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MicroRNAs and Benign Biliary Tract Diseases

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Abstract

Cholangiocytes, the epithelial cells lining the biliary tree, represent only a small portion of the total liver cell population (3–5%), but they are responsible for the secretion of up to 40% of total daily bile volume. In addition, cholangiocytes are the target of a diverse group of liver diseases affecting the biliary tract, the cholangiopathies; for most of these conditions, the pathological mechanisms are unclear. MicroRNAs (miRNAs) are small, noncoding RNAs that posttranscriptionally regulate gene expression. Thus, it is not surprising that altered miRNA profiles underlie the dysregulation of several proteins involved in the pathobiology of the cholangiopathies, as well as showing promise as diagnostic and prognostic tools. Here the authors review recent work relevant to the role of miRNAs in the etiopathogenesis of several of the cholangiopathies (i.e., fibroinflammatory cholangiopathies and polycystic liver diseases), discuss their value as prognostic and diagnostic tools, and provide suggestions for further research.

Keywords

cholangiocytes; microRNAs; cholangiopathies; cholangiocarcinoma; polycystic liver disease; primary biliary cirrhosis; primary sclerosing cholangitis; biliary atresia

Cholangiocytes, the epithelial cells lining the biliary tree, represent only a small portion of the total liver cell population, but are responsible for the secretion of up to 40% of the total daily bile volume. Cholangiocytes are the target of a diverse group of diseases now known as the cholangiopathies.¹ The cholangiopathies can be subdivided into malignant, immune-mediated, drug- or toxin-induced, infectious, genetic, ischemic, and idiopathic. Except for cholangiocarcinoma (CCA), a malignant tumor of the biliary tree, the common outcome for most cholangiopathies is the destruction of the bile ducts (i.e., ductopenia), with features of cholestasis, inflammation, and ultimately fibrosis and cirrhosis. In general, the pathological mechanisms underlying the cholangiopathies remain unclear and new insights into their etiopathogenesis are needed.

MicroRNAs (miRNAs) are short noncoding RNAs (20–22 nucleotides) that have a critical role in the posttranscriptional regulation of gene expression. MiRNAs are transcribed as primary miRNAs that are recognized and processed inside the nucleus by the RNase III endonuclease, Drosha. The resultant precursor miRNA (60–90 nucleotides) is transported from the nucleus to the cell cytoplasm predominantly by a mechanism involving Exportin-5, in a RAN-GTP dependent manner. This precursor is further processed in the cytoplasm by the RNase III endonuclease, Dicer, resulting in a RNA duplex molecule of 20–23 nucleotides in length. To exert its regulatory effects, the miRNA duplex is loaded into the miRNA-associated RNA-induced silencing complex (miRISC) and separated into a functional guide strand and passenger strand. The guide strand or mature miRNA directs the RISC complex to the target mRNA by base complementarity, mainly between its 5' seed region and the 3'-UTR region of the target messenger. The end result of this interaction is either the transcriptional suppression or the degradation of the target mRNA.^{2,3} Liver specific *dicer1* knockout illustrates the regulatory role of miRNA in liver function. Even though the hepatic function is preserved in the absence of mature miRNAs, disruption of *dicer1* affects proper liver zonation and promotes hepatocarcinogenesis. Even more, the knockout mice showed significant ductular proliferation and inflammation suggesting the potential role of miRNAs in the development of biliary tract diseases.^{4–6}

Therefore, miRNAs are regulatory molecules that directly and precisely modulate gene expression, and it is not surprising that altered miRNA profiles underlie the dysregulation of several proteins involved in the pathobiology of the cholangiopathies including polycystic liver diseases, fibroinflammatory cholangiopathies, and CCA.

