REVIEW

The Role of miRNA and Long Noncoding RNA in Cholestatic Liver Diseases

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Cholestatic liver diseases encompass a range of organic damages, metabolic disorders, and dysfunctions within the hepatobiliary system, arising from various pathogenic causes. These factors contribute to disruptions in bile production, secretion, and excretion. Cholestatic liver diseases can be classified into intrahepatic and extrahepatic cholestasis, according to the location of occurrence. The etiology of cholestatic liver disease is complex, and includes drugs, poisons, viruses, parasites, bacteria, autoimmune responses, tumors, and genetic metabolism. The pathogenesis of cholestatic liver disease is not completely clarified; also, there is still a lack of effective therapy methods. It is urgent to clarify its mechanism to find more effective therapeutic targets and drugs. Increasing evidence demonstrates that miRNA and long noncoding RNA are involved in the progression of cholestatic liver diseases. This review provides a comprehensive summary of the research progress on the roles of miRNA and long noncoding RNA in cholestatic liver diseases. The aim is to enhance the understanding of their potential diagnostic, therapeutic, and prognostic value for patients with cholestasis. (Am J Pathol 2024, 190:1–15; https://doi.org/10.1016/j.ajpath.2024.02.006)

Cholestatic liver diseases result from a variety of disorders associated with the formation, secretion, and excretion of bile. Cholestasis is defined as a pathologic condition with obstruction of bile flow, and intrahepatic bile acid accumulation can result in hepatocyte injury, cholangiocyte damage, and inflammation, then leading to liver fibrosis, cirrhosis, liver failure, malignant proliferation of cholangiocytes, hepatocellular carcinoma, and cholangiocarcinoma. Common cholestatic liver diseases mainly include primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), biliary atresia (BA), intrahepatic cholestasis of pregnancy (ICP), and drug-induced cholestatic liver injury (DILI). The pathogenesis of cholestatic liver diseases is complicated and not completely explained, and the therapeutic effect is still unsatisfactory. At present, ursodeoxycholic acid (UDCA) is an effective drug for the treatment of cholestatic liver diseases, but nearly half of the patients have insufficient response or intolerance to UDCA. For the latter, only obeticholic acid is available. Therefore, there is an urgent need to elucidate the mechanisms underlying cholestatic liver diseases and to explore more effective therapeutic targets and drugs.

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Noncoding RNA (ncRNA) is the RNA that does not encode a protein. ncRNA accounts for 90% of all human RNAs and is verified to play a crucial role in diverse cellular processes, such as angiogenesis, apoptosis, and immune response. Among of them, miRNAs are small ncRNAs composed of 19 to 24 bp that can modulate gene expression by degrading target mRNA or through their translational inhibition. Long ncRNA (lncRNA) is a subtype of ncRNA >200 nucleotides, which has high tissue specificity and low sequence conservation, and it regulates gene expression at different levels through a variety of mechanisms. Recently, it has been reported that miRNAs and lncRNAs are involved in the development of cholestatic liver diseases. In particular, miRNAs may become potential biomarkers for cholestatic liver diseases. lncRNAs also exert many effects in cholestatic liver diseases, including regulation of various signal pathways to participate in cell proliferation, apoptosis, and other pathophysiological processes.

For the common cholestatic liver diseases (eg, PBC and PSC), there are new promising therapeutic targets related to miRNAs and lncRNAs, such as miR-506 and lncRNA-H19. Therefore, based on current research, this review summarizes recent knowledge about miRNAs and lncRNAs in cholestatic liver diseases, especially for their potential values in diagnosis, treatment, and prognosis evaluation.

Common Cholestatic Liver Diseases

PBC is one kind of chronic cholestatic liver disease associated with autoimmune-mediated destruction of small and medium intrahepatic bile ducts. It is characterized by progressive damage of biliary epithelial cells, leading to increased inflammation and fibrosis in the portal vein. PBC is a multifactorial disease, and the etiology is unclear. PBC mainly affects middle-aged women, most patients with PBC (approximately 90%) produce anti-mitochondrial autoantibodies against pyruvate dehydrogenase complex-E2 components. UDCA is a liver protective bile acid that can promote bile secretion through increasing the secretion of bicarbonate, thus preventing the development of cholestatic, or mixed DILI. The diagnosis of DILI depends on the serum levels of alanine transaminase and alkaline phosphatase. Aberrantly altered miRNAs in the DILI process may become the new diagnostic markers.

The Role of miRNAs in Cholestatic Liver Diseases

miRNA is a type of endogenous short-chain ncRNA. A single miRNA may regulate hundreds of protein-coding genes and plays an important role in cell biology. As a diagnosis biomarker or potential therapeutic target, miRNA has been widely studied in cholestatic liver diseases. In this section, the effects of miRNAs in cholestatic liver diseases and the underlying mechanisms are discussed.

Regulation of Bile Acid Metabolism and Excretion

Common features observed in patients with PBC include impaired biconarate secretion in bile and down-regulation of anion exchanger 2 (AE2) protein expression. miR-506 is a miRNA overexpressed in cholangiocytes of PBC liver. Notably, miR-506 may mediate the characteristic down-regulation of AE2 and type 3 inositol 1,4,5-trisphosphate receptor in cholangiocytes by directly targeting AE2 and type 3 inositol 1,4,5-trisphosphate receptor mRNA, resulting in damaged bile secretion because of impaired Cl\(^{-}\)/HCO\(_3\) exchange activity and reduced Ca\(^{2+}\) release from endoplasmic reticulum (Figure 1). Inhibition of miR-506 expression in cultured cholangiocytes from patients with PBC led to an increase in AE2-related exchange activity, thus emphasizing the critical role of miR-506 in the pathogenesis and treatment of PBC.

