Xiong et al., 2009; Eismann et al., 2023). Therefore, directing therapeutic interventions toward these epigenetic modifications may be a viable approach.

The crosstalk between the Wnt signaling pathway and other signaling pathways, such as the androgen receptor (AR) signaling pathway, is gaining recognition as a key factor in the pathogenesis of PCa (Pisano et al., 2021). This presents a challenge in the targeting of the Wnt signaling pathway in PCa treatment (Pisano et al., 2021). The critical role of AR signaling in PCa progression is well-known, and recent studies

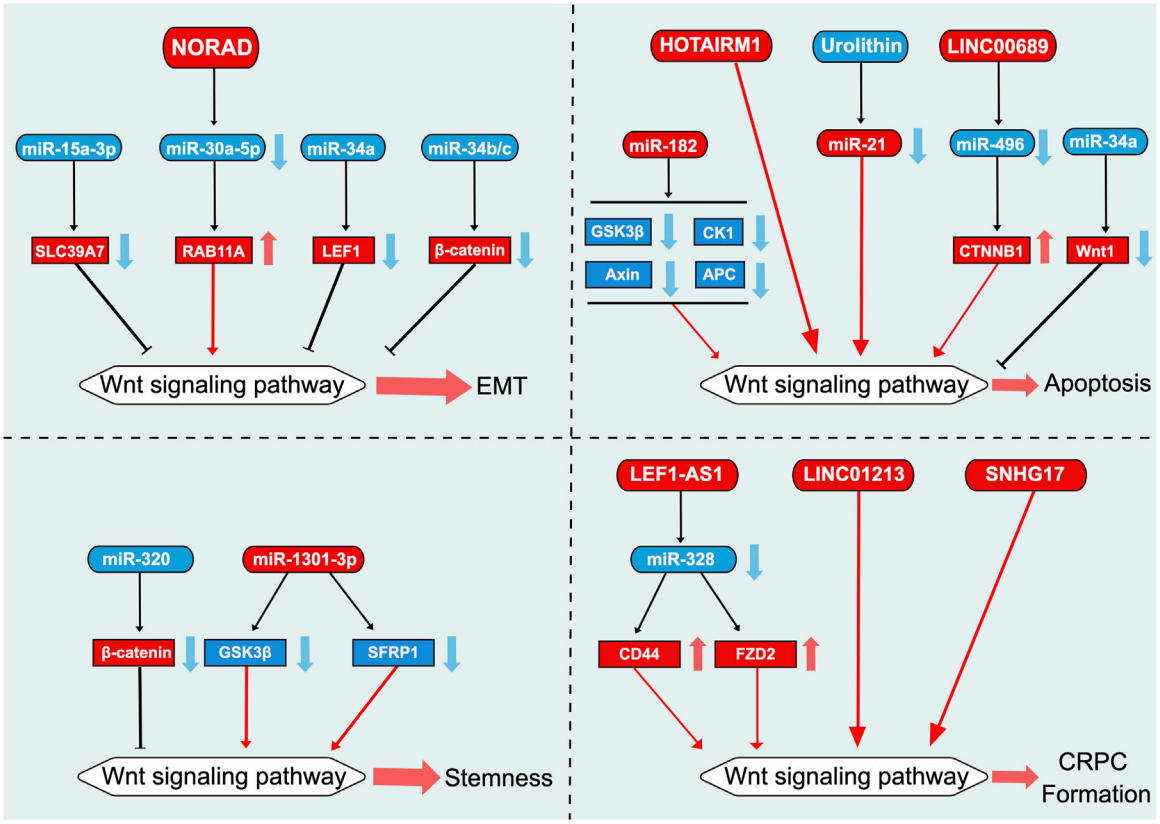

FIGURE 4
Role of Wnt signaling pathway-related ncRNAs in the regulation of EMT, apoptosis, stemness, and CRPC formation in prostate cancer. This diagram illustrates the role of Wnt signaling pathway-related ncRNAs in the regulation of EMT, apoptosis, stemness, and CRPC formation in PCa. Blue represents the role of suppressing the Wnt signaling pathway. Red represents roles that facilitate the Wnt signaling pathway.

indicate that Wnt and AR signals interact in complex ways, regulating each other's activities (Pisano et al., 2021). Thus, disrupting this crosstalk may be necessary to achieve desired treatment outcomes. Overall, a comprehensive examination of the Wnt/β-catenin signaling pathway may provide novel insights and avenues for the diagnosis, treatment, and prevention of PCa.

3 The involvement of NcRNAs in modulating the Wnt signaling pathway in PCa

The dysregulation of ncRNAs has been associated with the occurrence and progression of PCa (Ramnarine et al., 2019). Recent studies have elucidated several types of ncRNAs, such as miRNAs, lncRNAs, and circRNAs, which play crucial roles in PCa by regulating the Wnt signaling pathway (Figure 2). Various types of ncRNAs interact with each other and with other regulatory factors, such as transcription factors, and induce epigenetic modifications, to activate the Wnt signaling pathway (Ramnarine et al., 2019; Orafidiya et al., 2022). For example, the lncRNA SOX2-OT downregulates transcription factor 7-like (TCF7L), a negative regulator of the Wnt signaling pathway, thereby activating the pathway (Song et al., 2020). Similarly, miR-182 regulates the Wnt signaling pathway by targeting APC and glycogen synthase kinase 3 beta (GSK3β), which are two key components of the destruction complex that regulates β-catenin stability (Wang et al., 2018). ncRNAs also activate the Wnt signaling pathway by directly regulating the expression of Wnt signaling pathway-related genes. For example, the lncRNA TUG1 is highly expressed in PCa tissues and cells and promotes cell proliferation, migration, and invasion through the miR-496/Wnt/β-catenin axis (Xiu et al., 2020). miRNAs, such as miR-653-5p, miR-182, miR-1301-3p, and miR-454, also activate the Wnt/β-catenin signaling pathway and promote the malignant progression of PCa cells (Guan et al., 2017; Fu et al., 2018; Wang et al., 2018; Fu et al., 2019). Although many ncRNAs activate the Wnt signaling pathway to promote PCa progression, certain ncRNAs inhibit the Wnt signaling pathway to exert an anti-cancer effect on PCa (Dong et al., 2020). For example, the well-characterized tumor suppressor miR-34a targets multiple Wnt signaling pathway-related genes, including those of Wnt1, LEF1, and β-catenin (Dong et al., 2020).

Despite the aforementioned findings, the molecular mechanism of the regulation of the Wnt signaling pathway by ncRNAs in PCa is not entirely understood, and additional investigations are required to elucidate the complex interplay between various types of ncRNAs and other regulatory factors that activate or inhibit the Wnt signaling pathway in PCa. Furthermore, the investigation of ncRNAs poses technical

TABLE 1 Type and role of Wnt signaling pathway-related ncRNAs in PCa progression.

	ncRNA	Role	Function	Signaling network	Ref.
miRNA	miR-34a	Suppressor	Inhibit proliferation, invasion, migration	Wnt1	Dong et al. (2020)
			Promote apoptosis		
			Inhibit EMT	LEF1	Liang et al. (2015)
			Inhibit proliferation, invasion, migration, and EMT	TCF7, BIRC5	Chen et al. (2015)
	miR-34b/c	Suppressor	Inhibit proliferation, invasion, migration, and EMT	β-catenin	Liu et al. (2015)
	miR-1297	Suppressor	Inhibit proliferation and invasion	AEG-1	Liang et al. (2016)
	miR-1271	Suppressor	Inhibit proliferation and invasion	DIXDC1	Zhong et al. (2017)
	miR-218	Suppressor	Inhibit proliferation and invasion	LGR4	Li et al. (2016b)
	miR-520b	Suppressor	Inhibit proliferation and invasion	Capn4	Ren et al. (2018)
	miR-138	Suppressor	Inhibit proliferation, invasion, and migration	—	Yu et al. (2018)
	miR-574-3p	Suppressor	Inhibit proliferation, invasion, and migration	RAC1	Chiyomaru et al. (2013)
	miR-15a-3p	Suppressor	Inhibit proliferation, invasion, and EMT	SLC39A7	Cui et al. (2019)
	miR-320	Suppressor	Inhibit stem cell- like characteristics	β-catenin	Hsieh et al. (2013)
	miR-653-5p	Oncogene	Promote proliferation and invasion	SOX30	Fu et al. (2019)
	miR-182	Oncogene	Promote proliferation, invasion, and migration	GSK-3β, APC, CK1, and Axin	Wang et al. (2018)
			Inhibit apoptosis		
	miR-454	Oncogene	Promote proliferation and invasion	NDRG2	Fu et al. (2018)
	miR-744	Oncogene	Promote proliferation, invasion, and migration	SFRP1, GSK3β, TLE3, and NKD1	Guan et al. (2017)
	miR-21	Oncogene	Inhibit apoptosis	—	Zhou et al. (2016)
	miR-1301-3p	Oncogene	Promote cancer stem cell expansion	GSK3β and SFRP1	Song et al. (2018)
LncRNA	CCAT2	Oncogene	promote proliferation, invasion, migration	miR-217/TCF7L/Wnt/β-catenin	He et al. (2020)
	TUG1	Oncogene	promote proliferation, invasion, migration	miR-496/Wnt/β-catenin	Li et al. (2020a)
	SOX2-OT	Oncogene	promote proliferation, invasion, migration	miR-452-5p/HMGB3/Wnt/β-catenin	Song et al. (2020)
	SNHG12	Oncogene	promote proliferation, invasion, migration	miR-195/Wnt/β-catenin	Wang et al. (2019)
	LINC00115	Oncogene	promote proliferation, invasion	miR-212-5p/FZD5/Wnt/β-catenin	Peng et al. (2021)
	LINC00689	Oncogene	promote proliferation, invasion, migration	miR-496/CTNNB1/Wnt/β-catenin	Meng et al. (2020)
			Inhibit apoptosis		
	NORAD	Oncogene	promote proliferation, invasion, and EMT	miR-30a-5p/RAB11/Wnt/β-catenin	Zhang and Li (2020)
	SNHG1	Oncogene	promote proliferation, invasion, migration	EZH2/Wnt/β-catenin	Chen et al. (2020)
	LncRNA625	Suppressor	Inhibit proliferation and cell cycle	miR-432/Wnt/β-catenin	Li et al. (2017)
			Promote apoptosis		
	HOTAIRM1	Oncogene	promote proliferation	—	Wang et al. (2021)
			Inhibit apoptosis		
	LEF1-AS1	Oncogene	promote proliferation, invasion, angiogenesis in AIPC	miR-328/FZD2/CD44/Wnt/β-catenin	Li et al. (2020d)
	LINC01213	Oncogene	Androgen-independent transformation	—	Luo et al. (2020)
CircRNA	circ-ITCH	Suppressor	Inhibit proliferation, invasion, and migration	—	Li et al. (2020c)

challenges, owing to their low abundance and high sequence variability (Huang et al., 2023). Advances in next-generation sequencing technologies and bioinformatics tools have improved our ability to identify and validate ncRNA targets and their interactions with the Wnt signaling pathway (Mattick et al., 2023).

4 Type and role of Wnt signaling pathway-related NcRNAs in PCa progression

The Wnt signaling pathway plays a key role in the progression of PCa, with ncRNAs as crucial regulatory factors of this pathway (Pakula et al., 2017; Sonawala et al., 2022). Recent studies have elucidated several types of Wnt signaling pathway-related ncRNAs, including miRNAs, lncRNAs, and circRNAs, which exhibit abnormal expression in PCa and are closely linked to its progression (Table 1).

4.1 miRNAs

miRNAs are RNA molecules that are 22 nucleotides in length and regulate gene expression by binding to complementary target messenger RNAs (mRNAs) (Lagos-Quintana et al., 2001). The tissue-specific expression of miRNAs provides a premise for their clinical application as diagnostic and prognostic markers in cancer (Kim and Croce, 2021). For example, miR-574-3p is significantly downregulated in PCa tissues, and low expression of miR-574-3p is associated with an advanced tumor stage and a high Gleason score (Chiyomaru et al., 2013). The interactions between miRNAs and the Wnt signaling pathway in PCa are of two types: direct targeting of key genes associated with the Wnt signaling pathway and indirect targeting of the pathway via other genes. Based on their function, miRNAs can be categorized as oncogenic miRNAs or tumor suppressor miRNAs. Most miRNAs, including miR-34a, miR-34b/c, miR-1297, miR-1271, miR-218, miR-520b, miR-138, miR-574-3p, miR-15a-3p, and miR-320, exert tumor-suppressive effects by directly or indirectly inhibiting the Wnt signaling pathway (Chiyomaru et al., 2013; Hsieh et al., 2013; Liu et al., 2015; Li et al., 2016b; Liang et al., 2016; Zhong et al., 2017; Ren et al., 2018; Yu et al., 2018; Cui et al., 2019; Dong et al., 2020). However, a few miRNAs, such as miR-653-5p, miR-182, miR-1301-3p, and miR-454, promote malignant progression of PCa cells, such as proliferation, invasion, and migration, by activating the Wnt signaling pathway and targeting tumor suppressor genes (Guan et al., 2017; Fu et al., 2018; Wang et al., 2018; Fu et al., 2019). Simultaneously, miRNAs can also suppress stemness in PCa by inhibiting the Wnt signaling pathway (Hsieh et al., 2013; Zhou et al., 2016; Song et al., 2018). For example, miRNAs, such as miR-320 and miR-1301-3p, inhibit the activation of the Wnt signaling pathway and suppress the proliferation of PCa stem cells (Hsieh et al., 2013; Song et al., 2018). In addition, certain naturally active chemical substances have been found to exert therapeutic effects on PCa by regulating miRNAs and the Wnt signaling pathway. For example, urolithin, an active metabolite produced by human colonic microbiota, has been found to inhibit miR-21 and its downstream Wnt signaling pathway, thereby promoting apoptosis of PCa cells and inhibiting tumor growth (Zhou et al., 2016). Therefore, miRNAs and the Wnt signaling pathway may become notable therapeutic targets in PCa treatment, and their in-depth examination may reveal the pathogenesis of PCa and aid in the development of novel drugs against PCa.

4.2 LncRNAs

LncRNAs are RNA molecules that are 200 nucleotides in length and do not encode proteins (Szaflik et al., 2022). Although most of the genome is comprised of ncRNAs that are encoded by "junk DNA," they were originally considered to lack physiological functions (Huang et al., 2023). However, as the potential roles of lncRNAs in biological processes have been unveiled, the aforementioned notion has gradually changed (Wang et al., 2022a). LncRNAs regulate gene expression at various levels, i.e., chromatin, transcriptional, and post-transcriptional levels (Bhattacharjee et al., 2023; Petrone et al., 2023). Mounting evidence suggests that lncRNAs play crucial roles in PCa cell invasion, migration, and apoptosis and in castration-resistant PCa (CRPC), thereby affecting the proliferation, migration, and response of cancer cells to treatment (Li et al., 2020d; Song et al., 2020; Liu et al., 2021; Tan et al., 2021; Wu et al., 2021). LncRNAs can serve as signals, baits, or scaffolds to modulate cellular functions (Yang et al., 2020; Yan and Bu, 2021). Similar to miRNAs, lncRNAs may exert tumor-suppressive or oncogenic effects, contingent upon their category and mode of regulation. For example, lncRNAs such as TUG1, SOX2-OT, LINC04080, LINC00115, LINC00689, NORAD, SNHG1, and LEF1-AS1 activate the Wnt signaling pathway and promote tumor growth and metastasis by regulating other ncRNAs or proteins (Wang et al., 2019; Li et al., 2020a; Chen et al., 2020; Meng et al., 2020; Song et al., 2020; Zhang and Li, 2020; Peng et al., 2021). Conversely, lncRNAs such as lncRNA625 and HOTAIRM1 inhibit the Wnt signaling pathway to suppress cancer, promote cell apoptosis, and inhibit cell proliferation in PCa (Li et al., 2017; Wang et al., 2021). Investigation of the role of lncRNA625 in cancers has revealed that it promotes tumor development in esophageal carcinoma; however, it exerts a significant tumor-suppressive effect on PCa, suggesting that lncRNA625 could potentially serve as a therapeutic target for PCa (Li et al., 2017; Guo-Wei et al., 2019). Given the lack of effective PCa treatments, the investigation of treatments based on Wnt signaling pathway-related lncRNAs is of great significance for developing novel therapeutic strategies for PCa. Therefore, in-depth research on lncRNAs is expected to yield novel treatment options for patients with PCa. In addition, Wnt signaling pathway-related lncRNAs may have notable implications for prognosis evaluation and diagnostic positioning for PCa, with diverse potential applications.

