Non-Coding RNA in Cholangiocarcinoma: An Update

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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 of 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 repress 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 IncRNAs 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-
oncogene in various cancers, particularly in CCA. Yes-associated protein 1 (YAP1) functions as a pro-cancer role in CCA cells. Survivin, 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 [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 proto-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 up-regulate 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 RhoA 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.

<table>
<thead>
<tr>
<th>LncRNA</th>
<th>Expression</th>
<th>miRNA sponge</th>
<th>mRNA</th>
<th>Function</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLEU1</td>
<td>up</td>
<td>miR-149-5p</td>
<td>YAP1</td>
<td>Promoting CCA cells proliferation, migration and EMT</td>
<td>[40]</td>
</tr>
<tr>
<td>NKILA</td>
<td>up</td>
<td>miR-582-3p</td>
<td>YAP1</td>
<td>Promoting cells proliferation, migration, invasion</td>
<td>[48]</td>
</tr>
<tr>
<td>TTN-AS1</td>
<td>up</td>
<td>miR-320a</td>
<td>NRP-1</td>
<td>Promoting CCA cells cycle progression, EMT, and angiogenesis</td>
<td>[42]</td>
</tr>
<tr>
<td>TTN-AS1</td>
<td>up</td>
<td>miR-513a-5p</td>
<td>SFN</td>
<td>Contributing to growth and metastasis of CCA</td>
<td>[49]</td>
</tr>
<tr>
<td>H19</td>
<td>up</td>
<td>let-7a/let-7b</td>
<td>IL-6</td>
<td>Regulating CCA cells migration and invasion</td>
<td>[44]</td>
</tr>
<tr>
<td>HULC</td>
<td>up</td>
<td>miR-372/miR-373</td>
<td>CXCR4</td>
<td>Regulating CCA cells migration and invasion</td>
<td>[44]</td>
</tr>
<tr>
<td>TMPO-AS1</td>
<td>up</td>
<td>let-7 g-5p</td>
<td>HMG1A</td>
<td>Improving CCA cells migration proliferation; impair cell apoptosis</td>
<td>[50]</td>
</tr>
<tr>
<td>SNHG12</td>
<td>up</td>
<td>miR-199a-5p</td>
<td>Klotho</td>
<td>Promoting iCCA cells growth and metastasis</td>
<td>[45]</td>
</tr>
<tr>
<td>LINCO0630</td>
<td>up</td>
<td>miR-199a</td>
<td>FGF7</td>
<td>Promoting CCA cells proliferation, migration and invasion</td>
<td>[51]</td>
</tr>
<tr>
<td>SNHG3</td>
<td>up</td>
<td>miR-3173-5p</td>
<td>ERG</td>
<td>Promoting CCA cells proliferation, migration, invasion</td>
<td>[52]</td>
</tr>
<tr>
<td>ST8SIA6-AS1</td>
<td>up</td>
<td>miR-145-5p</td>
<td>MAL2</td>
<td>Facilitating CCA cells growth and migration</td>
<td>[53]</td>
</tr>
<tr>
<td>PCAT1</td>
<td>up</td>