Hepatic miR-210 levels were observed to be enhanced >10-fold in patients with PBC along with markedly increased mRNA expression of Krüppel-like factor-4, decreased nuclear expression of mixed lineage leukemia 4, and small heterodimer chaperones (SHPs) in the liver. Mixed lineage leukemia 4 is a coactivator of farnesoid X receptor, which helps the induction of SHP and bile salt...
miRNA/IncRNA in Cholestatic Liver Diseases

**Figure 1**  The role of miRNAs in cholestatic liver injury. Ablant expression of miRNAs may contribute to cholestatic liver injury through several mechanisms, including the following: i) regulating bile acid metabolism; ii) increasing inflammatory response; iii) regulating oxidative stress; and iv) influencing liver fibrosis. AE2, anion exchanger 2; BA, biliary atresia; BCL-2, B-cell lymphoma 2; CLD, cholestatic liver disease; EMT, epithelial-mesenchymal transition; HLA-G, human leukocyte antigen G; HSC, hepatic stellate cell; ICP, intrahepatic cholestasis of pregnancy; InsP3R3, type 3 inositol 1,4,5-triphosphate receptor; IRAK1, IL-1 receptor-associated kinase 1; MLL4, mixed lineage leukemia 4; NF-κB, nuclear factor erythroid 2-related factor 2; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; RASGRP1, RAS guanyl-releasing protein 1; SOCS1, suppressor of cytokine signaling 1; TGF-β, transforming growth factor-β; TGF-βR2, transforming growth factor-β receptor 2; VCAM-1, vascular cell adhesion molecule 1; TLR, toll-like receptor; TNF-α, tumor necrosis factor-α; TRAF6, TNF receptor-associated factor 6; VCAM-1, vascular cell adhesion molecule 1.

The miR-148a levels were found to be significantly increased in the peripheral blood and placenta of patients with ICP. Moreover, in the placenta, both mRNA and protein expression of human leukocyte antigen G were inversely correlated with the miR-148 levels, but such correlation was not observed in the serum. The expression of human leukocyte antigen G was inversely correlated with serum total bile acid contents, although it was reported that human leukocyte antigen G was involved in the reprogramming of maternal immune response. The miR-148a levels in the placenta were positively correlated with serum bile acid contents. Therefore, the elevated expression of miR-148a may promote the progression of ICP by increasing serum total bile acid levels via reducing human leukocyte antigen G expression in the placenta.

Additionally, the expression of miR-221/miR-222 was reported to be increased along with the down-regulation of B-cell lymphoma 2 (BCL-2) expression in the placenta of patients with ICP, which resulted in apoptosis of placenta trophoblast cells and decreased bile acid transport of the placenta. Vascular cell adhesion molecule 1 is a critical regulator in neonatal angiogenesis. In patients with ICP, elevated levels of bile acids inhibited vascular cell adhesion molecule 1 expression in vascular endothelial cells of placenta and induced vasoconstriction in the fetal umbilical vein, leading to excessive accumulation of bile acids and even fetal death (Figure 1). In vitro, bile acids suppressed vascular cell adhesion molecule 1 expression by up-regulating miR-590-3p in umbilical endothelial cells. Therefore, either up-regulation of vascular cell adhesion molecule 1 or inhibition of miR-590-3p expression may be an effective treatment for ICP.

**Regulation of Inflammatory Response**

Both adult and pediatric liver diseases involve immune activation in the pathogenesis. BA is the most common pediatric chronic liver disease. In the progression of BA and adult cholestatic liver disease, such as PSC and PBC, the...
innate immune response and adaptive immune response can contribute to the pathogenesis of cholestatic liver injury.\textsuperscript{25–27} Cholangiocytes, neutrophils, macrophages, and Kupffer cells are involved in innate immune response. For example, macrophages may tend to differentiate into proinflammatory macrophage (M1) rather than restorative macrophage (M2), producing mediators like IL-6 and IL-8, which are involved in inflammation and mediate liver injury.\textsuperscript{26} The adaptive immune response in BA includes the role of T-helper cells, macrophages, natural killer cells, and the antibodies (IgG, IgM) against the biliary epithelium.\textsuperscript{27,28} BA typically manifests in infancy, and its rapid progression requires prompt diagnosis and early surgical intervention, such as the Kasai procedure. After surgical treatment, most young people born with BA will survive into adulthood. But these survivals may have signs of recurrent cholangitis or cirrhosis, which may be the long-term consequences of inflammatory insults experienced during pediatric liver disease. This emphasizes the importance of monitoring and managing liver health throughout the life of survivors with a multidisciplinary team involving pediatricians and hepatologists.\textsuperscript{29} In the adult cholestatic liver diseases, intrahepatic and extrahepatic bile ducts are damaged along with chronic inflammation, which is different from the extrahepatic bile duct stenosis in infants or children with BA. Additionally, it has been reported the aberrant proliferation and senescence of cholangiocytes due to chronic injury may be involved in the pathogenesis of adult cholestatic liver diseases.\textsuperscript{30} miRNAs have been also reported to influence the progression of cholestatic liver disease through regulating immune response.