4.3 CircRNAs

CircRNAs were first discovered in plant viruses and the Sendai virus through electron microscopy in 1976 (Kolakofsky, 1976). However, it is a widely held belief that circRNAs are the result of splicing errors and exhibit low expression levels (Memczak et al., 2013). With the advancement of bioinformatics and sequencing technologies, various types of circRNAs have been implicated in tumors (Zhou et al., 2021). For example, cir-znf215 has been found to promote the growth and metastasis of cholangiocarcinoma by inhibiting the AKT pathway (Liao et al., 2023). circRNAs also exhibit tissue- and cell-specific expression (Wang et al., 2023). Therefore, circRNAs could potentially serve as diagnostic and prognostic markers as well as therapeutic targets for PCa. cir-

ITCH is typically downregulated in PCa tissues and cell lines compared with normal adjacent tissues and normal RWPE-1 cells, indicating the potential of cir-ITCH as a diagnostic and prognostic marker for PCa (Li et al., 2020c). Moreover, cir-ITCH exerts an anti-cancer effect on PCa by inhibiting the Wnt signaling pathway (Li et al., 2020c). In addition, circRNAs interact with ncRNAs and regulate each other's expression levels (Li et al., 2020c; Ghafouri-Fard et al., 2021). For example, mutual inhibition of expression has been observed between cir-ITCH and miR-17 in PCa (Li et al., 2020c; Ghafouri-Fard et al., 2021).

5 Molecular mechanisms and functions of Wnt signaling pathway-related NcRNAs in PCa

5.1 Invasion and migration

Metastasis is the primary cause of most cancer-related deaths (Fares et al., 2020). Invasion and migration are critical steps in the cascade of tumor metastasis (Fares et al., 2020). miR-34a is a key regulatory factor in tumor suppression, modulating the expression of numerous target proteins involved in cell cycle, differentiation, epithelial-to-mesenchymal transition (EMT), and apoptosis, among others, and antagonizing processes such as cancer cell activity, stemness, metastasis, and chemoresistance (Misso et al., 2014). The expression of miR-34a is significantly lower in PCa tissues than in normal tissues (Lichner et al., 2015). The overexpression of miR-34a significantly reduces the proliferation and migration abilities of PCa cell lines, sush as PC3 cells (Lichner et al., 2015) (Figure 3). These effects are achieved by inhibiting the Wnt signaling pathway through the regulation of Wnt1 transcriptional activity (Dong et al., 2020). Notably, bones are the most common site of metastasis in PCa (Coleman et al., 2020). In PCa exhibiting activated Ras signaling, bone metastasis associated with low expression of miR-34a has been observed (Chen et al., 2015). miR-34a knockdown has been observed to induce the expression of TCF7 and BIRC5 by activating the Wnt signaling pathway, thereby promoting cell survival (Chen et al., 2015). These findings suggest that miR-34a could serve as a potential target in the treatment of metastatic PCa.

Li et al. demonstrated that miR-1297 directly targets the 3′-untranslated region of AEG-1 and regulates its mRNA and protein expression levels (Liang et al., 2016). In addition, they found that miR-1297 inhibits the Wnt signaling pathway by targeting AEG-1 in PCa, thereby suppressing cell proliferation and invasion (Liang et al., 2016). DIXDC1 is involved in the regulation of the proliferation and invasion of various tumors (Zhong et al., 2017; Xin et al., 2018). In PCa, it can be directly targeted and inhibited by miR-1271 (Zhong et al., 2017). MiR-1271 exhibits low expression in PCa and inhibits cell proliferation, invasion, and Wnt signal transduction by targeting DIXDC1 (Zhong et al., 2017).

Epidemiological and histopathological evidence suggests a correlation between inflammation and PCa incidence (De Nunzio et al., 2011). LGR4, which is induced by IL-6 during cancer progression, has been recently identified as a response gene associated with PCa progression (Liu et al., 2013). Yang et al. found that miR-218 directly targets LGR4 to inhibit the Wnt signaling pathway in LNCaP-IL-6^{+} cells during IL-6-induced PCa cell progression, thereby suppressing cell proliferation, cell cycle progression, and invasion (Li et al., 2016b). CapnS1 has been found to have a negative correlation with disease progression in various solid tumors (Zheng et al., 2020). In PCa, CapnS1 expression is regulated by miR-520b, and it exerts an oncogenic effect by promoting Wnt signal transduction (Ren et al., 2018). miR-520b is significantly downregulated in PCa (Ren et al., 2018). Inhibition of CapnS1 by miR-520b suppresses the growth and invasion of PCa cells associated with the downregulation of Wnt signal transduction (Ren et al., 2018). miR-138 has been observed to be downregulated in invasive PCa cell lines and promote PCa cell invasion and migration through the Wnt signaling pathway, whereas its overexpression has been observed to suppress these functions (Yu et al., 2018). miR-574-3p is significantly downregulated in PCa, and its low expression is associated with advanced tumor stage and a high Gleason score (Chiyomaru et al., 2013). The overexpression of miR-574-3p significantly inhibits the proliferation, migration, and invasion of PCa cells, which is associated with the inhibition of the Wnt signaling pathway via RAC1 (Chiyomaru et al., 2013). SOX30 is a recently identified cancer-related member of the SOX family that has a significant role in various types of cancer (Fu et al., 2019). miR-653-5p is highly expressed in PCa tissues and promotes the proliferation and invasion of PCa cells by targeting and upregulating β-catenin expression via SOX30 and activating the Wnt signaling pathway (Fu et al., 2019). miR-182 expression is higher in PCa tissues than in non-cancerous tissues, and miR-182 significantly activates the Wnt signaling pathway by targeting multiple negative regulators of Wnt signaling, thereby promoting cell proliferation, colony formation, migration, and invasion (Wang et al., 2018). miR-454 is highly expressed in PCa tissues and cell lines and promotes PCa cell proliferation and invasion by upregulating the Wnt signaling pathway, which is achieved by inhibiting NDRG2 expression (Wei et al., 2020). miR-744 significantly activates the Wnt signaling pathway by targeting multiple negative regulators of the pathway and promotes PCa cell proliferation, migration, and invasion (Guan et al., 2017).

TUG1, a 7.1 kb lncRNA, was first discovered to be upregulated in mouse retinal cells in response to taurine treatment (Young et al., 2005). TUG1 is highly expressed in PCa tissues and cells and promotes cell proliferation, migration, and invasion through the miR-496/Wnt/β-catenin axis (Li et al., 2020b; Xiu et al., 2020). LncRNA SOX2-OT plays crucial roles in psychiatric disorders, cancer, and diabetic complications (Li et al., 2020c). In PCa tissues and cells as well, SOX2-OT is highly expressed. Regulation of the miR-452-5p/HMGB3 axis and inactivation of the Wnt signaling pathway have been shown to inhibit PCa cell proliferation and metastasis, thereby suppressing tumor growth *in vivo* (Song et al., 2020). SNHG12, also known as LINC04080, is a lncRNA spanning approximately 1867 nucleotides and is located in the 1p35.3 region (Lan et al., 2017). In PCa, SNHG12 expression is upregulated in serum and tissues and is associated with RFS, biochemical recurrence, and Gleason scores of 8–10 in patients (Wang et al., 2019). This lncRNA activates the Wnt signaling pathway through the sponging effect of miR-195, thereby promoting cell proliferation, invasion, and migration in PCa (Song et al., 2019). LINC00115 was first identified as a notable pro-cancer lncRNA in lung cancer (Li et al., 2016a). In PCa, it is highly expressed in tissues and closely associated with a poor prognosis (Peng et al., 2021). LINC00115 promotes PCa cell

proliferation and invasion by targeting the miR-212-5p/FZD5/Wnt axis (Peng et al., 2021). LINC00689, first found to be associated with obesity susceptibility genes in the Han Chinese population of northern China, exerts a pro-cancer effect in multiple solid tumors, including gastric cancer, breast cancer, and liver cancer (Liu et al., 2019b; Du et al., 2020; Lu et al., 2020). This lncRNA activates the Wnt signaling pathway by regulating miR-496/CTNNB1, thereby promoting PCa cell proliferation, migration, and invasion (Meng et al., 2020). LEF1 is a key component of the Wnt/β-catenin signaling pathway. It is highly expressed in PCa and is associated with its malignant progression (Fakhr et al., 2021). The recently identified lncRNA LEF1-AS1 is encoded by the LEF1 locus and is associated with poor prognosis in multiple cancer types (Li et al., 2020d). LEF1-AS1 promotes PCa metastasis and serves as a competing endogenous RNA (ceRNA) for miR-328, thereby modulating Wnt/β-catenin pathway activity by regulating FZD2 and CD44, ultimately promoting androgen-independent PCa (AIPC) cell proliferation, migration, invasion, angiogenic ability, and tumor growth (Li et al., 2020d). In addition, lncRNAs also play crucial roles in PCa metastasis by directly binding to EZH2 (Chen et al., 2020). For example, both the lncRNAs SNHG1 and EZH2 are highly expressed in PCa tissues and cells, and their expression is positively correlated. SNHG1 regulates the Wnt signaling pathway through the EZH2 gene, modulating PCa cell proliferation, invasion, and migration (Chen et al., 2020). These findings further enrich our understanding of the mechanism of action of Wnt signaling pathway-related lncRNAs in PCa. The aforementioned lncRNAs are associated with the Wnt signaling pathway and play vital roles in the growth and progression of PCa, thereby presenting as novel targets for PCa treatment.

In addition to the aforementioned Wnt signaling pathway-related lncRNAs, the role of the lncRNA CCAT2 in PCa metastasis should be considered (Zheng et al., 2016; He et al., 2020). Studies have shown that complex feedback loops exist between CCAT2 and the Wnt signaling pathway (Zheng et al., 2016; He et al., 2020). Moreover, CCAT2 plays a key role in the invasion and migration of PCa (Zheng et al., 2016; He et al., 2020). Notably, CCAT2 is not only aberrantly expressed in PCa but also exhibits similar expression patterns in many other cancers (Ling et al., 2013). In colorectal cancer, it is a downstream target of the Wnt signaling pathway, indicating that TCF7L2 is also involved in this feedback loop (Ling et al., 2013). Therefore, an in-depth investigation of the role of lncRNAs in the Wnt signaling pathway is of great significance for the prognosis and treatment of cancer as well as the recovery from cancer. In summary, the key role of the lncRNA CCAT2 in PCa metastasis indicates its potential as a therapeutic target.

In contrast to miRNAs and lncRNAs, circRNAs associated with the Wnt signaling pathway in PCa have been the subject of comparatively less investigation. The expression of cir-ITCH is downregulated in PCa tissues and cell lines (Li et al., 2020c), and its overexpression significantly inhibits the proliferation, migration, and invasion of human PCa cells. Cir-ITCH and miR-17 function as mutual expression inhibitory factors (Li et al., 2020c). Cir-ITCH contributes to the suppression of PCa progression by inhibiting the Wnt/β-catenin signaling pathway, which may be achieved through the inhibition of miR-17 (Li et al., 2020c).

5.2 EMT

The metastasis of solid tumors is also influenced by the characteristics and plasticity of cancer cells, such as EMT (Babaei et al., 2021). During EMT, epithelial cells transform into highly mobile mesenchymal cells, thereby increasing the migration ability of cancer cells (Hao et al., 2019). Inhibiting EMT is key to preventing cancer metastasis and improving prognosis (Fedele et al., 2022). miR-15a-3p is downregulated in PCa tissues and cell lines, whereas its overexpression inhibits cell proliferation, invasion, and EMT by downregulating the Wnt signaling pathway, with SLC39A7 as its direct downstream target (Cui et al., 2019) (Figure 4). LEF1 is a key transcription factor in the Wnt signaling pathway that regulates cell proliferation and invasion (Fakhr et al., 2021). miR-34a can regulate the level of LEF1 to inhibit EMT in PCa cells (Liang et al., 2015). Additionally, two other members of the miR-34 family, namely miR-34b/c, when overexpressed, can target β-catenin mRNA expression, thereby inhibiting cell migration and EMT in PCa (Liu et al., 2015).

The lncRNA NORAD exerts a pro-cancer effect in melanoma, pancreatic cancer, and glioblastoma (Soghli et al., 2021). In PCa as well, NORAD is highly expressed in cells and tissues and promotes cell proliferation, invasion, and EMT (Zhang and Li, 2020; Hu et al., 2021; Fletcher et al., 2022). miR-30a-5p attenuates NORAD-mediated promotion of cell proliferation, invasion, and EMT by targeting RAB11A (Zhang and Li, 2020).

5.3 Apoptosis

Cell apoptosis is a key self-regulation mechanism in multicellular organisms, serving to eliminate unwanted or abnormal cells (Kerr et al., 1972). Dysregulation of cell apoptosis has been implicated in various diseases, including cancer, autoimmune diseases, cardiovascular diseases, and neurological diseases (Chen et al., 2021). In recent years, a growing body of evidence has shown that ncRNAs play a crucial role in PCa cell apoptosis (Tamtaji et al., 2021). The Wnt signaling pathway is associated with PCa cell apoptosis, and ncRNAs associated with this pathway also play key roles in PCa cell apoptosis (Tamtaji et al., 2021). The overexpression of miR-34a inhibits the Wnt signaling pathway by regulating the transcriptional activity of Wnt1, thereby significantly increasing the rate of cell apoptosis (Dong et al., 2020) (Figure 4). Compared with non-cancerous tissues, PCa tissues exhibit upregulated expression of miR-182 (Wang et al., 2018). The upregulation of miR-182 activates the Wnt signaling pathway by targeting negative regulatory factors of the pathway, such as GSK3β, APC, CK1, and Axin, ultimately inhibiting cell apoptosis (Wang et al., 2018). Urolithin, a bioactive metabolite derived from ellagic acid, has been observed to inhibit miR-21 and its downstream Wnt/β-catenin signaling pathway to reduce cell viability and promote caspase-dependent cell apoptosis in DU145 cells (Zhou et al., 2016; Singh et al., 2019).

LINC0689 exerts a pro-oncogenic effect in multiple parenchymal tumors, where its expression is elevated (Liu et al., 2019b; Du et al., 2020). LINC00689 is upregulated in end-stage PCa tissues and inhibits apoptosis through miR-496/CTNNB1 (Meng et al., 2020). In addition, the lncRNA HOTAIRM1 is highly expressed in mature bone marrow cells (Zhang et al., 2014).