<td>miR-216a-3p</td>
<td>BCL3</td>
<td>Promoting CCA cells proliferation, migration, invasion</td>
<td>[54]</td>
</tr>
<tr>
<td>LINCO00184</td>
<td>up</td>
<td>miR-23b-3p</td>
<td>ANXA2</td>
<td>Facilitating CCA cells proliferation, metastasis, and adenine metabolism</td>
<td>[55]</td>
</tr>
<tr>
<td>FAM66C</td>
<td>up</td>
<td>miR-23b-3p</td>
<td>KCND2</td>
<td>Promoting progression and glycolysis in iCCA</td>
<td>[56]</td>
</tr>
<tr>
<td>H19</td>
<td>up</td>
<td>miR-612</td>
<td>Bcl-2</td>
<td>Promoting CCA cells proliferation, migration, invasion</td>
<td>[57]</td>
</tr>
<tr>
<td>HOTAIR</td>
<td>down</td>
<td>miR-204-5p</td>
<td>HMGB1</td>
<td>Suppressing CCA cells apoptosis, autophagy and inducing cell proliferation</td>
<td>[58]</td>
</tr>
<tr>
<td>HEIH</td>
<td>up</td>
<td>miR-98-5p</td>
<td>HECTD4</td>
<td>Promoting CCA cells proliferation, migration, invasion</td>
<td>[59]</td>
</tr>
<tr>
<td>Linc00473</td>
<td>up</td>
<td>miR-506</td>
<td>DDX5</td>
<td>Promoting CCA cells proliferation and invasion</td>
<td>[60]</td>
</tr>
<tr>
<td>HOXD-AS1</td>
<td>up</td>
<td>miR-520e-3p</td>
<td>MYCN</td>
<td>Promoting CCA cells proliferation, migration, EMT, stemness maintenance</td>
<td>[61]</td>
</tr>
<tr>
<td>PSMA3-AS1</td>
<td>up</td>
<td>miR-376a-3p</td>
<td>LAMC1</td>
<td>Promoting CCA cells proliferation, migration, invasion</td>
<td>[62]</td>
</tr>
<tr>
<td>SPRY4-IT1</td>
<td>up</td>
<td>miR-101-3p</td>
<td>EZH2</td>
<td>Raising EZH2 expression and mediating epigenetic in CCA</td>
<td>[35]</td>
</tr>
<tr>
<td>PCAT6</td>
<td>up</td>
<td>miR-326</td>
<td>RhoA</td>
<td>Activating RhoA-ROCK signaling pathway and modulating macrophages</td>
<td>[46]</td>
</tr>
<tr>
<td>PAICC</td>
<td>up</td>
<td>miR-141-3p, miR-27A-3p</td>
<td>YAP1</td>
<td>Promoting tumorigenesis of iCCA cells and activating the Hippo signaling pathway</td>
<td>[47]</td>
</tr>
<tr>
<td>UCA1</td>
<td>up</td>
<td>miR-122</td>
<td>CLIC1</td>
<td>Activating the ERK/MAPK signaling pathway</td>
<td>[63]</td>
</tr>
<tr>
<td>SNHG1</td>
<td>up</td>
<td>miR-140</td>
<td>TLR4</td>
<td>Activating the NF-κB signaling pathway</td>
<td>[64]</td>
</tr>
<tr>
<td>NEAT1</td>
<td>up</td>
<td>miR-186-5p</td>
<td>PTP4A1</td>
<td>Activating the PI3K/AKT signaling pathway</td>
<td>[65]</td>
</tr>
<tr>
<td>MEG3</td>
<td>down</td>
<td>miR-361-5p</td>
<td>TRAF3</td>
<td>Inhibiting the NF-κB signaling pathway</td>
<td>[66]</td>
</tr>
<tr>
<td>UC.158−</td>
<td>up</td>
<td>miR-193b</td>
<td>-</td>
<td>As a mediator downstream of the Wnt/β-catenin pathway</td>
<td>[67]</td>
</tr>
</tbody>
</table>