The overexpression of many proinflammatory cytokines, such as IL-8, IL-12, IL-17, IL-18, and tumor necrosis factor-\(\alpha\), in PBC liver promoted the increased miR-506 expression in cholangiocytes, resulting in the appearance of PBC-like features (eg, stress, cell dedifferentiation, and apoptosis induced by bile salts).\textsuperscript{31} All of these features, mediated by up-regulation of miR-506 in cholangiocytes, may contribute to loss of bile ducts in patients with PBC. Changes in pyruvate dehydrogenase complex-E2 overexpression\textsuperscript{3,31} and mitochondrial function mediated by miR-506 ultimately led to immune activation in PBC, which was partly due to direct down-regulation of AE2 and Ins3PR3 by miR-506 (Figure 1).\textsuperscript{32} But the immunomodulation role of miR-506 in the pathophysiology of cholangiocytes is not completely elucidated. In future studies, it is imperative to investigate and ascertain whether miR-506 may regulate the expression of other genes involved in the progression of PBC. Therefore, miR-506 emerges as a pivotal player and a potential therapeutic target for the treatment of PBC.

In many types of cells, miR-21 plays an anti-apoptotic and growth-promoting role, which may be also involved in the occurrence of PBC.\textsuperscript{32} miR-21 expression was found to be elevated in the livers of patients with PBC and bile duct ligation (BDL) mice.\textsuperscript{32} miR-21 expression was also found to be higher in the cholangiocytes and liver samples from patients with PSC than in control liver samples.\textsuperscript{33} In research on the circulating miRNAs in 22 healthy children and 58 children with chronic liver disease, the children with BA demonstrated significantly higher miR-21 levels compared with healthy control or age-matched children with other cholestatic disorders, such as PSC.\textsuperscript{34} So, serum miR-21 may be used as a noninvasive diagnostic indicator to distinguish BA from other cholestatic disorders in children.\textsuperscript{34}

Furthermore, elevated levels of miR-21 were observed in peripheral blood mononuclear cells from anti-mitochondrial autoantibody—negative patients with PBC compared with healthy control. This increase in miR-21 was accompanied with decreased expression of its target gene RAS guanyl-releasing protein 1, which is involved in linking T-cell receptor signal transduction to Ras and mitogen-activated protein kinase activation. Down-regulation of RAS guanyl-releasing protein 1 contributed to cell hypomethylation via reduction of DNA methyltransferase 1, followed by higher expression of methylation-sensitive autoimmune-related genes, thus driving PBC progression (Figure 1). Hence, these studies present a novel perspective that miR-21 may become a therapeutic target in cholestatic liver disease.

The miR-146a and miR-155 expression was observed to be dramatically increased in peripheral blood mononuclear cells from patients with PBC compared with healthy people.\textsuperscript{36} Lipopolysaccharide stimulated expression of miR-146a by interacting with toll-like receptor 4 and triggering NF-\(\kappa\)B, and in turn miR-146a negatively regulated toll-like receptor signaling by targeting IL-1 receptor-associated kinase 1 and tumor necrosis factor receptor-associated factor 6.\textsuperscript{37} Enhanced hepatic miR-155 expression was reported to be accompanied by the hepatic down-regulation of suppressor of cytokine signaling 1 protein in patients with PBC (Figure 1). Meanwhile, the mRNA and protein expression of vitamin D receptor were decreased up to 51% and 59%, respectively, in PBC livers. Moreover, vitamin D receptor/miR-155—modulated reduction of suppressor of cytokine signaling 1 expression may result in insufficient negative regulation of cytokine secretion, which was involved in the pathogenesis of PBC.\textsuperscript{38} Meanwhile, highly elevated miR-155 expression was detected in BA histologic samples, mainly located in the cholangiocytes.\textsuperscript{39,40} Overexpression of miR-155 contributed to expression of CXCL1, CXCL9, CXCL10, and monocyte chemotactic protein 1 in human intrahepatic biliary epithelial cells (HIBECs) stimulated by interferon-\(\gamma\) via down-regulating suppressor of cytokine signaling 1 expression.\textsuperscript{41}

Furthermore, through single-end deep sequencing and RT-PCR, it was confirmed that the serum level of miR-139-5p is reduced in patients with advanced PBC compared with healthy individuals. The miR-139-5p expression was observed to be much higher in lymphocytes than that in hepatocytes in PBC liver samples. Meanwhile, the expression of miR-139-5p was significantly higher in PBC-derived
lymphocytes than autoimmune hepatitis— or chronic hepatitis C–derived lymphocytes. Up-regulation of miR-139-5p may induce the expression of tumor necrosis factor-α in lymphocytes of patients with PBC and enhanced liver inflammation by inhibiting the transcription of c-FOS that can act on NF-κB p65 subunit and inhibit the NF-κB signaling pathway. miR-181a is one of the miRNAs regulating lymphocyte development and homeostasis. Song et al found that the proportion of type 17 helper T (Th17) cells in peripheral blood was highly elevated in patients with PBC compared with healthy people and patients with hepatitis B, and miR-181a expression in T lymphocytes was down-regulated. Further research found that the expression of BCL-2, an anti-apoptotic protein, was significantly increased in patients with PBC; BCL-2 protein expression was not only negatively correlated with the miR-181a level, but also positively related to the percentage of Th17 cells. Therefore, the down-regulation of miR-181a may inhibit Th17 cell apoptosis by increasing BCL-2 expression, thereby promoting the activation of T lymphocytes in the process of PBC (Figure 1).