Silencing HOTAIRM1 in PC3 cells promotes PCa cell apoptosis by downregulating the Wnt/β-catenin signaling pathway; however, the exact mechanism remains unknown (Wang et al., 2021).

5.4 Stemness

Cancer stem cells play a crucial role in the survival, proliferation, metastasis, and recurrence of tumors (Murota et al., 2022). miR-320 inhibits the activation of the Wnt/β-catenin signaling pathway by targeting β-catenin mRNA expression, thereby suppressing PCa stem cell characteristics such as tumor sphere formation, chemoresistance, and tumorigenicity (Hsieh et al., 2013) (Figure 4). In contrast, miR-320 knockdown significantly enhances the aforementioned characteristics (Hsieh et al., 2013). miR-1301-3p is significantly upregulated in PCa cells and tissues and targets inhibitors of the Wnt signaling pathway, namely GSK3β and SFRP1, thereby promoting the proliferation of PCa stem cells by activating the Wnt/β-catenin signaling pathway (Song et al., 2018).

5.5 CRPC formation

Targeting the Wnt/β-catenin signaling pathway may be an attractive therapeutic strategy for treating CRPC (Shafi et al., 2013). The potential of Wnt signaling pathway-related lncRNAs in the treatment of CRPC is currently gaining increasing attention (Yap et al., 2011) (Figure 4). Androgen deprivation therapy has become the mainstay for the treatment of patients with advanced PCa (Shafi et al., 2013). However, most patients eventually progress to CRPC, leading to poor prognosis (Yap et al., 2011). Bone metastasis is a notable issue in patients with CRPC (Beltran et al., 2016; Lin et al., 2020). Luo et al. elucidated that crosstalk between the AR and Wnt/β-catenin signals promotes the androgen-independent transformation of PCa (Luo et al., 2020). Although androgens can inhibit the Wnt/β-catenin signaling pathway in androgen-dependent PCa cells, this inhibitory effect is not observed in AIPC cells (Luo et al., 2020). Moreover, LEF1-AS1 has been observed to promote PCa metastasis through the Wnt/β-catenin signaling pathway and function as a ceRNA for miR-328, thereby regulating the activity of the Wnt/β-catenin signaling pathway by regulating FZD2 and CD44, ultimately enhancing proliferation, migration, invasion, and angiogenic ability of AIPC cells and tumor growth (Li et al., 2020d). The Wnt/β-catenin signaling pathway inhibits AIPC cell proliferation by promoting the cell cycle process and inhibiting apoptosis (Luo et al., 2020). Therefore, targeting the Wnt/β-catenin signaling pathway may be a viable strategy for the treatment of CRPC.

Exosomes are small vesicles that measure approximately 40–160 nm (typically approximately 100 nm) in diameter and originate from endosomes (Pegtel and Gould, 2019). Many studies have demonstrated the importance of several lncRNAs in exosomes across various cancers (Kalluri and LeBleu, 2020). For example, the exosomal lncRNA HOXD-AS1 has been observed to promote the metastasis of PCa through the miR-361-5p/FOXM1 axis (Jiang et al., 2021). Wnt signaling pathway-related lncRNAs loaded in exosomes could potentially serve as diagnostic and therapeutic tools for the treatment of CRPC (Guo et al., 2022). For example, LINC01213 plays a role in the transition of PCa cells from an androgen-dependent to an androgen-independent state (Guo et al., 2022). Additionally, it induces androgen deprivation tolerance by activating the Wnt signaling pathway through exosome-mediated intercellular communication in PCa (Guo et al., 2022). The lncRNA SNHG17 is one of the four significantly upregulated lncRNAs in metastatic PCa and AIPC cells, wherein it promotes tumor cell proliferation, survival, invasion, and resistance to chemotherapy by upregulating the Wnt/β-catenin signaling pathway (Bai et al., 2020; Zhao et al., 2021).

6 Wnt signaling pathway-related NcRNAs in the diagnosis and treatment of PCa

Early detection of PCa is crucial for effective treatment and improved survival rates (Sung et al., 2021). For example, in the event of early detection, the five-year survival rate of patients with localized PCa is nearly 100% (Sandhu et al., 2021). In contrast, the median survival duration for patients with metastatic PCa is approximately 3 years (Sandhu et al., 2021). Therefore, early diagnosis of PCa is essential. Additionally, because PCa is prone to metastasis and chemoresistance, it has become one of the leading causes of cancer-related mortality worldwide (He et al., 2022). Due to the lack of symptoms in the early stages of PCa, despite technological advancements, the discovery of novel tumor biomarkers remains crucial (Wang et al., 2022b). This is necessary to address the challenges associated with the diagnosis and treatment of prostate cancer. NcRNAs exhibit tissue-specific expression and are detectable at all stages of PCa development, making them potential biomarkers and therapeutic targets (Mugoni et al., 2022). Mounting evidence suggests that Wnt signaling pathway-related ncRNAs are closely associated with PCa progression (Doghish et al., 2022), rendering them promising biomarkers for the diagnosis, prognosis, and treatment of PCa (Table 2). Therefore, investigating Wnt signaling pathway-related ncRNAs in the context of early diagnosis, prognosis prediction, cancer treatment, and resolution of treatment resistance is an effective strategy to improve the survival of PCa patients.

6.1 Potential PCa diagnostic biomarkers

Early screening and diagnosis of cancer are crucial for patient survival (Sung et al., 2021). The identification of suitable biomarkers has consistently posed a notable challenge in the field of cancer research (Movahedpour et al., 2022; Xie et al., 2022). Wnt signaling pathway-related ncRNAs aid in the early diagnosis of PCa. In patients with PCa, certain Wnt signaling pathway-related ncRNAs, such as SNHG17 and LINC00115, are upregulated (Peng et al., 2021), whereas other ncRNAs, such as miR-34a, are downregulated (Chiyomaru et al., 2013). Additionally, certain ncRNAs are aberrantly expressed in various stages or special subtypes of PCa (Li et al., 2020d; Meng et al., 2020). For instance, LINC00689 is upregulated in end-stage PCa tissues and LEF1-AS1 is significantly overexpressed in AIPC (Li et al., 2020d;

TABLE 2 Clinical applications of ncRNAs and Wnt/β-catenin pathway in PCa.

NcRNA		Expression	Prognosis	Diagnosis	Clinical significance	Ref.
miRNA	miR-34a	Down	Poor	Profitable	Bone metastasis, gleason score	Chen et al. (2015)
	miR-34b/c	Down	—	Profitable	—	Liu et al. (2015)
	miR-1297	Down	—	Profitable	—	Liang et al. (2016)
	miR-1271	Down	—	Profitable	—	Zhong et al. (2017)
	miR-218	Down	Poor	Profitable	Bone Metastasis, TNM stage, T stage, N stage, M stage and gleason score	Li et al. (2016b)
	miR-520b	Down	Poor	Profitable	—	Ren et al. (2018)
	miR-138	Down	Poor	Profitable	N stage, M stage and gleason score	Yu et al. (2018)
	miR-574-3p	Down	Poor	Profitable	T stage and gleason score	Chiyomaru et al. (2013)
	miR-653-5p	High	—	Profitable	—	Fu et al. (2019)
	miR-182	High	—	Profitable	—	Wang et al. (2018)
	miR-454	High	—	Profitable	—	Fu et al. (2018)
	miR-744	High	Poor	Profitable	CRPC progression	Guan et al. (2017)
	miR-15a-3p	Down	—	Profitable	—	Cui et al. (2019)
	miR-21	High	—	Profitable	DDP Chemoresistance, and pathological stage, N stage, capsular invasion, organ confined disease, gleason score, and biochemical recurrence	Zhou et al. (2016)
	miR-320	Down	Poor	Profitable	DDP chemoresistance, serum PSA levels, TNM stage	Hsieh et al. (2013)
	miR-1301-3p	Down	—	Profitable	—	Song et al. (2018)
	miR-425-5p	Down	Poor	Profitable	DDP chemoresistance, residual tumor, T stage, N stage, and TP53 status	Liu et al. (2019a)
LncRNA	CCAT2	High	Poor	Profitable	Histological grade and M stage	He et al. (2020)
	TUG1	High	Poor	Profitable	TNM stage, gleason score, TNM stage, preoperative PSA level, and N stage	Li et al. (2020a)
	SOX2-OT	High	Poor	Profitable	—	Song et al. (2020)
	SNHG12	High	Poor	Profitable	Biochemical recurrence and gleason score 8–10	Wang et al. (2019)
	LINC00115	High	Poor	Profitable	—	Peng et al. (2021)
	LINC00689	High	Poor	Profitable	TNM stage	Meng et al. (2020)
	NORAD	High	Poor	Profitable	Bone metastasis	Zhang and Li (2020)
	SNHG1	High	Poor	Profitable	TNM stage, Gleason Score, N stage, and long-term metastasis mortality rate	Chen et al. (2020)
	LncRNA625	Down	—	Profitable	—	Li et al. (2017)
	HOTAIRM1	High	—	Profitable	—	Wang et al. (2021)
	LEF1-AS1	High	—	Profitable	—	Li et al. (2020d)
	HOXD-AS1	High	Poor	Profitable	Highly expressed in serum exosomes from metastatic PCa patients, Gleason Score, and N stage	Jiang et al. (2021)
	LINC01213	—	—	—	—	Guo et al. (2022)
	HOTTIP	High	Poor	Profitable	DDP chemoresistance, T stage, presence of extra prostatic extension, seminal vesicle invasion, perineural invasion, and the tumor involvement of resection margin	Jiang et al. (2019)
	SNHG17	High	Poor	Profitable	Docetaxel chemoresistance, Histological grade, T stage, N stage, and M stage	Bai et al. (2020), Zhao et al. (2021)
CircRNA	circ-ITCH	Down	Poor	Profitable	T stage, N stage, Gleason score, and surgical margin status	Li et al. (2020c)

Meng et al., 2020). These findings suggest that Wnt signaling pathway-related ncRNAs could potentially serve as diagnostic biomarkers for PCa. Notably, ncRNAs present in plasma will be relatively non-invasive and more convenient as diagnostic tools. Wnt signaling pathway-related lncRNAs loaded in exosomes could also potentially serve as diagnostic and therapeutic tools for PCa (Guo et al., 2022). For example, exosomal LINC01213 can serve as a diagnostic biomarker for CRPC (Guo et al., 2022).

6.2 Potential PCa prognostic biomarkers

Patient prognostic information is essential in the process of making informed treatment decisions (Lin and Farooqi, 2021). Mounting evidence suggests that the Wnt signaling pathway-related ncRNAs may hold significant potential for predicting patient prognosis (Chiyomaru et al., 2013; Dong et al., 2020; Xiu et al., 2020; Peng et al., 2021; Zhao et al., 2021). These ncRNAs are closely associated with overall survival, disease-free survival, recurrence-free survival, five-year survival rates, and progression-free survival of patients with PCa (Peng et al., 2021). For example, high expression of LINC00115 is associated with shorter overall survival and recurrence-free survival in patients with PCa (Peng et al., 2021). Additionally, Wnt signaling pathway-related ncRNAs are also associated with other prognostic factors (Chiyomaru et al., 2013; Dong et al., 2020; Xiu et al., 2020; Zhao et al., 2021). For instance, miR-34a is associated with bone metastasis of Ras-activated PCa cells (Dong et al., 2020). Furthermore, miR-574-3p is associated with advanced tumor stage and higher Gleason scores (Chiyomaru et al., 2013). In contrast, high expression of SNHG17 is associated with grade, stage, and metastasis (Zhao et al., 2021). Additionally, TUG1 is associated with Gleason score, clinical stage, preoperative PSA level, and lymph node metastasis (Xiu et al., 2020). These findings have crucial implications for the prognostic assessment and treatment selection in PCa. Therefore, Wnt signaling pathway-related ncRNAs could potentially serve as vital indicators for the prognostic evaluation and treatment selection in PCa.

6.3 Potential therapeutic targets

Cancer treatment has long been regarded as one of the most formidable challenges worldwide, despite the progress made in treatment modalities (Sung et al., 2021). Targeted therapy strategies based on ncRNAs have yielded novel insights into cancer treatment (Paunovska et al., 2022). NcRNAs regulate cell proliferation, invasion, migration, apoptosis, and stemness in PCa and conversion of PCa to CRPC by directly or indirectly interacting with the Wnt signaling pathway (Guan et al., 2017; Wang et al., 2018). Therefore, modulating the expression of Wnt signaling pathway-related ncRNAs could be an effective strategy for treating PCa and improving patient prognosis. For example, silencing miR-182 using inhibitors has been observed to significantly reduce the growth of PCa xenograft tumors, whereas silencing miR-744 using short hairpin RNA (shRNA) has been observed to significantly reduce the growth of PCa xenograft tumors (Guan et al., 2017; Wang et al., 2018). However, identifying targeted drugs that modulate ncRNA expression and stably transmit this effect remains a challenge, and necessites an enhanced understanding of the structure and function of Wnt signaling pathway-related ncRNAs. Most lncRNAs and circRNAs function as "sponges" for miRNAs to activate or deactivate the Wnt signaling pathway (Li et al., 2020d). Therefore, the regulation of target miRNA of Wnt signaling pathway-related lncRNA and circRNA or the interfering with upstream lncRNA and circRNA associated with Wnt signaling pathway-related miRNA could also be a viable treatment strategy. For example, miR-496 intervention has been observed to effectively reverse the growth-promoting effect of TUG1 on PCa xenografts (Li et al., 2020d).

Targeting ncRNAs has been considered an attractive strategy for cancer treatment (Damase et al., 2021; Garbo et al., 2022; Zogg et al., 2022). In addition to using the above approach, there are other methods that can be employed to intervene in the expression of ncRNAs involved in the Wnt pathway, which may provide therapeutic benefits for patients (Damase et al., 2021; Garbo et al., 2022; Zogg et al., 2022). In the field of Wnt pathway-related miRNAs, miRNA mimics (miRNA-like dsRNA) have been found to enhance the expression and function of certain miRNAs; meanwhile, antagomiRs can serve as tools to inhibit oncogenic miRNAs associated with the Wnt pathway, thereby blocking the specific functions of these miRNAs (Neumeier and Meister, 2020; Xu et al., 2023). In recent years, strategies based on lncRNAs for cancer treatment have gained widespread recognition. Currently, the main therapeutic approaches for managing lncRNAs involve modulating their expression levels to decrease oncogenic lncRNAs (through RNA interference methods) or increase tumor-suppressive lncRNAs (Garbo et al., 2022). It is worth noting that targeting strategies for lncRNAs need to take into account their cellular localization. Antisense oligonucleotides (ASOs) are the most effective method for targeting nuclear lncRNAs (Adewunmi et al., 2023). However, small interfering RNAs (siRNAs) are preferred for cytoplasmic lncRNAs (Li et al., 2022). Additionally, other strategies such as aptamers, nucleases, and miRNAs can be developed to disrupt lncRNA activit (Damase et al., 2021; Zogg et al., 2022). Due to their wide biological activity and stability, circRNAs have emerged as a potential and powerful therapeutic strategy that can significantly impact cancer occurrence and progression (Zong et al., 2023). However, limiting off-target effects remains a challenge in this field (Loan Young et al., 2023). Addressing this issue, specific carriers for synthetic circRNAs or siRNAs targeting junction sequences could offer substantial benefits to patients. All in all, targeted therapeutic strategies based on ncRNAs hold promise as a novel approach for PCa treatment.

