Review

The Role of microRNAs in Cholangiocarcinoma

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Abstract: Cholangiocarcinoma (CCA), an aggressive malignancy, is typically diagnosed at an advanced stage. It is associated with dismal 5-year postoperative survival rates, generating an urgent need for prognostic and diagnostic biomarkers. MicroRNAs (miRNAs) are a class of non-coding RNAs that are associated with cancer regulation, including modulation of cell cycle progression, apoptosis, metastasis, angiogenesis, autophagy, therapy resistance, and epithelial–mesenchymal transition. Several miRNAs have been found to be dysregulated in CCA and are associated with CCA-related risk factors. Accumulating studies have indicated that the expression of altered miRNAs could act as oncogenic or suppressor miRNAs in the development and progression of CCA and contribute to clinical diagnosis and prognosis prediction as potential biomarkers. Furthermore, miRNAs and their target genes also contribute to targeted therapy development and aid in the determination of drug resistance mechanisms. This review aims to summarize the roles of miRNAs in the pathogenesis of CCA, their potential use as biomarkers of diagnosis and prognosis, and their utilization as novel therapeutic targets in CCA.

Keywords: microRNA; cholangiocarcinoma; diagnostic biomarker; prognostic biomarker

1. Introduction

Cholangiocarcinoma (CCA) includes a diverse group of biliary epithelial malignancies that involve all points of the biliary tree. Depending on the anatomic location, CCAs are classified into three subtypes: intrahepatic (iCCA), perihilar (pCCA), and distal (dCCA) [1,2]. Among them, pCCA and dCCA are also referred to as "extrahepatic CCA" (eCCA). pCCA, the most common CCA, accounts for 50–60% of all CCAs, followed by dCCA, which accounts for 20–30% of all cases [1]. iCCA is the second most common primary liver cancer after hepatocellular carcinoma (HCC) and accounts for 10–15% of all primary hepatic malignancies [3]. Additionally, a rare type of primary liver cancer, mixed hepatocellular cholangiocarcinoma (HCC-CCA), accounts for <1% of all cases according to the World Health Organization (WHO) [4] (Figure 1).

CCAs are aggressive tumors that account for approximately 3% of all gastrointestinal cancers [5]. CCAs are usually asymptomatic in the early stages and are typically diagnosed at an advanced stage. Although surgery is a therapeutic strategy for patients with CCAs, the 5-year postoperative survival rate (7–20%) remains low because of the challenge of diagnosing patients at an early stage [1,6]. Therefore, developing advanced diagnostic techniques and exploring the mechanisms underlying CCA development and progression can be effective approaches to improve the outcomes for patients with CCA.

Risk factors

❖ Lithiasis
    Cholecystolithiasis
    Hepatolithiasis
    Choledocholithiasis
❖ Cholestatic liver diseases
    Primary sclerosing cholangitis
    Fibropolycystic liver diseases
❖ Parasitic infection
❖ Cirrhosis with any etiology
❖ Infections
    Hepatitis B, Hepatitis C
❖ Inflammatory disorders
    Inflammatory bowel disease
    Chronic pancreatitis
❖ Metabolic abnormalities
    Diabetes
    Non-alcoholic fatty liver disease
    Obesity
❖ Lifestyle
    Alcohol
    Tobacco

intrahepatic   10–20%
(10–15% of primary liver cancer)

perihilar   50–60%

distal   20–30%

extrahepatic
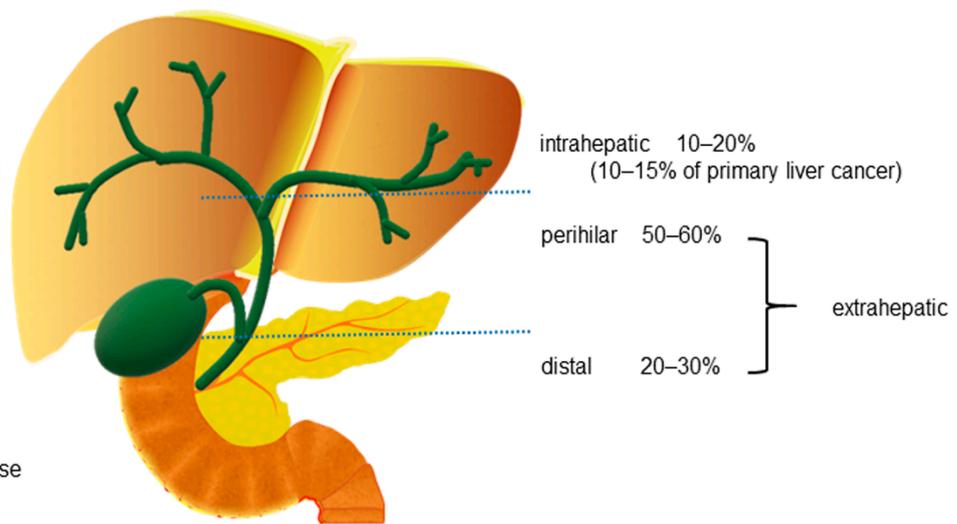
Figure 1. Anatomical classification of cholangiocarcinoma and its risk factors.