**Abbreviations:** YAP1, Yes-associated protein 1; NRP-1, neuropilin-1; IL-6, interleukin 6; CXCR4, CXC-chemokine receptor 4; HMG1A, 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.
Fig. 2. Mechanism of dysregulated lncRNAs regulating cancer-related signaling pathways, including PI3K/AKT, ERK/MAPK, Wnt/β-catenin, NF-κB, Hedgehog, TGF/β/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, Cyclin D1; 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; 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.

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 circHMCGS1-016 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 circHMCGS1-016 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 CircSEED3 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/β-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.

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>
<thead>
<tr>
<th>CircRNA</th>
<th>Expression</th>
<th>Source</th>
<th>Pathway</th>
<th>Functions</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>circHMGCS1-016</td>
<td>up</td>
<td>tissue</td>
<td>circHMGCS1-016/miR-1236-3p/CD73</td>
<td>Inducing iCCA cells invasion and reshaping the tumor immune microenvironment</td>
<td>[87]</td>
</tr>
<tr>
<td>circSETD3</td>
<td>down</td>
<td>cell, tissue</td>
<td>circSETD3/miR-421/BMF</td>
<td>Inhibiting proliferation and inducing apoptosis in CCA cells</td>
<td>[88]</td>
</tr>
<tr>
<td>circ0021205</td>
<td>up</td>
<td>cell, tissue</td>
<td>circ0021205/miR-204-5p/RAB22A</td>
<td>Promoting CCA cells proliferation, migration, and invasion</td>
<td>[89]</td>
</tr>
<tr>
<td>circ-ZNF609</td>
<td>up</td>
<td>tissue</td>
<td>YY1/eIF4A3/circ-ZNF609/miR-432-5p/LRRC1</td>
<td>Promoting CCA cells proliferation, migration, and invasion</td>
<td>[90]</td>
</tr>
<tr>
<td>circ-LAMP1</td>
<td>up</td>
<td>cell, tissue</td>
<td>circ-LAMP1/miR-556-5p and miR-567/YY1</td>
<td>Contributing to the growth and metastasis of CCA</td>
<td>[91]</td>
</tr>
<tr>
<td>circ_0005230</td>
<td>up</td>
<td>cell, tissue</td>
<td>circ_0005230/miR-1238 and miR-1299</td>
<td>Facilitating CCA cells growth and metastasis</td>
<td>[92]</td>
</tr>
<tr>
<td>circDNM3OS</td>
<td>up</td>
<td>cell, tissue</td>
<td>circDNM3OS/miR-145-5p/MORC2</td>
<td>Accelerating CCA growth and glutamine metabolism</td>
<td>[93]</td>
</tr>
<tr>
<td>circ_0059961</td>
<td>down</td>
<td>cell, tissue</td>
<td>circ_0059961/miR-629-5p/SFRP2</td>
<td>Suppressing CCA cells proliferation, migration, and invasion</td>
<td>[94]</td>
</tr>
<tr>
<td>circRTN4P1</td>
<td>up</td>
<td>cell, tissue</td>
<td>circRTN4P1/miR-541-5p/HIF1A</td>
<td>Regulating iCCA cells malignancy and glucose metabolism</td>
<td>[95]</td>
</tr>
<tr>
<td>circNFIB</td>
<td>down</td>
<td>tissue</td>
<td>circNFIB/MEK1/ERK2</td>
<td>Inhibiting tumor growth and metastasis</td>
<td>[96]</td>
</tr>
<tr>
<td>circACTN4</td>
<td>up</td>
<td>tissue</td>
<td>circACTN4/miR-424-5p/YAP1, circACTN4/YBX1/FZD7</td>
<td>Promoting iCCA proliferation and metastasis</td>
<td>[84]</td>
</tr>
<tr>
<td>cGGNBP2</td>
<td>up</td>
<td>cell, tissue</td>
<td>IL-6/cGGNBP2/cGGNBP2-184aa/JAK/STAT3</td>
<td>Promoting iCCA cells growth and metastasis</td>
<td>[97]</td>
</tr>
<tr>
<td>CDR1as</td>
<td>up</td>
<td>cell, tissue</td>
<td>CDR1as/miR-641/AKT3/mTOR</td>
<td>Promoting CCA cells proliferation, migration, and invasion</td>
<td>[98]</td>
</tr>
<tr>
<td>circ_0000591</td>
<td>up</td>
<td>cell, tissue</td>
<td>Circ_0000591/miR326/TLR4/MyD88/IL6</td>
<td>Promoting CCA cells proliferation, migration, and invasion</td>
<td>[99]</td>
</tr>
<tr>
<td>circ_0020256</td>
<td>up</td>
<td>serum Exos</td>
<td>E-circ_0020256/miR-432-5p/E2F3</td>
<td>Promoting CCA cells proliferation, migration, and invasion</td>
<td>[100]</td>
</tr>
<tr>
<td>circ-0000284</td>
<td>up</td>
<td>cell, tissue, serum Exos</td>
<td>circ0000284/miR637/LY6E</td>
<td>Promoting CCA cells proliferation, migration, and invasion</td>
<td>[101]</td>
</tr>
<tr>
<td>circ-CCAC1</td>
<td>up</td>
<td>tissue, bile EVs</td>
<td>circ-CCAC1/miR-514a-5p/YY1/CAMLG</td>
<td>Promoting CCA tumorigenesis and metastasis</td>
<td>[102]</td>
</tr>
<tr>
<td>hsa_circ_0001649</td>
<td>down</td>
<td>cell, tissue</td>
<td>-</td>
<td>Promoting CCA cells proliferation, migration, and invasion</td>
<td>[103]</td>
</tr>
<tr>
<td>SMARCA5</td>
<td>down</td>
<td>tissue</td>
<td>-</td>
<td>Inhibiting proliferation and increasing chemotherapy sensitivity</td>
<td>[104]</td>
</tr>
</tbody>
</table>

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, recombiant 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 mRNA 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-cadherin 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 chromatins 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-independent and cap-independent way. It has been shown that circ-SMO encodes the carcinogenic protein SMO-193a.a., which represses 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 nomenclature 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 IncRNAs 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 IncRNAs, ENST00000588480.1, and ENST00000517758.1, were highly expressed in bile-derived exosomes of CCA patients were highly expressed in CCA patients. When these two IncRNAs 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 IncRNAs in CCA patients, the worse their survival and can be used as predictors for monitoring CCA [127]. It was reported that IncRNA-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 IncRNA-NEF as a diagnostic marker for iCCA. In addition, overall survival (OS) was significantly lower in iCCA patients with low IncRNA-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-circRNAs 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 IncRNA 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. s as markers of CCA. Overall survival (OS); recurrence free survival (RFS).