Liang et al analyzed the miRNAs in the plasma and peripheral monocytes of patients with PBC and found that there were decreased serum levels of miR-92a and miR-92a expression in the peripheral blood mononuclear cells, which were negatively correlated with the Th17 cell percentage in patients with PBC. Th17 cells are proinflammatory T-helper cells, which exacerbate inflammation by secreting IL-17A. miR-92a and IL-17A are co-expressed in peripheral blood mononuclear cells, indicating that miR-92a may participate in the up-regulation of Th17 cells, thus contributing to the development of PBC.

miR-425 regulates the production of inflammatory cytokines, such as IL-2 and interferon-γ, in CD4 T cells through modulating N-Ras expression in PBC. N-Ras is a regulator of the T-cell receptor signaling pathway. The expression of miR-181a, miR-181b, miR-374b, and miR-425 was identified to be significantly down-regulated in PBC, which was associated with the T-cell receptor signaling pathway in CD4 T cells. miR-425 can target N-Ras, and the decreased miR-425 expression was involved in the regulation of inflammation via increasing N-Ras expression in the progression of PBC (Figure 1). So, inhibition of N-Ras may be a novel and potential immunotherapy method for the treatment of PBC. The inhibitory function of miR-425 on N-Ras is unique in contrast to other Ras inhibitors.

IL-6 is a pleiotropic cytokine that can regulate cholangiocyte growth and proliferation. During cholestasis, IL-6 may be secreted by cholangiocytes. Decreased expression of miR-124 was observed in plasma and liver tissue of patients with BA and BDL rats. Down-regulation of miR-124 resulted in increased IL-6 secretion by targeting the STAT3 gene and further mediating cholangiocyte proliferation. Meanwhile, the elevation of miR-200 family members during cholestasis enhanced IL-6 expression through suppressing forkhead box A2 expression in cholangiocytes. In summary, the reduced expression of miR-124 and elevated expression of miR-200 may collectively contribute to the development of cholestasis, accompanied by increased IL-6 secretion.

Regulation of Oxidative Stress

miR-34a is controlled by p53 expression and plays a variety of roles in the modulation of cell differentiation, migration, and apoptosis. Increased miR-132 and miR-34a levels were observed in the peripheral blood and liver tissues from patients with PBC. PBC is associated with impaired liver response to oxidative stress. Oxidative stress induced by either hydrogen peroxide or tert-butylhydroquinone enhanced miR-132 and miR-34a expression in H69 cholangiocytes, whereas melatonin can suppress the elevation of these two miRNAs, thus reducing cholangiocyte injury due to oxidative stress—mediated apoptosis and inflammation. Meanwhile, nuclear factor erythroid 2—related factor 2, an important regulator of oxidation-reduction homeostasis, was observed to be inhibited by miR-132 and miR-34a over-expression. Hence, down-regulation of nuclear factor erythroid 2—related factor 2 may lead to defective response to oxidative stress, which may be another mechanism resulting in liver dysfunction in PBC. Furthermore, Kelch-like ECH-associated protein 1, which can regulate nuclear factor erythroid 2—related factor 2 activity, was found to be increased with p62 in H69 cholangiocytes, but it could not fulfill its normal function. These findings demonstrated that hepatic miR-34a up-regulation and p62 accumulation provided new insights into PBC treatment because they suggested there may be a correlation between miRNA expression and autophagy regulation in PBC.

An miRNA-sequencing analysis identified miR-4645-3p, one of the recently identified miRNAs, may be involved in age-associated endoplasmic reticulum stress and disorders, which was dramatically increased up to fourfold in circulating extracellular vesicles isolated from patients with PSC compared with control subjects (Figure 1).

Regulation of Liver Fibrosis

As mentioned above, enhanced miR-34a expression was found in the serum of patients with PBC. miR-34a increased epithelial-mesenchymal transition (EMT) and fibrogenesis activity of HIBECs and led to up-regulation of transforming growth factor-β1 (TGF-β1), TGF-β1 receptor, and phosphorylated smad2/3 by inhibiting TGF-β—induced factor homeobox 2, which can inhibit the TGF-β1/Smad signaling pathway (Figure 1). Meanwhile, miR-34a induced IL-6 and IL-17 expression in HIBECs, and IL-6 and IL-17 not only exerted inflammatory functions but also stimulated HIBEC EMT. After the stimulation with IL-17A, HIBECs changed into a fibroblastic morphology, and the miRNA and
protein expression of vimentin was up-regulated, along with down-regulation of E-cadherin in HIBECs. These findings suggest that miR-34a may contribute to EMT through pathways other than the TGF-β1/Smad pathway.

