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miRNA IN PATHOBIOLOGY (JUSTIN MOTT, SECTION EDITOR)

MicroRNAs in Cholangiopathies

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Abstract Cholangiocytes, the cells lining bile ducts, comprise a small fraction of the total cellular component of the liver, yet perform the essential role of bile modification and transport of biliary and blood constituents. Cholangiopathies are a diverse group of biliary disorders with the cholangiocyte as the target cell; the etiopathogenesis of most cholangiopathies remains obscure. MicroRNAs are small non-coding RNAs that post-transcriptionally regulate gene expression. These small RNAs may not only be involved in the etiopathogenesis of disease, but are also showing promise as diagnostic and prognostic tools. In this brief review, we summarize recent work regarding the role of microRNAs in the etiopathogenesis of several cholangiopathies, and discuss their utility as prognostic and diagnostic tools.

Keywords Cholangiocytes · MicroRNAs, cholangiopathies · Cholangiocarcinoma · Polycystic liver disease · Primary biliary cirrhosis · Primary sclerosing cholangitis · Biliary atresia

Introduction

Cholangiocytes, the epithelial cells lining bile ducts, comprise a small proportion of the total cellular component of the liver, but perform the essential role of bile modification and transport of biliary and blood constituents. Cholangiocytes are the target of a diverse group of disorders collectively referred to as cholangiopathies [1]. Cholangiopathies can be broadly categorized into malignant, immune-mediated, drug- or toxin-induced, infectious, genetic, ischemic, and idiopathic. A common end result for most cholangiopathies is cholestasis, inflammation, fibrosis, and ultimately, the destruction of the bile ducts, yet the etiopathogenesis of most cholangiopathies remains poorly defined. The purpose of this brief review is to present current knowledge and recent discoveries regarding the role of microRNAs in the etiopathogenesis of several cholangiopathies, as well as the diagnostic, prognostic, and therapeutic potential of these small non-coding RNAs in this important group of diseases.

MicroRNAs are one class of small non-coding RNAs that have a demonstrated role in post-transcriptional gene expression. These RNA molecules are transcribed as primary microRNAs that are recognized and processed by the RNase III endonuclease, Drosha. The resultant 60–90 nucleotide precursor microRNA is shuttled from the nucleus in a RAN-GTP/exportin-5-dependent manner, where further cytoplasmic processing by the RNase III endonuclease, Dicer, results in a RNA duplex molecule ~20–23 nucleotides in length. The microRNA duplex is loaded into the microRNA-associated RNA-induced silencing complex (miRISC) and separated into a functional guide strand and passenger strand. Through complementary base-pairing, the guide strand (mature microRNA) directs the RISC complex to target mRNA for transcriptional suppression or mRNA degradation [2, 3]. Hence, microRNAs are transacting gene regulatory molecules that directly and precisely regulate gene expression and cellular function. It is not surprising; therefore, that altered microRNA expression affects gene expression of several targets underlying the pathobiology of different diseases. Furthermore, differentially expressed microRNAs in diseased tissues or circulating microRNAs in blood or

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Table 1 MicroRNAs, functions, and target genes associated with cholangiopathies

miRNA	Function	Target	References
Cholangiocarcinoma			
Upregulated			
Let-7a	Cell survival	NF2	[75]
miR-21	Apoptosis, proliferation, invasion, metastasis	MBD2, 15-PGDH/HPGD, PTEN, PDCD4, TIMP3	[12, 17, 18, 76, 77]
miR-25	Apoptosis	DR4	[78]
miR-26a	Proliferation, colony formation, tumor growth	GSK-3b	[79]
miR-31	Proliferation, apoptosis	RASA1	[15]
miR-141	Proliferation, circadian rhythm	CLOCK	[12]
miR-200b	Chemoresistance	PTPN12	[12]
miR-210	Proliferation	Mnt	[80]
miR-421	Proliferation, migration, colony formation	FXR	[81]
Downregulated			
miR-29b	Gemcitabine sensitivity, apoptosis	PIK3R1, MMP-2, Mcl1	[87, 11••]
miR-34a	Cell cycle, proliferation	c-Myc	[80]
miR-124	Migration, invasion	SMYD3	[16]
miR-138	Proliferation, cell cycle, migration, invasion	RhoC	[14]
miR-148a	Proliferation	DNMT-1	[82]
miR-200b/c	Migration, invasion	Rho-kinase2, SUZ12	[9]
miR-204	EMT, migration, invasion, apoptosis	Slug, Bcl-2	[7, 83]
miR-214	EMT, metastasis	Twist	[8]
miR-320	Apoptosis	Mcl-1	[83]
miR-370	Proliferation	MAP3K8	[84]
miR-373	Epigenetics	MBD2	[85]
miR-376c	Migration	GRB2	[13]
miR-494	Proliferation, cell cycle	CDK6	[86]
Polycystic liver diseases			
Downregulated			
miR-15a	Proliferation, cell cycle	Cdc25a	[23]
miR-17	Cyst development	Pkd2	[22, 29, 30]
Fibro-obliterative cholangiopathies			
PBC upregulated			
miR-506	Secretion	AE2	[49•]
Biliary atresia upregulated			
miR-29	Epigenetics, cell survival, inflammation	Dnmt3a, Dnmt3b, Igf1, Igf2bp2	[60•]