MicroRNAs (miRNAs) are small, non-coding RNAs with a 17–25 nucleotide length [7]. miRNA biogenesis is a multistep process that is categorized into: transcription, nuclear cropping, export to cytoplasm, and cytoplasmic dicing [8]. miRNA genes are transcribed as primary RNA (pri-miRNA) by RNA polymerase II (pol-II) and are processed by Drosha, a nuclear enzyme of the RNase III family, in the nucleus to release a hairpin-shaped precursor called "pre-miRNA". Pre-miRNA is recognized by Exportin 5/Ran-GTP transporter and is exported from the nucleus to the cytoplasm. The pre-miRNA is then cleaved by Dicer and the TAR RNA-binding protein to produce a miRNA duplex, which is then loaded onto the Argonaute (AGO) protein to assemble the RNA-induced silencing complex (RISC). One strand remains on the AGO protein to form the mature miRNA, while the other strand is degraded. The mature miRNA represses gene expression by interacting with the complementary sequences in the 3′-untranslated region of the target mRNAs [8–10] (Figure 2). Over 5000 miRNAs from diverse organisms are registered in online databases, such as the miRBase (www.mirbase.org, accessed on 9 May 2021). In humans, approximately one-third of the miRNAs are organized in clusters and contain two or more miRNAs with similar sequences [11], possibly leading to combinatorial diversity and synergy in the biological effects of the miRNAs. Furthermore, approximately 30% of the human genes are regulated by miRNAs via signaling pathways [12].

Cancer is a complex genetic disease associated with gene mutations and deregulation of the gene expression. During the last decade, many studies have focused on miRNAs and cancer and have highlighted the impact of miRNAs on gene expression. In this review, we have comprehensively discussed the association between miRNAs and CCA; we have also summarized the roles of miRNAs in the pathogenesis of CCA, their potential use as biomarkers of diagnosis or prognosis, and their possible use as novel therapeutic targets in CCA.
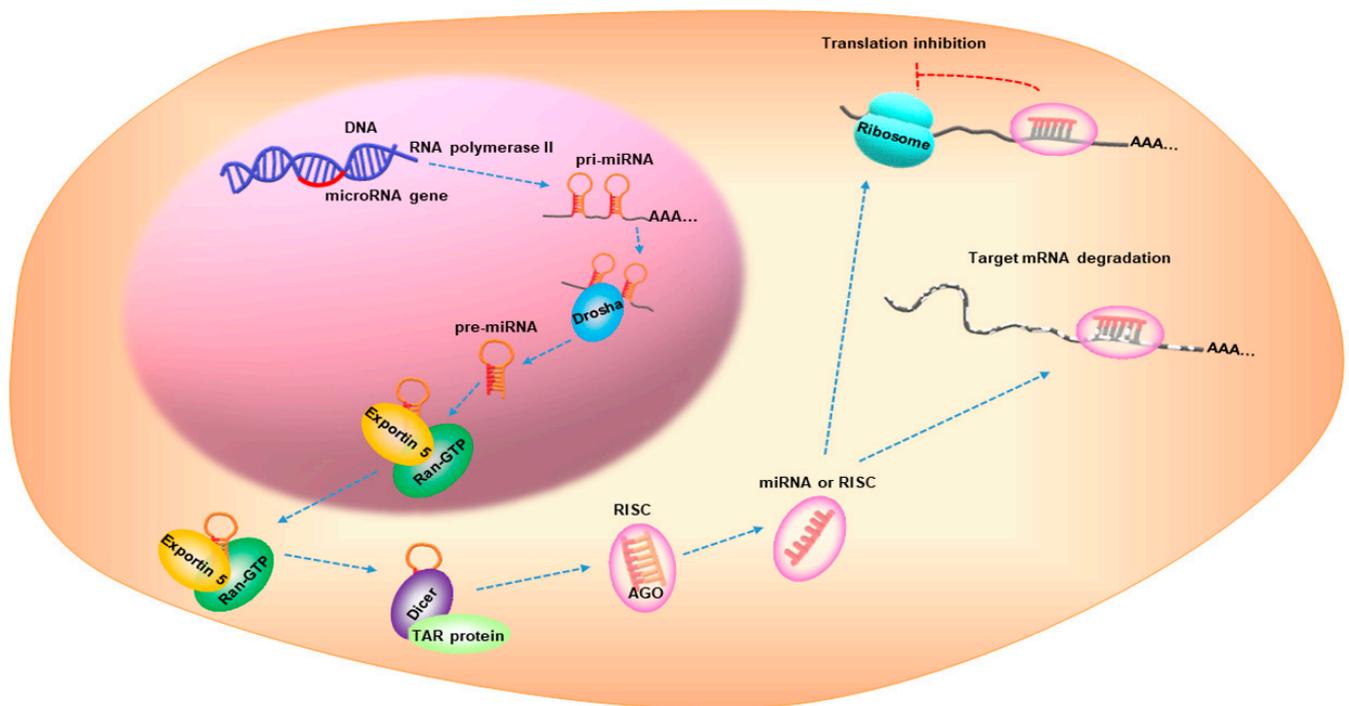
Figure 2. microRNA biogenesis. AGO, Argonaute; RISC, RNA-induced silencing complex.

2. Epidemiology

The mortality rates of iCCA have increased globally in recent years, with the highest rates reported from 2010 to 2014 (1.5–2.5/100,000 in men and 1.2–1.7/100,000 in women) based on the data of 32 selected countries from the WHO and Pan American Health Organization databases [13]. In addition, in Japan, the mortality rate associated with eCCA is 2.8/100,000 in men and 1.4/100,000 in women [13]. The data from the National Center for Health Statistics between 1999 and 2014 in the USA showed that CCA mortality was 36% higher in patients with age >25 years, and the mortality was lower in females than in males (risk ratio [RR] = 0.78, 95% confidence interval [CI] = 0.77–0.79) [14]. Differences in CCA incidence rates have been reported among different racial and ethnic groups, with the highest rates reported in Southeast Asia and the lowest in Australia [15]. A study in Western Europe indicated that the incidence rates of iCCA increased considerably between 1999 and 2009, especially in the population in the age group of 45–59 years [16]. In contrast, research from the USA has shown that the incidence of iCCA has remained stable from 1992 to 2007; whereas, the incidence of eCCA has been increasing considerably [17]. In Japan, from 1976 to 2013, a total of 14,287 cases of CCA have been identified, and iCCA was more likely to develop in younger patients. The prognosis of iCCA was poorer in comparison to that of eCCA; however, the prognosis of both iCCA and eCCA cases improved after 2006 [18].