6.4 Potential chemoresistance targets

Chemoresistance is a notable concern in cancer treatment, and enhancing chemosensitivity in PCa through ncRNA intervention has become a strategy that is increasingly being recognized and investigated (Chen et al., 2022). Targeting Wnt signaling pathway-related ncRNAs may help reverse chemoresistance in PCa (Liu et al., 2019a). Cisplatin, a platinum-based chemotherapeutic drug commonly used in the treatment of PCa, works by forming covalent bonds with DNA,

leading to the formation of DNA cross-links and inhibiting DNA replication and transcription (Li et al., 2021). In PCa, cisplatin plays a role in inhibiting tumor growth by damaging the DNA of cancer cells and triggering apoptosis (programmed cell death) (Dhar et al., 2011). However, the development of resistance to cisplatin remains a major challenge in PCa treatment. Mechanisms underlying cisplatin resistance in PCa include enhanced DNA repair mechanisms, altered drug uptake and efflux, increased drug inactivation, and alterations in cell death pathways (Kalathil et al., 2023). miR-425-5p is downregulated in PCa and is further downregulated in cisplatin-resistant PCa (Liu et al., 2019a). Therefore, upregulating miR-425-5p by targeting the Wnt signaling pathway could potentially enhance the sensitivity of PCa to cisplatin (Liu et al., 2019a). Additionally, HOTTIP, a known oncogene, is upregulated in patients with PCa and PCa cell lines, promoting PCa cell proliferation and reducing sensitivity to cisplatin by activating the Wnt signaling pathway (Jiang et al., 2019). As a member of a class of chemotherapy drugs known as taxanes, docetaxel acts by disrupting microtubule dynamics, ultimately inhibiting cell division and inducing cell death (Sanchez-Hernandez et al., 2023). Docetaxel exerts its anti-cancer effects by targeting rapidly dividing cancer cells, inhibiting tumor growth, and promoting cancer cell death (Sanchez-Hernandez et al., 2023). In the treatment of PCa, docetaxel is particularly effective in advanced or metastatic CRPC (Gebrael et al., 2023). However, similar to cisplatin, resistance to docetaxel of PCa can develop over time. Mechanisms of docetaxel resistance in PCa involve alterations in microtubule dynamics, activation of cell survival pathways, and increased drug efflux (Gebrael et al., 2023). SNHG17 promotes chemotherapeutic resistance to docetaxel in PCa tumor cells by upregulating the Wnt signaling pathway, thereby leading to increased chemoresistance (Zhao et al., 2021). Therefore, modulation of Wnt signaling pathway-related ncRNAs may be an effective strategy to reverse chemoresistance in PCa. However, identifying targeted drugs that can regulate ncRNA expression and stably transmit this effect remains a challenge (Jaiswal et al., 2023). Therefore, an enhanced understanding of the structure and function of Wnt signaling pathway-related ncRNAs can aid the development of novel treatment strategies to reverse chemoresistance in PCa.

7 Conclusion

This review provided a comprehensive overview of the role of the Wnt signaling pathway and related ncRNAs (miRNAs, lncRNAs, and circRNAs) in PCa. MiRNAs affect the expression of target genes associated with the Wnt signaling pathway. Additionally, the Wnt signaling pathway establishes feedback mechanisms and functions as an upstream mediator of miRNAs. In most cases, lncRNAs regulate the expression of proteins in the Wnt signaling pathway by serving as sponges for miRNAs. CircRNAs also regulate the expression of the Wnt signaling pathway; however, similar to lncRNAs, they primarily regulate the expression of the Wnt signaling pathway by targeting miRNAs. We also discussed the novel avenues for the development of siRNAs and shRNAs that target the Wnt signaling pathway and their potential clinical applications. However, the limited efficacy of siRNAs and shRNAs *in vivo* has hindered their potential clinical application, necessitating the exploration of more reliable strategies to target ncRNAs.

Although the regulation of the Wnt signaling pathway through ncRNA intervention is a promising avenue for the treatment of PCa, certain issues need to be addressed. First, the Wnt signaling pathway is highly intricate, consisting of 19 distinct types of Wnt-secreted glycoproteins and over 15 types of Wnt receptors in humans, which activate various downstream pathways. Second, the differences and balance between the classical and non-classical Wnt signals are difficult to capture, making targeting the Wnt signaling pathway even more challenging. Third, the Wnt signaling pathway plays a fundamental role in the dynamic balance of systems, such as the digestive and hematopoietic systems; therefore, blocking the Wnt signaling pathway may lead to systemic toxicity. Hence, modulation of the Wnt signaling pathway as a therapeutic strategy for PCa is both an opportunity and a challenge, warranting further research. In the future, it will be necessary to conduct additional investigations of the interplay between the Wnt signaling pathway and ncRNAs and develop more reliable methods for targeting ncRNAs for their clinical application. Simultaneously, alternative therapeutic strategies for PCa should also be explored to improve the efficacy of treatment and the quality of life of patients.

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Long non-coding RNA H19 enhances the pro-apoptotic activity of ITF2357 (a histone deacetylase inhibitor) in colorectal cancer cells

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Introduction: Long non-coding RNA H19 (lncH19) is highly expressed in colorectal cancer (CRC) and plays critical roles in tumor development, proliferation, metastasis, and drug resistance. Indeed, the expression of lncH19 usually affects the outcomes of chemo-, endocrine, and targeted therapies. ITF2357 (givinostat) is a histone deacetylase inhibitor (HDACi) that revealed a significant anti-tumor action by inducing apoptosis in different tumor models, including leukemia, melanoma, and glioblastoma. However, no data are present in the literature regarding the use of this compound for CRC treatment. Here, we investigate the role of lncH19 in ITF2357-induced apoptosis in CRC cells.

Methods: The HCT-116 CRC cell line was stably silenced for H19 to investigate the role of this lncRNA in ITF2357-induced cell death. Cell viability assays and flow cytometric analyses were performed to assess the anti-proliferative and pro-apoptotic effects of ITF2357 in CRC cell lines that are silenced or not for lncH19. RT-PCR and Western blot were used to study the effects of ITF2357 on autophagy and apoptosis markers. Finally, bioinformatics analyses were used to identify miRNAs targeting pro-apoptotic factors that can be sponged by lncH19.

Results: ITF2357 increased the expression levels of H19 and reduced HCT-116 cell viability, inducing apoptosis, as demonstrated by the increase in annexin-V positivity, caspase 3 cleavage, and poly (ADP-ribose) polymerase (PARP-1) degradation. Interestingly, the apoptotic effect of ITF2357 was much less evident in lncH19-silenced cells. We showed that lncH19 plays a functional role in the pro-apoptotic activity of the drug by stabilizing TP53 and its transcriptional targets, NOXA and PUMA. ITF2357 also induced autophagy in CRC cells, which was interpreted as a pro-survival response not correlated with lncH19 expression. Furthermore, ITF2357 induced apoptosis in 5-fluorouracil-resistant HCT-116 cells that express high levels of lncH19.

Conclusion: This study shows that lncH19 expression contributes to ITF2357-induced apoptosis by stabilizing TP53. Overall, we suggest that lncH19 expression

may be exploited to favor HDACi-induced cell death and overcome 5-fluorouracil chemoresistance.

KEYWORDS

lncH19, colorectal cancer, histone deacetylase inhibitor, apoptosis, drug resistance

Introduction

Accumulating evidence indicates that long non-coding RNAs (lncRNAs) profoundly influence cancer development through intricate networks based on their interplay with DNA, RNAs, and proteins. LncRNA-H19 (lncH19) is one of the first lncRNAs identified and exerts multiple functions in various diseases, including cancers (Bao et al., 2018; Bitarafan et al., 2019; He et al., 2020; Yang et al., 2021). LncH19 is canonically considered to exert an oncogenic function since it is upregulated in many forms of tumors and is associated with tumor transformation, progression, and malignancy (Shima et al., 2018; Corrado et al., 2019; Mahmoudian-Sani et al., 2019; Zhou et al., 2019). LncH19 may also act through the production of intragenic microRNAs, miR-675-5p and miR-675-3p, which also display a pro-tumor activity (Lo Dico et al., 2016; Muller et al., 2019). LncH19 and its intragenic miRNAs are upregulated in colon tumors and correlate with poor prognosis in patients (Costa et al., 2017; Feng et al., 2017; Zhang et al., 2017; Dai et al., 2019; Yang et al., 2020; O'Brien et al., 2022).

In colorectal cancer, lncH19 overexpression affects cell proliferation (Yang et al., 2017; Saieva et al., 2020) and cell motility (Ding et al., 2018; Yang et al., 2018), and more recently, scientific evidence correlates the expression levels of lncH19 with the reduced sensitivity to 5-FU, suggesting that lncH19 may function as a marker for prediction of the chemotherapeutic response to this drug (Wang et al., 2018; Zhang et al., 2022).

Wang and collaborators demonstrated that lncH19, functioning as a competitive endogenous RNA, mediates 5-FU resistance in CRC via SIRT1-mediated autophagy (Wang et al., 2018).

We have recently demonstrated that lncH19-derived miR-675-5p enforces hypoxia-induced chemoresistance to 5-FU by targeting pro-caspase-3 and inhibiting the pro-apoptotic effects of 5-FU (Zichittella et al., 2022).

Numerous studies propose the therapeutic use of histone deacetylase inhibitors (HDACis) for the treatment of several diseases, including metabolic, inflammatory, autoimmune, and neurodegenerative diseases, and not least for the treatment of cancer (Eckschlager et al., 2017; Vagapova et al., 2021; Squarzoni et al., 2022).

HDACis are well-known epigenetic drugs with widely recognized anti-tumor activity (Zhao et al., 2020). HDACis target the aberrant activity of histone deacetylases (HDACs), which are often overexpressed in tumor cells, restoring or increasing histone acetylation, thereby promoting transcriptional activation of tumor suppressor and pro-apoptotic genes (Singh et al., 2018; Patra et al., 2019; Ramaiah et al., 2021). Therefore, inhibition of HDACs represents a valid basis for new anti-tumor therapies (Dasko et al., 2022).

To date, the Food and Drug Administration has approved some HDACis such as vorinostat (SAHA), belinostat (PXD-101), panobinostat (LBH-589), and romidepsin (FK-228) for the treatment of cancer (Squarzoni et al., 2022). Clinical and pre-clinical studies have also shown that these compounds can be used as adjuvants to traditional chemotherapeutics in different types of cancer (Suraweera et al., 2018; Psilopatis et al., 2021; Pramanik et al., 2022). More recently, it has been shown that epigenetic targeting of colon cancer based on combined HDACis with DNA methyltransferase (DNMT) inhibitors has revealed clinical relevance (Tang et al., 2023).

ITF2357 (givinostat) is a potent HDAC inhibitor belonging to the hydroxamic acid class. This compound is currently used in the therapy for the treatment of Duchenne muscular dystrophy, and in clinical trials for Becker muscular dystrophy and juvenile idiopathic arthritis (Vojinovic and Damjanov, 2011; Vojinovic et al., 2011; Spreafico et al., 2021; Comi et al., 2023; Sandona et al., 2023).

The compound has also revealed a significant anti-tumor action by inducing apoptosis in different tumor models, including leukemia, melanoma, and glioblastoma cells (Li et al., 2016; Celesia et al., 2022; Taiarol et al., 2022).

In addition, it has been widely demonstrated that ITF2357 can also act as an adjunct to conventional chemotherapy, increasing sensitivity to demethylating or chemotherapeutic agents such as pemetrexed in lung cancer, doxorubicin in sarcoma cells, and temozolomide in glioma stem cells (Di Martile et al., 2018; Cui et al., 2023; Nakagawa-Saito et al., 2023).

ITF2357 has recently been reported to exert a targeting effect on oncogenic BRAF in melanoma cells (Celesia et al., 2022) and affect oncogenic BRAF and p53 interplay, thus representing a promising candidate for melanoma-targeted therapy (Celesia et al., 2023).

To date, the only data present in the literature on the effects of ITF2357 in colon cancer are described in a manuscript that discusses the use of the compound for the prevention of colitis-associated cancer in mice (Glauben et al., 2008). Here, we describe the pro-apoptotic effect of ITF2357 in CRC cells and show that lncH19 plays a functional role in apoptosis execution by stabilizing TP53, probably by exerting its action as a miRNA sponge. Moreover, the paper provides evidence that lncH19-expressing CRC cells, resistant to 5-FU treatment, nicely respond to ITF2357, thus supporting a possible therapeutic application of this compound to overcome colon drug resistance.

Materials and methods

Cell culture

HCT-116 cells (ATCC–LGC Standards S.r.L., Italy) were cultured in McCoy's 5A medium (Euroclone, United Kingdom) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin (10,000 U/mL penicillin and 10 mg/mL streptomycin), and 200 mM L glutamine (all sourced from Euroclone, United Kingdom).

5-Fluorouracil (5-FU)-resistant HCT-116 cells (HCT-116-5-FU-R) were cultured in DMEM (Euroclone, United Kingdom) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin (10,000 U/mL penicillin and 10 mg/mL streptomycin), and 200 mM L glutamine (all sourced from Euroclone, United Kingdom), and additionally, the culture medium contained 5-fluorouracil (5-FU, cat. n°F6627, Sigma-Aldrich, St. Louis, MO, United States) at concentrations up to 70 μM.

Cells were maintained in a humidified atmosphere containing 5% CO_2 at 37°C and used at early passages for all experiments. The culture medium was changed every 2–3 days, and cells were split at 70%–80% confluence.

Infection with lentiviral vectors to stably silence lncH19

HCT-116 cells were stably silenced for lncH19 by lentiviral infection with H19 human shRNA lentiviral particles (Cat. n° TL318197V, OriGene Technologies, Inc., Rockville, MD, United States), while relative control cells were infected with control shRNA lentiviral particles (Cat. n° TR30021V, OriGene Technologies, Inc., Rockville, MD, United States). Subsequently, infected cells were selected by cell sorting (BD FACSAria™ III Sorter, ATeN Center) and maintained in culture under selective pressure with 1 mg/mL of puromycin (Gibco™ puromycin dihydrochloride, cat. n°A1113802, Thermo Fisher® Scientific, United States). Silencing efficiency was regularly tested by qRT-PCR and fluorescence microscopy.

Selection of HCT-116-5-FU-resistant cells

The 5-FU-resistant HCT-116 cell line (HCT-116-5-FU-R) was established after sequential treatments with 5-FU during an 8-month period starting from 1 μM to 70 μM concentrations. Control parental cells were split in parallel. Viable cells treated with 70 μM 5-FU were considered stably resistant when the morphology resembled that of parental HCT-116.

Chemicals and reagents

ITF2357 (givinostat) was synthesized and supplied by the pharmaceutical company Italfarmaco S.p.A. (Cinisello Balsamo, MI, Italy). For *in vitro* experiments, ITF2357 was dissolved in DMSO (20 mM stock solution) and stored at −20°C. Before use, the stock solution was thawed and diluted in McCoy's 5A or DMEM culture media, not exceeding 0.01% (v/v) DMSO, to realize the proper final concentrations.

The autophagy inhibitor bafilomycin A1 (Cat. n° B1793-2UG, Sigma-Aldrich, United States) was solubilized in DMSO, according to the data sheet instructions and used for the experiments at 20 nM and 50 nM final concentrations.