bile have potential as diagnostic and/or prognostic tools. In the following brief review, we discuss recent discoveries regarding microRNAs with respect to cholangiocarcinoma (CCA), polycystic liver diseases, primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), and biliary atresia (BA).

Cholangiocarcinoma

CCA is a malignancy thought to be derived from cholangiocytes whose incidence and mortality have increased in recent decades [4]. As in other malignancies, microRNA dysregulation in CCA has been associated with the repression of tumor suppressor genes and the upregulation of oncogenes, affecting a myriad of cellular processes and phenotypes including proliferation, apoptosis and stress resistance, migration, invasion, and epithelial–mesenchymal transition (EMT). Indeed, multiple microRNAs are dysregulated in CCA [5, 6] and several have validated molecular targets (Table 1). Much of the recent work has focused on examining molecular targets and pathophysiological outcomes of altered microRNA expression. In the following paragraphs, we summarize the most recent

reports describing the contribution of microRNAs to the pathobiology of CCA.

Two recent reports have described the functional relevance of diminished microRNA expression in migration and loss of the epithelial phenotype through upregulated EMT-associated transcription factor expression. For example, miR-204 is decreased, as determined by qPCR, in CCA compared to normal adjacent tissue [7]. In vitro, overexpression of miR-204 inhibits migration and invasion of CCA, induces expression of E-cadherin, and reduces the mesenchymal marker, vimentin, likely due to post-transcriptional suppression of Slug, a transcription factor that represses E-cadherin expression. Another microRNA potentially involved in EMT, miR-214, is downregulated in CCA compared to normal tissue; further data stratification has shown that this microRNA is remarkably downregulated in metastatic compared to non-metastatic CCA, and in vitro depletion of miR-214 induces cell migration through direct suppression of the EMT-associated transcription factor Twist [8].

A critical yet poorly understood feature of CCA is the highly aggressive nature and resistance to standard chemotherapy. Utilizing microRNA microarray and validation by RT-qPCR, the miR-200 family members (miR-200a/b/c/429) were found to be downregulated in perihilar and distal biliary CCA compared to corresponding normal ductal tissue [9]. Both in vitro and in vivo models demonstrate that miR-200b and -c regulate migration and invasion, tumor initiating capacity, 5-FU chemoresistance, and expression of the stem cell marker CD133. Furthermore, these microRNAs target rho-kinase 2 (ROCK2), a downstream mediator of cytoskeleton remodeling, Rho GTPase, and the polycomb repressive complex 2 subunit, SUZ12, a known mediator of breast cancer cell stemness [10]. To directly address microRNAs and CCA chemoresistance, the microRNA expression profile of a gemcitabine resistant cell line (HuH28) was recently compared to a sensitive cell line (HuCCT1) [11••]. Bioinformatics analysis and subsequent in vitro experiments revealed three downregulated microRNAs, miR-29b, miR-205, and miR-221, and one upregulated microRNA, miR-125a-p, associated with gemcitabine resistance. Potential targets of these microRNAs include PI3KRI and MMP-2, but direct suppression of these targets remains to be confirmed. Altogether, these new findings confirm and expand the previously shown role of miRNAs in response to chemotherapy [12].