<table>
<thead>
<tr>
<th>sRNA</th>
<th>Source</th>
<th>Expression</th>
<th>Clinical application</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Prognostic indicator</th>
<th>p value</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLEU1</td>
<td>tissue</td>
<td>up</td>
<td>prognostic marker</td>
<td>0.747</td>
<td>72.4%</td>
<td>65.4%</td>
<td>OS</td>
<td>p &lt; 0.001</td>
<td>[40]</td>
</tr>
<tr>
<td>PSMA3-AS1</td>
<td>tissue</td>
<td>up</td>
<td>prognostic marker</td>
<td>0.793</td>
<td>70.8%</td>
<td>79.2%</td>
<td>OS</td>
<td>p &lt; 0.001</td>
<td>[62]</td>
</tr>
<tr>
<td>ZEB1-AS1</td>
<td>tissue</td>
<td>up</td>
<td>prognostic marker</td>
<td>0.749</td>
<td>65.5%</td>
<td>80.0%</td>
<td>OS</td>
<td>p &lt; 0.001</td>
<td>[130]</td>
</tr>
<tr>
<td>HOXD-AS1</td>
<td>tissue</td>
<td>up</td>
<td>prognostic marker</td>
<td>0.786</td>
<td>80.0%</td>
<td>65.4%</td>
<td>OS</td>
<td>p &lt; 0.001</td>
<td>[61]</td>
</tr>
<tr>
<td>SNHG20</td>
<td>tissue</td>
<td>up</td>
<td>prognostic marker</td>
<td>0.748</td>
<td>73.4%</td>
<td>75.5%</td>
<td>OS</td>
<td>p &lt; 0.001</td>
<td>[131]</td>
</tr>
<tr>
<td>LINC00667</td>
<td>tissue</td>
<td>up</td>
<td>prognostic marker</td>
<td>0.830</td>
<td>87.5%</td>
<td>72.0%</td>
<td>OS</td>
<td>p &lt; 0.001</td>
<td>[132]</td>
</tr>
<tr>
<td>lncRNA-NEF</td>
<td>plasma</td>
<td>down</td>
<td>diagnostic marker, prognostic factor</td>
<td>0.864</td>
<td>-</td>
<td>-</td>
<td>OS</td>
<td>p = 0.0198</td>
<td>[128]</td>
</tr>
<tr>
<td>FOXD2-AS1</td>
<td>tissue</td>
<td>up</td>
<td>prognostic marker</td>
<td>0.741</td>
<td>82.0%</td>
<td>60.0%</td>
<td>OS</td>
<td>p &lt; 0.001</td>
<td>[133]</td>
</tr>
<tr>
<td>cGNNBP2</td>
<td>tissue</td>
<td>up</td>
<td>prognostic factor</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>OS, RFS</td>
<td>p &lt; 0.001</td>
<td>[97]</td>
</tr>
<tr>
<td>circNFIB</td>
<td>tissue</td>
<td>down</td>
<td>prognostic factor</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>OS, RFS</td>
<td>p &lt; 0.001</td>
<td>[96]</td>
</tr>
<tr>
<td>Cdr1as</td>
<td>tissue</td>
<td>up</td>
<td>prognostic marker</td>
<td>-</td>
<td>83.3%</td>
<td>58.3%</td>
<td>OS</td>
<td>p = 0.0001</td>
<td>[129]</td>
</tr>
<tr>
<td>circ-CCAC1</td>
<td>bile, serum</td>
<td>up</td>
<td>diagnostic marker, prognostic factor</td>
<td>0.857, 0.759</td>
<td>-</td>
<td>-</td>
<td>OS, DFS</td>
<td>p &lt; 0.001</td>
<td>[102]</td>
</tr>
</tbody>
</table>

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 (IncRNAs and circRNAs) regulate the occurrence and progression of CCA by mediating epigenetic modifications, acting as cRNA 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 miRNAs 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.

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Conflict of Interest
The authors declare no conflict of interest.

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