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide] assay

Cell viability was determined by MTT assay, following the manufacturer's instructions (Cat. n° M6494, Thermo Fisher®, United States), and the absorbance was measured using a biophotometer at 540 nm (BioTek Elisa ELx800 Absorbance Microplate Reader, BioTek Instruments, United States).

HCT-116 cells (wild type, silenced for lncH19, or 5-FU-resistant) were seeded in at least three technical replicates at 5×10^4 cells/cm^2; then, 24 h post-seeding, cells were treated with different concentrations of ITF2357 (0.25–0.5–1–2 μM or 4 μM) and maintained in a humidified atmosphere of 5% CO_2 at 37°C. The MTT assay was performed at different time points, as indicated in the results.

For the experiments with the autophagy inhibitor bafilomycin A1, HCT-116 cells were pretreated for 1 h with bafilomycin A1 (20 nM and 50 nM concentrations), and then, ITF2357 was added at different concentrations (0.25–0.5 μM or 1 μM) for 48 h.

Colony formation assay

LncH19-silenced HCT-116 cells and control cells were seeded at 40 cells/cm^2 in six-well plates. After 48 h, cells were treated with different concentrations of ITF2357 (0.05–0.1–0.25 μM and 0.5 μM) and maintained in culture for 8 days to allow clone formation. Clones were then washed once with phosphate buffer solution (PBS), fixed, and stained with methylene blue 1% in PBS/ethanol 50% for 1 min at room temperature. Following air-drying, clones were observed under a light microscope (LeicaDMR, Microsystems S.r.l, Wetzlar, Germany). Only clones containing more than 50 cells were considered and counted. For counting, each well was divided into four quadrants, and the media of the number of clones in each quadrant was estimated. The total number of clones per well was then obtained.

Annexin V/PI apoptosis detection assay

Annexin V/PI apoptosis detection assay (APC Annexin V Apoptosis Detection Kit with PI, cat. n° 640932, BioLegend®) was used to identify early and late apoptotic cells. LncH19-silenced HCT-116 cells and respective control cells were seeded at 1.87×10^4 per cm^2, allowed to adhere overnight, and then treated with 1 μM ITF2357 for 48 h.

Briefly, following the manufacturer's instructions, cells were harvested, and after centrifugation, cell pellets were washed twice with the cold BioLegend cell staining buffer (Cat. n° 420201), resuspended in annexin V binding buffer, and labeled with APC annexin V and propidium iodide.

Approximately 50,000 events were acquired for each sample on a FACSCanto cytometer (Becton Dickinson, Franklin Lakes, NJ, United States). Flow cytometry data were analyzed using FlowJo software (v10; TreeStar, Ashland, OR, United States).

Western blotting

H19-silenced HCT-116 cells and control HCT-116 cells were lysed using a lysis buffer (15 mM Tris/HCl pH 7.5, 120 mM NaCl, 25 mM KCl, 1 mM EDTA, and 0.5% Triton X-100) supplemented with phosphatase inhibitor cocktail (Cat. N° 37492, Active Motif, United States) for 1.30 h on ice. Cell debris was removed by centrifugation at 17,000 × g for 15 min at 4°C, and the supernatant, containing the protein lysate, was quantified using the Bradford assay method (Pierce™ Coomassie Plus Assay Kit, cat. N° 23236, Thermo Fisher Scientific, United States) using bovine serum albumin (BSA, cat. n° A2153, Sigma-Aldrich, United States) as a standard. A measure of 15 µg of protein from each sample was separated using Bolt Bis–Tris gel 4%–12% (Cat. n° NP0326BOX, Thermo Fisher Scientific, United States) and transferred onto a nitrocellulose blotting membrane (Amersham Protran Premium 0.45 µm NC by GE Healthcare Life Science, United Kingdom). The membranes were stained with 0.1% red Ponceau in 5% acetic acid to evaluate the correct loading of all samples. The membranes were incubated for 1 h in a blocking solution (5% milk or 5% BSA in 20 mM Tris, 140 mM NaCl, and 0.1% Tween-20) and at 4°C overnight with the following primary antibodies: anti-SQSTM1/p62 (1:500, cat. n° 39749S, Cell Signaling Technology, United States), anti-LC3B (1:500, cat. n° 2775S, Cell Signaling Technology, United States), anti-poly ADP-ribose polymerase-1 (Anti-PARP-1, 1:500, cat. n° sc-8007, Santa Cruz Biotechnology, United States), anti-cleaved caspase-3 (1:400, cat. n° 9664S, Cell Signaling Technology, United States), and anti-p53 (DO-1, 1:200, cat. n° sc-126, Santa Cruz Biotechnology, United States).

After washing with Tris-buffered saline-Tween-20 (TBS-T, 20 mM Tris, 140 mM NaCl, 0.1% Tween-20) three times, the membrane was incubated with appropriate secondary antibodies such as HRP-conjugated goat anti-rabbit IgG (1:10.000, cat. n° 31460, Invitrogen™, Thermo Fisher® Scientific, United States) and anti-mouse IgG (1:10.000, cat. n° 7076, Cell Signaling Technology, United States) at room temperature for 1 h. The chemiluminescent signal was visualized using a chemiluminescence solution (ECL™ Prime Western Blotting System, Cytiva, RPN2232) and detected using the ChemiDoc acquisition instrument (Bio-Rad, United States). The images were analyzed using Image Lab software (Bio-Rad, United States).

Depending on the molecular weight of the protein, if required, the membranes were subjected to a stripping protocol before proceeding with further incubation with other antibodies. This involved a brief incubation of 10–15°min with a stripping solution (Restore™ PLUS Western Blot Stripping Buffer, Cat. n° 46,430, Thermo Fisher® Scientific, United States) at 37°C, followed by subsequent washes in TBS-T.

LC3-B assay

HCT-116 cells were seeded at 5 × 10^4 cells/cm^2 in cell culture chamber slides (Cat. n° 94.6190.802, Sarstedt, Germany), and the LC3B assay (Cat. n°L10382, LC3B Antibody Kit for Autophagy, Invitrogen™ by Thermo Fisher® Scientific, United States) was performed following the manufacturer's instructions.

Briefly, 24 h after seeding, HCT-116 cells were treated for 24 h with 50 µM chloroquine diphosphate (CQ, provided by the LC3B Antibody Kit for Autophagy) alone or co-treated with 50 µM chloroquine and 1 µM of ITF2357. Chloroquine blocks autophagosome–lysosome fusion, thus allowing autophagosome visualization. After treatments, cells were fixed with 4% paraformaldehyde for 15 min, permeabilized with 0.1% Triton X-100 for 15 min, and incubated with diluted LC3B rabbit polyclonal primary antibody (0.5 µg/mL according to the manufacturer's instructions) for 1 h. DyLight™ 594 was used as a secondary antibody (Goat anti-Rabbit IgG Secondary Antibody, DyLight™ 594, 1:300, cat n°35560, Invitrogen™ by Thermo Fisher Scientific, United States).

Finally, cells have been counterstained with Hoechst (Hoechst 33342, trihydrochloride, trihydrate, 1:1000, cat n°H3570, Molecular Probes, Life Technologies by Thermo Fisher Scientific, United States) and ActinGreen (ActinGreen™ 488 ReadyProbes™ Reagent, 1:125, cat n°R37110, Invitrogen™ by Thermo Fisher Scientific, United States). All steps have been performed at room temperature. The samples were analyzed using a Nikon A1 confocal microscope.

RNA extraction and real-time PCR (qRT-PCR)

Total RNA was extracted using the commercially available Macherey–Nagel™ NucleoSpin™ miRNA Kit (Cat. n°740971.250, Macherey–Nagel, Germany), according to the manufacturer's instructions. The total RNA concentration was detected with the Nanodrop spectrophotometer (Thermo Fisher®, United States) and reverse-transcribed to cDNA using the High-Capacity cDNA Reverse Transcription kit (Cat. n° 4368814, Applied Biosystem™, United States).

Quantitative real-time polymerase chain reactions (qRT-PCR) were carried out using the SYBR™ Green PCR Master Mix (Cat. n° 4309155, Applied Biosystems™, United States), following the manufacturer's instructions in a Step One™ Real-time PCR System Thermal Cycling Block (Applied Biosystems, Waltham, MA, United States).

The primers' sequences used for expression analysis of the genes of interest are reported in Table 1. Gene expression levels were normalized using β-actin as an endogenous control. Finally, the data are presented as $2^{-\Delta\Delta Ct}$ compared with the untreated control.

Bioinformatic analysis

For predicting interactions between ncRNAs and their targets, bioinformatic analyses were performed using DIANA tools (Rincon-Riveros et al., 2021). Specifically, lncH19–miRNA interactions were identified using DIANA-LncBase v.3, while miRNA–TP53 interactions were identified using DIANA-TarBase v.8.

In *Homo sapiens*, we identified 159 validated miRNAs that lncH19 directly binds to and 42 validated miRNAs that directly bind to the TP53 gene.

TABLE 1 Primers' sequences of the genes analyzed.

Primer	Forward	Reverse
H19	TCGTGCAGACAGGGCGACATC	CCAGCTGCCACGTCCTGTAACC
SQSTM1/p62	TGTGTAGCGTCTGCGAGGGAAA	AGTGTCCGTGTTTCACCTTCCG
MAP1LC3A	GCTACAAGGGTGAGAAGCAGCT	CTGGTTCACCAGCAGGAAGAAG
ATG16L	CTACGGAAGAGAACCAGGAGCT	CTGGTAGAGGTTCCTTTGCTGC
LAMP1	CGTGTCACGAAGGCGTTTTCAG	CTGTTCTCGTCCAGCAGACACT
LAMP2	GGCAATGATACTTGTCTGCTGGC	GTAGAGCAGTGTGAGAACGGCA
TP53	CCTGGATTGGCCAGACTGC	TTTTCAGGAAGTAGTTTCCATAGGT
NOXA	AGCTGGAAGTCGAGTGTGCT	ACGTGCACCTCCTGAGAAAA
PUMA	GGAGCAGCACCTGGAGTC	TACTGTGCGTTGAGGTCGTC
β-ACTIN	TCCCTTGCCATCCTAAAAGCCACCC	CTGGGCCATTCTCCTTAGAGAGAAG

TABLE 2 Twenty six miRNAs sponged from lncH19 that directly target the pro-apoptotic TP53 gene.

hsa-let-7a-5p	hsa-miR-17-5p	hsa-miR-107
hsa-let-7b-5p	hsa-miR-19a-3p	hsa-miR-125b-5p
hsa-let-7c-5p	hsa-miR-19b-3p	hsa-miR-181a-5p
hsa-let-7d-5p	hsa-miR-22-3p	hsa-miR-218-5p
hsa-let-7e-5p	hsa-miR-24-3p	hsa-miR-522-5p
hsa-let-7f-5p	hsa-miR-30a-5p	hsa-miR-940
hsa-let-7g-5p	hsa-miR-34a-5p	—
hsa-let-7i-5p	hsa-miR-93-5p	—
hsa-miR-10b-5p	hsa-miR-98-5p	—
hsa-miR-15a-5p	hsa-miR-103a-3p	—

By overlaying the two datasets from DIANA-LncBase v.3 (lncH19–miRNA interactions) and DIANA-TarBase v.8 (miRNA–TP53 interactions), we found that lncH19 can bind to 26 miRNAs that directly target the pro-apoptotic TP53 gene (Table 2).

Statistical analysis

Data reported in all graphs are expressed as the mean ± standard deviation (SD) of at least three independent biological replicates. The following tests have been performed: Student's t-test to compare two groups, one-way ANOVA for comparisons among three or more groups, and two-way ANOVA for comparison of multiple variables among two groups. Analyses were performed using GraphPad Prism software (GraphPad Software, United States).

p-values were indicated in the graphs as follows: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; and **** = $p < 0.0001$. A *p*-value ≤0.05 was considered significant.

Results

ITF2357 reduces CRC cell viability and increases the expression levels of lncH19

Initially, to evaluate the sensitivity of the HCT-116 CRC cell line to ITF2357, cells were treated with different concentrations of ITF2357 for 16 h, 24 h, 48 h, and 72 h. Evaluation of cell morphology indicated that the drug exerted a cytotoxic effect, which appeared after 24 h in cells treated with 1 μM ITF2357 and was clearly evident after 48 h either with 1 μM or 2 μM (Figure 1A). Morphological data were confirmed by the MTT assay (Figure 1B). As expected, ITF2357 treatment reduced the viability of HCT-116 cells in a dose- and time-dependent manner. Approximately 50% reduction in viability was observed after 48 h of treatment with 1 μM ITF2357.

LncH19 is known to display the oncogenic activity in CRC, promoting cell proliferation (Yang et al., 2017), epithelial-to-mesenchymal transition (Ding et al., 2018), and 5-FU drug resistance (Wang et al., 2018). To elucidate whether HDACi modifies the expression levels of lncH19, we performed qRT-PCR analyses. Interestingly, the results revealed that ITF2357 promoted lncH19 expression in HCT-116, determining a two-fold increase in the level of lncRNA after 24 h of treatment and almost three-fold increase at 48 h (Figure 1C). Therefore, we hypothesized that lncH19 induction could somehow be functional to ITF2357 to exert its cytotoxic effect.

To verify this hypothesis, HCT-116 cells were stably silenced for lncH19, and the silencing efficiency was confirmed through gene expression analysis (Figure 2A). Cell viability assays in H19-silenced cells revealed that ITF2357 displayed much less efficacy under lncH19 knockdown. Indeed, the effect of ITF2357 was reduced by approximately 15%, suggesting that lncH19 plays a role in ITF2357-induced cytotoxicity in CRC cells (Figures 2B, C).