The hepatic levels of miR-19b were much lower in patients with BA compared with controls. miR-19b can inhibit profibrotic TGF-β signaling by targeting TGF-β receptor 2. miR-19b was expressed in hepatic stellate cells (HSCs), down-regulated during their activation, and accompanied by increased expression of fibrosis markers, such as α-smooth muscle actin and type I collagen. Decreased miR-19b led to up-regulation of its target gene TFG-β receptor 2, which may be involved in BA-associated fibrosis due to activation of TFG-β signaling. 53

The Other Altered miRNAs during Cholestatic Liver Diseases

The serum levels of miR-126 and miR-1281 were reported to be significantly increased in patients with PSC by applying deep sequencing and quantitative RT-PCR assay, suggesting that these two miRNAs may reflect a PSC-specific disease process and malignant transformation.13 miRNA analysis in 11 patients with PSC has shown that miR-378a-5p was substantially increased in PSC liver compared with healthy controls, along with reduced mRNA expression of sulfotransferase 2A1 in ileum of patients with PSC.54 Additionally, miR-122 enriched in circulating extracellular vesicles was isolated from patients with PSC compared with healthy control,51 which may act as a novel and promising prognostic indicator related to improved liver transplant-free survival in patients with PSC.55 The serum miR-122 level was also up-regulated in patients with gallstones, which was related to severe liver injury.56

By using a microfluidic array platform, miR-200b and miR-429 were identified to be elevated in sera of patients with BA, and the miR-200b/miR-429 cluster may be a promising diagnostic index for BA.57 By using next-generation sequencing and RT-PCR, it has been confirmed that the plasma levels of miR-100-5p and miR-122-5p were increased, whereas levels of miR-126-3p and miR-140-3p were decreased, in patients with BA compared with healthy controls.58 But only miR-140-3p expression was much lower in patients with BA than that in patients with other cholestatic disease; thus, it may serve as a promising diagnostic marker for BA.58

The levels of miR-1275, miR-4271, and miR-6891-5p in the serum exosomes from patients with ICP were reported to be lower than those in healthy pregnant women. The combination of these three miRNAs may increase the diagnostic accuracy of ICP.59 Bioinformatics analysis showed that these three miRNAs were related to lipid metabolism, apoptosis, oxidative stress, and the mitogen-activated protein kinase signaling pathway, suggesting they may be used as novel noninvasive indicators for the diagnosis of ICP.

In addition, it was reported that chlorpromazine, cyclosporine A, and α-naphthylisothiocyanate induced the expression of miR-21-3p, miR-21-5p, miR-22-3p, miR-27a-5p, miR-34a-5p, miR-98-5p, and miR-1260b, but suppressed the expression of miR-16-5p, miR-122-5p, miR-192-5p, and miR-424-5p in hepatocytes, thus discovering the specificity of miRNAs for DILI.60 The translation of these miRNAs into potential targets for the therapy of DILI is not yet clear, and further studies are necessary to elucidate their roles in cholestatic DILI.

In summary, miRNAs are involved in the pathogenesis of cholestatic liver diseases, such as PBC, PSC, BA, and ICP (Table 1), which may become biomarkers or therapeutic targets for these diseases.

The Role of miRNAs in Experimental Cholestatic Models

The Up-Regulated miRNAs in BDL Animal Models

miR-122 is a tiny RNA highly specifically expressed in the liver that is involved in liver physiology and disease, especially in regulating cholesterol in the body.61 Under pathologic conditions, miR-122 can participate in the progression of diverse liver diseases.62 Serum miR-122 level may serve as a promising new indicator of cholestasis because serum miR-122 significantly increased and displayed a similar time course to alanine transaminase concentration during BDL-induced cholestatic liver injury.63,64

Consistent with higher hepatic expression of miR-21 in patients with PBC, elevation of miR-21 was observed in the liver and cholangiocytes of BA mice.33 Down-regulation of miR-21 decreased BDL-induced cholangiocyte proliferation and intrahepatic biliary mass. miR-21 deficiency reduced oxidative stress, hepatocellular degeneration, HSC activation, collagen deposition, and fibrotic marker expression, such as Tgf-β1 and α-smooth muscle actin, in BA mice.33,34 In vitro, treatment of miR-21 inhibitor reduced cell proliferation and fibrotic marker expression and enhanced apoptosis in immortalized murine biliary cell lines and human HSCs. In addition, lack of miR-21 resulted in elevated Smad-7 expression in BDL mice, which may be the reason for the reduced biliary hyperplasia and liver fibrosis.33,34 Furthermore, rosuvastatin interference may exhibit hepatoprotective effect in BDL rats by reducing miR-21 expression and the activation of high-mobility group box 1—toll-like receptor 4 signaling axis.65

Yang et al66 observed that c-Myc expression was up-regulated in BDL and lithocholic acid–treated mice. C-Myc mediated up-regulation of miR-27a/b, which targeted prohibitin 1 and nuclear factor erythroid 2–related factor 2 to decrease their expression, thus contributing to cholestatic liver injury.

miR-183-5p expression was reported to be elevated in the liver samples from patients with hepatolithiasis as well as BDL rat liver and activated HSCs (LX-2).57 Down-
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<th>Target</th>
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Table 1 (continued)