The Cholangiopathies and miRNAs

Polycystic Liver Disease

Polycystic liver disease (PLD) is a group of genetic disorders characterized by the presence of multiple cysts derived from cholangiocytes. Polycystic liver disease can be inherited as an isolated entity (i.e., autosomal dominant polycystic liver disease [ADPLD]), but most frequently is associated with autosomal dominant (AD-) or autosomal recessive (AR-) polycystic kidney disease (PKD).^{7–9} Formation of hepatic cysts is initiated by mutations in disease-related genes: (1) *SEC63* and *PRKCSH* (ADPLD), (2) *PKD1* and *PKD2* (ADPKD), and (3) *PKHD1* (ARPKD). Once formed, cysts continue to grow involving many intracellular signaling pathways.^{7–10} Recent evidence suggests that cystic cholangiocytes and renal epithelial cells are characterized by global changes in miRNA patterns suggesting a novel regulatory mechanism of cyst progression.^{11–14} Several studies showed that miRNAs contribute to cystogenesis by regulating the dosage of PLD-related genes.¹⁵ Experimental manipulations with two miRNA families (miR-17–92 and miR-200) that target *Pkd1* and *Pkd2* genes result in cyst development in both liver and kidney.^{16,17} The role of miRNAs in the regulation of *PKHD1*, *PRKSCH*, and *SEC63* has yet to be demonstrated. However, by in silico analysis, we detected that miR-1, -17, -20, -23, -31, -106, -130, -150, -194, -218, and -342 are predicted to target the *PKHD1*, *PRKSCH*, and *SEC63* transcripts. All of these miRNAs are aberrantly expressed in cystic cholangiocytes.^{13,14} In addition, the aforementioned miRNAs are predicted to bind to

mRNAs of proteins involved in cell-cycle progression, cAMP and calcium signaling, cell proliferation, MAPK/ERK pathway, fluid secretion, and cell-matrix interactions (see below) further emphasizing the emerging role of miRNAs in the regulation of network of molecules involved in cystogenesis.^{9,13,18}

Fibroinflammatory Cholangiopathies

Several cholangiopathies are characterized by chronic inflammation, cholestasis, and biliary fibrosis. Two examples, primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC), follow a course that generally progresses to cirrhosis, portal hypertension, and liver failure. Biliary atresia (BA), unlike PBC and PSC, is a disorder exclusively diagnosed in the neonatal period and is the leading indication for pediatric liver transplantation worldwide.¹⁹

Primary Biliary Cirrhosis and Primary Sclerosing Cholangitis—The autoimmune nature of PBC is fairly well established and supported by antimitochondrial antibodies (AMAs) and autoreactive T-cells; yet the specific cellular mechanisms that result in the initiation and progress of PBC still remain unclear. A recent miRNA microarray identified 35 differentially expressed miRNAs (11 upregulated and 24 down-regulated) in PBC compared with normal tissue.²⁰ Furthermore, a bioinformatics approach demonstrated that the predicted upregulated genes (i.e., predicted targets of downregulated miRNAs) clustered into the biological processes of inflammatory response, calcium ion homeostasis, and negative regulation of hormone secretion. Further investigations are needed to validate altered target gene expression and identify cell types involved.

There is currently no effective pharmacotherapy for PSC, which exhibits a median liver transplantation- (LT-) free survival of 12 years.^{21,22} A feared complication of this disease is CCA, which occurs in approximately 10% of patients within 10 years of diagnosis.^{23,24} The diagnostic potential of miRNAs to monitor disease progression in PSC patients is discussed below.

Biliary Atresia—Biliary atresia progresses to fibro-obstruction of the extrahepatic bile ducts.^{25,26} Early diagnosis of BA is essential for good outcomes. Following diagnosis, the Kasai procedure (hepatoportoenterostomy) should be performed promptly to restore bile flow.²⁶ Moreover, patients must be carefully monitored as nearly half will gradually develop chronic liver disease and require liver transplantation. In an animal model of BA (i.e., the Rhesus rotavirus- (RRV-) BALB/c model), multiple miRNAs exhibited altered expression, including upregulated miR-29a and miR-29b1.²⁷

Similarly, a miRNA expression array using RNA isolated from extrahepatic bile ducts (EHBDs) of RRV-BALB/c mice revealed a similar overall expression pattern of miRNA.²⁸ However, miR-29b, but not miR-29a was elevated in the EHBDs. Despite the discrepancy, which may be due to the tissue source of RNAs, the results support a possible functional role of miR-29 family members in the etiopathogenesis of biliary atresia. Intriguingly, decreased miR-29 expression has been implicated in rodent models of fibrosis including carbon tetrachloride- treated and bile duct-ligated mice,²⁹ which correlated with decreased miR-29 expression in livers from patients with advanced liver fibrosis. Decreased miR-29 in hepatic stellate cells in the hepatic fibrosis models was mediated by TGF β and NF κ B and associated

with increased expression of extracellular matrix molecules. These results suggest that individual miRNAs may have different functional roles depending on the cell type where they are expressed. Whether manipulation of miR-29 in the RRV model of BA modifies disease course has yet to be determined.