Moreover, colony formation assay further confirmed a direct role of lncH19 to sustain the efficacy of HDACi in CRC cells. Specifically, as shown in Figure 2D, treatment with ITF2357 affected the clonogenicity of HCT-116 control cells in a dose-dependent manner, while this effect was significantly weaker in H19-silenced

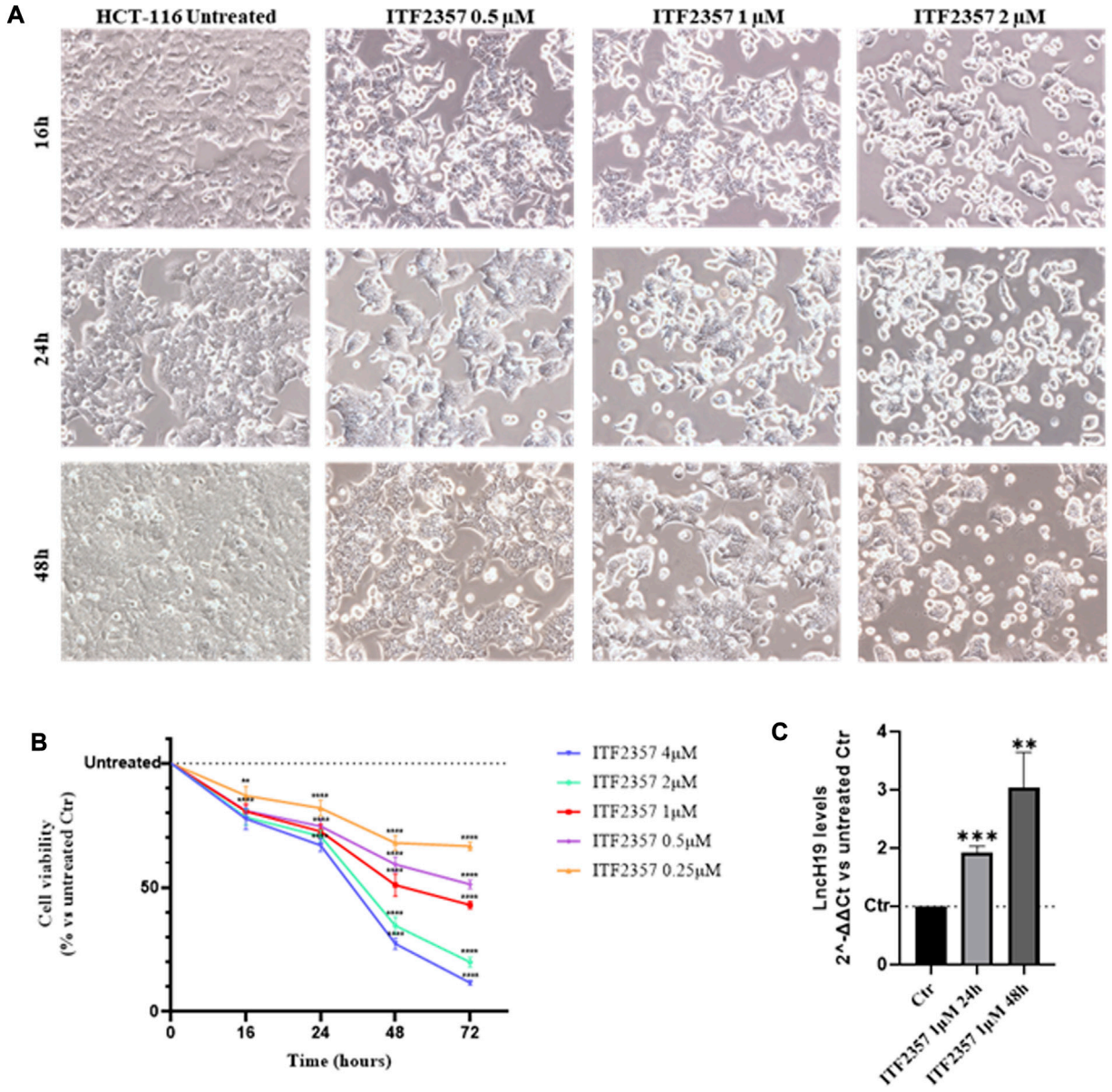

FIGURE 1
Effects of ITF2357 on HCT-116 cell viability and lncH19 expression. **(A)** Phase contrast images of HCT-116 cells treated with different concentrations of ITF2357 (0.5–1 µM and 2 µM) for 16 h, 24 h, and 48 h. The cells were visualized under a light microscope at ×20 magnification, and the pictures were acquired using NISA1 Leica software. **(B)** Cell viability assay (MTT assay) in HCT-116 cells treated with different concentrations of ITF2357 (0.25–0.5–1–2 µM and 4 µM) for 16 h, 24 h, 48 h, and 72 h. Data are expressed as cell viability percentages compared to untreated cells (Ctr). The results reported in the graph are expressed as the mean ± SD of three independent biological replicates. Statistical analyses were performed using ordinary two-way ANOVA with Bonferroni's multiple comparison test; $^{**}p < 0.01$ and $^{****}p < 0.0001$. **(C)** Analysis of the expression level (qRT-PCR) of lncH19 in HCT-116 cells treated with 1 µM ITF2357 for 24 h and 48 h. LncH19 expression levels are reported as $2^{-\Delta\Delta Ct}$ compared to untreated cells (Ctr), and the threshold cycle (Ct) was normalized against β-actin. The results reported in the graph are expressed as the mean ± SD of three independent biological replicates. Statistical analyses were performed using Student's t-test; $^{*}p < 0.05$ and $^{**}p < 0.01$.

cells, as also revealed by the quantification of the number of clones in the two cell types (Figure 2E).

ITF2357 induces pro-survival autophagy in CRC cells

It is well known that both HDACis and lncH19 induce autophagy in different tumor cells (Xu et al., 2018; Mrakovcic and Frohlich, 2019; Zhao et al., 2021). Therefore, we hypothesized that ITF2357, enforced by H19 expression, induces autophagy-dependent cell death. To verify this hypothesis, the transcriptional levels of some autophagy markers (ATG16L, SQSTM1/p62, MAP1LC3B/LC3, and LAMP1/2) were analyzed. As shown in Figure 3A, ITF2357 upregulated all the autophagy genes analyzed, an effect that was already evident after 24 h. This effect was maintained after 48 h of treatment (data not shown).

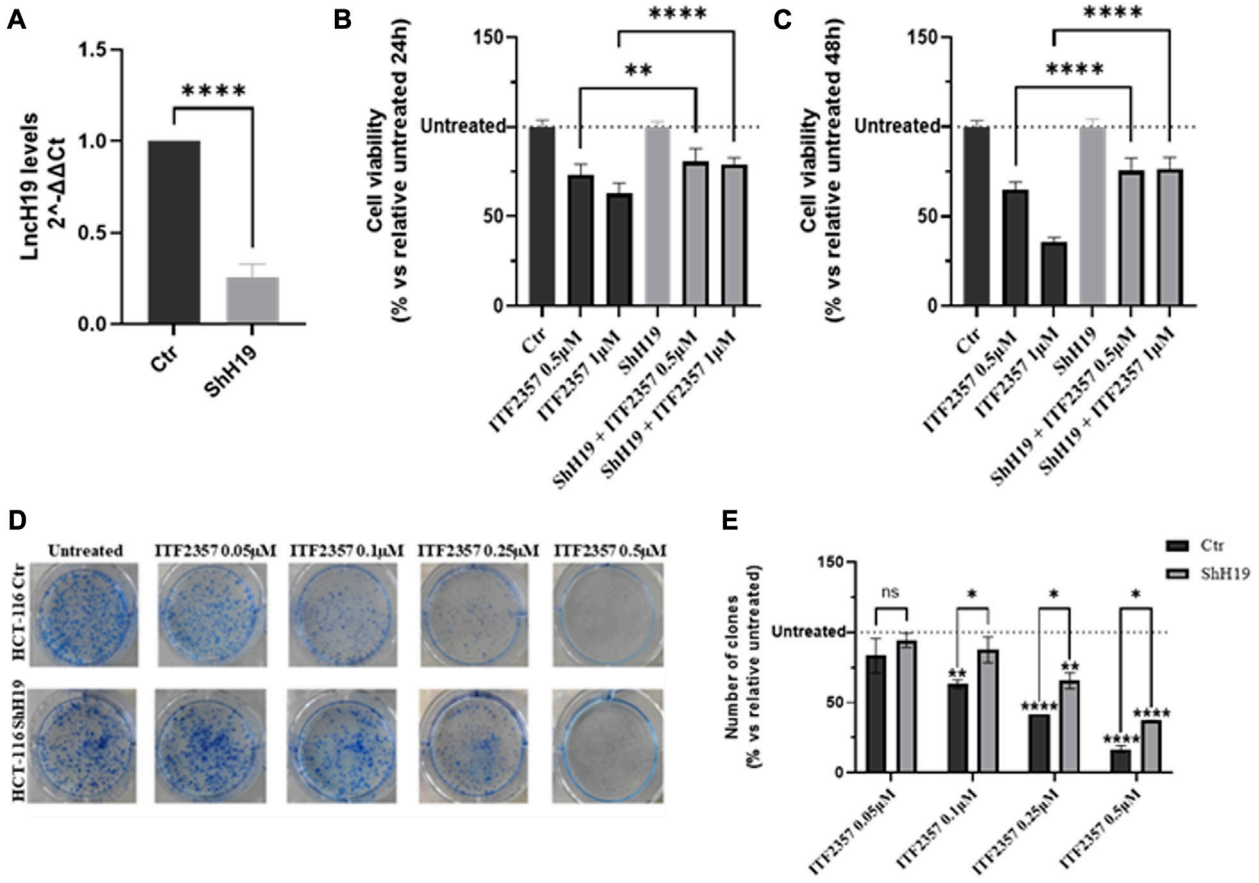

FIGURE 2
Effects of silencing lncH19 in HCT-116-silenced cells treated with ITF2357. **(A)** Analysis of the expression level (qRT-PCR) of lncH19 in HCT-116-silenced cells with respect to control cells (Ctr). LncH19 expression levels are reported as $2^{-\Delta\Delta Ct}$ compared to control cells (Ctr); Ct was normalized against β-actin. Data are expressed as the mean ± SD of three independent biological replicates. Statistical analyses were performed using Student's t-test, ****$p < 0.0001$. **(B, C)** Cell viability assay (MTT assay) in HCT-116 cells (that are silenced or not) for lncH19 and treated with two different concentrations of ITF2357 (0.5 and 1 μM) for 24 h (left graph) and 48 h (right graph). Data are expressed as the cell viability percentage compared to untreated cells. Data are expressed as the mean ± SD of three independent biological replicates. Statistical analyses were performed using ordinary one-way ANOVA with Bonferroni's multiple comparison test; **$p < 0.01$ and ****$p < 0.0001$. **(D, E)** Clonogenic assay in HCT-116 cells with silenced or unsilenced lncH19 cells, untreated or treated with indicated concentrations of ITF2357, and maintained in culture for 8 days to allow clone formation. In the histogram, data are expressed as a percentage of the number of clones compared to relative untreated cells. Data are expressed as the mean ± SD. Statistical analyses were performed using ordinary two-way ANOVA with Bonferroni's multiple comparison test; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

The activation of autophagy was confirmed by an increase in the LC3B signal in autophagosomes, as revealed by immunofluorescence (Figure 3B). These data were confirmed by Western blot analysis, showing a much higher level of LC3II-cleaved form in ITF2357-treated cells. Moreover, further confirmation of the autophagic process induced by ITF2357 was sustained by the significant decrease in the levels of p62 protein (Figures 3C, D). This marker is usually considered to monitor the autophagic flux, and it is associated with completed autophagy when decreasing since it is degraded by the autophagosome (Emanuele et al., 2020).

To investigate whether the activation of autophagy in HCT-116 cells could promote cell death, cell viability was evaluated in cells treated with ITF2357 in the presence of the autophagy inhibitor bafilomycin A1.

As shown in Figure 3E, the cytotoxic effect exerted by three different doses of ITF2357 was enhanced when co-treated with either 20 nM or 50 nM bafilomycin A1. These data suggest that autophagy induced by the HDAC inhibitor represents a pro-survival adaptive response to the effects of the compound. Moreover, we provided evidence that H19 silencing did not affect ITF2357-induced autophagy (Supplementary Figure S1).

ITF2357 induces apoptosis in HCT-116 cells, and lncH19 is functional to this effect

To further characterize cell death activated in response to ITF2357 and elucidate the role of lncH19, apoptosis was investigated in H19-silenced cells in comparison with the respective control cells. Specifically, an annexin V/PI apoptotic assay was performed at early (16 h) and late (48 h) treatment time points to properly detect the process over time. The results shown in Figures 4A, B indicate that ITF2357 stimulated early and late apoptosis to a different extent in control and H19-silenced cells.

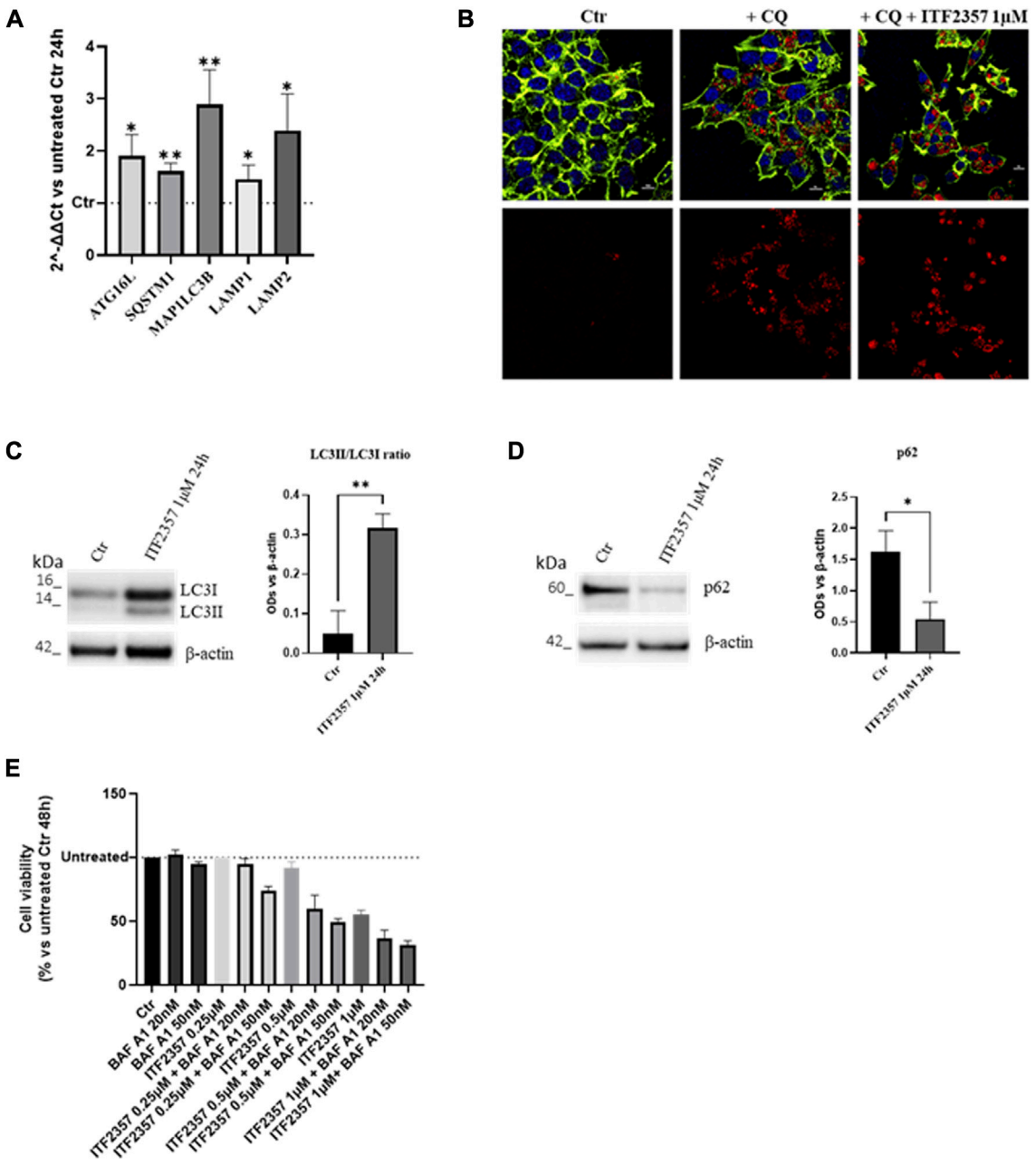

FIGURE 3
HDAC inhibitor ITF2357 induces survival autophagy in CRC cells. **(A)** Analysis of the expression level (qRT-PCR) of autophagic genes in HCT-116 cells treated with 1 μM concentration of ITF2357 for 24 h. The expression levels of genes are reported as $2^{-\Delta\Delta Ct}$ compared to untreated cells (Ctr), and Ct was normalized against β-actin. Data are expressed as the mean ± SD of three independent biological replicates. Statistical analyses were performed using Student's t-test; *$p < 0.05$ and **$p < 0.01$. **(B)** Immunofluorescence for LC3B on HCT-116 cells, untreated or treated with 50 μM chloroquine diphosphate (CQ) alone or in combination with 1 μM of ITF2357 for 24 h. LC3B is represented in red, counterstained with Hoechst and ActinGreen, for nuclei in blue and cytoskeleton in green, respectively. Nuclear focal plane; the scale bar is 10 μm. **(C)** Representative images and densitometric analysis of Western blots for LC3II/LC3I in HCT-116 cells treated or not with ITF2357 1 μM for 24 h. The graph shows the ratio of the normalized optical density (OD). Housekeeping β-actin was used as a loading control. Data are expressed as the mean ± SD of three independent biological replicates. Statistical analyses were performed using Student's t-test, **$p < 0.01$. **(D)** Representative images and densitometric analysis of Western blots for p62 in cells treated or not with ITF2357 1 mM concentration for 24 h. The graph shows the normalized OD. Housekeeping β-actin was used as a loading control. Data are expressed as the mean ± SD of three independent biological replicates. Statistical analyses were performed using Student's t-test, *$p < 0.05$. **(E)** Cell viability assay (MTT assay) in HCT-116 cells co-treated with different concentrations of ITF2357 (0.25–0.5 μM and 1 μM) and two different concentrations of bafilomycin A1 (20 nM and 50 nM) for 48 h. Data are expressed as cell viability percentages compared to untreated cells (Ctr). Data are expressed as the mean ± SD.