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<td>Liver</td>
<td>SOCS1</td>
<td>miR-155 amplifies inflammatory responses via suppression of SOCS1.</td>
<td>39,40</td>
</tr>
<tr>
<td></td>
<td>miR-124</td>
<td>Down</td>
<td>Liver (cholangiocyte)</td>
<td>IL-6R, STAT3</td>
<td>Decreased miR-124 expression promotes IL-6-induced cholangiocyte proliferation.</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>miR-200</td>
<td>Up</td>
<td>Liver (cholangiocyte)</td>
<td>FOXA2</td>
<td>miR-200 family members enhance IL-6 expression, miR-19b inhibits HSC activation.</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>miR-19b</td>
<td>Down</td>
<td>Liver (HSC)</td>
<td>TGF-βR2</td>
<td></td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>miR-429</td>
<td>Down</td>
<td>Serum</td>
<td>Unknown</td>
<td>Unknown</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>miR-140-3p</td>
<td>Down</td>
<td>Serum</td>
<td>Unknown</td>
<td>Unknown</td>
<td>58</td>
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<table>
<thead>
<tr>
<th>Disease</th>
<th>miRNA</th>
<th>Expression</th>
<th>Source</th>
<th>Target</th>
<th>Effect</th>
<th>Reference(s)</th>
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<tbody>
<tr>
<td>BA</td>
<td>miR-21</td>
<td>Up</td>
<td>Serum</td>
<td>Unknown</td>
<td>Unknown</td>
<td>34</td>
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<tr>
<td>miR-155</td>
<td>Up</td>
<td>Liver</td>
<td>SULT2A1</td>
<td>miR-378a-5p may lead to impaired hepatoprotection by reducing SULT2A1 expression in the ileum.</td>
<td>54</td>
<td></td>
</tr>
</tbody>
</table>

The Down-Regulated miRNAs in BDL Animal Models

Current studies have shown that the overexpression of miR-29a alleviated BDL-induced cholestatic liver damage and fibrosis through decreasing Tgf-β expression and regulating Wnt/β-catenin. Overexpression of miR-29a decreased HSC proliferation and migration via inhibiting enhancer of zeste homolog 2 and bromodomain-containing 4, thus mitigating BDL-induced liver fibrosis. Treatment of miR-29a improved cholestatic hepatitis, liver fibrosis, and protein homeostasis through blocking the p85 α subunit of phosphoinositide 3-kinase. These findings highlight the potential value of miR-29a during cholestatic liver diseases. Meanwhile, miR-33 expression was found to be decreased in BDL rats along with the up-regulation of carnitine palmitoyltransferase 1A, a key enzyme controlling mitochondrial β-oxidation, which was restored by transplantation of chorionic plate-derived mesenchymal stem cells. Meng et al. observed that miR-125b expression was substantially reduced in BDL mice. In vitro, up-regulation of miR-125b or knockdown of histidine decarboxylase can reduce the expression of histidine decarboxylase and increase the expression of SULT2A1, a biomarker of PSC.

Regulation of miR-183-5p expression decreased HSC proliferation and fibrotic marker expression. miR-183-5p may promote liver fibrosis through decreasing the expression of forkhead box protein O1 that can inhibit the TGF-β pathway in HSCs. Recently, 151 miRNAs were verified to be enriched in circulating extracellular vesicles from BDL mice, of which 66 miRNAs were conserved in humans. Among of them, miR-22-3p, miR-29a-3p, miR-192-5p, and miR-194-5p were also observed to elevate in extracellular vesicles isolated from BA miR-29a alleviated BDL-induced cholestatic liver damage and fibrosis. Meanwhile, miR-125b expression was substantially reduced in BDL mice. In vitro, up-regulation of miR-125b or knockdown of histidine decarboxylase can reduce the expression of histidine decarboxylase and increase the expression of SULT2A1, a biomarker of PSC.
## Table 2 miRNAs Involved in Cholestatic Animal Models