Several recent reports reveal that microRNAs likely contribute to CCA survival, growth, and metastasis via regulation of receptor tyrosine kinase and MAPK signaling. One of these microRNAs, mir-376c, is downregulated in CCA cell lines compared to a normal bile duct epithelial cell line [13]. Proteomic analysis and molecular approaches identify GRB2, an essential adaptor for EGFR signaling and Ras/MAPK activation, as a target and potential mediator of the reduced miR-376c cellular phenotype. Additionally, the 'Ras-like' superfamily member, RhoC, is targeted by a microRNA which is reduced in CCA. The reduced expression of miR-138 in CCA correlates with malignant progression of the disease, and in vitro manipulation of miR-138 regulates cell proliferation, G1/S transition, migration, and invasion, likely through direct targeting of RhoC [14]. Another microRNA potentially associated with EGFR signaling and Ras/MAPK activation, miR-31, is upregulated in CCA, targets the negative regulator of Ras, RAS p21 GTPase activating protein 1 (RASA1), and induces increased proliferation and decreased apoptosis via activation of RAS/MAPK signaling [15].

In chronic hepatitis C-related intrahepatic cholangiocarcinoma, miR-124, is downregulated. Overexpression of miR-124 decreased cell migration and invasion [16], likely through the identified target, SMYD3, a histone methyltransferase. Indeed, experimental suppression of SMYD3 represses c-Myc and MMP9 and consequently cell migration and invasion. While dysregulation of miR-21 in CCA was previously described [5], a recent manuscript provides new insight into the functional role for this microRNA in CCA [17]. In *O. viverrini*-associated CCA, miR-21 is overexpressed and mediates 15-PGDH repression, a physiologic antagonist of COX2/PGE2 signaling. Indeed, miR-21-dependent inhibition of 15-PGDH induces PGE2 accumulation. Interestingly, miR-21 expression is induced by PGE2 signaling, suggesting a positive feed-forward loop. Additionally, miR-21 overexpression correlates with decreased expression of the tumor suppressor PDCD4, cell growth and migration in vitro, and shorter survival and lymph node metastasis in *O. viverrini*-associated CCA [18].

When examining intrahepatic tumors, microRNA expression is usually compared to adjacent normal liver tissue; in many cases, the cellular source of this control tissue is not well defined. A standardized approach, including microRNA isolation techniques, and laser capture microdissection of normal adjacent cholangiocytes as control tissue may enhance accurate detection of specific microRNAs altered in CCA compared to normal tissue. Nonetheless, as demonstrated here, the expression of multiple microRNAs is altered in CCA; the challenge remains to determine which if any of these microRNAs drive the malignant phenotype in a given tumor, and whether molecular manipulation can change the course of disease. Furthermore, whether microRNAs can be used as markers of clinical chemosensitivity needs to be explored further.

Polycystic Liver Diseases

Polycystic liver disease refers to a group of inherited cholangiopathies resulting from mutation of specific disease-related genes whose products typically localize to primary cilia. Polycystic liver disease seldomly exists as an isolated entity (i.e., autosomal dominant polycystic liver disease, ADPLD), but occurs as an extra-renal manifestation of autosomal dominant (AD-) or autosomal recessive (AR-) polycystic kidney disease (PKD) [19–21]. Several major pathobiological events contribute to hepatic cystogenesis including: (i) mutations in disease-causative genes—*PRKCSH* and *SEC63* (ADPLD-related), *PKD1* and *PKD2* (ADPKD-related), and *PKHD1* (ARPKD-related); (ii) defective remodeling of the ductal plate; and iii) aberrant signaling and cellular function. An increasing number of studies suggest that cystic cholangiocytes and renal epithelial cells are characterized by global changes in microRNA expression patterns which may also contribute to the pathobiology of cystogenesis [22–25].