MiRNAs in Animal Models of Cholestasis

A recent study has demonstrated that serum levels of several miRNAs are altered following hepatocellular injury, cholestasis, and steatosis in rats.³⁰

These studies reinforced that miRNAs make ideal potential candidate biomarkers due to high tissue specificity and stability in sera. Moreover, the expression profile of plasma miRNAs differed depending on the insult, suggesting that miRNAs could eventually be used as specific and sensitive biomarkers for several types of liver injury. The two cholestatic models assessed were a-naphthyl isothiocyanate (ANIT) treatment and bile duct ligation (BDL) representing both intrahepatic and extrahepatic cholestatic models. None of the upregulated miRNAs were specific to the models of cholestasis, whereas two miRNAs, miR-190 and miR-743b, were specifically downregulated in both of these models. In a functional analysis of miRNAs in cholestasis using the BDL model, it was determined that miR-125b and Let-7a were decreased in cholangiocytes, in a secretin-dependent manner.³¹ The translation of these promising findings to the human disease remains to be determined.

Cholangiocarcinoma

Cholangiocarcinoma is a lethal malignancy with limited therapeutic options; the cancer is derived from cholangiocytes, and its incidence and mortality have increased in recent decades.³² There are several seminal studies profiling miRNAs expression in CCA^{33–35}; the dysregulation of miRNAs, as in other tumors, has been linked to the repression of tumor suppressor genes (oncomiRs) and the upregulation of oncogenes (tumor suppressor miRs). The dysregulation of miRNAs in CCA is discussed in detail in the article, “Micro-RNAs in Cholangiocarcinoma” in this issue of *Seminars*.

MicroRNAs in the Pathobiology of the Cholangiopathies

Despite the heterogeneity among the cholangiopathies, they share several fundamental pathogenetic mechanisms, including altered proliferation, secretion, epithelial–mesenchymal transition, and apoptosis among others, even though the contribution of these processes may vary between the different cholangiopathies. We next present recent discoveries regarding the role of miRNAs in the regulation of the common basic cellular mechanisms altered in several cholangiopathies (Fig. 1 and Table 1).

Cholangiocyte Proliferation

During the past decades, several miRNAs have been described to modulate cholangiocyte proliferation, like let-7a, miR-21, -26a, -34a, -421, and -494.^{62–64} Some recent reports reveal that miRNAs likely promote cell growth via regulation of receptor tyrosine kinase and MAPK signaling, particularly in CCA. One of these miRNAs, miR-376c, was found to be significantly downregulated in CCA cell lines compared with a normal bile duct epithelial

cell line.⁵⁵ Utilizing proteomics analysis of control and pre-miR-376c transfected HuCCT1 cells and further in silico analysis, GRB2, an essential adaptor for epidermal growth factor receptor signaling and Ras/MAPK activation, was identified and validated as the potential mediator of miR-376c downregulation effect in CCA cell phenotype, even though the significance of these observations needs to be validated in vivo using human CCA samples.

Additionally, the expression level of miR-138 in CCA is reduced compared with adjacent nontumor tissues, and the lower expression of this miRNA correlates with the malignant progression of the diseases.⁴⁸ The mRNA of “Ras-like” superfamily member, RhoC, was found as a direct target of miR-138, suggesting that this miRNA function is a repressor of RhoC expression. Furthermore, in vitro manipulations of miR-138 regulate cell proliferation, likely through RhoC and its downstream effector ERK, but no evidence has been provided beyond the direct binding of miR-138 to the 3'UTR region of RhoC messenger.