<table>
<thead>
<tr>
<th>Model</th>
<th>Species</th>
<th>miRNA</th>
<th>Expression</th>
<th>Source</th>
<th>Target</th>
<th>Effect</th>
<th>Reference no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDL</td>
<td>Mouse</td>
<td>miR-21</td>
<td>Up</td>
<td>Serum/liver</td>
<td>Smad7</td>
<td>Promoting liver fibrosis</td>
<td>32,33</td>
</tr>
<tr>
<td>BDL</td>
<td>Rat</td>
<td>miR-200</td>
<td>Up</td>
<td>Liver</td>
<td>Foxa2</td>
<td>miR-200 enhances IL-6 expression.</td>
<td>46</td>
</tr>
<tr>
<td>BDL</td>
<td>Rat</td>
<td>miR-124</td>
<td>Down</td>
<td>Liver</td>
<td>IL-6R, Stat3</td>
<td>Down-regulation of miR-124 increases IL-6 expression and cholangiocyte proliferation.</td>
<td>46</td>
</tr>
<tr>
<td>BDL</td>
<td>Mouse</td>
<td>miR-122</td>
<td>Up</td>
<td>Serum</td>
<td>Unknown</td>
<td>Acting as specific biomarkers</td>
<td>63,64</td>
</tr>
<tr>
<td>BDL</td>
<td>Rat</td>
<td>miR-21</td>
<td>Up</td>
<td>Liver</td>
<td>Hmgb1</td>
<td>Increasing the levels of NF-κB, TNF-α, and IL-6</td>
<td>65</td>
</tr>
<tr>
<td>BDL</td>
<td>Mouse</td>
<td>miR-27a/b</td>
<td>Up</td>
<td>Liver</td>
<td>Phb1/Nrf2</td>
<td>Decreasing reduced glutathione synthesis and antioxidant capacity activation</td>
<td>66</td>
</tr>
<tr>
<td>BDL</td>
<td>Rat</td>
<td>miR-183-5p</td>
<td>Down</td>
<td>Liver</td>
<td>Foxo1</td>
<td>Promoting the activation of Tgf-β signaling pathway</td>
<td>67</td>
</tr>
<tr>
<td>BDL</td>
<td>Mouse/rat</td>
<td>miR-29a</td>
<td>Down</td>
<td>Liver</td>
<td>Ezh2, Brd4</td>
<td>miR-29a reduces HSC proliferation and activation.</td>
<td>69—71</td>
</tr>
<tr>
<td>BDL</td>
<td>Rat</td>
<td>miR-33</td>
<td>Down</td>
<td>Liver</td>
<td>Cpt1a</td>
<td>miR-33 is involved in free fatty acid oxidation metabolism.</td>
<td>72</td>
</tr>
<tr>
<td>BDL</td>
<td>Mouse</td>
<td>miR-125b</td>
<td>Down</td>
<td>Cholangiocytes</td>
<td>Hdc, Vegfa</td>
<td>Down-regulation of miR-125b promotes cholangiocyte proliferation.</td>
<td>73</td>
</tr>
<tr>
<td>BDL</td>
<td>Mouse</td>
<td>miR-200c-3p</td>
<td>Down</td>
<td>Cholangiocytes</td>
<td>Sesn1</td>
<td>miR-200c prevents neuroendocrine-like activation of cholangiocytes and inhibits liver fibrosis.</td>
<td>74</td>
</tr>
<tr>
<td>BA model</td>
<td>Mouse</td>
<td>miR-29a/miR-29b1</td>
<td>Up</td>
<td>Liver (hepatocyte, cholangiocyte)</td>
<td>Igf1/Il1rap</td>
<td>Increasing the death of cholangiocytes and promoting inflammation</td>
<td>75</td>
</tr>
<tr>
<td>MDA/ANIT</td>
<td>Rat</td>
<td>miR-218a-5p</td>
<td>Up</td>
<td>Cholangiocyte</td>
<td>Ppp1cb, Ppp2r5a</td>
<td>Promoting cholangiocyte proliferation and collagen deposition</td>
<td>76</td>
</tr>
</tbody>
</table>

(table continues)
Table 2 (continued)

<table>
<thead>
<tr>
<th>Model</th>
<th>Species</th>
<th>miRNA</th>
<th>Expression</th>
<th>Source</th>
<th>Target</th>
<th>Effect</th>
<th>Reference no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mdr2&lt;sup&gt;−/−&lt;/sup&gt; Mouse</td>
<td>miR-125b</td>
<td>Down</td>
<td>Liver</td>
<td>Vegfa</td>
<td>miR-125b negatively regulates TGF-β/Smad signaling. miR-191 can inhibit bile acid synthesis.</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>Mdr2&lt;sup&gt;−/−&lt;/sup&gt; Mouse</td>
<td>miR-191-3p</td>
<td>Down</td>
<td>Liver</td>
<td>Lrh-1</td>
<td>miR-191 can inhibit bile acid synthesis.</td>
<td>79</td>
<td></td>
</tr>
</tbody>
</table>

Cholangiocyte proliferation, which suggested that miR-125b may be a potent target for the therapy of cholestatic liver disease.73

Song et al74 observed abundant miR-200c-3p expression in normal cholangiocytes, but it was down-regulated in BDL mice. Stimulation of bile acids down-regulated miR-200c-3p expression in cultured cholangiocytes. miR-200c-3p may suppress cholangiocyte proliferation and neuroendocrine-like secretory phenotype transition by targeting sestrin 1 and the IL-6/AKT feedback signaling, thus mitigating cholestatic liver fibrosis.74

The Aberrant Expression of miRNAs in Other Animal or Cellular Models

It was reported that miR-29a/29b1 expression was elevated in the Rhesus rotavirus—BALB/c mouse BA model. Furthermore, insulin-like growth factor 1 and IL-1 receptor accessory protein are identified as the targets of miR-29 by performing luciferase assays in NIH3T3 cells.75

In 4,4-methylenedianiline— and α-naphthylisothiocyanate—induced rat cholestatic models, Oda et al76 found that plasma miR-143-3p and miR-218a-5p levels were increased dose dependently. But the hepatic expression of protein phosphatase 1 catalytic subunit β and protein phosphatase 2 regulatory subunit B′β decreased in cholestatic rats. miR-218a-5p was expressed in cholangiocytes, and it suppressed cholangiocyte proliferation by down-regulating protein phosphatase 1 catalytic subunit β and protein phosphatase 2 regulatory subunit B′β expression, thus promoting the progression of cholestasis.76

Zhuyu pill is an effective Chinese medicine consisting of Huang Lian, which shows potential anti-cholestatic effects.77 The expression of miR-29b-3p, miR-147, miR-20b-5p, and miR-3586-3p was found to be significantly up-regulated in cholestasis and down-regulated after zhuyu pill intervention. Indeed, these miRNAs may also be potential targets for the therapy of cholestatic liver disease.77

