Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2023**; 28(8): 173
https://doi.org/10.31083/j.fbl2808173

Review

Non-Coding RNA in Cholangiocarcinoma: An Update

Jiehan Li[1], Haolin Bao[1], Ziyue Huang[1], Zixin Liang[1], Ning Lin[2], Chunjie Ni[3], Yi Xu[1,2,3,4,5,*]

[1]Hepatopancreatobiliary Surgery, Second Affiliated Hospital of Harbin Medical University, 150086 Harbin, Heilongjiang, China
[2]Key Laboratory of Functional and Clinical Translational Medicine, Fujian Province University, Xiamen Medical College, 361008 Xiamen, Fujian, China
[3]Jiangsu Province Engineering Research Center of Tumor Targeted Nano Diagnostic and Therapeutic Materials, Yancheng Teachers University, 224002 Yancheng, Jiangsu, China
[4]Department of Pathology, School of Clinical Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China
[5]Key Laboratory of Myocardial Ischemia, Ministry of Education, Harbin Medical University, 150088 Harbin, Heilongjiang, China
*Correspondence: xuyihrb@pathology.hku.hk (Yi Xu)

Academic Editor: Amancio Carnero Moya
Submitted: 28 October 2022 Revised: 27 December 2022 Accepted: 5 January 2023 Published: 18 August 2023

Abstract

Cholangiocarcinoma (CCA) is one of the most common tumors with high malignancy. Its incidence is increasing year by year, and it is insidious and easily metastasized, and most patients are already in advanced stages when they are diagnosed. Surgery is an essential treatment for CCA, but the 5-year survival rate is still unsatisfactory due to the low early diagnosis rate and high malignancy of CCA. Therefore, exploring the molecular mechanisms of CCA to find reliable biomarkers and effective therapeutic targets is essential to improve the early diagnosis and survival rate of CCA. Non-coding RNA (ncRNA) is a class of RNA without protein-coding ability, mainly including microRNA (miRNA), long non-coding RNA (lncRNA), and circular RNA (circRNA). In recent years, numerous pieces of evidence have shown that aberrantly expressed ncRNAs can regulate the occurrence and development of CCA through various mechanisms such as mediating epigenetic, sponge miRNAs regulating the expression of target genes and participating in regulating cancer-related signaling pathways, which provides new approaches and ideas for early diagnosis, prognosis assessment and therapeutic targeting of CCA. In this paper, we review the molecular mechanisms of lncRNAs and circRNAs regulating the progression of CCA in recent years and discuss their potential clinical value in CCA.

Keywords: cholangiocarcinoma; non-coding RNA; long non-coding RNA; circular RNA; epigenetic; competitive endogenous RNA; signaling pathways

1. Introduction

As the second most common primary hepatic malignancy after hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA) is a malignant tumor originating from biliary epithelial cells or hepatic progenitor cells [1]. Cholangiocarcinoma can be classified by anatomical site into three different tumor types: intrahepatic cholangiocarcinoma (iCCA), perihilar cholangiocarcinoma (pCCA), and distal cholangiocarcinoma (dCCA) [2]. CCA is an aggressive malignancy with a meager 5-year survival rate of less than 10% [3]. Currently, laboratory tests, imaging tests, and tissue biopsies are usually used to diagnose CCA, but early specific markers are lacking [4]. Surgery is the only means to cure CCA. However, due to the lack of early specific clinical signs and the tumor's rapid growth, most patients are already in the middle to a late stage when diagnosed, and the best time for surgery is lost [1]. The standard of care for patients with advanced or surgically incurable CCA is systemic chemotherapy with gemcitabine and cisplatin [5]. However, available systemic therapies are limited, and 5-year survival rates for CCA patients remain below 20–40% [6]. There is increasing evidence that CCA is a complex biological process [7], while complex molecular mechanisms and tumor microenvironments are the current hotspots for exploring CCA. Therefore, to improve the diagnosis and cure rate of CCA, there is an urgent need to understand the molecular pathogenesis of CCA.

Non-coding RNA (ncRNA) is a general term for a class of genomic transcription products that do not have the function of coding proteins, including microRNA (miRNA), long non-coding RNA (lncRNA), and circular RNA (circRNA), which account for 98% of the human genome [8]. Despite the inability of ncRNAs to encode proteins, they can serve as regulatory molecules that mediate cellular processes such as chromatin remodeling, transcription, post-transcriptional changes, and signal transduction [9,10]. As ncRNA functions continue to be explored, researchers have discovered that ncRNA can participate in the biological processes of cancer as oncogenes or suppressors and play a critical role in regulating the progression of various cancers [11]. miRNAs are small RNAs o f about 22 nucleotides in length [12], and reviews of their molecular mechanisms in CCA have been extensively reported [13,14]. Therefore, this paper will not summarize in detail the mechanism of action of miRNAs involved in the regulation of CCA.

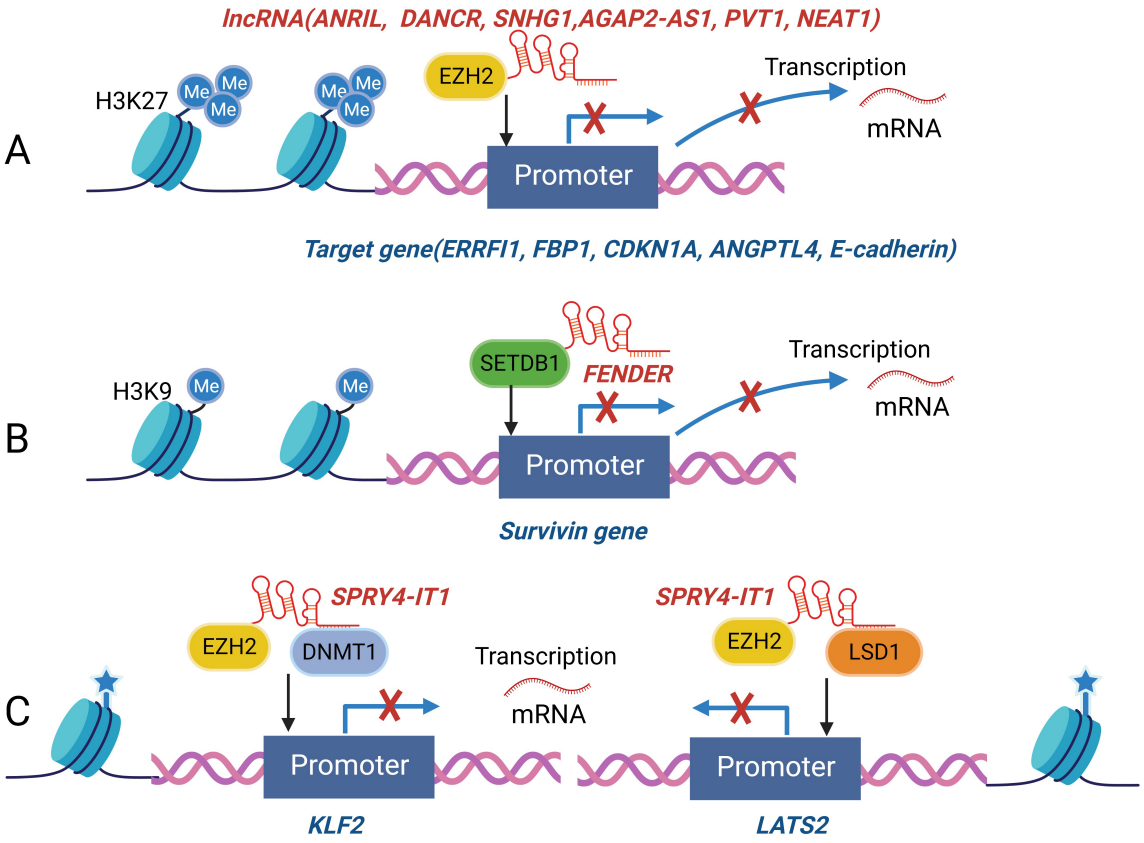

Fig. 1. LncRNAs mediate epigenetic modification in three different ways. (A) In cholangiocarcinoma (CCA) cells, LncRNAs could recruit EZH2 to its target gene promoter region and directly repress target gene expression by mediating H3K27me3 demethylation modifications. (B) In CCA cells, FENDER could recruit SET domain bifurcated 1 (SETDB1) to the survivin promoter region and represses survivin expression by inducing H3K9 methylation. (C) In cholangiocarcinoma (CCA) cells, SPRY4-IT1 could recruit EZH2 and DNMT1 to the KLF2 promoter region and recruit EZH2 and LSD1 to the LATS2 promoter region, respectively, thereby inhibiting transcription of KLF2 and LATS2. Abbreviations: EZH2, enhancer of zeste 2; H3K27, histone H3 lysine 27; ERRFI1, ERBB receptor feedback inhibitor 1; FBP1, Fructose-1, 6-biphosphatase; CDKN1A, cyclin-dependent kinase inhibitor 1; ANGPTL4, angiopoietin-like 4; SETDB1, SET domain bifurcated histone lysine methyltransferase 1; H3K9, histone H3 lysine 9; LSD1, lysine specific demethylase 1; DNMT1, DNA methyltransferase 1; KLF2, Kruppel-like factor 2; LATS2, large tumor suppressor 2.

2. Mechanism of LncRNAs Involvement in CCA Progression

LncRNA is a class of non-coding or limited protein-coding RNAs with transcripts more significant than 200 nucleotides in length that can regulate gene expression at multiple levels [15]. In recent decades, lncRNA has been considered transcriptional biological noise [16]. However, as researchers have learned more about it, lncRNA could regulate gene expression through shear regulation, exogenous silencing, interaction with miRNA or proteins, and many other ways [17]. Numerous studies have demonstrated that aberrantly expressed lncRNAs function as oncogenes or tumor suppressors through epigenetic, transcriptional regulation, and interaction with miRNAs and other modes of action involved in the biological behavior of a variety of malignancies, including CCA [18,19]. There is no doubt that lncRNAs play an increasingly crucial role in the incidence and progression of CCA.

2.1 Regulating Epigenetic Modifications

Epigenetics refers to the phenomenon that DNA sequences do not change, allowing heritable changes in gene expression, including DNA methylation, histone modifications, and chromatin modifications [20,21]. LncRNAs could play a key role in epigenetic regulation and promote CCA development by mediating multiple epigenetic modifications [22]. The polycomb repressor complex 2 (PRC2) has histone methyltransferase activity and can silence gene expression by methylating lysine 27 on histone H3 [23,24]. The enhancer of histidine-lysine N-methyltransferase (EZH2) is the catalytic subunit of the PRC2 complex [24,25]. It was shown that specific lncRNAs could recruit EZH2 to the promoter region of its target genes and directly repress the expression of target genes by mediating H3K27me3 demethylation modifications, thus promoting the progression of CCA [26–30]. LncRNA ANRIL was reported to accelerate CCA tumorigenesis by bind-

ing to EZH2, recruiting to the promoter region of ERRFI1, the tumor suppressor gene of CCA, and then directly suppressing ERRFI1 expression by mediating H3K27me3 demethylation modifications [26]. LncRNA DANCR promotes CCA cell growth and migration by binding to EZH2 to catalyze the FBP1 promoter region H3K27me3, which can partially inhibit FBP1 expression [27]. Furthermore, in CCA, SNHG1 [28]/AGAP2-AS1 [31], PVT1 [29], and NEAT1 [30] are also able to recruit EZH2 at the promoter regions of CDKN1A, ANGPTL4, and E-cadherin, respectively, resulting in elevated H3K27me3 levels at these loci. In addition to recruiting EZH2 to its target gene promoter, lncRNAs can also recruit SET domain bifurcated 1 (SETDB1) to bind to the survivin gene promoter [32]. Survivin is a bifunctional protein that acts as an inhibitor of apoptosis, and its expression is elevated in most tumors [33]. LncRNA FENDER could recruit SETDB1 to bind to the survivin gene promoter and induce H3K9 methylation to suppress survivin expression, thereby inhibiting proliferation, migration, and invasion of CCA cells [32].