As mentioned before, in a cholestatic model, very recent findings demonstrate that bile duct ligation induces the down-regulation of let-7i and miR-125b. The downregulation of these miRNAs is mediated by the increased hormone secretin and is secretin receptor-dependent. Moreover, let-7i and miR-125b directly target the 3'-UTR region of the messengers for nerve growth factor (NGF) and vascular endothelial growth factor (VEGF), respectively, important mediators of cholangiocyte proliferation.³¹ Hence, secretin-mediated miRNA suppression appears to be an essential component of the molecular network activated in cholestasis-induced hepatobiliary reparative mechanisms. Whether this signaling has direct relevance to human disease remains to be determined.

Cholangiocyte Cell-Cycle Regulation

Cell-cycle dysregulation is a common feature of several cholangiopathies. For example, we found by miRNA micro-array that the majority of miRNAs are downregulated in cystic cholangiocytes from the PCK (polycystic kidney) rat (an animal model of ARPKD) compared with normal rats, and experimentally proved that manipulations with one of the most downregulated miRNAs, miR-15a, affect hepatic cystogenesis in vitro.¹² This miRNA promotes cell-cycle progression and cyst expansion through increased expression of cell-division cycle 25A (Cdc25A), an important cell-cycle regulator.¹² Using a similar approach, substantial changes in miRNA profiles were described in renal epithelia of PKD/mhm (cy/+) rats, a model of ADPKD.¹⁴ Importantly, despite the differences between these two animal models and tissue analyzed, several miRNAs (miR-21, -31, -125, and 196a) were identically downregulated in both renal and hepatic epithelia, suggesting either shared regulation by disease-associated signaling or a common role for these miRNAs in cell-cycle regulation of renal and hepatic epithelia.

Cholangiocyte Secretion

Bile is modified by cholangiocytes via absorptive and secretory processes. Bile flows through the intrahepatic bile ducts lumen, where cholangiocytes reabsorb solutes, mainly bile salts and glucose, and secrete ions, such as Cl^- and HCO_3^- , leading to water secretion through aquaporin water channels. A key biological feature of PBC is the decreased biliary

expression of anion exchanger 2 (AE2/SLC4A2), which in turn induces a reduced secretin-stimulated bicarbonate secretion.^{65,66} AE2 is a $\text{Cl}^-/\text{HCO}_3^-$ exchanger mainly located in the apical domain of cholangiocytes. This exchanger participates in the regulation of intracellular pH and the alkalization of bile secretion.^{67,68} Two different groups reported that miR-506 is upregulated in PBC cholangiocytes.^{20,58} Interestingly, in silico analysis identified the AE2 transcript as a target of miR-506, and in vitro functional analyses demonstrated that this miRNA targets the 3'UTR of AE2, decreases AE2 protein expression, and modulates bicarbonate secretion. Moreover, isolated human PBC cholangiocytes exhibit increased miR-506 expression and diminished AE2 activity, and the transfection of these cells with a miR-506 antagomir rescues AE2 activity.⁵⁸ These data suggest an etiopathogenetic role of miR-506 in the downregulation of AE2 and the repression of bicarbonate secretion into bile in PBC.

Ductal Plate Formation

Development of hepatic cysts is linked to ductal plate malformation—embryological arrest of ductal plate development.^{7,8,69–73} During development, cholangiocyte precursor cells form a single layered sheath of cells, the ductal plate; each ductal plate originates usually a couple of bile ducts per portal tract. But only the minority of these cells is involved in the generation of the bile ducts, and the rest of the ductal plate precursors regress by apoptosis or may generate peri-portal hepatocytes and adult liver progenitor cells.⁶⁹

MiRNAs have recently emerged as critical regulators of liver development.⁷⁴ Comprehensive gene and miRNA profiling of human liver reveals miRNAs enriched in embryonic liver (i.e., miR-106a, miR-18a, miR17–92, and miR-574–3p) and in adult liver (i.e., let-7a and c, miR-23b, and miR-22). Moreover, the expression patterns of these miRNAs negatively correlate with levels of their predicted target genes.⁷⁴ Though miRNA profiling was performed on whole liver tissue, it is important to emphasize that the aforementioned miRNAs are significantly downregulated in cystic cholangiocytes of animal model of PLD, the PCK rats,¹³ and in patients with ADPKD (unpublished observation). Arising evidence shows that miR-30a plays an important role in ductal plate formation. Indeed, abnormal bile duct development was detected in zebrafish due to specific depletion of miR-30a.⁷⁵ This observation is of particular interest because miR-30a is decreased in human cystic cholangiocytes.^{13,14} Moreover, multiple transcriptional factors known to regulate ductal plate remodeling are predicted targets of miRNAs that are negatively expressed in cystic cholangiocytes.¹³ Collectively, these studies suggest that miRNAs are involved in the maintenance of bile duct integrity, and aberrant miRNA expression contributes to cyst formation and cyst growth.