We found, by microRNA microarray, that the vast majority of microRNAs are downregulated in cystic compared to normal cholangiocytes in an animal model of ARPKD, the PCK rat [23]. In cultured normal rat cholangiocytes, experimental suppression of one of the highly suppressed microRNAs, miR-15a, promotes cell cycle progression and cyst expansion through increased expression of cell division cycle 25A (Cdc25A), an important cell-cycle regulator [23]. Substantial changes in microRNA profiles are also observed in renal epithelia of PKD/mhm (cy/+) rats, a model of ADPKD [25]. Importantly, despite the differences between these two animal models, several microRNAs (miR-21, -31, -125, and 196a) are downregulated in both renal and hepatic (i.e., biliary) epithelia, suggesting either shared regulation by disease-associated signaling or a common role for these microRNAs in cystogenesis of renal and hepatic epithelia.

Target prediction algorithms reveal that the known genes involved in cytogenesis are likely under control of multiple microRNAs; some of these microRNA–mRNA target interactions have been evaluated experimentally [26–28]. Indeed, microRNAs contribute to cystogenesis via regulation of polycystic liver disease-related genes, *Pkd1* and *Pkd2*. Differentially expressed in both renal epithelia of $Pkd1^{-/-}$ mice and in cystic cholangiocytes of PCK rats, miR-17 is predicted to target *PKD2* mRNA, [23–25, 29] and recent experimental evidence demonstrates that *Pkd2* is regulated by miR-17 [22, 29, 30]. Moreover, transgenic mice expressing artificial microRNAs to target *Pkd1* developed PKD [31, 32]. These results suggest that abnormally expressed PKD1 and PKD2 as a result of microRNA regulation might contribute to cyst development in both liver and kidney. To date, no experimental data exist regarding regulation of *PKHD1, PRKSCH,* and *SEC63* by microRNAs; however, miR-1, -17, -20, -31, -106, -130, -194, and -342 are predicted to target the *PKHD1, PRKSCH, and SEC63* transcripts. All of these microRNAs are aberrantly expressed in cystic cholangiocytes [24] and all potentially target disease-associated transcripts, further emphasizing the unexplored potential of post-transcriptional gene regulation in hepatic cystogenesis.

A major event associated with hepatic cystogenesis, as mentioned above, is embryological arrest of ductal plate development, i.e., ductal plate malformation [19, 20, 27, 33–36]. Ductal plate malformation is controlled by a network of signaling pathways (i.e., TGFβ1, WNT, and FGF) and transcription factors (e.g., hepatocyte nuclear factors [HNF] 1β, 4 and 6, homeobox factor [Hhex], CCAAT/enhancer binding protein α [C/EBPα]), many of which are aberrantly expressed in cystic cholangiocytes [27, 33, 37–41]. New findings suggest that microRNAs should be considered as regulators of ductal plate remodeling. Indeed, in zebrafish larvae, specific depletion of miR-30a, a microRNA depleted in human cystic cholangiocytes and renal epithelia [24, 25] results in defective bile duct morphogenesis [42]. Whether depletion of ductal plate malformation-associated microRNAs in mammalian systems promotes cystogenesis has yet to be explored; however, several of the known mediators of ductal plate malformation are predicted targets of microRNAs aberrantly expressed in cystic cholangiocytes [24].

Despite interesting observations, the expression, regulation, and role of microRNAs in polycystic liver and kidney diseases are still in an early stage. A comprehensive, integrative genomics approach using both mRNA and microRNA microarrays of the same samples might provide insight into interactions between microRNAs and their targets in cystic cholangiocytes. Such parallel profiling might also help to identify novel mediators of hepatic and/or renal cystogenesis and reveal novel therapeutic targets.

Fibro-Inflammatory Cholangiopathies

PBC and PSC are rare but important, chronic, cholestatic liver diseases. Both are characterized by chronic inflammation of the bile ducts, cholestasis, and biliary fibrosis and follow a course that generally progresses to cirrhosis, portal hypertension, and liver failure. BA is also a progressive, fibro-inflammatory, cholestatic liver disease, but unlike PBC and PSC, is a disorder exclusively diagnosed in the neonatal period. BA is the leading indication for pediatric liver transplantation (LT) worldwide [43]. A more comprehensive understanding of the etiology and pathogenesis of these diseases is necessary for the development

of non-invasive predictive and prognostic biomarkers as well as targeted therapies that improve outcomes.