3. Risk Factors

Although the occurrence and etiology of CCA have not been determined, there are several well-established risk factors associated with chronic biliary epithelium inflammation and cholestasis. These include lithiasis [19]; cholestatic liver diseases, such as primary sclerosing cholangitis (PSC) [20] and fibropolycystic liver diseases [21]; parasitic infections by *Opisthorchis viverrini*, *Clonorchis sinensis*, and *Schistosomiasis japonica* [19,22]; cirrhosis with any etiology [23]; infections, such as hepatitis [23,24]; inflammatory disorders, such as inflammatory bowel disease (IBD) [25] and chronic pancreatitis [25]; metabolic abnormalities [25,26]; and lifestyle [27,28] (Figure 1).

A Chinese study has indicated that biliary stone diseases, including choledocholithiasis (odds ratio [OR] = 2.704, 95% CI = 1.054–6.941), hepatolithiasis (OR = 3.278, 95% CI = 1.226–8.766), and cholecystolithiasis (OR = 4.499, 95% CI = 2.990–6.769) are risk factors for hilar CCA [19]. Approximately 7% of patients (325 cases) with hepatolithiasis developed CCA as per the data from a nationwide survey in Japan in 2006 [29].

PSC, a chronic cholestatic liver disease with an unclear etiology, is characterized by inflammation and fibrosis with multifocal biliary strictures. Additionally, PSC is closely associated with IBD, and about two-thirds of the patients in Northern Europe and the USA have PSC concurrently with IBD [30]. The incidence of CCA in patients with PSC is approximately 0.6–1.5% per year [31]. A genetic study of 186 patients with PSC-biliary tract cancer showed eCCA with high genomic alterations in *TP53* (35.5%), *KRAS* (28.0%), *CDKN2A* (14.5%), and *SMAD4* (11.3%), and even in underlying druggable mutation genes, such as *HER2/ERBB2* [32]. Moreover, a 10-year nationwide population-based study from the UK suggested that development of PSC increases the risk of CCAs (hazard ratio [HR] = 28.46, *p* < 0.001) in patients with PSC-IBD, and it also increases the risk of HCC (HR = 21.00, *p* < 0.001), gallbladder cancer (HR = 9.19, *p* < 0.001), pancreatic cancer (HR = 5.26, *p* < 0.001), and colorectal cancer (HR = 2.43, *p* < 0.001) [33].

Certain regions of Southeast Asia, such as North and Northeast Thailand, where *Opisthorchis viverrini* infestation is prevalent, show high CCA burden, with 19.3% and 15.7% of the population having the infection, and CCA incidence rates of 14.6/100,000 and 85/100,000, respectively [34,35]. Southeastern and Northeastern China, Korea, Northern Vietnam, and Eastern Russia show a prevalence of human *Clonorchis sinensis* infections [36]. Furthermore, the prevalence of liver fluke (OR = 10.088, 95% CI = 1.085–93.775) is reportedly higher in patients with hilar CCA than in healthy controls [19]. Additionally, there are reports of CCA associated with *Schistosomiasis japonica* infection [37].

Approximately 57% of global cirrhosis cases are induced by chronic infection with hepatitis B (HBV) and hepatitis C viruses (HCV) [38]. Several meta-analyses have indicated that HBV or HCV infection is associated with an increased risk of CCA, especially iCCA [39–41]. Cirrhosis, diabetes, and obesity are also risk factors for CCA [41]. A case-control study showed that cirrhosis is a major risk factor for iCCA; other risk factors include nonspecific cirrhosis (adjusted OR = 27.2), HCV infection (adjusted OR = 6.1), diabetes (adjusted OR = 2.0), and alcoholic liver disease (adjusted OR = 7.4) [23]. Furthermore, another study indicated that risk factors associated with iCCA and eCCA were nonspecific cirrhosis, chronic pancreatitis, diabetes, alcoholic liver disease, biliary cirrhosis, and cholelithiasis. Factors associated with iCCA include non-alcoholic fatty liver disease (NAFLD), obesity, and smoking [42].

4. The Role of miRNAs in CCA

In recent decades, several studies have focused on the role of miRNAs in cancers. miRNAs play a key role in cancer cell regulation, and are associated with the progression of the cell cycle, apoptosis, metastasis, angiogenesis, glycolysis/Warburg effect, autophagy, therapy resistance, and epithelial–mesenchymal transition (EMT).

4.1. miRNAs Associated with CCA Risk Factors

miRNAs play an important role in regulating physiological and pathophysiological functions. In gallstone disease, upregulated miR-210 reduces the expression of its target gene, *ATP11A*, in human gallbladder epithelial cells to regulate the ABC transporter pathway [43]. The pathological mechanism of hepatolithiasis is closely related to chronic inflammation and overexpression of mucin 5AC (MUC5AC). miR-130b inhibits the expression of specificity protein 1 (Sp1), which is followed by a decrease in the expression of MUC5AC [44]. In addition, a clinical control study indicated that the expression levels of miR-21 and miR-221 were upregulated in CCA associated with hepatolithiasis [45]. In PSC, increased melatonin reduces biliary hyperplasia and liver fibrosis by overexpressing arylalkylamine N-acetyltransferase (*AANAT*) in the pineal gland. Moreover, inhibition

of miR-200b reduces the expression of fibrotic and angiogenic genes, such as *Col1a1*, *Fn-1*, *Vegf-a/c*, *Vegfr-2/3*, *Angpt1/2*, and *Tie-1/2* [46]. In schistosomiasis, miR-21, miR-96, miR-351, miR-146a/b, and miR-27b promote hepatic fibrosis by regulating signaling pathways [47,48]. During liver cirrhosis progression, miR-378 plays a key role in promoting hepatic inflammation and fibrosis via the NF-κB-TNFα axis in non-alcoholic steatohepatitis [49]. Increased miR-30a expression downregulates extracellular matrix-related gene expression, such as that of *α-SMA*, *TIMP-1*, and collagen I, and prevents liver fibrogenesis by directly targeting the Beclin1-mediated autophagy [50]. Activation of hepatic stellate cells (HSCs) is a major step in the initiation and progression of hepatic fibrosis and overexpression of miR-214-3p suppresses the expression of suppressor-of-fused homolog (Sufu) to promote HSC activation and fibrosis development [51].