Notably, lncRNAs can also act as scaffolds for at least two different histone modification complexes to mediate epigenetic modifications of genes [34]. Xu *et al*. [35] confirmed that LncRNA SPRY4-IT1 could act as a scaffold to recruit EZH2, LSD1, and DNMT1 to the tumor suppressor KLF2 and LATS2 promoter regions for epigenetic silencing, respectively. Further studies showed that EZH2 directly binds to the LATS2 and KLF2 promoter regions and mediates H3K27 trimethylation; DNMT1 directly binds to the KLF2 promoter region; LSD1 directly binds to the LATS2 promoter region and induces H3K4 demethylation modification, thus promoting the aggressiveness of CCA cells. These studies confirm that lncRNAs can mediate epigenetic modifications to promote the progression of CCA in multiple ways, as shown in Fig. 1 [26–32,35].

2.2 The CeRNA Regulatory Network of LncRNAs

The competitive endogenous RNA (ceRNA) mechanism was first proposed by Salmena [36], revealing that lncRNAs can act as natural miRNAs sponge competing miRNAs to regulate miRNA-targeted mRNAs and participate in the physiological processes of cell proliferation, differentiation, and apoptosis, which play an essential role in cancer development and progression [37]. Increased expression of lncRNA DLEU1 has been reported in CCA tissues and cells, and the transcription factor YY1 transcriptionally promotes its expression. Yes-associated protein 1 (YAP1) is a transcriptional co-activator that has an essential regulatory role in cell signaling and functions as a pro-oncogene in various cancers, particularly in CCA [38,39]. DLEU1 promotes oncogene YAP1 expression by acting as a sponge to bind miR-149-5p competitively. In addition, YAP1/TEAD2 promotes stemness maintenance through transcriptional upregulation of SOX2. YY1/DLEU1/miR-149-5p/YAP1 axis plays a critical pro-cancer role in CCA progression [40]. NRP-1 is a transmembrane protein [41] that functions as a co-receptor for multiple cellular signaling pathways associated with the advancement of cancer and interacts with the HGF/c-Met and TGF-/TGF-RI pathways in CCA cells [42,43]. The lncRNA TTN-AS1 was reported to sponge miR-320a in an Ago2-dependent manner and down-regulate miR-320a expression in CCA cells. Through the downregulation of miR-320a, TTA-AS1 promotes cell cycle progression, Epithelial-mesenchymal transition (EMT), and angiogenesis via NRP-1. However, after transfection of miR-320a mimics into CCA cells, miR-320a could partially counteract the effect of TTN-AS1 overexpression [42]. Wang *et al*. [44] confirmed that lncRNA H19 and HULC were increased by oxidative stress and regulated CCA cell migration and invasion by elevating the levels of IL-6 and CXCR4 through sponges let-7a/let-7b and miR-372/miR-373, respectively. Yang *et al*. [45] demonstrated that lncRNA SNHG12 could sponge miR-199a-5p and upregulate Klotho expression in iCCA cells, promoting iCCA cell growth and metastasis and providing a potential marker and therapeutic target for iCCA patients. In addition, specific lncRNAs can also influence epigenetic and signaling pathways through sponge miRNAs, thus promoting the progression of CCA. Xu *et al*. [35] demonstrated that SPRY4-IT1 acts as a molecular sponge for miR-101-3p and antagonizes its ability to inhibit EZH2 protein translation, leading to increased EZH2 protein levels, which in turn mediates epigenetic promotion of CCA progression. Patients with CCA had considerably higher PCAT6 levels, whereas PCAT6 was highly expressed in macrophages. PCAT6 contributed to the immunological response of CCA by influencing macrophages, including ROS generation, mitochondrial stress response, and M2 polarization, via sponging miR-326 and activating RohA signaling [46]. Furthermore, the oncogenic lncRNA PAICC activates the Hippo pathway by competitively binding miR-141-3p and miR-27A-3p and down-regulating YAP1 expression [47]. According to the most recent findings, Table 1 (Ref. [35,40,42,44–67]) summarizes the ceRNA regulation mechanisms of some lncRNAs in CCA.

2.3 LncRNAs and Cancer-Associated Signaling Pathways

Many studies have shown that lncRNAs can act as carcinogens or tumor suppressors and regulate tumor progression by participating in multiple signaling pathways. Phosphatidylinositol 3-kinases (PI3Ks) are often over-activated in tumors and usually accompanied by the inactivation of the tumor suppressor gene PTEN. By promoting the PI3K/AKT signaling pathway, dysregulated LncRNA expression can accelerate the development of CCA. Peroxiredoxin 2 (PRDX2) is an essential protein in cancer cell proliferation, invasion, and apoptosis. It is a member of the peroxiredoxin family [68]. LncRNA CASC15 may promote the development of iCCA by binding to PRDX2 and suppressing its degradation, thereby activat-

Table 1. LncRNAs act as ceRNAs.

LncRNA	Expression	miRNA sponge	mRNA	Function	Ref.
DLEU1	up	miR-149-5p	YAP1	Promoting CCA cells proliferation, migration, invasion and EMT	[40]
NKILA	up	miR-582-3p	YAP1	Promoting cells proliferation, migration, invasion	[48]
TTN-AS1	up	miR-320a	NRP-1	Promoting CCA cells cycle progression, EMT, and angiogenesis	[42]
TTN-AS1	up	miR-513a-5p	SFN	Contributing to growth and metastasis of CCA	[49]
H19	up	let-7a/let-7b	IL-6	Regulating CCA cells migration and invasion	[44]
HULC	up	miR-372/miR-373	CXCR4	Regulating CCA cells migration and invasion	[44]
TMPO-AS1	up	let-7 g-5p	HMGA1	Improving CCA cells migration proliferation; impaire cell apoptosis	[50]
SNHG12	up	miR-199a-5p	Klotho	Promoting iCCA cells growth and metastasis	[45]
LINC00630	up	miR-199a	FGF7	Promoting CCA cells proliferation, migration and invasion	[51]
SNHG3	up	miR-3173-5p	ERG	Promoting CCA cells proliferation, migration, invasion	[52]
ST8SIA6-AS1	up	miR-145-5p	MAL2	Facilitating CCA cells growth and migration	[53]
PCAT1	up	miR-216a-3p	BCL3	Promoting CCA cells proliferation, migration, invasion	[54]
LINC00184	up	miR-23b-3p	ANXA2	Facilitating CCA cells proliferation, metastasis, and adenine metabolism	[55]
FAM66C	up	miR-23b-3p	KCND2	Promoting progression and glycolysis in iCCA	[56]
H19	up	miR-612	Bcl-2	Promoting CCA cells proliferation, migration, invasion	[57]
HOTAIR	down	miR-204-5p	HMGB1	Suppressing CCA cells apoptosis, autophagy and inducing cell proliferation	[58]
HEIH	up	miR-98-5p	HECTD4	Promoting CCA cells proliferation, migration, invasion	[59]
Linc00473	up	miR-506	DDX5	Promoting CCA cells proliferation and invasion	[60]
HOXD-AS1	up	miR-520c-3p	MYCN	Promoting CCA cells proliferation, migration, invasion, EMT, stemness maintenance	[61]
PSMA3-AS1	up	miR-376a-3p	LAMC1	Promoting CCA cells proliferation, migration, invasion	[62]
SPRY4-IT1	up	miR-101-3p	EZH2	Raising EZH2 expression and mediating epigenetic in CCA	[35]
PCAT6	up	miR-326	RhoA	Activating RhoA-ROCK signaling pathway and modulating macrophages	[46]
PAICC	up	miR-141-3p, miR-27A-3p	YAP1	Promoting tumorigenesis of iCCA cells and activating the Hippo signaling pathway	[47]
UCA1	up	miR-122	CLIC1	Activating the ERK/MAPK signaling pathway	[63]
SNHG1	up	miR-140	TLR4	Activating the NF-κB signaling pathway	[64]
NEAT1	up	miR-186-5p	PTP4A1	Activating the PI3K/AKT signaling pathway	[65]
MEG3	down	miR-361-5p	TRAF3	Inhibiting the NF-κB signaling pathway	[66]
UC.158−	up	miR-193b	-	As a mediator downstream of the Wnt/β-catenin pathway	[67]

Abbreviations: YAP1, Yes-associated protein 1; NRP-1, neuropilin-1; IL-6, interleukin 6; CXCR4, CXC-chemokine receptor 4; HMGA1, high mobility group A1; SFN, stratifin; FGF7, fibroblast growth factor 7; ERG, ETS-related gene; MAL2, Mal, T cell differentiation protein 2; BCL3, B-cell lymphoma 3; ANXA2, annexin A2; KCND2, potassium voltage-gated channel, Shal-related subfamily, member 2; Bcl-2, B cell lymphoma 2; HMGB1, high mobility group B1; HECTD4, HECT domain e3 ubiquitin protein ligase 4; DDX5, DEAD box protein 5; MYCN, MYCN Proto-Oncogene, BHLH Transcription Factor; LAMC1, laminin subunit gamma 1; CLIC1, chloride intracellular channel 1; TLR4, Toll-like receptor 4; PTP4A1, phosphatase of regenerating liver 1; TRAF3, TNF receptor-associated factor 3.

ing the PI3K/AKT/c-Myc signaling pathway [69]. As mentioned, lncRNAs can act as oncogenes and activate the CCA-related signaling pathway by reducing miRNA levels through the ceRNA mechanism. It was found that miRNA-122 inhibits the phosphorylation of ERK/MAPK protein, thus blocking the ERK/MAPK pathway. LncRNA UCA1 promotes the activation of the ERK/MAPK pathway and the metastasis of CAA cells by sponging miR-122 and regulating the expression of its downstream gene CLIC1 [63]. Similarly, SNHG1, a ceRNA for miR-140, increases TLR4 expression and activates the NF-κB signaling pathway, influencing CCA development and carcinogenesis [64]. PCAT1 promotes extrahepatic CCA progression by upregulating Wnt1 levels and activating the Wnt/β-catenin signaling pathway through sponge miRNA-122 [70]. Besides, in CCA, lncRNAs can regulate multiple signaling pathways in addition to one signaling pathway alone, which enhances the proliferation, migration, and