Primary Biliary Cirrhosis

PBC is characterized by immune destruction of small intrahepatic cholangiocytes. While the autoimmune nature of disease is established and supported by the highly specific anti-mitochondrial antibodies (AMAs) and autoreactive T-cells, the etiology of PBC remains unknown. Recent analysis of microRNA expression in diseased livers, isolated cells, and patient sera has provided insight into both the molecular pathogenesis of disease and the utility of microRNAs as biomarkers of disease.

A microRNA microarray approach was recently utilized to gain preliminary insight into the potential role of microRNAs in the etiopathogenesis of PBC. Thirty-five microRNAs were differentially expressed (11 upregulated, and 24 downregulated) in PBC compared to normal tissue, a subset of which were validated by real-time PCR (RT-PCR) [44•]. A bioinformatics approach was then used to characterize the predicted cellular phenotype; many of the predicted upregulated genes (i.e., predicted targets of downregulated microRNAs) clustered into the biological processes of inflammatory response, calcium ion homeostasis, and negative regulation of hormone secretion. While this study demonstrated that there are differences in the microRNA profiles between PBC and normal livers, further investigations are needed to identify cell types and pathways involved in and modified by this altered microRNA expression profile, and whether the observations are related to cirrhosis in general or PBC specifically.

A key biological feature of PBC is diminished secretin-stimulated bicarbonate secretion and decreased biliary expression of anion exchanger 2 (AE2/SLC4A2) [45, 46]. AE2 is a Cl-/HCO3- exchanger on hepatocyte canalicular and cholangiocyte apical membranes that controls intracellular pH and promotes alkalization of bile [47, 48]. It was recently proposed that microRNAs may contribute to the observed AE2 suppression in PBC patients [49•]. Target prediction algorithms identified the AE2 transcript as a target of miR-506, previously identified as upregulated in PBC tissue [44•]. Cell culture-based functional analyses demonstrated that mir-506 targets the 3′UTR of AE2, decreases AE2 protein expression, and modulates anion exchange. Moreover, isolated human PBC cholangiocytes exhibit increased mir-506 expression and diminished AE2 activity; transfection of these cells with a mir-506 antagomir rescues AE2 activity. Hence, this series of experiments demonstrates the logical extension from high-throughput identification of candidate microRNAs through hypothesis-driven validation of target-gene function in a specific cell type.

The microRNA expression profile in peripheral blood mononuclear cells (PBMCs) from patients with PBC has also been assessed by microarray [50]. This was initially performed using microarray and followed by RT-PCR, which validated six microRNAs, miR-15, -20a, -106b, -140, -181a, and -3654, as being altered in PBC compared to healthy controls. A bioinformatics approach identified predicted targets which were categorized into GO biological processes, and pathway analyses were performed. A microRNA-gene interaction network placed three upregulated microRNAs (miR-20a, -106b, and -93) at the core of a gene network potentially regulating endocytosis, MAPK, TGF-b, Wnt, and p53 signaling pathways. The microRNA expression profile of sera from a small cohort of patients with PBC has also been assessed [51]. Based on Illumina deep sequencing, expression of two microRNAs, miR-505 and miR-197-3p, was decreased in PBC patients compared to normal healthy and disease controls. While these high-throughput approaches can identify putative molecular pathways affected in disease, these observations and predictions have yet to be validated in cell culture and animal models of disease or evaluated for relevance in human disease.

Primary Sclerosing Cholangitis

PSC is a progressive cholangiopathy characterized by biliary tract inflammation and fibrosis, no effective pharmacotherapy, and a median LT-free survival of 12 years [52, 53]. CCA is a feared complication of this disease and occurs in approximately 10 % of PSC patients within 10 years of initial PSC diagnosis [54, 55]. A better understanding of the pathogenesis and identification of new therapeutic molecular targets are needed. Currently, no studies have addressed the role of microRNAs in the etiology or pathogenesis of PSC. To date, two manuscripts address the utility of microRNA expression profiles in bile as a diagnostic tool for CCA detection [56••, 57], yet only one utilizes PSC patients without CCA as a control [56••]. The role of microRNAs as a tool for detection of CCA in PSC patients is discussed below.

Biliary Atresia

BA remains an idiopathic disorder, but several mechanisms, including genetic, infectious, immunologic, and toxin-induced have been implicated and may be interrelated [58]. Regardless of type, BA universally progresses to fibro-obliteration of the extrahepatic bile ducts [58, 59]. Early diagnosis of BA is essential for good outcomes, and once established, the Kasai procedure (hepatoportoenterostomy) should be performed promptly in an attempt to relieve biliary obstruction and restore bile flow [58].