HBV infection induces a spectrum of liver diseases ranging from acute infection to chronic hepatitis, cirrhosis, and HCC [52]. Wang et al. have indicated that miR-98, miR-375, miR-335, miR-199a-5p, and miR-22 are involved in HBV infection [53]. The expression of miR-192-3p is negatively associated with increased levels of HBV DNA in the serum of patients with HBV. HBV induces autophagy to promote its replication by the miR-192-3p-XIAP axis via the NF-κB signaling [54]. Additionally, miR-224 [55] and miR-1231 [56] suppress HBV replication by inhibiting SIRT1-mediated autophagy and targeting the core mRNA, respectively. Other studies have reported that the miR-99 family promotes replication by enhancing autophagy through the mTOR/ULK1 signaling [57].

HCV infection is a global health problem that leads to chronic carriage in 70–80% of all cases and presents a high risk of cirrhosis and cancer [58]. miR-215 [59] promotes HCV replication by targeting *TRIM22* and miR-21-5p [60], and it enhances the HCV life cycle and steatosis induced by the viral core 3a protein and other promoters such as miR-122 [61]. Overexpression of miR-199a suppresses HCV genome replication [62], and miR-130a [63] inhibits HCV replication via an Atg5-dependent autophagy pathway.

Inflammation is associated with cancer origin and is based on an environment rich in inflammatory cells and factors, such as activated stroma and DNA-damage-promoting agents. Moreover, activation of the inflammation signaling pathway enhances cell proliferation [64]. In IBD, miR-301a is overexpressed in peripheral blood mononuclear cells and inflamed mucosa, which promotes mucosal inflammation by inducing IL-17A and TNF-α expression [65]. Increased miR-31 expression in colon tissues of patients with IBD reduces the inflammatory response by inhibiting the expression of IL-7R, IL-17RA, and signaling proteins (GP130). In addition, miR-31 promotes epithelial regeneration via the Wnt and Hippo signaling pathways [66]. In chronic pancreatitis, upregulated miR-15 and miR-16 expression can alleviate apoptosis and fibrosis by targeting both *BCL-2* and *SMAD5* [67].

Diabetes, a metabolic disease, is characterized by high blood sugar and insulin resistance, which are risk factors for HCC and CCA. Various miRNAs have been implicated in the regulation of diabetes [68]. For instance, miR-7 and miR-375 are essential for pancreatic β-cell differentiation and development; miR-184, miR-33, miR-187, miR-29a, and miR-30a are involved in insulin secretion; and miR-15b, miR-199a, miR-181a, and miR-143 are associated with insulin resistance [68]. miR-10b-5p regulates diabetes via the KLF11-KIT pathway [69], and exosomal-derived miR-690 improves insulin sensitivity [70]. NAFLD is caused by triglyceride accumulation, which increases the risk of various liver diseases, such as steatosis, non-alcoholic steatohepatitis, fibrosis, cirrhosis, and ultimately HCC [71]. miR-122 is involved in triglyceride and cholesterol metabolism by targeting the expression of *SREBP-1c*, *SREBP-2*, and *HMGCR*. miR-34a is involved in hepatic lipid metabolism, fatty acid β-oxidation, and apoptotic pathways, and miR-29 is associated with fibrosis [71]. Overexpression of miR-132 alters serum and hepatic lipid profiles, thereby inducing hepatic steatosis [72].

Alcohol consumption is closely associated with liver injury, especially alcoholic liver disease. A recent study indicated that alcohol decreases the expression of miR-148a in hepatocytes through FoxO1, thereby facilitating *TXNIP* overexpression and NLRP3 inflammasome activation-induced hepatocyte apoptosis [73]. miR-155 promotes alcohol-induced

steatohepatitis and fibrosis [74]. Tobacco smoke induces miR-25-3p maturation via m6A modification, which in turn promotes the development and progression of cancers [75,76] (Table 1).

Table 1. microRNAs (miRNAs) associated with cholangiocarcinoma risk factors.