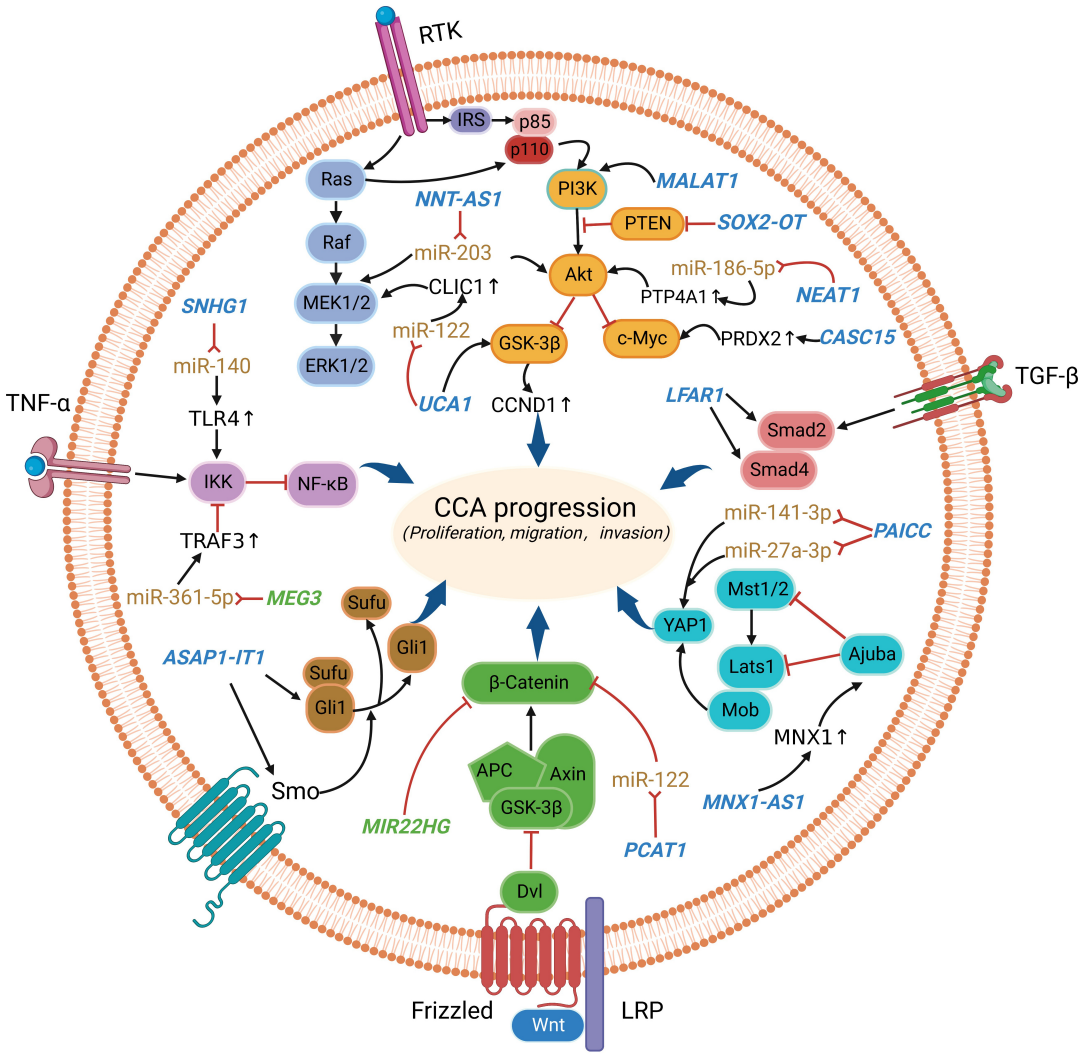

Fig. 2. Mechanism of dysregulated lncRNAs regulating cancer-related signaling pathways, including PI3K/AKT, ERK/MAPK, Wnt/β-catenin, NF-κB, Hedgehog, TGFβ/Smad, and Hippo pathways. From Fig. 2, it can be concluded that most dysregulated lncRNAs promote the proliferation, migration, and invasion of cholangiocarcinoma, except for MEG3 and MIR22HG, which inhibit the progression of CCA. LncRNAs that promote or inhibit CCA progression are shown in blue or green, respectively. An arrow represents activation, a T-shaped arrow represents inhibition, and a V-shaped arrow represents the lncRNA sponge microRNA (miRNA). Abbreviations: PRDX2, peroxiredoxin 2; PTP4A1, phosphatase of regenerating liver 1; CCND1, CyclinD1; CLIC1, chloride intracellular channel 1; TRAF3, TNF receptor-associated factor 3; TLR4, Toll-like receptor 4; MNX1, motor neuron and pancreas homeobox protein 1; IRS, insulin receptor substrates; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; Akt, protein kinase B; GSK3β, glycogen synthase kinase 3 beta; MEK1/2, MAP kinase/ERK kinase 1/2; ERK1/2, extracellular regulated MAP kinase 1/2; Dvl, segment polarity protein disheveled homolog; LRP, LDL-receptor-related protein; APC, adenomatous polyposis coli; Axin, axis inhibition protein; TGF-β, transforming growth factor-beta; Smo, smoothened, frizzled class receptor; Sufu, Sufu negative regulator of hedgehog signaling; Gli1, gli family zinc finger 1; TNF-α, Tumour Necrosis factor-Alpha; IKK, inhibitor of kappa B kinase beta; NF-κB, nuclear factor kappa B; SMAD2/4, drosophila mothers against decapentaplegic protein 2/4; Mst1/2, the STE20-like serine/threonine kinases Mst1 and Mst2; Lats1, large tumor suppressor 1; Mob, mps one binder.

invasion of CCA cells in a synergistic relationship. Recent studies have shown that NNT-AS1 overexpression in CCA tissues and cell lines increases CCLP1 and TFK1 cell proliferation and EMT through modulating the activation of PI3K/AKT and ERK1/2 signaling pathways via miR-203 downregulation [71]. Interestingly, this study showed that PI3K/AKT and ERK1/2 signaling pathways were co-regulated by NNT-AS1 and miR-203 and synergistically promoted the proliferation and EMT of CCA cells. In addition, we summarize the mechanisms by which dysregulated lncRNAs regulate cancer-related signaling pathways, as shown in Fig. 2 [47,63–66,69–78].

3. Mechanisms of CircRNAs Involved in CCA Progression

CircRNA is a class of circular non-coding RNAs that do not have 5′ and 3′ terminal head and tail structures and is between several hundred and several thousand nucleotides in length [79,80]. Compared with linear RNAs, circRNA exhibits higher stability and conservation and is widely present in eukaryotic cells [81]. As the research progressed, researchers discovered that circRNAs possess various properties, such as miRNA biological sponges, interact with RNA-binding proteins, encode proteins or peptides and regulate the transcription and translation of genes [82,83]. Besides, aberrantly expressed circRNAs can be localized not only in the cytoplasm and nucleus [84] but also in extracellular vesicles [85], playing an essential regulatory role in tumor cell proliferation, migration, invasion, and apoptosis [86]. Compared with lncRNAs, cirCRNAs are still in the initial stage of investigation in CCA, but their essential role in CCA has been paid more and more attention by scholars, as shown in Table 2 (Ref. [84,87–104]).

3.1 As CeRNAs

Like lncRNAs, circRNAs could directly sponge miRNA and play an essential role in the progression of CCA by competing with mRNA as ceRNA to bind miRNA and regulate its activity. CD73 is a new immunoinhibitory protein that promotes the growth of tumors by inhibiting anti-tumor immune responses [87,105]. GAL-8 induces the death of activated T cells and promotes the differentiation of immunosuppressive Tregs. It also indirectly affects tumor cells by stimulating the oncogenic-like transformation of epithelial cells via partial and reversible EMT [87,106]. Xu *et al*. [87] found that circHMGCS1-016 could sponge miR-1236-3p, down-regulate miR-1236-3p, and upregulate CD73 and GAL-8 expression in iCCA cells. Horizontal upregulation of CD73 and GAL-8 resulted in a suppressed immune environment and, in turn, induced immune escape of iCCA cells, promoting iCCA progression. Interestingly, the study also confirmed that circHMGCS1-016 enhances iCCA resistance to anti-PD1 therapy. Circ-LAMP1 binds directly to miR-556-5p and miR-567, which could regulate YY1 expression. Furthermore, transfection of miR-556-5p and miR-567 mimicked into CCA cells decreased YY1 expression and inhibited the oncogenic effect of circ-LAMP1 [91]. Xu *et al*. [102] demonstrated that circ-CCAC1 promotes CCA tumorigenesis and metastasis through spongy miR-514a-5p upregulating YY1 expression and further activating GAMLG expression. In a separate study, the transcription factor YY1 binds to the promoter of circ-ZNF609, thereby enhancing its transcription. circ-ZNF609 promotes CCA cell proliferation, migration, and invasion by upregulating LRRC1 through sponge miR-432-5p [90]. Interestingly, YY1 plays a crucial role in circ-LAMP1, circ-CCAC1, and circ-ZNF609 in promoting CCA progression, and whether these three circRNAs have a synergistic role in the mechanism of CCA occurrence and development remains to be further explored. In addition, circRNAs can also act as ceRNAs for miRNAs to inhibit the progression of CCA. It was shown that CircSETD3 competitively binds miR-421 and negatively regulates miR-421 expression, thereby inhibiting the proliferation of CCA cells and inducing apoptosis, but miR-421 mimics can reverse these effects [88].

3.2 CircRNAs and Cancer-Associated Signaling Pathways

Dysregulated circRNAs can be closely associated with the progression of CCA by participating in multiple cancer-related signaling pathways. Recent studies have shown that a limited number of translatable circRNAs can encode proteins or functional peptides that activate cancer-related signaling pathways, which regulate cancer progression [97,107,108]. cGGNBP2-184aa, a protein encoded by the GGNBP2 induced by IL-6, was reported to promote iCCA cell proliferation and metastasis *in vitro* and *in vivo*. Mechanistically, cGGNBP2-184aa interacts directly with the DBD of STAT3 and phosphorylates STAT3 at the Tyr705 site, which is then translocated into the nucleus to activate the transcription of target genes. Thus, IL-6/cGGNBP2-184aa/STAT3 forms a positive feedback loop to maintain constitutive activation of IL-6/STAT3 signaling to promote iCCA progression [97]. In addition, certain circRNAs can act as miRNA sponges and protein scaffolds [109] to localize transcription factors in the nucleus and thus activate cancer-related pathways. CircACTN4 has been reported to sponge miR-424-5p in the cytoplasm, activating the Hippo pathway by upregulating the level of the oncogenic transcriptional cofactor YAP1. In the nucleus, the YBX1 transcription factor is recruited to induce transcription of FZD7, a positive regulator of the Wnt/-catenin signaling pathway that promotes the growth and metastasis of iCCA cells. Importantly, circACTN4 acts as a signaling nexus, enhancing the interaction between YAP1 and β-catenin, which also confirms the synergistic effect of the Hippo pathway and Wnt/β-catenin pathway in the growth and metastasis of iCCA cells [84]. Furthermore, there are unanswered questions from this study. Whether circACTN4 acts as a scaffold to recruit YBP1 into the FZD7 promoter by interacting with DNA or other cofactors remains to be determined [110]. The mechanism of circACTN4-mediated interaction between YAP1 and β-catenin still needs further exploration [84]. Interestingly, circRNAs not only activate cancer signaling pathways but also inhibit CCA progression by suppressing cancer signaling pathways. cNFIB was shown to bind directly to the NTD region of MEK1, blocking the binding of the kinase (MEK1) to the substrate (ERK2) and preventing the phosphorylation of ERK2, thereby inhibiting iCCA proliferation and metastasis by inhibiting MEK1/ERK signaling. Notably, further research is required to determine which binding site on cNFIB mediates the interaction between cNFIB