Cholangiocyte Apoptosis

Apoptosis is the process of programmed cell death characterized by a series of morphologic changes that plays an important role in the development and tissue homeostasis of the biliary tract. Disturbances in apoptotic pathways may lead to uncontrolled cell proliferation or abnormal cell death. The tight control of cholangiocyte apoptosis by miRNAs has been suggested in several reports. For example, a role for miR-21 has been postulated in

cholangiocarcinogenesis by suppressing the expression of programmed cell death 4 (PDCD4).³⁷

Furthermore, miR-31 was involved in the pathogenesis of cholangiocarcinoma by directly inhibiting the protein expression of RAS p21 GTPase-activating protein 1 (RASA1).

Downregulation of RASA1 by miR-31 inhibited cellular apoptosis, partially due to upregulation of RAS-mitogen-activated protein kinase (MARK) signaling pathway activity in CCA.⁴⁵ Another miRNA overexpressed in CCA, miR-25, shows an anti-apoptotic role by protecting cholangiocytes from TNF-related apoptosis-inducing ligand- (TRAIL-) induced apoptosis by decreasing the expression of death receptor 4.⁴¹ Conversely, decreased expression of miR-29 in CCA promotes the overexpression of cellular Mcl-1 protein levels, a key antiapoptotic protein, and helps cells evade cell death.⁷⁶ Likewise, miR-320 and miR-204 are downregulated in CCA, which could fully explain the overexpression of their targets Mcl-1 or Bcl-2, respectively, raising possible mechanisms by which malignant cholangiocyte cells resist apoptosis.³⁵

Cholangiocyte Pathogen Recognition and Inflammatory Response

Cholangiocytes form a simple epithelial layer separating the bile duct lumen from the liver parenchyma. Although under normal conditions microorganisms are undetected in bile by conventional culture methods, cholangiocytes are periodically exposed to potentially pathogenic organisms or products derived from these microbes.^{77–80} Indeed, the liver is a major organ for lipopolysaccharide (LPS) clearance, and though LPS undergoes metabolism in Kupffer cells and hepatocytes, it is excreted in bile where it remains bioactive.^{81,82} Moreover, in cholestatic liver diseases, cholangiocytes are exposed to elevated levels of LPS.⁸² Cholangiocytes express a variety of pathogen pattern recognition receptors (PRRs), such as the Toll-like receptors (TLRs) and nucleotide binding and oligomerization domain-like receptors (NLRs), which recognize pathogens or pathogen-associated molecular patterns (PAMPs). Activation of these receptors in cholangiocytes has been demonstrated in response to bacterial, viral, and parasitic infections.^{77,83} NF- κ B (nuclear factor kappa beta) signal pathway activation via TLRs/NLRs is a common response of epithelial cells following detection of microbial products and NF- κ B induces the expression of proinflammatory and antimicrobial molecules.^{77,84} We focus here on the role of miRNAs in the cholangiocyte response to microbial pathogens.