Risk Factor	Related microRNA	Expression	Functions	References
Lithiasis				
Cholecystolithiasis	miR-210	upregulated	Targets *ATP11A* to regulate the ABC transporter pathway	[43]
Hepatolithiasis	miR-130b	downregulated	miR-130b-Sp1-MUC5AC signaling pathway	[44]
Choledocholithiasis	unknown	unknown	unknown	
Cholestatic liver diseases				
PSC	miR-200b	upregulated	Promotes biliary hyperplasia and liver fibrosis	[46]
FPLD	unknown	unknown	unknown	
Parasitic infection				
	miR-21 miR-96 miR-351 miR-146a/b miR-27b	unknown	Promotes fibrosis	[47,48]
Cirrhosis				
	miR-378	upregulated	NF-κB-TNFα axis	[49]
	miR-30a	downregulated	Targets Beclin1-mediated autophagy	[50]
	miR-214-3p	upregulated	Decreases Sufu and promotes HSC activation	[51]
Infections				
HBV	miR-192-3p	downregulated	Inhibits autophagy and suppresses HBV replication	[54]
	miR-224, miR-1231	unknown	Suppresses HBV replication	[55,56]
	miR-99 family	unknown	Promotes HBV replication	[57]
HCV	miR-215	unknown	Promotes HCV replication via targeting *TRIM22*	[59]
	miR-21-5p	upregulated	Promotes HCV replication	[60]
	miR-199a	unknown	Suppresses HCV replication	[62]
	miR-130a	unknown	Suppresses HCV replication	[63]
Inflammatory disorders				
IBD	miR-301a	upregulated	Promotes mucosal inflammation	[65]
	miR-31	upregulated	Reduces inflammatory response	[66]
Chronic pancreatitis	miR-15/16	downregulated	Alleviates apoptosis and fibrosis	[67]
Metabolic abnormalities				
Diabetes	miR-10b-5p	unknown	Regulates diabetes by KLF11-KIT pathway	[69]
	miR-690	unknown	Improves insulin sensitivity	[70]
NAFLD	miR-132	upregulated	Alters serum and hepatic lipid profiles	[72]
Lifestyle				
Alcohol	miR-148a	downregulated	Regulates hepatocyte apoptosis	[73]
Tobacco	miR-25-3p	unknown	Promotes the development and progression of cancers	[75,76]

Abbreviations: PSC, primary sclerosing cholangitis; FPLD, fibropolycystic liver diseases; HBV, hepatitis B virus; HCV, hepatitis C virus; IBD, inflammatory bowel disease; NAFLD, non-alcoholic fatty liver disease; HSC, hepatic stellate cell.

4.2. Dysregulation of miRNAs in CCA

The expression of miRNAs can be correlated with the cancer type and other clinical variables. As each miRNA has multiple target genes, it regulates the target gene expression in a complex manner. This suggests that miRNAs are involved in almost all aspects of cancer biology [7–9]. During CCA development, some miRNAs are upregulated, i.e.,

they act as "oncogenic miRNAs", whereas, other miRNAs are downregulated and act as "suppressors" (Table 2).

Table 2. Reported miRNAs acting as oncogenic or suppressor miRNAs in cholangiocarcinoma.

miRNA	Target Gene	Mechanism	References
Oncogenic miRNA			
miR-383	Interferon regulatory factor 1 (*IRF1*)	Proliferation, migration, and invasion	[77]
miR-221	Phosphatase and tensin homolog (*PTEN*)	Epithelial–mesenchymal transition (EMT)	[78]
miR-31	RAS p21 GTPase activating protein 1 (*RASA1*)	Proliferation and apoptosis	[79]
miR-361-5p	TNF receptor-associated factor 3 (*TRAF3*)	Apoptosis	[80]
miR-30a-5p	Suppressor of cytokine signaling 3 (*SOCS3*)	Proliferation and apoptosis	[81]
miR-21	15-hydroxyprostaglandin dehydrogenase (*15-PGDH/HPGD*)	Cell growth	[82]
miR-18a-5p	Fructose-bisphosphatase 1 (*FBP1*)	Proliferation, invasion, and apoptosis	[83]
miR-421	Farnesoid X receptor (*FXR*)	Proliferation and migration	[84]
miR-191	Ten-eleven translocation 1 (*TET1*)	Proliferation, invasion, and migration	[85]
miR-181c	N-myc downstream-regulated gene 2 (*NDRG2*)	Proliferation, chemoresistance, and metastasis	[86]
miR-193-3p	Transforming growth factor-β receptor type 3 (*TGFBR3*)	Proliferation, migration, and invasion	[87]
Suppressor miRNA			
miR-876	B-cell lymphoma-extra large (*BCL-XL*)	Proliferation and apoptosis	[88]
miR-451a	Activating transcription factor 2 (*ATF2*), ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 4 (*ST8SIA4*)	Migration, invasion, and proliferation	[89,90]
miR-34a	Notch receptor 1 (*NOTCH1*), Notch receptor 2 (*NOTCH2*), Jagged canonical Notch ligand 1 (*JAG1*)	Proliferation	[91]
miR-186	Twist family BHLH transcription factor 1 (*TWIST1*)	Proliferation, migration, invasion, and EMT	[92]
miR-15a	Plasminogen activator inhibitor type-2 (*PAI-2*)	Migration	[93]
miR-612	*BCL-2*	Proliferation, migration, and invasion	[94]
miR-424-5p	AMPK-related protein kinase 5 (*ARK5*)	Metastasis, invasion, migration, and EMT	[95]
miR-124-3p	Ubiquitin-like, containing PHD and RING finger domains 1 (*UHRF1*)	Proliferation and cell cycle arrest	[96]
miR-551b-3p	Cyclin D1 (*CCND1*)	Proliferation and apoptosis	[97]
miR-186-5p	Microchidia family CW-type zinc finger 2 (*MORC2*)	Cell growth and metastasis	[98]
miR-137	Wnt family member 2B (*WNT2B*)	Proliferation, migration, and invasion	[99]
miR-490-3p	*AKIRIN2*	Proliferation, migration, invasion, and angiogenesis	[100]
miR-329	Laminin subunit beta 3 (*LAMB3*)	Proliferation, migration, and invasion	[101]
miR-122-5p	Aldolase, Fructose-Bisphosphate A (*ALDOA*)	Proliferation, invasion, apoptosis, and EMT	[102,103]
miR-410	X-linked inhibitor of apoptosis protein (*XIAP*)	Apoptosis	[104]
miR-22	Histone deacetylase 6 (*HDAC6*)	Proliferation and migration	[105]
miR-433	Histone deacetylase 6 (*HDAC6*)	Proliferation and migration	[105]
miR-144	Platelet-activating factor acetylhydrolase isoform 1b (*LIS1*), ST8SIA4	Proliferation and invasion	[90,106]
miR-590-3p	Sphingosine-1-phosphate receptor 1 (*SIP1*)	Invasion, migration, and EMT	[107]
miR-101	Vascular endothelial growth factor (*VEGF*), Cyclooxygenase-2 (*COX-2*), E2F transcription factor 8 (*E2F8*)	Angiogenesis and proliferation	[108,109]
miR-26b-5p	S100 calcium-binding protein A7 (*S100A7*)	Proliferation, migration, and invasion	[110]
miR-1182	*NUAK1* (also known as *ARK5*)	Invasion, migration, and autophagy	[111]
let-7a	*NUAK1*	Invasion, migration, and autophagy	[111]
miR-874	Cyclin E1 (*CCNE1*)	Invasion and EMT	[112]
miR-885-5p	Insulin like growth factor 2 mRNA binding protein 1 (*IGF2BP1*), Polypeptide N-acetylgalactosaminyltransferase 3 (*GALNT3*)	Proliferation and metastasis	[113]
miR-622	*C-MYC*	Proliferation, migration, and invasion	[114]
miR-320	Neuropilin-1 (*NRP-1*)	Proliferation, invasion, and angiogenesis	[115]
miR-200b/c	SUZ12 Polycomb repressive complex 2 subunit (*SUZ12*)/Rho associated coiled-coil containing protein kinase 2 (*ROCK2*)	Tumorigenesis and metastasis	[116]