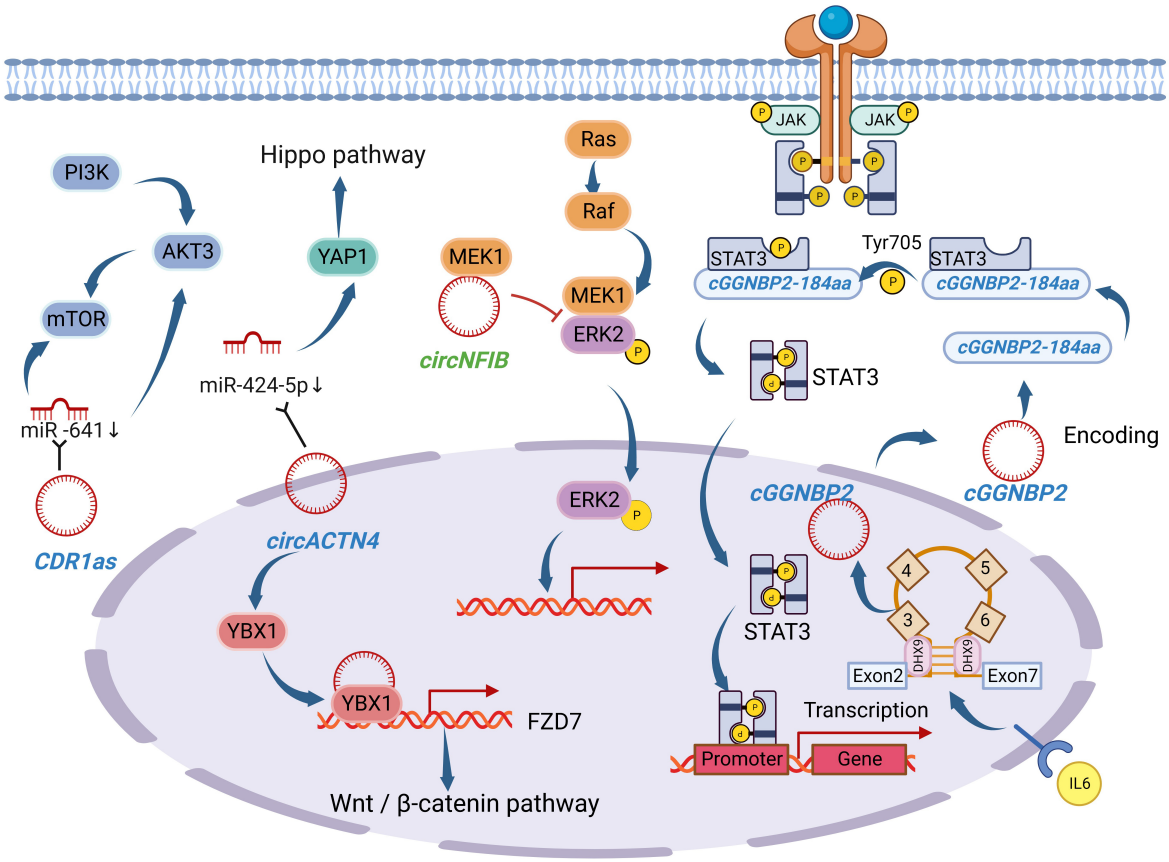

Fig. 3. CircRNAs regulate cancer-related signaling pathways, including AKT3/mTOR, Hippo, Wnt/β-catenin, ERK/MAPK, and JAK/STAT3 signaling pathways. CircRNAs that promote or inhibit CCA progression are shown in blue or green, respectively. An arrow denotes activation, a T-shaped arrow denotes inhibition, and a V-shaped arrow denotes circRNA sponge miRNA. Abbreviations: PI3K, phosphatidylinositol 3-kinase; AKT3, AKT Serine/Threonine Kinase 3; mTOR, mammalian target of rapamycin; YAP1, Yes-associated protein 1; YBX1, Y-box binding protein 1; FZD7, Frizzled-7; MEK1, mitogen-activated protein kinase 1; ERK2, extracellular regulated MAP kinase 2; IL6, interleukin- 6; STAT3, signal transducers and activators of transduction-3.

and MEK1 [96]. For the mechanism of circRNA regulation of cancer-related signaling pathways, see Fig. 3 [84,96–98].

3.3 Regulating CCA Progression via EVs

Extracellular vesicles (EVs) are vesicle-like vesicles with a bilayer membrane structure, ranging from 40 nm to 1000 nm in diameter, that are detached from the cell membrane or secreted by the cell into the extracellular matrix [111,112]. EVs, which are mainly composed of microvesicles (MVs) and exosomes [113], are abundantly found in various body fluids and cell supernatants [112] and transport signaling molecules such as non-coding RNA, DNA fragments and proteins in a stable manner [114]. They could play an essential role in tumor development as transmitters of transduction signals between cancer cells and as crucial mediators of intercellular communication by shifting their contents and altering biological responses in other cells [115,116]. However, few studies have focused on the mechanism of action of EVs and EV-circRNAs in CCA. Recent studies have shown that CCA-derived EVs can act as cancer migration and invasion mediators by transferring oncogenic circRNAs to normal bile duct cells [101]. It has been reported that circ-0000284 promotes the development and progression of CCA by competitively binding to miR-637 and upregulating the expression of LY6E. This study also found that the expression of circ-0000284 in exosomes was approximately three times higher than that in producing cells. Importantly, circ-0000284 may transfer directly from CCA cells to surrounding normal cells via exosomes, evoke the malignant phenotype of CCA cells through cellular communication, and regulate the biological functions of peripheral cells [101]. Notably, this is the first EVs-mediated circRNA identified in CCA, suggesting that EV-circRNAs can act as novel players in regulating the progression of CCA. Furthermore, exosomal circRNAs can also play an essential role in cellular communication as a significant mediator. A recent study showed that circ_0020256 was delivered to CCA tumor cells via exosomes secreted by tumor-associated macrophages (TAMs) to enhance the biological activity of CCA cells by regulating the expression of transcription factor E2F3 through interaction with miR-432-5p [100]. Several studies have shown that circRNAs

Table 2. Dysregulated circRNAs in CCA.

CircRNA	Expression	Source	Pathway	Functions	Ref.
circHMGCS1-016	up	tissue	circHMGCS1-016/miR-1236-3p/CD73 and GAL-8	Inducing iCCA cells invasion and reshaping the tumor immune microenvironment	[87]
circSETD3	down	cell, tissue	circSETD3/miR-421/BMF	Inhibiting proliferation and inducing apoptosis in CCA cells	[88]
circ0021205	up	cell, tissue	circ0021205/miR-204-5p/RAB22A	Promoting CCA cells proliferation, migration, and invasion	[89]
circ-ZNF609	up	tissue	YY1/eIF4A3/circ-ZNF609/miR-432-5p/LRRC1	Promoting CCA cells proliferation, migration, and invasion	[90]
circ-LAMP1	up	cell, tissue	circ-LAMP1/miR-556-5p and miR-567/YY1	Contributing to the growth and metastasis of CCA	[91]
circ_0005230	up	cell, tissue	circ_0005230/miR-1238 and miR-1299	Facilitating CCA cells growth and metastasis	[92]
circDNM3OS	up	cell, tissue	circDNM3OS/miR-145-5p/MORC2	Accelerating CCA growth and glutamine metabolism	[93]
circ_0059961	down	cell, tissue	circ_0059961/miR-629-5p/SFRP2	Suppressing CCA cells proliferation, migration, and invasion	[94]
circRTN4IP1	up	cell, tissue	circRTN4IP1/miR-541-5p/HIF1A	Regulating iCCA cells malignancy and glucose metabolism	[95]
circNFIB	down	tissue	circNFIB/MEK1/ERK2	Inhibiting tumor growth and metastasis	[96]
circACTN4	up	tissue	circACTN4/miR-424-5p/YAP1, circACTN4/YBX1/FZD7	Promoting iCCA proliferation and metastasis	[84]
cGGNBP2	up	cell, tissue	IL-6/cGGNBP2/cGGNBP2-184aa/JAK/STAT3	Promoting iCCA cells growth and metastasis	[97]
CDR1as	up	cell, tissue	CDR1as/miR-641/AKT3/mTOR	Promoting CCA cells proliferation, migration, and invasion	[98]
circ_0000591	up	cell, tissue	Circ_0000591/miR326/TLR4/MyD88/IL6	Promoting CCA cells proliferation, migration, and invasion	[99]
circ_0020256	up	serum Exos	E-circ_0020256/miR-432-5p/E2F3	Promoting CCA cells proliferation, migration, and invasion	[100]
circ-0000284	up	cell, tissue, serum Exos	circ0000284/miR637/LY6E	Promoting CCA cells proliferation, migration, and invasion	[101]
circ-CCAC1	up	tissue, bile EVs	circ-CCAC1/miR-514a-5p/YY1/CAMLG	Promoting CCA tumorigenesis and metastasis	[102]
hsa_circ_0001649	down	cell, tissue	-	Promoting CCA cells proliferation, migration, and invasion	[103]
SMARCA5	down	tissue	-	Inhibiting proliferation and increasing chemotherapy sensitivity	[104]

Abbreviations: CD73, ecto-5′-nucleotidase; GAL-8, a member of -glycan-binding protein family; BMF, B-cell lymphoma-2 modifying factor; RAB22A, a member of the proto-oncogene RAS family; LRRC1, leucine-rich repeat-containing protein 1; YY1, Yin and Yang 1; MORC2, MORC Family CW-Type Zinc Finger 2; ULK1, unc-51 like kinase 1; SFRP2, secreted frizzled related protein 2; HIF1A; hypoxia inducible factor 1 subunit alpha; MEK1, mitogen-activated protein kinase 1; YBX1, Y-box binding protein 1; FZD7, Frizzled-7; STAT3, signal transducers and activators of transduction-3; mTOR, mechanistic target of rapamycin; TLR4, toll like receptor 4; MyD88, myeloid differentiating factor 88; IL6, interleukin- 6; E2F3, recombinant E2F transcription factor 3; Exs, exosomes; LY6E, lymphocyte antigen 6E; EVs , extracellular vesicles; CAMLG , calcium-modulating cyclophilin ligand.

can be transported by tumor-secreted EVs to reshape the tumor microenvironment by inducing immunosuppression and angiogenesis and providing a supportive microenvironment for metastatic cancer cells [102,117,118]. Xu *et al*. [102] confirmed that circ-CCAC1 was transported from CCA cell-derived EVs to endothelial monolayer cells, enhancing endothelial monolayer permeability and causing angiogenesis by breaking the vascular endothelial barrier, which in turn promoted CCA progression. Significantly, circ-CCAC1 in the EVs of this study was highly expressed in bile samples from CCA patients. The abundance and ease of detection of bile-derived EV-circRNA compared to cellular free nucleic acids detected by conventional liquid biopsies, which can be repeatedly sampled for large-scale testing, opens up new perspectives on EV-circRNAs as a noninvasive biomarker for CCA.

3.4 "Sponge" Other Factors

The ceRNA hypothesis is a currently well-studied mode of gene expression regulation. Currently, some circRNAs have been shown to act as sponges for miRNAs and regulate the ability of miRNAs to target mRNAs by competitively binding to miRNAs [36]. However, the abundance of circRNAs is low in most mammals. In order to function as sponge miRNAs, circRNAs need to be highly expressed in the cytoplasmic matrix or EVs and contain many miRNA binding sites, so most circRNAs cannot act as miRNA sponges [119]. For example, although the level of cNFIB is upregulated in both iCCA tissues and cell lines, there are no binding sites and interactions detected between cNFIB and miRNAs, so it cannot act as a miRNA sponge. Therefore, the adaptation of the ceRNA hypothesis for circRNA is becoming increasingly controversial. Furthermore, a study demonstrated that brain circRNA sponge miRNA did not exhibit a more robust ability than linear mRNA. Instead, it was able to interact with RNA binding protein (RBP) [120]. Interestingly, some researchers have proposed that some circRNAs bind, sequester, or store molecules such as transcription factors into specific subcellular locations by "sponging" RBP, which act as dynamic scaffolds for assembling other components [121]. For example, circ-Foxo3 can prevent CDK2 function and block cell cycle progression by interacting with CDK2 and p21 to form the circ-Foxo3-p21-CDK2 ternary complex [122]. Xu *et al*. [102] demonstrated that circ-CCAC1 enters endothelial cells via EVs, binds strongly to EZH2, and sequesters it in the cytoplasm without affecting its overall expression. Because circ-CCAC1 restricts the nuclear localization of EZH2, it increases the expression of SH3GL2 to inhibit the expression of intercellular linker proteins ZO-1 and Occludin, ultimately increasing cell permeability. Thus, after circRNA binding to RBP, circRNAs could dissociate protein interactions or alter intracellular protein distribution, which in turn regulates the progression of CCA.

Interestingly, Du *et al*. [96] proposed an effective circRNA sponge miRNA-like mode of action in a study exploring the inhibition of iCCA proliferation and metastasis. Mechanistically, cNFIB could bind directly to the NTD region of MEK1 and disrupt the interaction between MEK1 and ERK2 by binding competitively to MEK1, thereby downregulating ERK phosphorylation to inhibit iCCA translocation. In addition, this study knocked down ERK2, performed cNFIB pull-down, and discovered that cNFIB enriched MEK1 more [96]. Notably, does this mode of action of circRNA in iCCA have similarities to circRNA sponge-like miRNAs?