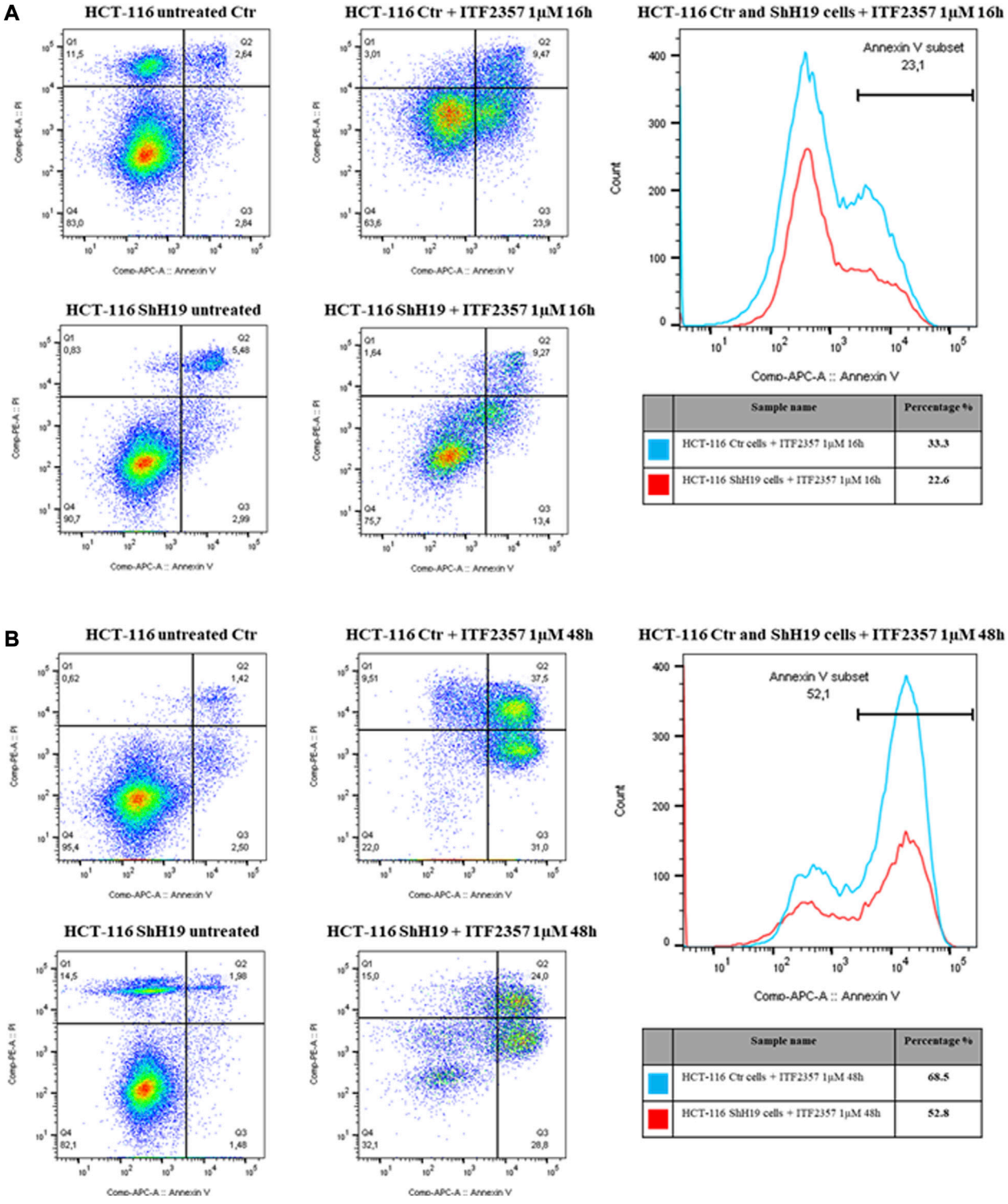

FIGURE 4
(Continued).

Indeed, the total percentage of annexin V positive cells after treatment with ITF2357 was approximately 33% in control cells, compared to 22.6% in H19-silenced cells at 16 h. Such a difference was maintained at 48 h (68.6% in control cells vs. 52.8% in H19-silenced cells), thus confirming that lncH19 knockdown reduces the apoptotic efficacy of ITF2357.

Morphological analysis of ITF2357-treated cells clearly showed the differential effect of HDACi in the two cell types (Figure 4C).

These data were confirmed by Western blot analysis of apoptotic markers, including cleaved caspase 3 and cleaved PARP-1, an analysis that was performed at late time points to evidence apoptosis execution. As shown in Figures 4D, E, although

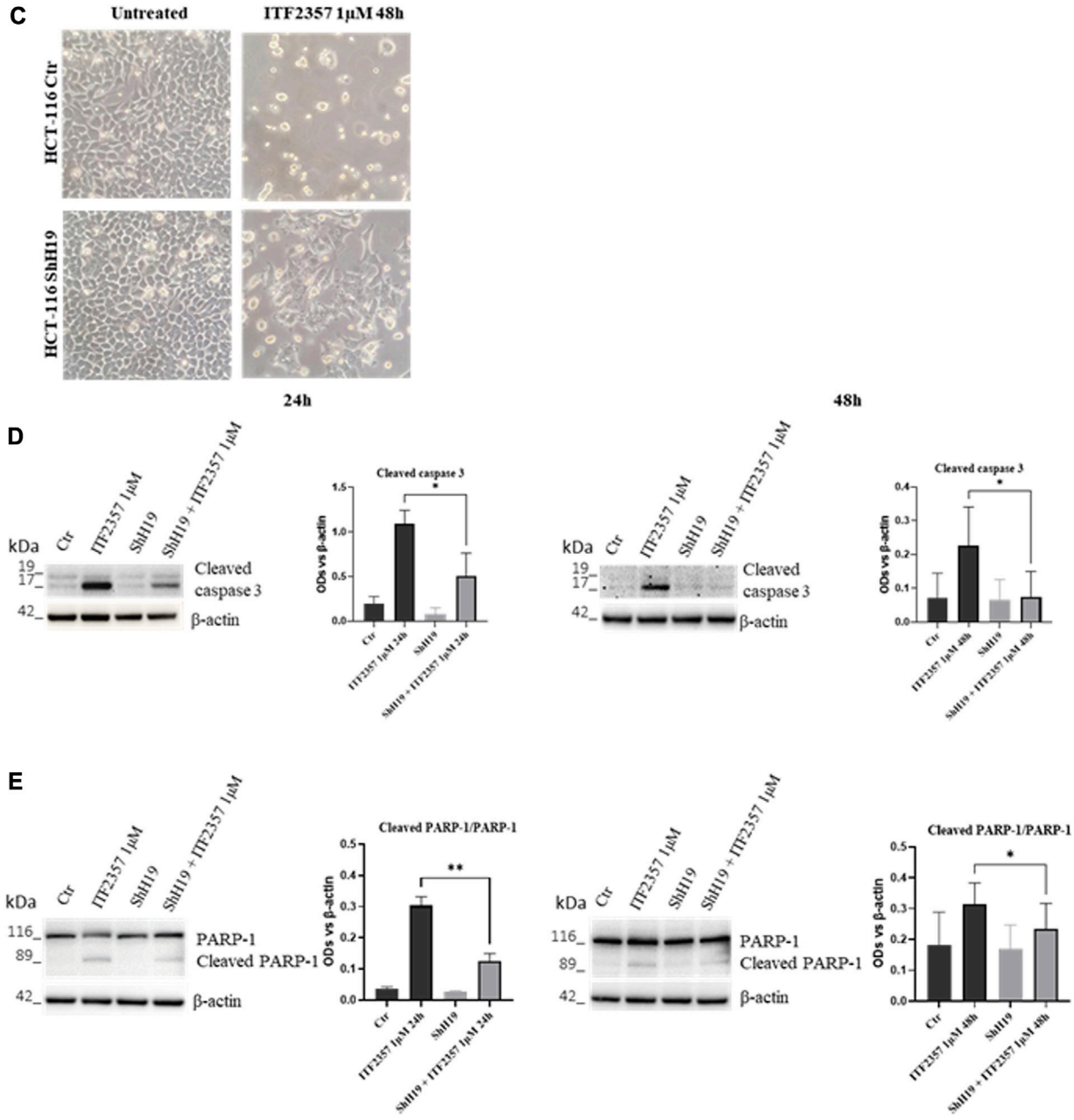

FIGURE 4
(Continued). Effects of lncH19 silencing on apoptosis markers. **(A, B)** Annexin V/PI apoptosis detection assay on HCT-116 cells silenced for lncH19 or unsilenced control cells (Ctr) treated with 1 μM concentration of ITF2357 for 16 h and 48 h. Data are expressed as the apoptotic cell percentage compared to untreated cells (silenced or unsilenced for lncH19). **(C)** Phase contrast images of HCT-116 cells with silenced lncH19 or unsilenced control cells (Ctr), untreated or treated with 1 μM of ITF2357 for 48 h. Cells were visualized under a light microscope at ×20 magnification, and the pictures were acquired using IM50 Leica software (Leica DMR Microsystems, Wetzlar, Germany). **(D, E)** Representative images and densitometric analysis of Western blots for cleaved caspase 3 **(D)** and cleaved PARP-1/PARP-1 **(E)** obtained from protein lysates of HCT-116 silenced for lncH19 or control cells (Ctr) were treated with 1 μM ITF2357 for 24 h or 48 h. The graphs show the OD of the indicated proteins normalized for the housekeeping's OD (β-actin). Data are expressed as the mean ± SD of three independent biological replicates. Statistical analyses were performed using Student's t-test in **(D, E)**; $*p < 0.05$ and $**p < 0.01$.

caspase 3 cleavage and PARP-1 degradation were evident in ITF2357-treated control cells, these effects were much less evident in H19-silenced cells. These data suggest that H19 expression somehow reinforces the pro-apoptotic action of ITF2357.

To investigate the molecular mechanism by which lncH19 promotes ITF2357-induced apoptosis, we focused on identifying putative miRNAs with a pro-apoptotic role that could be targeted by lncH19. Similar to other lncRNAs, H19 can also behave as an endogenous competitive sponge for

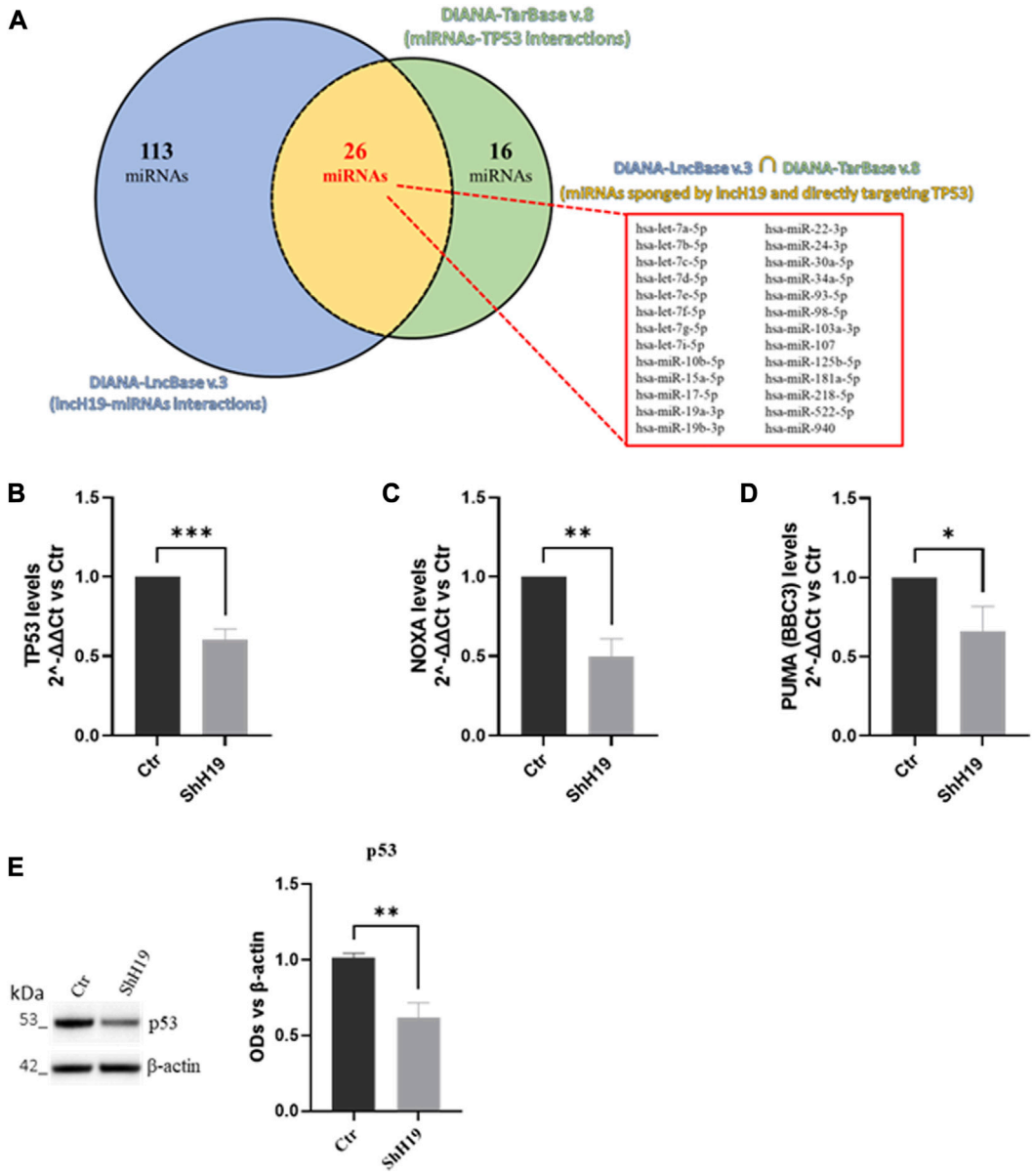

FIGURE 5
Identification of lncH19 miRNAs that target TP53. **(A)** Venn diagram obtained by bioinformatic analysis using DIANA tools, illustrating the intersection (in yellow) between the dataset of validated direct miRNAs that lncH19 binds to (DIANA-LncBase v.3, in blue) and the dataset of validated miRNAs that directly bind to TP53 (DIANA-TarBase v.8, in green). The intersection shows 26 miRNAs (listed in the panel) sponged from lncH19 that directly target the pro-apoptotic TP53 gene. **(B–D)** Analysis of the expression levels (qRT-PCR) of TP53 **(B)**, NOXA **(C),** and PUMA **(D)** in HCT-116 cells with respect to control cells (Ctr). Gene expression levels are reported as $2^{-\Delta\Delta Ct}$ compared to control cells (Ctr); Ct was normalized against β-actin. Data are expressed as the mean ± SD of three independent biological replicates. Statistical analyses were performed using Student's t-test; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. **(E)** Representative images and densitometric analysis of Western blots for p53 in HCT-116 with respect to control cells (Ctr). The graphs show the OD of the indicated proteins normalized for the housekeeping's OD(β-actin). Data are expressed as the mean ± SD of three independent biological replicates. Statistical analyses were performed using Student's t-test, **$p < 0.01$.

miRNAs (Ye et al., 2019). By using DIANA tools (Rincon-Riveros et al., 2021), we identified 159 validated human miRNAs sponged by lncH19, and among these, 26 validated human miRNAs directly target the pro-apoptotic TP53 gene (Figure 5A). Real-time PCR in Figure 5B confirmed a positive correlation between the expression of lncH19 and TP53. The transcriptional analyses revealed that cells silenced for lncH19 express lower levels of TP53 and its targets, PUMA and NOXA (Figures 5B–D). The reduction of p53 in shH19 cells was further confirmed at the protein level (Figure 5E). Overall, these data indicate that ITF2357 induces TP53-mediated apoptosis in colorectal cancer cells, and the expression of lncH19 plays a functional role in regulating p53 expression.