4.2.1. Oncogenic miRNAs in CCA

Upregulated miRNAs in CCA tissues or cells are known as oncogenic miRNAs. Oncogenic miRNAs, such as miR-21 [82] and miR-191 [85], promote CCA cell proliferation, metastasis, angiogenesis, autophagy, and EMT. miR-21 is a well-known oncogenic miRNA that is overexpressed in various cancers such as HCC [117] and breast cancer [118]. In CCA, miR-21 promotes cancer cell proliferation by inhibiting the expression of programmed cell death 4 (PDCD4) and tissue inhibitor of metalloproteinase 3 (TIMP3) [119] by targeting *15-PGDH/HPGD* [82]. Moreover, miR-21 promotes iCCA cell growth by targeting *PTPN14* and *PTEN* as its functional targets [120]. A meta-analysis showed that miR-21 expression is associated with poor prognostic outcomes in patients with CCA (HR = 1.88, 95% CI = 1.41–2.51), and it could predict shorter overall survival in patients with CCA [121]. In addition, a recent study indicated that plasma miR-21 and miR-122 levels were significantly higher in patients with iCCA than in controls (healthy, benign liver lesions, other malignant liver tumors), and the increased plasma miR-21 level was correlated with a larger tumor size [122]. Meanwhile, a novel three-marker model comprising plasma miR-21, miR-122, and CA19-9 has been used to increase the diagnostic sensitivity (operating characteristic curve (AUC) = 0.853; 95% CI = 0.824–0.879; sensitivity = 73.0%; specificity = 87.4%) [122].

A meta-analysis suggested that elevated expression levels of miR-21, miR-26a, miR-29a, miR-181c, miR-191, miR-192, miR-200c, and miR-221 are associated with poor survival in patients with CCA; whereas, decreased expression of miR-34a, miR-106a, miR-203, and miR-373 are associated with poor prognosis [121]. Li et al. [85] demonstrated that miR-191 was significantly overexpressed in iCCA tissues, and it promoted cell proliferation, invasion, and migration by targeting *TET1* both in vitro and in vivo. Additionally, miR-191 is an independent risk factor for poor prognosis in patients with iCCA (HR = 3.742, 95% CI = 2.080–6.733). Further, miR-181c promotes CCA cell proliferation, chemoresistance, and metastasis by targeting *NDRG2*, and overexpression of miR-181c is associated with poor overall survival in patients with CCA [86].

4.2.2. Tumor Suppressor miRNAs in CCA

The association of tumor suppressor miRNAs, such as miR-34a [91], miR-122 [102], miR-22 [105], and miR-101 [108], is well established in many cancer types. miR-34a expression is often decreased in cancers. It is transcriptionally controlled by *TP53* and regulated by multiple p53-independent mechanisms. miR-34a, a candidate tumor suppressor miRNA, regulates multiple targets, such as *MYC*, *MET*, *CDK4/6*, *NOTCH1*, *NOTCH2*, *BCL2*, and *CD44*, all of which have been implicated in tumorigenesis and cancer progression [123].

miR-122 is a tumor suppressor in various cancer types, including CCA. It inhibits proliferation and metastasis by targeting *ALDOA* [103] and chloride intracellular channel 1 (*CLIC1*) [124]. Moreover, miR-122 is a regulator in various liver diseases, including HCC [125].

miR-22 plays an important role in many cancer types and has been shown to modulate some oncogenic processes, such as proliferation, apoptosis, angiogenesis, immune response, and metastasis [105]. In an in vitro study on CCA, overexpression of miR-433 and miR-22 was demonstrated to suppress cell proliferation and cellular migration by targeting *HDAC6* [105]. In addition, a survival analysis indicated that *DEPDC1*, *FUT4*, *MDK*, *PACS1*, *PIWIL4*, miR-22, miR-551b, and cg27362525 and cg26597242 CpGs can be used as prognostic markers of CCA. Although miR-22 is a known tumor suppressor, its high expression is correlated with poor survival of patients with CCA [126].