3.5 As a Protein Recruiter

As the functions of circRNAs continue to be explored, circRNAs have been demonstrated to act as protein recruiters and as scaffolds to regulate protein-protein interactions. It has been reported that circMRPS35 could serve as a recruiter to recruit histone acetyltransferase KAT7 to the promoters of the FOXO1 and FOXO3a genes, thereby altering the expression of the downstream genes p21, p27, Twist1, and E-calmodulin and inhibiting the proliferation and invasion of gastric cancer cells [123]. In another study, circACTN4 was confirmed to initiate FZD7 transcription by recruiting the transcription factor YBX1 to the FZD7 promoter region in the nucleus, ultimately promoting iCCA cells proliferation and invasion [84]. These studies illustrate that circRNAs recruit them to chromatin for chromatin remodeling or regulate the transcription of target genes by acting as protein recruiters. Through this mechanism, circRNAs are strongly associated with the occurrence and development of CCA. However, there are relatively few studies on this aspect, and it is hoped that shortly, this mode of action of circRNAs as recruiters to recruit functional proteins may provide new ideas for the targeted treatment of cholangiocarcinoma.

3.6 Coding Proteins

CircRNA has long been considered a class of endogenous non-coding RNA. However, as research continues, this concept seems increasingly skeptical. Legnini *et al*. [124] and Pamudurti *et al*. [125] found that circ-ZNF609 and circMbl3 could translate proteins in mouse myoblasts and fly heads, respectively. Importantly, circ ZNF609 contains an infinite open reading frame (ORF) that spans the start codon and is translated into the protein in a splicing-dependent and cap-independent way. It has been shown that circ-SMO encodes the carcinogenic protein SMO-193a.a., which de-repressed SMO from PTCH1 during Shh stimulation via enhancing SMO cholesterol modification. Moreover, Shh/Gli1/FUS/SMO-193a.a. establishes a positive feedback loop to maintain Hedgehog signaling activation in glioblastoma [126]. Indeed, there is growing evidence that some circRNAs can be involved in carcinogenesis through internal ribosome entry sites, N6-methyladenosine

modification, or rolling circle amplification for non-cap-dependent translation of circRNAs encoding proteins. In another study, Li *et al*. [97] found a highly conserved ORF that can encode a 184-amino-acid protein when investigating the protein-coding potential of cGGNBP2. This study further identifies a key role for IRES in recruiting ribosomes to initiate the translation of circRNAs lacking the 5′ cap. Notably, a predicted protein consisting of 184 amino acids (cGGNBP2-184aa) can be translated by cGGNBP2 driven by this IRES. Furthermore, the cGGNBP2-edited protein cGGNBP2-184aa was shown to promote iCCA growth and metastasis by activating the JAK-STAT signaling pathway. With the deepening of research, the discovery of the hidden functions of CircRNAs encoding proteins or functional peptides has aroused the curiosity of many researchers. As a relatively new research field, it not only enriches the connotation of translationomics and proteomics but also provides a new perspective for researchers to study the mechanism of circRNAs in CCA.

4. Clinical Application of NcRNAs in CCA

4.1 NcRNAs as Biomarkers of CCA

A growing number of studies have discovered that most lncRNAs are significantly expressed in the tissues, cells, and bile of CCA and have tremendous diagnostic and prognostic potential as CCA biomarkers. Ge *et al*. [127] found that two lncRNAs, ENST00000588480.1, and ENST00000517758.1, were highly expressed in bile-derived exosomes of CCA patients were highly expressed in CCA patients. When these two lncRNAs were combined for diagnosis, their area under the curve (AUC), sensitivity, and specificity were 0.709, 82.9%, and 58.9%, respectively. Their sensitivity was superior to serum CA19-9 (82.9% *vs*. 74.3%). It was confirmed that the higher the expression of these two lncRNAs in CCA patients, the worse their survival and can be used as predictors for monitoring CCA [127]. It was reported that lncRNA-NEF was downregulated in the plasma of iCCA patients and had good diagnostic properties, significantly distinguishing iCCA patients from healthy controls (AUC = 0.8642). However, unfortunately, this study did not mention the sensitivity and specificity of lncRNA-NEF as a diagnostic marker for iCCA. In addition, overall survival (OS) was significantly lower in iCCA patients with low lncRNA-NEF expression (p = 0.0198) [128]. DLEU1 was shown to be associated with advanced Tumor Node Metastasis (TNM) stage and lymph node infiltration, and CCA patients with high DLEU1 expression had poorer OS. More importantly, DLEU1 can be used as a prognostic marker for CCA (AUC = 0.747, specificity = 65.4%, sensitivity = 72.4%) to predict the prognosis of CCA [40]. In the clinical correlation study of circRNAs and CCA, the expression of circRNA Cdr1as was markedly elevated in tumor tissues relative to neighboring normal tissues and was highly related to lymph node infiltration, progressed TNM, and postoperative recurrence. In addition, Cdr1as could be used as a novel independent prognostic biomarker to predict overall survival in CCA patients (sensitivity = 83.3%, specificity = 58.3%) [129]. Notably, researchers found that circRNAs were highly expressed in bile and serum-derived EVs from CCA patients, implying that EV-cirRNAs could serve as potential non-invasive biomarkers for CCA. For example, Xu *et al*. [102] found upregulated levels of circ-CCAC1 in bile and serum-derived EVs of CCA patients, both of which have good diagnostic properties. The diagnostic value of serum EVs (AUC = 0.759) was almost equal to that of serum CA19-9 (AUC = 0.757), but bile EVs (0.857) was superior to CA19-9. Interestingly, the combination of both bile- or serum-derived EV-circ-CCAC1 and serum CA19-9 had better diagnostic performance than alone. Furthermore, this study confirmed that high expression (p = 0.001) of circ-CCAC1 was an independent prognostic marker for iCCA and that circ-CCAC1 expression was a predictor of postoperative recurrence of iCCA (p = 0.002). In Table 3 (Ref. [40,61,62,96,97,102,128–133]), we summarize some relevant studies of ncRNAs as markers of CCA.

4.2 NcRNAs as Therapeutic Targets in CCA

In non-surgical biliary malignancies, gemcitabine in combination with cisplatin is considered the standard chemotherapy regimen. However, they have poor chemoresistance and limited efficiency, with a median survival of only 11.7 months [5]. Therefore, finding more effective drug treatment options and improving the sensitivity of CCA to chemotherapeutic agents is crucial to improve the survival rate of CCA patients. As a chromatin regulator, BRCA-1 associated protein-1 (BAP1) has been reported to regulate the expression of lncRNA NEAT-1 and, thus, the sensitivity of gemcitabine in CCA cells through epigenetic regulation. Interestingly, by understanding the expression of BAP1, the researchers found that both olaparib and GSK126 have synergistic effects with gemcitabine in CCA cells and could be combined to enhance sensitivity to gemcitabine. However, this synergistic combination needs to be validated by extensive clinical studies before it can be applied to the clinic [134]. Lu *et al*. [135] reported that LINC00665 was substantially expressed in gemcitabine-resistant CCA cell lines and was linked to a poor prognosis in CCA patients. When the expression level of LINC00665 was downregulated in gemcitabine-resistant CCA cell lines, the drug resistance effect of CCA cells was reduced; conversely, LINC00665 overexpression increased gemcitabine resistance in CCA-sensitive cells, suggesting the ability of LINC00665 to increase the chemoresistance of CCA cells. Mechanistically, LINC00665 regulates BCL9L expression by sponging miR-424-5p, which activates Wnt/β-Catenin signaling and ultimately promotes Wnt/β-Catenin signaling-mediated EMT and stemness properties [135]. In addition, HOTTIP could enhance the resistance of gemcitabine and cisplatin in CCA by sponging miR-637 [136].

Table 3. NcRNAs as markers of CCA. Overall survival (OS); recurrence free survival (RFS).

NcRNA	Source	Expression	Clinical application	AUC	Sensitivity	Specificity	Prognostic indicator	*p* value	Ref.
DLEU1	tissue	up	prognostic marker	0.747	72.4%	65.4%	OS	$p < 0.001$	[40]
PSMA3-AS1	tissue	up	prognostic marker	0.793	70.8%	79.2%	OS	$p < 0.001$	[62]
ZEB1-AS1	tissue	up	prognostic marker	0.749	65.5%	80.0%	OS	$p < 0.001$	[130]
HOXD-AS1	tissue	up	prognostic marker	0.786	80%	65.4%	OS	$p < 0.001$	[61]
SNHG20	tissue	up	prognostic marker	0.748	73.4%	75.5%	OS	$p < 0.001$	[131]
LINC00667	tissue	up	prognostic marker	0.830	87.5%	72.0%	OS	$p < 0.001$	[132]
lncRNA-NEF	plasma	down	diagnostic marker, prognostic factor	0.864	-	-	OS	$p = 0.0198$	[128]
FOXD2-AS1	tissue	up	prognostic marker	0.741	82.0%	60.0%	OS	$p < 0.001$	[133]
cGGNBP2	tissue	up	prognostic factor	-	-	-	OS,RFS	$p < 0.001$	[97]
circNFIB	tissue	down	prognostic factor	-	-	-	OS,RFS	$p < 0.001$	[96]
Cdr1as	tissue	up	prognostic marker	-	83.3%	58.3%	OS	$p = 0.0001$	[129]
circ-CCAC1	bile, serum	up	diagnostic marker, prognostic factor	0.857, 0.759	-	-	OS, DFS	$p < 0.001$	[102]

Abbreviations: OS, overall survival; RFS, recurrence free survival; DFS, disease free survival.

The expression level of Circ-SMARCA5 was reported to be downregulated in tumor tissues, and its upregulation increased the sensitivity of cisplatin and gemcitabine in iCCA cells [104]. Upregulation of cNFIB or trametinib (MEK inhibitor) treatment alone has been reported to inhibit tumor progression. The combination of cNFIB overexpression and trametinib enhanced this inhibition by *in vivo* experiments [96]. This suggests that synergistic effects between cNFIB and trametinib may enhance pit tumor efficacy and that iCCA cells with high levels of cNFIB may potentially enhance the sensitivity of trametinib, providing a new idea for the treatment of iCCA.

5. Conclusions

CCA is a kind of tumor with high molecular diversity and gene heterogeneity regulated by various molecules such as pro-oncogenes and tumor suppressor genes. Therefore, an in-depth exploration of the molecular mechanisms of CCA is essential to find reliable biomarkers and effective therapeutic targets. In recent years, ncRNAs have gradually become a hot spot for CCA research, and they play a vital role in the occurrence and development of CCA. In this paper, we review that aberrantly expressed ncRNAs (lncRNAs and circRNAs) regulate the occurrence and progression of CCA by mediating epigenetic modifications, acting as ceRNA sponge miRNAs, participating in regulating cancer-related pathways, recruiting or encoding proteins, and other mechanisms.

In the current research results, ncRNAs do not regulate the occurrence and progression of CAA alone but interact with miRNAs or proteins to regulate mRNAs of genes associated with cancer. Notably, the primary function of current ncRNAs is to participate in the progression process of CCA as ceRNAs. We can use the ncRNA-miRNA-mRNA axis as a new therapeutic target to guide clinical drug use. In addition, EVs possess a stable structure and mediate intercellular communication. We can assemble ncRNA molecules that exert oncogenic effects into EVs and transport them through body fluids to CCA cells and be taken up for subsequent treatment of diseases. This could provide new ideas for developing effective novel anti-cancer molecular drugs. However, the research of ncRNAs in CCA is still in the preliminary stage, and the target genes of many ncRNAs and their mechanisms of regulating target genes are not well understood, and there are many problems to be solved. For example, are there any common targets of ncRNAs in CCA, and are there any synergistic or antagonistic effects among the many signaling pathways involved in regulation? Whether the identified ncRNAs that exert oncogenic effects through ceRNA mechanisms can encode functional proteins to regulate the progression of CCA. For ncRNAs identified to be aberrantly expressed in CCA, the results obtained from intervention experiments in vitro or animal models still require long clinical translation. Therefore, conducting more large and controlled clinical studies to translate ncRNAs to clinical applications has also become a future research direction. Furthermore, due to the simplicity of bile extraction and the structural stability of EVs, more research should focus on exploring EV-ncRNAs in the bile of CCA patients as reliable biomarkers and effective therapeutic targets. With the development of bioinformatics, genomics, and proteomics, the molecular mechanisms of ncRNAs in CCA progression will be gradually clarified, more effective molecular markers for early diagnosis and treatment of CCA will be validated, and the prognosis and survival quality of CCA patients will be better improved.