Finally, to assess whether ITF2357 can overcome the resistance to 5-FU chemotherapeutics, we used the HCT-116-5-FU-R, a 5-FU-resistant HCT-116 cell line properly selected in our laboratory. Interestingly, HCT-116-5-FU-R cells express high levels of lncH19 compared to parental HCT-116 cells (Figure 6A). It is

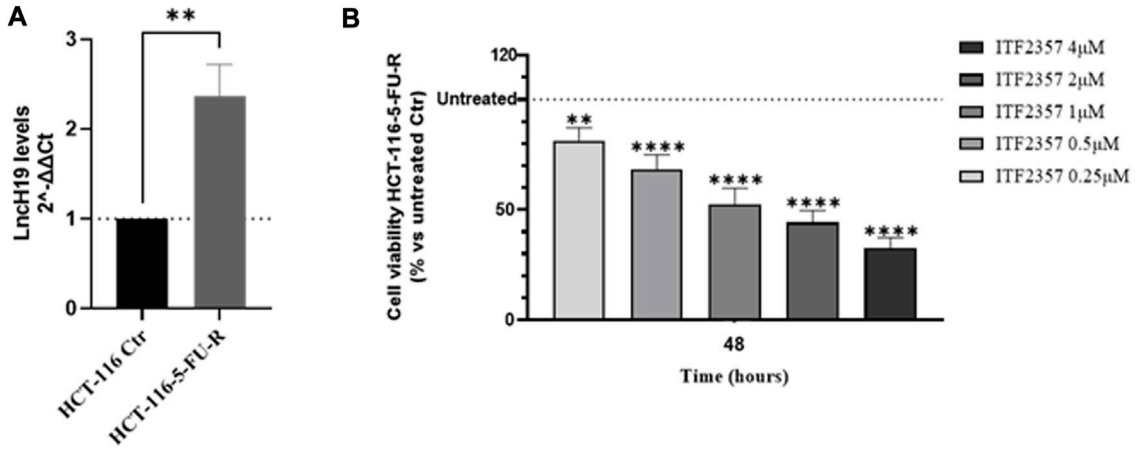

FIGURE 6
HCT-116 cells resistant to 5-fluorouracil (5-FU) express high levels of lncH19 and respond to treatment with ITF2357. **(A)** Analysis of the expression level (qRT-PCR) of lncH19 in HCT-116-5-FU-R cells compared to untreated cells (HCT-116 Ctr). LncH19 expression levels are reported as $2^{-\Delta\Delta Ct}$ compared to HCT-116 Ctr cells, and Ct was normalized against β-actin. The results reported in the graph are expressed as the mean ± SD of three independent biological replicates. Statistical analyses were performed using Student's t-test, $^{**}p < 0.01$. **(B)** Cell viability assay (MTT assay) in HCT-116-5-FU-R cells treated with different concentrations of ITF2357 (0.25–0.5–1–2 μM and 4 μM) for 48 h. Data are expressed as cell viability percentages compared to untreated cells (Ctr). The results reported in the graph are expressed as the mean ± SD of three independent biological replicates. Statistical analyses were performed using ordinary one-way ANOVA with Bonferroni's multiple comparison test; $^{**}p < 0.01$ and $^{****}p < 0.0001$.

noteworthy that these cells nicely respond to ITF2357, as indicated by the cell viability evaluation reported in Figure 6B, which revealed a dose-dependent effect of the compound.

Discussion

This paper shows, for the first time, that lncH19 supports apoptosis induced by HDACi ITF2357 in colon cancer cells. Although some papers sustain the potential of HDACis in colon cancer treatment (Garmpis et al., 2022; Lee et al., 2022), to date, no evidence has been provided about the efficacy of this pan-HDACi in colon cancer cells. Our data indicate that ITF2357 is active in colon cancer cells at micromolar concentrations, in line with the findings of other authors in different tumor cell lines (Angeletti et al., 2016; Di Martile et al., 2018; Celesia et al., 2022; Celesia et al., 2023).

We also provided evidence that ITF2357 upregulates lncH19 in colon cancer cells. Similarly, Di Fazio et al. found increased lncH19 levels in adrenocortical carcinoma, following treatment with pan-HDACis such as panobinostat, trichostatin A (TSA), and SAHA, correlated with autophagy induction (Di Fazio et al., 2022).

To understand the role of lncH19 in ITF2357-induced cytotoxicity in colon cancer cells, both autophagy and apoptosis induction were examined in H19 stably silenced HCT-116 cells in comparison with control HCT-116 cells. It is well known that HDACis can promote autophagy in different tumor types (Bai et al., 2019; Xiao et al., 2020; Korholz et al., 2021). However, it is well known that autophagy can exert a dual role in tumor cells. Indeed, the process can be activated as a pro-survival response, which is frequently associated with tumor progression and chemoresistance, or it can serve a death-inducing function, thereby representing an alternative form of cell death to target tumor cells that have developed apoptosis resistance (Patra et al., 2019). This paper shows that ITF2357 promoted the expression of autophagy markers, including ATG16L, SQSTM1/p62, MAP1LC3B/LC3, and LAMP1/2. HDACi also induced the conversion of LC3I into active LC3II and a reduction in the levels of p62. Our data support the hypothesis that ITF2357-induced autophagy is correlated with a pro-survival cell response since the autophagy inhibitor bafilomycin A1 markedly potentiated the cytotoxic effect of the compound and the p62 protein marker decreased, indicating autophagy completion (Emanuele et al., 2020). Our findings are in accordance with the observation of Angeletti et al., who found that inhibition of autophagy potentiates the effect of ITF2357 in glioblastoma cells (Angeletti et al., 2016). However, our Supplementary Material indicates that lncH19 silencing does not significantly modify the levels of autophagy markers.

Therefore, we concluded that the cytotoxic effect of ITF2357 does not depend on autophagy-induced cell death, and subsequently, caspase-dependent apoptosis was considered.

Evaluation of apoptosis by annexin V/PI double staining and analysis of apoptotic markers revealed that lncH19 plays a role in this event. Indeed, ITF2357-induced apoptosis was reduced in H19-silenced cells compared to the respective control cells. We consider these results relevant since they imply that lncH19 can be exploited to favor apoptosis induction and that HDACi may promote a H19-dependent targeted effect in colon cancer cells. In accordance with our results, other authors have previously found a correlation between lncH19 and apoptosis.

In particular, Hou et al. have shown that overexpressed lncH19 alleviates induced lung injury in mice, as well as lipopolysaccharide (LPS)-induced apoptosis, oxidative stress, and inflammation (Hou et al., 2022). Similarly, Yang provided evidence that H19 silencing alleviates LPS-induced apoptosis and inflammation by regulating the miR-140-5p/TLR4 axis in cell models of pneumonia (Yang, 2023). In a more specific tumoral context, lncH19 has been shown to participate in triptolide/TNF-α-

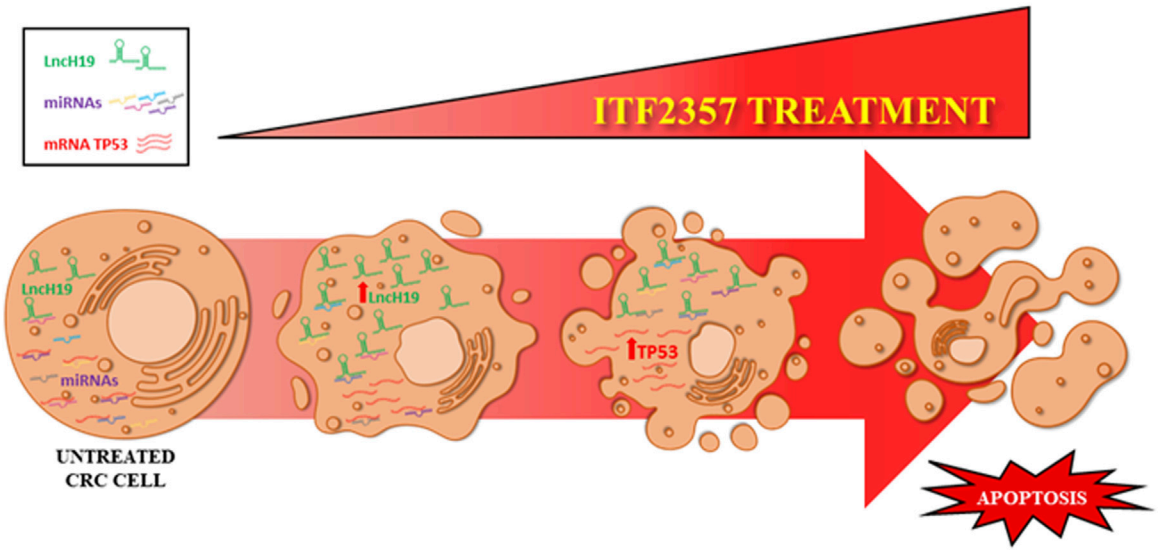

FIGURE 7
Schematic representation of the proposed model. The levels of lncH19 increase in CRC cells treated with HDACi ITF2357. This increases the sponge effect by lncH19 on miRNAs targeting pro-apoptotic genes, including TP53. Overall, treatment with ITF2357 increases lncH19 levels and promotes activation of apoptosis, thus leading to increased expression of TP53.

induced apoptosis via binding miR-204-5p in gastric cancer models (Yuan et al., 2022). In addition, Liu et al. demonstrated that lncH19 inhibits proliferation and enhances apoptosis of nephroblastoma cells by regulating the miR-675/TGFBI axis (Liu et al., 2022). Accordingly, lncH19 has also been implicated in sensitization to X-ray and carbon ion irradiation of non-small cell lung cancer (Zhao et al., 2021), and positively modulates the sensitivity of glioma cells to radiation-favoring apoptosis (Kuang et al., 2021). However, some controversial data are present in the literature regarding the pro-apoptotic role of lncH19. For instance, the knockdown of H19 in resveratrol-treated cancer cells has been shown to enhance the effects of resveratrol on apoptosis (Li et al., 2022). Other evidence of an antiapoptotic role of lncH19 was provided by Wang et al., who showed that it promotes proliferation, migration, and invasion, and inhibits apoptosis of breast cancer cells by targeting the miR-491-5p/ZNF703 axis (Wang et al., 2020). It is clear that lncRNA H19 and many other cellular factors may exert a dual role in regulating cell fate (Shermane Lim et al., 2021).

Our data strongly suggest a pro-apoptotic role of lncH19 in CRC cells treated with HDACi ITF2357 since lncH19 silencing profoundly reduced the effects of the compound on cell viability and apoptosis. To explain the pro-apoptotic role of lncH19 in HDACi-treated cells, we hypothesized that it may act as an endogenous competitive sponge for miRNAs (Zhang et al., 2022), antagonizing miRNAs targeting pro-apoptotic genes. Bioinformatic analysis revealed that lncH19 sponged 26 validated human miRNAs directly targeting the pro-apoptotic gene TP53 (Figure 7).

Our data provide evidence that lncH19 knockdown reduces the expression of TP53 and its pro-apoptotic targets, PUMA and NOXA. The relationship between lncH19 and TP53 is controversial in the literature since some papers sustain a negative control of TP53 by H19 (Yang et al., 2012; Li et al., 2020; Gan et al., 2022), while others support that lncH19 may activate the tumor suppressor. Specifically, in accordance with our findings, we have shown that overexpression of lncH19 enhanced TP53 expression, whereas H19 silencing exerted the opposite effect (Zhuang et al., 2021). In addition, Du et al. have found that lncH19 promotes p53 phosphorylation by a direct interaction, an effect that results in increased NOTCH-mediated angiogenesis in mesenchymal stem cells (Du et al., 2023).

Interestingly, our paper also provided evidence that lncH19 is overexpressed in HCT-116-5-FU-R cells, and we consider it relevant that the HDACi ITF2357 was capable of overcoming 5-FU resistance in these cells. Other authors have associated 5-FU resistance with lncH19 expression (Wang et al., 2018; Yokoyama et al., 2019; Zhang et al., 2022); here, we suggest that this condition may be exploited to promote TP53-dependent apoptosis using HDACi. To date, several lines of evidence indicate that HDACi can sensitize different tumor types to the effects of diverse chemotherapeutic agents (Perego et al., 2012; Almeida et al., 2017; Minegaki et al., 2018; Rodrigues Moita et al., 2020; Roca et al., 2022).

It has to be considered that the present study refers to CRC cell lines, with all the limitations to an *in vitro* study; however, it represents a molecular basis to proceed with translational studies. In particular, we provided evidence for the first time that HDACi ITF2357 is efficacious in a colon cancer model by upregulating lncH19 and is capable of overcoming 5-FU resistance in highly H19-expressing CRC cells. These findings need to be validated *in vivo* for a possible clinical application in CRC patients displaying 5-FU drug resistance. In our opinion, the relevant finding was that lncH19, which canonically acts as an oncogene, may be exploited to favor apoptosis induced by ITF2357. This implies that high expression of lncH19 in CRC, especially in conditions of 5-FU resistance, may facilitate apoptosis induction.

Overall, our data suggest that lncH19 levels may be a useful parameter to promote epigenetic targeting of colon cancer and propose ITF2357 as a promising epi-drug in colon cancer treatment.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the studies on humans and animals in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used.

Author contributions

CZ: conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing–original draft, and review and editing. ML: investigation, methodology, and writing–review and editing. AdC: investigation, methodology, and writing–review and editing. DD: investigation, methodology, and writing–review and editing. CC: methodology and writing–review and editing. RA: supervision and writing–review and editing. SE: conceptualization, supervision, visualization, writing–original draft, and review and editing. AlC: conceptualization, funding acquisition, project administration, supervision, visualization, writing–original draft, and review and editing.

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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 authors declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1275833/full#supplementary-material

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