miR-101 has been shown to be a tumor suppressor in certain cancers. For instance, miR-101 overexpression considerably inhibits CCA cell proliferation and angiogenesis by targeting vascular endothelial growth factor (*VEGF*), cyclooxygenase-2 (*COX-2*) [108], and *E2F8* [109]. *EZH2* is also a target gene of miR-101 that regulates CCA cell proliferation [127] (Figure 3).
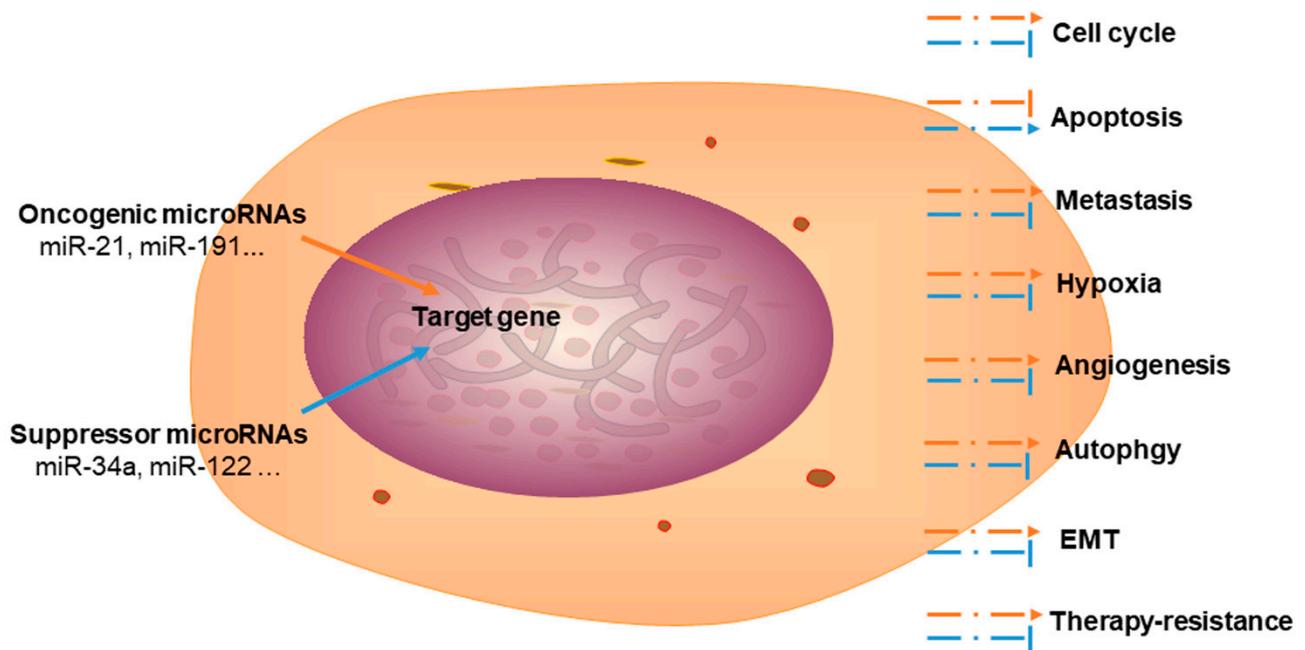
Figure 3. The functions of oncogenic and suppressor microRNAs in cholangiocarcinoma. EMT, epithelial–mesenchymal transition.

4.3. miRNAs as Biomarkers for CCA

CCA is commonly diagnosed through a combination of clinical details, biochemical information, radiological imaging, and histology. Histology is usually considered as the "golden standard" to confirm a diagnosis. Radiological imaging techniques, such as ultrasound, computed tomography, magnetic resonance imaging/magnetic resonance cholangiopancreatography, and positron emission tomography, have been used to diagnose CCA subtypes.

Carbohydrate antigen 19-9 (CA19-9), a non-specific tumor biomarker, helps in the diagnosis of CCA; however, this biomarker lacks sensitivity and specificity, particularly in the early stages of CCA [2]. Most patients with early-stage CCA are usually asymptomatic, and are thus, diagnosed at an advanced stage. Tumor biomarkers have been widely used to improve early-stage diagnosis and prognosis prediction. In recent years, many studies have examined miRNAs as potential biomarkers for the early diagnosis and prognosis prediction in case of CCA (Table 3).

Numerous studies have reported the use of miRNAs as biomarkers in CCA. miR-21, an oncogenic miRNA, [82], is a potential biomarker for both diagnosis [45] and prognosis [121]. In addition, miR-21 and miR-221 aid in the diagnosis of CCA associated with hepatolithiasis with an increased accuracy (AUC = 0.911) and a sensitivity and specificity of 77.42% and 97.50%, respectively [45]. Serum miR-26a is upregulated in patients with CCA, and its expression levels are associated with the tumor-node-metastasis stage. miR-26a can be clinically beneficial as a diagnostic biomarker for CCA, as it has previously shown an AUC = 0.899, 84.8% sensitivity and 81.8% specificity [128]. Moreover, high expression of serum miR-26a is also an independent predictor of poor overall (HR = 3.461, 95% CI = 1.331–5.364) and progression-free survival in patients with CCA (HR = 4.226, 95% CI = 1.415–10.321) [128]. The expression of serum miR-150-5p is downregulated in patients with CCA, demonstrating a 91.43% sensitivity and 80% specificity for diagnosis. When combined with CA19-9 expression, the sensitivity increases to 93.33% and specificity to 96.88% [129]. To discriminate dCCA from pancreatic ductal adenocarcinoma and improve diagnosis, a recent study revealed that miR-16 is downregulated and miR-877 is upregulated in patients with dCCA. The combination of the two miRNAs—miR-16 and miR-877—has shown an AUC = 0.90, 79% sensitivity, and 90% specificity for diagnosis,

and an AUC = 0.88, 71% sensitivity, and 90% specificity for discriminating dCCA from pancreatic ductal adenocarcinoma [138].