Author Contributions

JL and HB: Substantial contributions to the conception of the manuscript, and literature research, manuscript writing, as well as reviewing. ZH and ZL: Analysis of data, interpretation of data and manuscript polishing. NL and CN: Analysis of data, drafting the figures and reviewing the manuscript. YX: Design of the conception, interpretation of data, drafting the manuscript and final approval of the version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This research was supported by Hong Kong Scholars Program (XJ2020012); National Natural Science Foundation of China (81902431); Excellent Young Scientists Project of Natural Science Foundation of Heilongjiang Province (YQ2019H007); Special Project of China Postdoctoral Science Foundation (2019T120279); Special Project of Heilongjiang Postdoctoral Science Foundation (LBH-TZ1016); China Postdoctoral Science Foundation (2018M641849); Heilongjiang Postdoctoral Science Foundation (LBH-Z18107); The Fundamental Research Funds for the Heilongjiang Provincial Universities (2018-KYYWF-0511); Beijing Xisike Clinical Oncology Research Foundation (Y-Young2022-0188); Chen Xiaoping Foundation for the Development of Science and Technology of Hubei Province (CXPJJH11800004-001);

2022 Higher Education Science Research Planning Project of China Association of Higher Education (22CX0405); Postgraduate Innovative Research Project of Harbin Medical University (YJSCX2016-21HYD); Opening Project of Key Laboratory of Myocardial Ischemia, Ministry of Education (KF201810); Opening Project of Key Laboratory of Functional and Clinical Translational Medicine, Fujian Province University (XMMC-FCTM202205); Opening Project of Jiangsu Province Engineering Research Center of Tumor Targeted Nano Diagnostic and Therapeutic Materials (JETNM202210); Medjaden Academy & Research Foundation for Young Scientists (MJR20220903).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Rizvi S, Gores GJ. Pathogenesis, diagnosis, and management of cholangiocarcinoma. Gastroenterology. 2013; 145: 1215–1229.
[2] Razumilava N, Gores GJ. Cholangiocarcinoma. Lancet. 2014; 383: 2168–2179.
[3] Rizvi S, Gores GJ. Emerging molecular therapeutic targets for cholangiocarcinoma. Journal of Hepatology. 2017; 67: 632–644.
[4] Buckholz AP, Brown RS. Cholangiocarcinoma: Diagnosis and Management. Clinics in Liver Disease. 2020; 24: 421–436.
[5] Valle J, Wasan H, Palmer DH, Cunningham D, Anthoney A, Maraveyas A, *et al*. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. The New England Journal of Medicine. 2010; 362: 1273–1281.
[6] Rizvi S, Borad MJ, Patel T, Gores GJ. Cholangiocarcinoma: molecular pathways and therapeutic opportunities. Seminars in Liver Disease. 2014; 34: 456–464.
[7] Rizvi S, Khan SA, Hallemeier CL, Kelley RK, Gores GJ. Cholangiocarcinoma - evolving concepts and therapeutic strategies. Nature Reviews Clinical Oncology. 2018; 15: 95–111.
[8] ENCODE Project Consortium, Birney E, Stamatoyannopoulos JA, Dutta A, Guigó R, Gingeras TR, *et al*. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature. 2007; 447: 799–816.
[9] Matsui M, Corey DR. Non-coding RNAs as drug targets. Nature Reviews Drug Discovery. 2017; 16: 167–179.
[10] Anastasiadou E, Jacob LS, Slack FJ. Non-coding RNA networks in cancer. Nature Reviews Cancer. 2018; 18: 5–18.
[11] Yan H, Bu P. Non-coding RNA in cancer. Essays in Biochemistry. 2021; 65: 625–639.
[12] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004; 116: 281–297.
[13] Shi T, Morishita A, Kobara H, Masaki T. The Role of microRNAs in Cholangiocarcinoma. International Journal of Molecular Sciences. 2021; 22: 7627.
[14] Howell JA, Khan SA. The role of miRNAs in cholangiocarcinoma. Hepatic Oncology. 2016; 3: 167-180
[15] Schmitt AM, Chang HY. Long Noncoding RNAs in Cancer Pathways. Cancer Cell. 2016; 29: 452–463.
[16] van Bakel H, Hughes TR. Establishing legitimacy and function in the new transcriptome. Briefings in Functional Genomics & Proteomics. 2009; 8: 424–436.
[17] Quinodoz S, Guttman M. Long noncoding RNAs: an emerging link between gene regulation and nuclear organization. Trends in Cell Biology. 2014; 24: 651–663.
[18] Sun M, Nie F, Wang Y, Zhang Z, Hou J, He D, *et al*. LncRNA HOXA11-AS Promotes Proliferation and Invasion of Gastric Cancer by Scaffolding the Chromatin Modification Factors PRC2, LSD1, and DNMT1. Cancer Research. 2016; 76: 6299–6310.
[19] Tan Y, Lin J, Li T, Li J, Xu R, Ju H. LncRNA-mediated posttranslational modifications and reprogramming of energy metabolism in cancer. Cancer Communications. 2021; 41: 109–120.
[20] Cedar H, Bergman Y. Linking DNA methylation and histone modification: patterns and paradigms. Nature Reviews. Genetics. 2009; 10: 295–304.
[21] Mattick JS. The hidden genetic program of complex organisms. Scientific American. 2004; 291: 60–67.
[22] Wilusz JE, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. Genes & Development. 2009; 23: 1494–1504.
[23] Shen X, Liu Y, Hsu Y, Fujiwara Y, Kim J, Mao X, *et al*. EZH1 mediates methylation on histone H3 lysine 27 and complements EZH2 in maintaining stem cell identity and executing pluripotency. Molecular Cell. 2008; 32: 491–502.
[24] Yamaguchi H, Hung M. Regulation and Role of EZH2 in Cancer. Cancer Research and Treatment. 2014; 46: 209–222.
[25] Conway E, Healy E, Bracken AP. PRC2 mediated H3K27 methylations in cellular identity and cancer. Current Opinion in Cell Biology. 2015; 37: 42–48.
[26] Yu Y, Chen Q, Zhang X, Yang J, Lin K, Ji C, *et al*. Long noncoding RNA ANRIL promotes the malignant progression of cholangiocarcinoma by epigenetically repressing ERRFI1 expression. Cancer Science. 2020; 111: 2297–2309.
[27] Wang N, Zhang C, Wang W, Liu J, Yu Y, Li Y, *et al*. Long noncoding RNA DANCR regulates proliferation and migration by epigenetically silencing FBP1 in tumorigenesis of cholangiocarcinoma. Cell Death & Disease. 2019; 10: 585.
[28] Yu Y, Zhang M, Wang N, Li Q, Yang J, Yan S, *et al*. Epigenetic silencing of tumor suppressor gene CDKN1A by oncogenic long non-coding RNA SNHG1 in cholangiocarcinoma. Cell Death & Disease. 2018; 9: 746.
[29] Yu Y, Zhang M, Liu J, Xu B, Yang J, Wang N, *et al*. Long Non-coding RNA PVT1 Promotes Cell Proliferation and Migration by Silencing ANGPTL4 Expression in Cholangiocarcinoma. Molecular Therapy Nucleic Acids. 2018; 13: 503–513.
[30] Zhang C, Li J, Tian F, Zhao G, Hu H, Ma Y, *et al*. Long Noncoding RNA NEAT1 Promotes Growth and Metastasis of Cholangiocarcinoma Cells. Oncology Research. 2018; 26: 879–888.
[31] Ji H, Wang J, Lu B, Li J, Zhou J, Wang L, *et al*. SP1 induced long non-coding RNA AGAP2-AS1 promotes cholangiocarcinoma proliferation via silencing of CDKN1A. Molecular Medicine. 2021; 27: 10.
[32] Qin X, Lu M, Zhou Y, Li G, Liu Z. LncRNA FENDRR represses proliferation, migration and invasion through suppression of survivin in cholangiocarcinoma cells. Cell Cycle. 2019; 18: 889–897.
[33] Pennati M, Folini M, Zaffaroni N. Targeting survivin in cancer therapy: fulfilled promises and open questions. Carcinogenesis. 2007; 28: 1133–1139.
[34] Tsai M, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, *et al*. Long noncoding RNA as modular scaffold of histone modification complexes. Science. 2010; 329: 689–693.
[35] Xu Y, Yao Y, Jiang X, Zhong X, Wang Z, Li C, *et al*. SP1-induced upregulation of lncRNA SPRY4-IT1 exerts oncogenic properties by scaffolding EZH2/LSD1/DNMT1 and sponging miR-101-3p in cholangiocarcinoma. Journal of Experimental & Clinical Cancer Research: CR. 2018; 37: 81.
[36] Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell. 2011; 146: 353–358.
[37] Ha M, Kim VN. Regulation of microRNA biogenesis. Nature Reviews. Molecular Cell Biology. 2014; 15: 509–524.