The role of miRNAs in NF- κ B-dependent innate immune responses was first demonstrated in human THP-1 monocytes.⁸⁵ In response to a variety of TLR agonists, miR-146a/b was induced in an NF- κ B dependent manner. It was further demonstrated that miR-146 targets both TNF receptor-associated factor 6 (TRAF6) and IL-1 receptor-associated kinase 1 (IRAK1) genes. In this instance, miRNAs function to repress the innate immune response through the targeting of central mediators of TLR-dependent NF- κ B signaling. It is now recognized that multiple miRNAs are induced in response to TLR activation in a variety of cell types, including cholangiocytes. Human cholangiocytes express numerous endogenous miRNAs.^{33,59} Activation of cultured cholangiocyte TLR4 via LPS treatment or in vitro infection with the parasitic protozoan, *Cryptosporidium parvum* activates NF- κ B in a Myd88-dependent manner⁸⁴ and promotes the expression of several miRNAs including

miR-125b, miR-21, miR-23b-27b-24-1, and miR-30b.⁸⁶ The precise function of these miRNAs is not known, yet molecular inhibition of these resulted in increased parasite burden in vitro, suggesting a potential role in antimicrobial defense. In contrast, the expression of several miRNAs is also repressed following NF- κ B activation. Using a cell culture model of the cholangiocyte response to microbial pathogens, it was demonstrated that following LPS treatment or *C. parvum* infection, transcription of the let-7i gene is suppressed through NF- κ B p50 subunit and C/EBP β interaction with the Let-7i promoter.⁸⁷ Decreased let-7i expression was associated with an upregulation of TLR4 in *C. parvum* infected cells, increased NF- κ B signaling, and diminished parasite numbers.⁵⁹ Moreover, functional manipulation of NF- κ B responsive miRNAs (e.g., let-7i and mir-27b) influenced *C. parvum* infection burden in vitro.^{59,86} Further investigations of let-7 miRNAs, including mir-98, revealed miRNA regulation of the cytokine-inducible Src homology 2-containing protein (CIS) and suppressor of cytokine signaling 4 (SOCS4), both members of the SOCS family of proteins.^{60,61} Again, using a human cultured cholangiocyte model of response to microbial insult (LPS or *C. parvum*), it was demonstrated, in contrast to the classical negative feedback regulation of cytokine signaling by SOCS family members, that decreased let-7 and miR-98 expression promoted the upregulation of CIS and enhanced NF- κ B signaling through CIS-dependent I κ B α degradation. The data raise the possibility that miRNA-mediated posttranscriptional pathways may contribute to host-cell responses to microbial infection by increasing inflammatory signaling in response to pathogens⁵⁹⁻⁶¹ or attenuation of the inflammatory response.⁸⁵ It is likely that miRNAs fine-tune the TLR/NF- κ B signaling cascade through regulation of both positive- and negative-feedback loops to ensure an appropriate epithelial response to microbial insult. As demonstrated here, miRNA regulation of cellular processes involves subtle manipulations of signaling circuitry. How the miRNAs function to fine-tune the inflammatory response in the cholangiopathies or in biliary repair processes needs to be explored further in both cell-culture models of infection and repair and in animal models of disease.

Diagnostic, Prognostic, and Therapeutic Potential of miRNAs in the Cholangiopathies

Early detection of CCA remains challenging; few are detected while still amenable to curative surgical intervention. Hence, more effective screening tools are desired as are reliable prognostic markers. Cholangiocarcinoma miRNA expression profiles have been investigated for utility as prognostic tools.⁸⁸⁻⁹⁰ In a retrospective study, utilizing paraffin-embedded samples, the expression of two miRNAs (overexpression of miR-151-3p or the downregulation of miR-126) showed promise as potential prognostic markers for CCA. Additionally, and similar to what has been shown in colon cancer and pancreatic cancer, miR-21 over-expression was associated with poor survival of CCA patients.⁸⁸ The prognostic value of these miRNAs should be explored further as they may be utilized for patient stratification for clinical trials and in identifying patients that may benefit from adjuvant therapies.⁹⁰

Cholangiocarcinoma is a dreaded outcome of PSC: Early detection is critical to patient outcome.^{91,92} The current surveillance modality is serum carbohydrate antigen 19-9 (CA