Table 3. The expression levels of potential miRNA-based biomarkers in cholangiocarcinoma.

miRNA	Expression	Detectable Location	Tumor Type (Background)	Biomarker Category	References
miR-21, miR-221	Upregulated	Plasma	Hepatolithiasis-CCA	Diagnosis/Prognosis	[45,121]
miR-26a	Upregulated	Serum	CCA	Diagnosis/Prognosis	[128]
miR-150-5p	Downregulated	Serum	CCA	Diagnosis	[129]
miR-29a	Upregulated	Tissue	CCA	Prognosis	[130]
miR-192	Upregulated	Serum	CCA	Prognosis	[131]
miR-151-3p	Upregulated	Tissue	Resected CCA	Prognosis	[132]
miR-126	Downregulated	Tissue	Resected CCA	Prognosis	[132]
miR-106a	Downregulated	Serum	CCA	Prognosis	[133]
miR-146a	Upregulated	Tissue	iCCA	Prognosis	[134]
miR-31	Upregulated	Tissue	CCA	Prognosis	[135]
miR-203	Downregulated	Tissue	CCA	Prognosis	[136]
miR-191	Upregulated	Tissue	iCCA	Prognosis	[85]
miR-195	Downregulated	Serum	CCA	Prognosis	[137]
miR-16	Downregulated	Plasma	dCCA	Diagnosis	[138]
miR-877	Upregulated	Plasma	dCCA	Diagnosis	[138]

Abbreviations: CCA, cholangiocarcinoma; iCCA, intrahepatic cholangiocarcinoma; dCCA distal cholangiocarcinoma.

4.4. miRNAs in CCA Therapy Resistance

For certain patients with advanced-stage CCA, for whom surgical resection or liver transplantation is not feasible, a combination of gemcitabine and cisplatin (GemCis) is used as a first-line systemic therapy irrespective of the CCA subtype [2]. Furthermore, a combination of 5-fluorouracil, leucovorin, oxaliplatin, and irinotecan (FOLFIRINOX) [139] or a combination of gemcitabine, cisplatin, and nab-paclitaxel [140] are associated with improved patient survival. In recent years, an increasing number of studies have focused on genetic pathophysiology, and carcinogenic and mutant genes have been identified in many malignancies, including CCA, which in turn promote the development of targeted therapy for CCA. However, drug resistance remains a key issue during such treatment. Numerous studies have reported the role of miRNAs in CCA therapy resistance. For instance, miR-210 sustains HIF-1α activity by targeting *HIF-3α*, which reduces the sensitivity to gemcitabine in CCA cells [141]. miR-130a-3p increases gemcitabine resistance by targeting *PPARG* [142]. Overexpression of miR-199a-3p enhances cisplatin sensitivity by inhibiting the mTOR signaling pathway in CCA cells [143]. miR-106b overexpression increases 5-fluorouracil sensitivity by targeting *ZBTB7A*, and miR-106b downregulation is related to poor prognosis in patients with CCA [144] (Table 4).

Table 4. miRNAs in cholangiocarcinoma therapy resistance.

Drug	miRNA	Resistance	Target	Reference
Gemcitabine	miR-210	increase	*HIF-3α*	[141]
	miR-130a-3p	increase	*PPARG*	[142]
Cisplatin	miR-199a-3p	decrease	mTOR signaling pathway	[143]
5-fluorouracil	miR-106b	decrease	*ZBTB7A*	[144]

4.5. miRNA-Based Therapies

As previously mentioned, miRNAs play a key role as oncogenic or suppressor miRNAs in the development and progression of CCA, and they act as regulators of drug resistance. miRNA-based therapy is a novel targeted therapy for cancers that is based on the concept of overexpressing suppressor miRNAs or decreasing the expression of

oncogenic miRNAs using miRNA sponges. Although numerous in vitro and xenograft model-based studies [82,84,91] that have used miRNA mimics, inhibitors, and plasmids have indicated the functions of miRNAs, miRNA-based therapy has not yet achieved clinical translation. Because of the heterogeneity of the tumor cells and the complexity of miRNA functions, the same miRNA can have opposite functions in different malignancies. Furthermore, one miRNA can target different genes to regulate the protein expression. Additionally, the complexity of the in vivo environment can also affect the therapy. Finally, determining the approach and accuracy of drug delivery, evaluating efficacious doses, and predicting off-target effects are aspects that need to be considered. Currently, these act as obstacles that prevent the use of miRNA-based therapy in clinical applications [145,146].

5. Conclusions

Studying the biological functions of miRNAs, especially their roles in malignancies, is a growing field of research. miRNAs have been reported to play key roles in tumorigenesis, cell proliferation, apoptosis, metastasis, angiogenesis, EMT, and autophagy. In this review, we summarized the functional roles and related target genes of oncogenic and suppressor miRNAs implicated in the development and progression of CCA. Dysregulated expression of miRNAs in CCA has been utilized as potential biomarker for clinical diagnosis and prognosis prediction. Furthermore, miRNAs and their target genes contribute toward the development of targeted therapy and determination of drug resistance mechanisms. Although accumulating evidence has demonstrated that miRNAs may be potential biological targets for CCA treatment in preclinical studies, they are not yet suitable for clinical practice because of tumor cell heterogeneity as well as the complexity of the in vivo environment and miRNA functions. Meanwhile, determining drug delivery approaches, evaluating efficacious doses, and predicting off-target effects remain obstacles that prevent the clinical application of miRNA-based therapy. Further research and analyses of miRNAs will provide more evidence and novel insights into the pathogenesis of CCA and will prove to be useful for the diagnosis, therapy, and prognosis prediction in patients with CCA.

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