[38] Sugihara T, Isomoto H, Gores G, Smoot R. YAP and the Hippo pathway in cholangiocarcinoma. Journal of Gastroenterology. 2019; 54: 485–491.
[39] Liu J, Zhao X, Wang K, Zhang X, Yu Y, Lv Y, *et al*. A novel YAP1/SLC35B4 regulatory axis contributes to proliferation and progression of gastric carcinoma. Cell Death & Disease. 2019; 10: 452.
[40] Li J, Jiang X, Xu Y, Kang P, Huang P, Meng N, *et al*. YY1-induced DLEU1/miR-149-5p Promotes Malignant Biological Behavior of Cholangiocarcinoma through Upregulating YAP1/TEAD2/SOX2. International Journal of Biological Sciences. 2022; 18: 4301–4315.
[41] Chaudhary B, Elkord E. Novel expression of Neuropilin 1 on human tumor-infiltrating lymphocytes in colorectal cancer liver metastases. Expert Opinion on Therapeutic Targets. 2015; 19: 147–161.
[42] Zhu H, Zhai B, He C, Li Z, Gao H, Niu Z, *et al*. LncRNA TTN-AS1 promotes the progression of cholangiocarcinoma via the miR-320a/neuropilin-1 axis. Cell Death & Disease. 2020; 11: 637.
[43] Li L, Jiang X, Zhang Q, Dong X, Gao Y, He Y, *et al*. Neuropilin-1 is associated with clinicopathology of gastric cancer and contributes to cell proliferation and migration as multifunctional co-receptors. Journal of Experimental & Clinical Cancer Research. 2016; 35: 16.
[44] Wang W, Ye H, Wei P, Han B, He B, Chen ZH, *et al*. LncRNAs H19 and HULC, activated by oxidative stress, promote cell migration and invasion in cholangiocarcinoma through a ceRNA manner. Journal of Hematology & Oncology. 2016; 9: 117.
[45] Yang H, Wang T, Hu S, Hu C, Jiang C, He Q. Long Noncoding RNA SNHG12, a New Therapeutic Target, Regulates miR-199a-5p/Klotho to Promote the Growth and Metastasis of Intrahepatic Cholangiocarcinoma Cells. Frontiers in Medicine. 2021; 8: 680378.
[46] Tu J, Wu F, Chen L, Zheng L, Yang Y, Ying X, *et al*. Long Non-Coding RNA PCAT6 Induces M2 Polarization of Macrophages in Cholangiocarcinoma *via* Modulating miR-326 and RhoA-ROCK Signaling Pathway. Frontiers in Oncology. 2021; 10: 605877.
[47] Xia L, Chen X, Yang J, Zhu S, Zhang L, Yin Q, *et al*. Long Non-Coding RNA-PAICC Promotes the Tumorigenesis of Human Intrahepatic Cholangiocarcinoma by Increasing YAP1 Transcription. Frontiers in Oncology. 2021; 10: 595533.
[48] Zheng H, Zhu M, Li W, Zhou Z, Wan X. m^5 C and m^6 A modification of long noncoding NKILA accelerates cholangiocarcinoma progression via the miR-582-3p-YAP1 axis. Liver International. 2022; 42: 1144–1157.
[49] Liu Y, Sun J, Qi P, Liu Y. Long non-coding RNA titin-antisense RNA1 contributes to growth and metastasis of cholangiocarcinoma by suppressing microRNA-513a-5p to upregulate stratifin. Bioengineered. 2021; 12: 12611–12624.
[50] Chang H, Yao Y. lncRNA TMPO antisense RNA 1 promotes the malignancy of cholangiocarcinoma cells by regulating let-7g-5p/ high-mobility group A1 axis. Bioengineered. 2022; 13: 2889–2901.
[51] Zhong B, Song C, He Q, Chen Z, Liao Q, Xiong Q, *et al*. LINC00630 promotes cholangiocarcinoma cell proliferation, migration and invasion by mediating the miR-199a/FGF7 axis. Journal of Cancer. 2022; 13: 975–986.
[52] Sun Z, Tan Z, Peng C, Yi W. LncRNA SNHG3 Facilitates the Malignant Phenotype of Cholangiocarcinoma Cells via the miR-3173-5p/ERG Axis. Journal of Gastrointestinal Surgery. 2022; 26: 802–812.
[53] He J, Yan H, Wei S, Chen G. LncRNA ST8SIA6-AS1 Promotes Cholangiocarcinoma Progression by Suppressing the miR-145-5p/MAL2 Axis. OncoTargets and Therapy. 2021; 14: 3209–3223.
[54] Sun D, Zhao Y, Wang W, Guan C, Hu Z, Liu L, *et al*. PCAT1 induced by transcription factor YY1 promotes cholangiocarcinoma proliferation, migration and invasion by sponging miR-216a-3p to up-regulate oncogene BCL3. Biological Chemistry. 2020; 402: 207–219.
[55] Sun H, Zhang G, Liu J, Nie C. Long noncoding RNA LINC00184 facilitates the proliferation, metastasis, and adenine metabolism of cholangiocarcinoma via modulating hsa-miR-23b-3p/ANXA2 axis. Environmental Toxicology. 2021; 36: 1576–1590.
[56] Lei G, Li Z, Li Y, Hong Z, Wang S, Bai Z, *et al*. Long noncoding RNA FAM66C promotes tumor progression and glycolysis in intrahepatic cholangiocarcinoma by regulating hsa-miR-23b-3p/KCND2 axis. Environmental Toxicology. 2021; 36: 2322–2332.
[57] Yu A, Zhao L, Kang Q, Li J, Chen K, Fu H. Transcription factor HIF1α promotes proliferation, migration, and invasion of cholangiocarcinoma via long noncoding RNA H19/microRNA-612/Bcl-2 axis. Translational Research. 2020; 224: 26–39.
[58] Lu M, Qin X, Zhou Y, Li G, Liu Z, Yue H, *et al*. LncRNA HOTAIR suppresses cell apoptosis, autophagy and induces cell proliferation in cholangiocarcinoma by modulating the miR-204-5p/HMGB1 axis. Biomedicine & Pharmacotherapy. 2020; 130: 110566.
[59] Wan T, Wang H, Gou M, Si H, Wang Z, Yan H, *et al*. LncRNA HEIH promotes cell proliferation, migration and invasion in cholangiocarcinoma by modulating miR-98-5p/HECTD4. Biomedicine & Pharmacotherapy. 2020; 125: 109916.
[60] Huang L, Jiang X, Li Z, Li J, Lin X, Hu Z, *et al*. Linc00473 potentiates cholangiocarcinoma progression by modulation of DDX5 expression via miR-506 regulation. Cancer Cell International. 2020; 20: 324.
[61] Li J, Jiang X, Li Z, Huang L, Ji D, Yu L, *et al*. SP1-induced HOXD-AS1 promotes malignant progression of cholangiocarcinoma by regulating miR-520c-3p/MYCN. Aging. 2020; 12: 16304–16325.
[62] Sun D, Li F, Liu L, Yu S, Wang H, Gao X, *et al*. PSMA3-AS1 induced by transcription factor PAX5 promotes cholangiocarcinoma proliferation, migration and invasion by sponging miR-376a-3p to up-regulate LAMC1. Aging. 2022; 14: 509–525.
[63] Kong L, Wu Q, Zhao L, Ye J, Li N, Yang H. Upregulated lncRNA-UCA1 contributes to metastasis of bile duct carcinoma through regulation of miR-122/*CLIC1* and activation of the ERK/MAPK signaling pathway. Cell Cycle. 2019; 18: 1212–1228.
[64] Li Z, Li X, Du X, Zhang H, Wu Z, Ren K, *et al*. The Interaction Between lncRNA SNHG1 and miR-140 in Regulating Growth and Tumorigenesis via the TLR4/NF-κB Pathway in Cholangiocarcinoma. Oncology Research. 2019; 27: 663–672.
[65] Li O, Jiang B, Yi W, Zhang Y, Yang P, Guo C, *et al*. LncRNA NEAT1 promotes cell proliferation, migration, and invasion via the miR-186-5p/PTP4A1 axis in cholangiocarcinoma. The Kaohsiung Journal of Medical Sciences. 2021; 37: 379–391.
[66] Lu W. Long non-coding RNA MEG3 represses cholangiocarcinoma by regulating miR-361-5p/TRAF3 axis. European Review for Medical and Pharmacological Sciences. 2019; 23: 7356–7368.
[67] Carotenuto P, Fassan M, Pandolfo R, Lampis A, Vicentini C, Cascione L, *et al*. Wnt signalling modulates transcribed-ultraconserved regions in hepatobiliary cancers. Gut. 2017; 66: 1268–1277.
[68] Xu J, Zhang S, Wang R, Wu X, Zeng L, Fu Z. Knockdown of PRDX2 sensitizes colon cancer cells to 5-FU by suppressing the PI3K/AKT signaling pathway. Bioscience Reports. 2017; 37: BSR20160447.

[69] Zhang Y, Zhang L, Lu S, Xiang Y, Zeng C, He T, *et al*. Long Non-coding RNA CASC15 Promotes Intrahepatic Cholangiocarcinoma Possibly through Inducing PRDX2/PI3K/AKT Axis. Cancer Research and Treatment. 2021; 53: 184–198.
[70] Zhang F, Wan M, Xu Y, Li Z, Leng K, Kang P, *et al*. Long noncoding RNA PCAT1 regulates extrahepatic cholangiocarcinoma progression via the Wnt/β-catenin-signaling pathway. Biomedicine & Pharmacotherapy. 2017; 94: 55–62.
[71] Gu Y, Zhu Z, Pei H, Xu D, Jiang Y, Zhang L, *et al*. Long non-coding RNA NNT-AS1 promotes cholangiocarcinoma cells proliferation and epithelial-to-mesenchymal transition through down-regulating miR-203. Aging. 2020; 12: 2333–2346.
[72] Wei C, Wong H, Xu F, Liu Z, Ran L, Jiang R. IRF4-induced upregulation of lncRNA SOX2-OT promotes cell proliferation and metastasis in cholangiocarcinoma by regulating SOX2 and PI3K/AKT signaling. European Review for Medical and Pharmacological Sciences. 2018; 22: 8169–8178.
[73] Xu Y, Yao Y, Leng K, Li Z, Qin W, Zhong X, *et al*. Long non-coding RNA UCA1 indicates an unfavorable prognosis and promotes tumorigenesis via regulating AKT/GSK-3β signaling pathway in cholangiocarcinoma. Oncotarget. 2017; 8: 96203–96214.
[74] Wang C, Mao ZP, Wang L, Wu GH, Zhang FH, Wang DY, *et al*. Long non-coding RNA MALAT1 promotes cholangiocarcinoma cell proliferation and invasion by activating PI3K/Akt pathway. Neoplasma. 2017; 64: 725–731.
[75] Hu X, Tan Z, Yang Y, Yang P. Long non-coding RNA MIR22HG inhibits cell proliferation and migration in cholangiocarcinoma by negatively regulating the Wnt/β-catenin signaling pathway. The Journal of Gene Medicine. 2019; 21: e3085.
[76] Chen C, Li H, Wang X, Wang L, Zeng Q. Lnc-LFAR1 affects intrahepatic cholangiocarcinoma proliferation, invasion, and EMT by regulating the TGFβ/Smad signaling pathway. International Journal of Clinical and Experimental Pathology. 2019; 12: 2455–2461.
[77] Guo L, Zhou Y, Chen Y, Sun H, Wang Y, Qu Y. LncRNA ASAP1-IT1 positively modulates the development of cholangiocarcinoma via hedgehog signaling pathway. Biomedicine & Pharmacotherapy. 2018; 103: 167–173.
[78] Li F, Chen Q, Xue H, Zhang L, Wang K, Shen F. LncRNA MNX1-AS1 promotes progression of intrahepatic cholangiocarcinoma through the MNX1/Hippo axis. Cell Death & Disease. 2020; 11: 894.
[79] Zhang X, Wang H, Zhang Y, Lu X, Chen L, Yang L. Complementary sequence-mediated exon circularization. Cell. 2014; 159: 134–147.
[80] Liang D, Wilusz JE. Short intronic repeat sequences facilitate circular RNA production. Genes & Development. 2014; 28: 2233–2247.
[81] Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, *et al*. Circular RNAs are abundant, conserved, and associated with ALU repeats. RNA. 2013; 19: 141–157.
[82] Ashwal-Fluss R, Meyer M, Pamudurti NR, Ivanov A, Bartok O, Hanan M, *et al*. circRNA biogenesis competes with pre-mRNA splicing. Molecular Cell. 2014; 56: 55–66.
[83] Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, *et al*. Circular RNAs are a large class of animal RNAs with regulatory potency. Nature. 2013; 495: 333–338.
[84] Chen Q, Wang H, Li Z, Li F, Liang L, Zou Y, *et al*. Circular RNA ACTN4 promotes intrahepatic cholangiocarcinoma progression by recruiting YBX1 to initiate FZD7 transcription. Journal of Hepatology. 2022; 76: 135–147.
[85] Li Y, Zheng Q, Bao C, Li S, Guo W, Zhao J, *et al*. Circular RNA is enriched and stable in exosomes: a promising biomarker for cancer diagnosis. Cell Research. 2015; 25: 981–984.
[86] Vo JN, Cieslik M, Zhang Y, Shukla S, Xiao L, Zhang Y, *et al*. The Landscape of Circular RNA in Cancer. Cell. 2019; 176: 869–881.e13.
[87] Xu Y, Dong Z, Wang S, Zheng Y, Zhang C, Zhou Y, *et al*. circHMGCS1-016 reshapes immune environment by sponging miR-1236-3p to regulate CD73 and GAL-8 expression in intrahepatic cholangiocarcinoma. Journal of Experimental & Clinical Cancer Research: CR. 2021; 40: 290.
[88] Xiong W, Zhang A, Xiao X, Liu W. CircSETD3 (hsa_circ_0000567) inhibits proliferation and induces apoptosis in cholangiocarcinoma cells via downregulation of microRNA-421 expression. Bioengineered. 2022; 13: 10191–10201.
[89] Wang J, Luo X, Lu J, Wang X, Miao Y, Li Q, *et al*. Rab22a promotes the proliferation, migration, and invasion of lung adenocarcinoma via up-regulating PI3K/Akt/mTOR signaling pathway. Experimental Cell Research. 2022; 416: 113179.
[90] Guan C, Liu L, Zhao Y, Zhang X, Liu G, Wang H, *et al*. YY1 and eIF4A3 are mediators of the cell proliferation, migration and invasion in cholangiocarcinoma promoted by circ-ZNF609 by targeting miR-432-5p to regulate LRRC1. Aging. 2021; 13: 25195–25212.
[91] Xu Y, Gao P, Wang Z, Su Z, Liao G, Han Y, *et al*. Circ-LAMP1 contributes to the growth and metastasis of cholangiocarcinoma via miR-556-5p and miR-567 mediated YY1 activation. Journal of Cellular and Molecular Medicine. 2021; 25: 3226–3238.
[92] Xu Y, Yao Y, Liu Y, Wang Z, Hu Z, Su Z, *et al*. Elevation of circular RNA circ_0005230 facilitates cell growth and metastasis via sponging miR-1238 and miR-1299 in cholangiocarcinoma. Aging. 2019; 11: 1907–1917.
[93] Su Y, Yu T, Wang Y, Huang X, Wei X. Circular RNA circDNM3OS Functions as a miR-145-5p Sponge to Accelerate Cholangiocarcinoma Growth and Glutamine Metabolism by Upregulating MORC2. OncoTargets and Therapy. 2021; 14: 1117–1129.
[94] Zhang X, Zhao Y, Wang W, Yu S, Liu L, Sun D, *et al*. Upregulation of circ_0059961 suppresses cholangiocarcinoma development by modulating miR-629-5p/SFRP2 axis. Pathology, Research and Practice. 2022; 234: 153901.
[95] Tang J, Wang R, Tang R, Gu P, Han J, Huang W. CircRTN4IP1 regulates the malignant progression of intrahepatic cholangiocarcinoma by sponging miR-541-5p to induce HIF1A production. Pathology, Research and Practice. 2022; 230: 153732.
[96] Du J, Lan T, Liao H, Feng X, Chen X, Liao W, *et al*. Circ-NFIB inhibits tumor growth and metastasis through suppressing MEK1/ERK signaling in intrahepatic cholangiocarcinoma. Molecular Cancer. 2022; 21: 18.
[97] Li H, Lan T, Liu H, Liu C, Dai J, Xu L, *et al*. IL-6-induced cGGNBP2 encodes a protein to promote cell growth and metastasis in intrahepatic cholangiocarcinoma. Hepatology. 2022; 75: 1402–1419.
[98] Li D, Tang Z, Gao Z, Shen P, Liu Z, Dang X. Circular RNA CDR1as Exerts Oncogenic Properties Partially through Regulating MicroRNA 641 in Cholangiocarcinoma. Molecular and Cellular Biology. 2020; 40: e00042–20.
[99] Xiao F, Xu F, Zhang H, Shuai X. Circ_0000591 served as endogenous RNA for miR-326 to promote progression of cholangiocarcinoma via the TLR4/MyD88/IL6 axis. Biochemical and Biophysical Research Communications. 2022; 600: 101–108.
[100] Chen S, Chen Z, Li Z, Li S, Wen Z, Cao L, *et al*. Tumor-associated macrophages promote cholangiocarcinoma progression via exosomal Circ_0020256. Cell Death & Disease. 2022; 13: 94.
[101] Wang S, Hu Y, Lv X, Li B, Gu D, Li Y, *et al*. Circ-0000284 arouses malignant phenotype of cholangiocarcinoma cells and regulates the biological functions of peripheral cells through cellular communication. Clinical Science. 2019; 133: 1935–1953.