19–9) coupled with imaging techniques (i.e., magnetic resonance imaging with magnetic resonance cholangiopancreatography). Establishing an accurate diagnosis of cancer using CA 19–9 in the clinical setting is often difficult—frequently resulting in delayed diagnosis and compromising therapeutic options and patient outcome.^{91,92} Improved diagnostic accuracy for CCA is needed and miRNAs obtained from bile have shown promise.^{93,94} Initially, small RNA library sequencing and reverse transcription polymerase chain reaction-based array identified an increase in biliary miRNAs in CCA patients.⁹³ One of these, miR-9, demonstrated the most reliable diagnostic specificity and sensitivity for biliary tract cancer. However, a recent analysis demonstrated that miRNAs derived from extracellular vesicles (i.e., exosomes) exhibit greater quality and quantity.⁹⁴ Using stringent RNA isolation methods from a patient cohort of 46 CCA and 50 control patients (including 13 with PSC but no CCA), it was determined that the combinatorial use of five miRNAs (miR-16, -486–3p, -484, -1274b, and -191) had the best predictive value. Ultimately, patient bile miRNAs and serum CA-19–9 may allow more reliable, earlier detection of CCA, particularly for those patients at high risk of CCA, such as PSC patients.

As with CCA, the development of a specific, feasible, noninvasive diagnostic marker is still needed for BA. Recently, serum miRNAs were assessed for their utility as a diagnostic tool for BA.⁹⁵ A miRNA array was performed on sera from BA patients and age- and sex-matched indeterminate cholestasis controls. The miR-200b/429 cluster of miRNAs could differentiate between BA and controls with sensitivity and specificity values ranging from 71% to 92%, comparable to serum γ -glutamyl transpeptidase. Though not improving on the current diagnostic methods, this study serves as a proof-of-principle and ultimately may complement the current serum biochemical parameters for early detection, intervention, and improved patient outcome. The current diagnostic approaches for PBC, PSC, and polycystic liver disease are accurate and efficient; hence, the utility of miRNA analysis for diagnosis is less clear. The utility of miRNA analyses in these cholangiopathies lies in their potential to serve as prognostic tools to detect more aggressive forms of the disease or those that will favorably respond to therapy; this is an area lacking published data.

The cholangiopathies represent a class of diseases with unique obstacles for effective, novel therapeutic strategies including drug delivery and enigmatic etiopathogenesis. While remaining an intensive area of research, curative therapies remain drastic (i.e., transplant) with modest, incremental advances in pharmacological therapies. The manipulation of miRNAs is a promising approach. An attractive feature of miRNA therapy is the potential to target multiple mediators of pathways that concertedly regulate cellular processes. Ideally, chemically modified miRNA mimics would restore aberrant expression of a diminished miRNA (replacement therapy), whereas antisense-modified oligonucleotides would inhibit an upregulated miRNA (miRNA inhibition therapy), restore cellular homeostasis, and delay progression of disease. RNA-based therapies are now feasible with the use of stable, chemically modified oligonucleotides^{96,97}; however, the critical hurdle of targeted delivery of these oligonucleotides remains an issue. Nonetheless, the delivery of molecules to the liver and “first-pass” metabolism may ultimately prove to be an advantage for RNA-based therapies. Many advances in oligonucleotide delivery have been realized since the discovery of RNA interference (RNAi),⁹⁸ yet whether any of these delivery methods can be utilized to specifically target the cells contributing to the cholangiopathies remains to be investigated.

Conclusions

In summary, miRNAs are promising as diagnostic, prognostic, and therapeutic tools, but their true value in these areas requires further study, including the validation of previous findings in larger cohorts of patients and the standardization of miRNA isolation, purification, and amplification protocols, as well as established normalization controls.

Furthermore, although there are many studies showing alterations in miRNAs in the cholangiopathies, the mechanisms underlying the modifications in these miRNAs remain obscure. More specifically, alterations of miRNA expression could be happening at gene expression levels, at the degradation level, and/or by alterations in the miRNA biogenesis machinery or nuclear transport. Understanding the mechanisms of miRNA dysregulation in the cholangiopathies is an area of research that definitely needs attention and may uncover novel therapeutic targets for biliary tract diseases.