Postoperatively, patients must be followed longitudinally as 50 % will gradually develop chronic liver disease and ultimately require LT. Given that early diagnosis portends better operative and survival outcomes, less-invasive biomarkers to diagnose and later to follow liver disease progression are needed. To this end, genetic, metabolomic, proteomic, and gene expression markers, including microRNAs have been investigated.

Initial inquiries into the role of microRNAs in BA etiopathogenesis were performed using the rhesus rotavirus (RRV)-BALB/c model of biliary atresia. MicroRNA microarray of liver explants demonstrated temporal alterations of microRNAs from 0, 3, 8, and 14 days post-infection [60•]. The mir-29 family of microRNAs (miR-29a and miR-29b-1) was upregulated 8 and 14 days post-infection. Intriguingly, this is in contrast to the decreased miR-29 expression observed in hepatic stellate cells in rodent models of fibrosis and in livers from patients with advanced liver fibrosis [61]. Indeed, overexpression of miR-29a in the RRV model was observed by in situ hybridization throughout the liver lobule, in hepatocytes and cholangiocytes, with increased expression in periportal regions. *In vivo* suppression of miR-29, using intraperitoneal injection of antisense oligonucleotides, results in a concomitant increase in DNA methyltransferases (Dnmt3a and Dnmt3b) as well as Igf1 and Igf2bp2, which were subsequently confirmed as targets of miR-29 using luciferase reporter assays. Whether manipulation of miR-29 in the RRV model of BA modifies disease course has yet to be determined. Nonetheless, this series of experiments demonstrates that the expression of a single microRNA or family of microRNAs may perform distinct functions depending on cell type.

A recent high-throughput microRNA expression array on RNA isolated from extrahepatic bile duct (EHBD) tissue of RRV-BALB/c mice revealed a similar overall pattern of microRNA repression [62••]. In contrast, however, miR-29b (but not miR-29a) was elevated in EHBD tissue. Despite the discrepancy in mir-29a expression between this study and the previous study, which may be due to the tissue source of RNAs, the results support a possible functional role for elevated miR-29 family members in BA pathogenesis. To predict possible functional roles of altered microRNAs, an integrative genomics approach was performed. A data set of upregulated mRNAs, identified by microarray [63], revealed 14 potential target genes harboring microRNA target sequences corresponding to eight microRNAs (miR-30b/c, -133a/b, -195, -200a, -320, and -365) consistently decreased at times of obstruction (7 days) and atresia (14 days). The predicted target genes had associations with the biological processes of hematology, inflammation, and organ and tissue development. This robust integrative genomics approach serves as a hypothesis-generating data set that is in need of validation by cell culture and animal models of disease. While predicted off-target effects of microRNA manipulation remains a concern in RNA-based therapeutics, this integrated data set demonstrates a potential advantage of RNAi-based therapeutics in that a single or few microRNAs, appropriately targeted to a specific cell type, may alter expression of gene clusters within signaling pathways and processes associated with disease.

Diagnostic, Prognostic, and Therapeutic Prospects

Early detection of CCA is challenging, and disappointingly few CCAs are detected while still amenable to curative surgical intervention. The current surveillance modalities for high-risk groups for CCA, including PSC, have limited sensitivity for the detection of CCA [64, 65] and establishing an accurate diagnosis of cancer is often difficult [66]. This can result in a delayed diagnosis of CCA, which can compromise therapeutic options and patient outcome [67, 68]. Thus, improvement in diagnostic methods for CCA is needed, and in this regard, microRNAs found in bile have shown promise [56••, 57]. Recently, Shigehera et al. assessed bile obtained from patients with CCA, gall bladder cancer, or choledocolithiasis, by small RNA library sequencing and microRNA RT-PCR-based arrays; one microRNA, miR-9, demonstrated the most reliable diagnostic specificity and sensitivity for biliary tract cancer [57]. A more recent analysis suggested that the quality and quantity of microRNAs derived from biliary exosomes are more predictable than those derived from whole bile [56••]. Using a highly standardized approach, 11 microRNAs were analyzed for their utility as biomarkers for CCA in 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 microRNAs (miR-16, -486-3p, -484, -1274b, and -191) had the best predictive value since together there were rarely false positive classifications, and because they were complimentary in making true-positive classifications. The 5-microRNA panel performed better than CA 19-9 in sensitivity (71 vs. 58 %) and was thus proposed to potentially facilitate earlier CCA detection. Ultimately, a combinatorial approach using serum CA 19-9 and bile microRNAs may improve patient outcomes by allowing more reliable, earlier detection and become a valuable diagnostic tool, particularly for those patients at high risk of CCA, e.g. PSC patients.