[102] Xu Y, Leng K, Yao Y, Kang P, Liao G, Han Y, *et al*. A Circular RNA, Cholangiocarcinoma-Associated Circular RNA 1, Contributes to Cholangiocarcinoma Progression, Induces Angiogenesis, and Disrupts Vascular Endothelial Barriers. Hepatology. 2021; 73: 1419–1435.
[103] Xu Y, Yao Y, Zhong X, Leng K, Qin W, Qu L, *et al*. Downregulated circular RNA hsa_circ_0001649 regulates proliferation, migration and invasion in cholangiocarcinoma cells. Biochemical and Biophysical Research Communications. 2018; 496: 455–461.
[104] Lu Q, Fang T. Circular RNA SMARCA5 correlates with favorable clinical tumor features and prognosis, and increases chemotherapy sensitivity in intrahepatic cholangiocarcinoma. Journal of Clinical Laboratory Analysis. 2020; 34: e23138.
[105] Jin D, Fan J, Wang L, Thompson LF, Liu A, Daniel BJ, *et al*. CD73 on tumor cells impairs antitumor T-cell responses: a novel mechanism of tumor-induced immune suppression. Cancer Research. 2010; 70: 2245–2255.
[106] Hasan SS, Ashraf GM, Banu N. Galectins - potential targets for cancer therapy. Cancer Letters. 2007; 253: 25–33.
[107] Wu P, Mo Y, Peng M, Tang T, Zhong Y, Deng X, *et al*. Emerging role of tumor-related functional peptides encoded by lncRNA and circRNA. Molecular Cancer. 2020; 19: 22.
[108] Zhang M, Huang N, Yang X, Luo J, Yan S, Xiao F, *et al*. A novel protein encoded by the circular form of the SHPRH gene suppresses glioma tumorigenesis. Oncogene. 2018; 37: 1805–1814.
[109] Cen J, Liang Y, Huang Y, Pan Y, Shu G, Zheng Z, *et al*. Circular RNA circSDHC serves as a sponge for miR-127-3p to promote the proliferation and metastasis of renal cell carcinoma via the CDKN3/E2F1 axis. Molecular Cancer. 2021; 20: 19.
[110] Louis C, Coulouarn C. One stone, two birds: circACTN4, a nexus for a coordinated activation of Hippo and Wnt/β-catenin pathways in cholangiocarcinoma. Journal of Hepatology. 2022; 76: 8–10.
[111] Bebelman MP, Smit MJ, Pegtel DM, Baglio SR. Biogenesis and function of extracellular vesicles in cancer. Pharmacology & Therapeutics. 2018; 188: 1–11.
[112] Tkach M, Théry C. Communication by Extracellular Vesicles: Where We Are and Where We Need to Go. Cell. 2016; 164: 1226–1232.
[113] van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nature Reviews Molecular Cell Biology. 2018; 19: 213–228.
[114] He C, Zheng S, Luo Y, Wang B. Exosome Theranostics: Biology and Translational Medicine. Theranostics. 2018; 8: 237–255.
[115] Takahashi K, Yan IK, Wood J, Haga H, Patel T. Involvement of extracellular vesicle long noncoding RNA (linc-VLDLR) in tumor cell responses to chemotherapy. Molecular Cancer Research. 2014; 12: 1377–1387.
[116] Maacha S, Bhat AA, Jimenez L, Raza A, Haris M, Uddin S, *et al*. Extracellular vesicles-mediated intercellular communication: roles in the tumor microenvironment and anti-cancer drug resistance. Molecular Cancer. 2019; 18: 55.
[117] Zhang H, Deng T, Liu R, Bai M, Zhou L, Wang X, *et al*. Exosome-delivered EGFR regulates liver microenvironment to promote gastric cancer liver metastasis. Nature Communications. 2017; 8: 15016.
[118] Becker A, Thakur BK, Weiss JM, Kim HS, Peinado H, Lyden D. Extracellular Vesicles in Cancer: Cell-to-Cell Mediators of Metastasis. Cancer Cell. 2016; 30: 836–848.
[119] Li X, Yang L, Chen L. The Biogenesis, Functions, and Challenges of Circular RNAs. Molecular Cell. 2018; 71: 428–442.
[120] You X, Vlatkovic I, Babic A, Will T, Epstein I, Tushev G, *et al*. Neural circular RNAs are derived from synaptic genes and regulated by development and plasticity. Nature Neuroscience. 2015; 18: 603–610.
[121] Du WW, Zhang C, Yang W, Yong T, Awan FM, Yang BB. Identifying and Characterizing circRNA-Protein Interaction. Theranostics. 2017; 7: 4183–4191.
[122] Du WW, Yang W, Liu E, Yang Z, Dhaliwal P, Yang BB. Foxo3 circular RNA retards cell cycle progression via forming ternary complexes with p21 and CDK2. Nucleic Acids Research. 2016; 44: 2846–2858.
[123] Jie M, Wu Y, Gao M, Li X, Liu C, Ouyang Q, *et al*. CircMRPS35 suppresses gastric cancer progression via recruiting KAT7 to govern histone modification. Molecular Cancer. 2020; 19: 56.
[124] Legnini I, Di Timoteo G, Rossi F, Morlando M, Briganti F, Sthandier O, *et al*. Circ-ZNF609 Is a Circular RNA that Can Be Translated and Functions in Myogenesis. Molecular Cell. 2017; 66: 22–37.e9.
[125] Pamudurti NR, Bartok O, Jens M, Ashwal-Fluss R, Stottmeister C, Ruhe L, *et al*. Translation of CircRNAs. Molecular Cell. 2017; 66: 9–21.e7.
[126] Wu X, Xiao S, Zhang M, Yang L, Zhong J, Li B, *et al*. A novel protein encoded by circular SMO RNA is essential for Hedgehog signaling activation and glioblastoma tumorigenicity. Genome Biology. 2021; 22: 33.
[127] Ge X, Wang Y, Nie J, Li Q, Tang L, Deng X, *et al*. The diagnostic/prognostic potential and molecular functions of long noncoding RNAs in the exosomes derived from the bile of human cholangiocarcinoma. Oncotarget. 2017; 8: 69995–70005.
[128] Liang Z, Zhu B, Meng D, Shen X, Li X, Wang Z, *et al*. Down-regulation of lncRNA-NEF indicates poor prognosis in intrahepatic cholangiocarcinoma. Bioscience Reports. 2019; 39: BSR20181573.
[129] Jiang X, Li Z, Li J, Xu Y, Leng K, Cui Y, *et al*. A novel prognostic biomarker for cholangiocarcinoma: circRNA Cdr1as. European Review for Medical and Pharmacological Sciences. 2018; 22: 365–371.
[130] Jiang X, Li J, Wang W, Hu Z, Guan C, Zhao Y, *et al*. AR-induced ZEB1-AS1 represents poor prognosis in cholangiocarcinoma and facilitates tumor stemness, proliferation and invasion through mediating miR-133b/HOXB8. Aging. 2020; 12: 1237–1255.
[131] Guan C, Zhao Y, Wang W, Hu Z, Liu L, Li W, *et al*. Knockdown of lncRNA *SNHG20* Suppressed the Proliferation of Cholangiocarcinoma by Sponging *miR-520f-3p*. Cancer Biotherapy & Radiopharmaceuticals. 2020. (online ahead of print)
[132] Li J, Guan C, Hu Z, Liu L, Su Z, Kang P, *et al*. Yin Yang 1-induced LINC00667 up-regulates pyruvate dehydrogenase kinase 1 to promote proliferation, migration and invasion of cholangiocarcinoma cells by sponging miR-200c-3p. Human Cell. 2021; 34: 187–200.
[133] Hu Z, Huang L, Wang W, Guan C, Zhao Y, Liu L, *et al*. Long Non-coding RNA FOXD2-AS1 Promotes Proliferation, Migration, and Invasion in Cholangiocarcinoma Through Regulating miR-760/E2F3 Axis. Digestive Diseases and Sciences. 2022; 67: 546–558.
[134] Parasramka M, Yan IK, Wang X, Nguyen P, Matsuda A, Maji S, *et al*. BAP1 dependent expression of long non-coding RNA NEAT-1 contributes to sensitivity to gemcitabine in cholangiocarcinoma. Molecular Cancer. 2017; 16: 22.
[135] Lu M, Qin X, Zhou Y, Li G, Liu Z, Geng X, *et al*. Long non-coding RNA LINC00665 promotes gemcitabine resistance of Cholangiocarcinoma cells via regulating EMT and stemness properties through miR-424-5p/BCL9L axis. Cell Death & Disease. 2021; 12: 72.
[136] Gao K, Chen S, Yang X. HOTTIP Enhances Gemcitabine and Cisplatin Resistance Through Sponging miR-637 in Cholangiocarcinoma. Frontiers in Oncology. 2021; 11: 2551.