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Abbreviations

AD	autosomal dominant
ADPKD	autosomal dominant polycystic kidney disease
ADPLD	autosomal dominant polycystic liver disease
AE2	anion exchanger 2
AMAs	antimitochondrial antibodies
ANIT	a-naphthyl isothiocyanate
AR	autosomal recessive
ARPKD	autosomal recessive polycystic kidney disease
BA	biliary atresia
BDL	bile duct ligation
CCA	cholangiocarcinoma
Cdc25A	cell division cycle 25A
CIS	cytokine-inducible src homology 2-containing protein
EHBD	extrahepatic bile ducts
HCV	hepatitis C virus
IRAK1	IL-1 receptor-associated kinase 1
LPS	lipopolysaccharide

LT	liver transplantation
MARK	mitogen-activated protein kinase
miRISC	miRNA-associated RNA-induced silencing complex
miR also miRNA	microRNA
mRNA	messenger RNA
NF-κB	nuclear factor kappa beta
NGF	nerve growth factor
PBC	primary biliary cirrhosis
PKD	polycystic kidney disease
PLD	polycystic liver disease
PRRs	pattern recognition receptors
PSC	primary sclerosing cholangitis
RISC	RNA-induced silencing complex
RNAs	ribonucleic acids
RNase	ribonuclease
RRV	Rhesus rotavirus
SOCS4	suppressor of cytokine signaling 4
TLRs	Toll-like receptors
TRAF6	TNF receptor-associated factor 6
TRAIL	TNF-related apoptosis-inducing ligand
UTR	untranslated region
VEGF	vascular endothelial growth factor

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Fig. 1. MicroRNAs in the cholangiopathies. Dysregulation of specific microRNAs induce abnormal expression of a myriad of targets that contribute to the different pathophysiological processes underlying the development of the cholangiopathies. BA, biliary atresia; CCA, cholangiocarcinoma; PBC, primary biliary cirrhosis; PLD, polycystic liver disease; PSC, primary sclerosing cholangitis.

Table 1

MicroRNAs dysregulation with confirmed targets in biliary tract diseases

miRNA	Expression	Function	Target	Reference
Cholangiocarcinoma				
let-7a	Up	Cell survival	NF2	36
miR-21	Up	Apoptosis, proliferation, invasion, metastasis	MBD2, 15-PGDH/HPGD, PTEN, PDCD4, TIMP3	33,37–40
miR-25	Up	Apoptosis	DR4	41
miR-26a	Up	Proliferation, colony formation, tumor growth	GSK-3b	42
miR-29b	Down	Gemcitabine sensitivity, apoptosis	PIK3R1, MMP-2, Mcl1	43,44
miR-31	Up	Proliferation, apoptosis	RASA1	45
miR-34a	Down	Cell-cycle, proliferation	c-Myc	46
miR-124	Down	Migration, invasion	SMYD3	47
miR-138	Down	Proliferation, cell cycle, migration, invasion	RhoC	48
miR-141	Up	Proliferation, circadian rhythm	CLOCK	33
miR-148a	Down	Proliferation	DNMT-1	49
miR-200b	Up	Chemoresistance	PTPN12	33
miR-200b/c	Down	Migration, invasion	rho-kinase2, SUZ12	50
miR-204	Down	EMT, migration, invasion, apoptosis	Slug, Bcl-2	35,51
miR-210	Up	proliferation	Mnt	46
miR-214	Down	EMT, metastasis	Twist	52
miR-320	Down	Apoptosis	Mcl-1	35
miR-370	Down	Proliferation	MAP3K8	53
miR-373	Down	Epigenetics	MBD2	54
miR-376c	Down	Migration, proliferation	GRB2	55
miR-421	Up	Proliferation, migration, colony formation	FXR	56
miR-494	Down	Proliferation, cell cycle	CDK6	57
Polycystic liver diseases				
miR-15a	Down	Proliferation, cell cycle	Cdc25a	12
miR-17	Down	Cyst development	Pkd2	11
Fibro-obliterative cholangiopathies				
Primary biliary cirrhosis				
miR-506	Up	Secretion	AE2	58
Biliary atresia				
miR-29	Up	Epigenetics, cell survival, inflammation	Dnmt3a, Dnmt3b, Igf1, Igf2bp2	27
Cholestatic/pathogen induced				
Let-7a, -7i	Down	Pathogen recognition, inflammation, proliferation	TLR4, NGF	31,59
miR-98	Down	Pathogen recognition, inflammation	CIS, SOCS4	60,61
miR-125b	Down	Proliferation	VEGFA	31

Abbreviations: EMT, epithelial–mesenchymal transition.

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