MicroRNAs have also recently shown promise as prognostic tools for CCA progression. In a retrospective study, the overexpression of miR-151-3p or the downregulation of miR-126 were identified as potential prognostic markers for CCA progression. Interestingly, these two

microRNAs were the only independent predictors of survival in that small group of patients [69•]. In a different study, the overexpression of miR-21 was associated with poor 3-year survival [70]. The prognostic value of these microRNAs may be important for the stratification of patients for clinical trials as well as in identifying which might benefit from adjuvant therapies.

As with CCA, the development of an inexpensive, relatively non-invasive, sensitive, and specific diagnostic marker that is feasible in routine practice is still needed for BA. In a recent study, serum microRNAs were assessed for their utility as a diagnostic tool to differentiate BA from other forms of neonatal hyperbilirubinemia [71]. A microRNA array was performed on sera from BA patients and age- and sex-matched indeterminate cholestasis controls. The microRNAs of the miR-200b/429 cluster displayed good diagnostic properties, with sensitivity and specificity values ranging from 71 to 92 %, comparable to serum γ-glutamyl transpeptidase. This study serves as a proof-of-principle for the use of microRNA detection as a sensitive non-invasive diagnostic biomarker in BA. Moreover, combination of microRNA analysis with other biochemical parameters from serum may facilitate early detection and thus early intervention, and improved patient outcome.

The utility of microRNAs as a primary diagnostic tool for PBC, PSC, and polycystic liver disease is less clear as the current approaches are accurate and efficient. For example, PBC can generally be diagnosed by positive AMA detection (or if needed, liver biopsy) and a cholestatic serum liver profile, PSC by cholangiography and a cholestatic profile (or liver biopsy in indeterminate or small duct PSC cases), and polycystic liver disease by physical examination coupled with abdominal imaging. Thus, the potential utility of microRNA 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.

Conclusions

While showing promise, and having various potential applications, the role of microRNAs as diagnostic, predictive, or prognostic biomarkers for cholangiopathies requires further investigation, including replication of previous findings in larger cohorts of patients. Furthermore, stringent protocol standardization with respect to the source of RNA, purification and amplification procedures, and microRNA expression normalization will be needed. Additionally, the search for more effective therapies for cholangiopathies is an intensive area of research, and the in vivo manipulation of microRNA expression is a promising approach. An attractive feature of microRNA therapy is the potential to target multiple mediators of pathways that concertedly regulate cellular processes. Ideally, chemically modified microRNA mimics would restore the expression of a diminished microRNA (replacement therapy), while antisense modified oligonucleotides would inhibit an upregulated microRNA (microRNA inhibition therapy) and restore cellular homeostasis. The issue of RNA stability in vivo has been addressed through the use of chemically modified oligonucleotides [72, 73]; however, the critical hurdle of targeted delivery of these oligonucleotides remains an issue. Many advances in oligonucleotide delivery have been realized since the discovery of RNAi [74], yet whether any of these delivery methods can be utilized to target cholangiocytes remains to be investigated. Moreover, the mechanisms underlying microRNA dysregulation remain understudied. Multiple mechanisms may account for alteration of microRNA expression, including transcription or epigenetic, RNA degradation, and altered microRNA biogenesis or nuclear transport. Understanding why microRNAs are dysregulated could aid in the identification of therapeutic targets and build a more versatile set of tools to restore normal expression and function of these important non-coding RNAs [12, 41, 75–87].

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Compliance with Ethics Guidelines

Conflict of Interest Steven O'Hara, Sergio Gradilone Tatyana Masyuk, James Tabibian, and Nicholas LaRusso declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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