The miR-125b expression was reported to be reduced in cholangiocytes isolated from multiple drug resistance gene knockout (Mdr2<sup>−/−</sup>) mice compared with wild-type mice, whereas deletion of secretin receptor gene restored miR-125b expression in Mdr2<sup>−/−</sup> mice to the levels similar to wild-type mice and alleviated cholestatic liver injury.78

Limb expression 1-like (LIX1L) is a possible RNA-binding protein. Increased LIX1L expression was observed in PBC, PSC, and three animal models of cholestasis (BDL, Mdr2<sup>−/−</sup>, and bile acid feeding).79 The critical regulation effect of LIX1L on bile acid synthesis during cholestasis was revealed, and the absence of Lix1l attenuated cholestatic liver damage in three animal models. At the same time, miR-191-3p expression was observed to be markedly reduced in cholestatic liver damage, and deletion of LIX1L restored miR-191-3p expression to attenuate liver injury. Adeno-associated virus—mediated hepatic delivery of miR-191-3p obviously reduced cholestatic liver injury in Mdr2<sup>−/−</sup> mice, which was via down-regulating liver receptor homolog-1, thus decreasing cholesterol 7 α-hydroxylase and cytochrome P450, family 8, subfamily b, polypeptide 1 expression.79 Hence down-regulation of LIX1L and overexpression of miR-191-3p may represent potential targets for the therapy of cholestatic liver injury.

In summary, the role of miRNAs during cholestasis is extensively studied in multiple animal models (Table 2), which helps to elucidate the promising diagnostic and therapeutic values of miRNAs in cholestatic liver diseases.

The Role of lncRNAs in Cholestatic Liver Diseases

lncRNAs are a type of ncRNAs that are composed of >200 nucleotides. Most lncRNAs are transcribed from RNA polymerase II, then followed by capping, splicing, and polyadenylation.80 Unlike miRNAs, there is poor conservation of lncRNAs between different species, and they have exquisite species- and tissue-specific expression, thus leading to their multiple functions. lncRNAs exert diverse

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functions by interacting with DNA, RNA, and protein, often through complex three-dimensional configurations.2

Recently, IncRNAs have become a focal point in liver disease research. With the aid of high-throughput gene sequencing technology, numerous studies on IncRNAs in various diseases have been conducted, and researchers are actively exploring the roles of IncRNAs in cholestatic liver injury. However, only a few IncRNAs, such as H19, maternally expressed gene 3 (MEG3), and LINC00663, have been the best characterized in cholestasis.3

H19

The IncRNA H19 is an imprinted and maternally expressed gene, which is conserved between humans and mice. H19 is involved in the regulation of cell differentiation and proliferation.8,2 It is abundantly expressed in fetal tissue, but seldom expressed in adult liver. However, its expression can be induced when there is liver injury.8 Since then, it has been widely studied in liver diseases.8,2,38 Recently, the role of H19 during cholestasis has been reported, which provided new insights into the pathogenesis of cholestatic liver disease.82,84

The anti-apoptotic protein Bcl-2 can activate caspase 8 and enhance the degradation of SHP in hepatocytes, which promotes the increased H19 expression and bile acid homeostasis, eventually resulting in liver fibrosis.85 The enhanced expression of H19 not only promotes liver fibrosis caused by BDL, carbon tetrachloride, and Mdr2 deficiency, but also participates in the progression of human PSC, PBC, and BA.83,84,86,87 The levels of H19 in the liver and serum exosome are positively correlated with severity of fibrotic liver damage in patients with BA.82 Cholangiocyte-derived H19 contributed to proliferation of cholangiocytes because it induced higher expression of sphingosine 1-phosphate receptor 2 and sphingosine kinase 2 and prohibited the effects of miRNA let-7 family, inducing elevated expression of high-mobility group AT-hook 2.82

It is well known that HSC activation is a key event during the progression of hepatic fibrosis. H19 was found to promote HSC activation during cholestatic liver fibrosis.83 The deficiency of H19 can protect BDL and Mdr2−/− mice from cholestatic liver fibrosis.83 The exosomes carrying H19 derived from cholangiocytes exacerbated liver fibrosis in BDL mice with depletion of H19 or Mdr2/H19 double-knockout mice.83 Furthermore, hepatic overexpression of H19 contributed to carbon tetrachloride−induced liver fibrosis because it markedly increased HSC activation and EMT of hepatocytes by activating TGF-β signaling pathway. The mechanism was related to the completion of H19 against miR-148a, maintaining the level of ubiquitin-specific protease 4, an identified target of miR-148a, which helped to stabilize TGF-β receptor 1.84

Cholangiocyte-derived exosomal H19 from wild-type mice inhibited the nuclear receptor Shp expression in hepatocytes, which may be the nuclear receptor Shp expression in hepatocytes, which may be the mechanism of the mechanisms associated with the role of H19 in cholestatic liver injury.10 H19-carrying exosomes from cholangiocytes can be rapidly taken up by Kupffer cells, which may promote activation of Kupffer cells and secretion of inflammatory cytokines, consequently exacerbating cholestatic liver injury.88 In addition, H19 derived from macrophages was involved in cholestasis. Selective clearance of macrophages alleviated cholestatic liver injury and fibrosis due to H19 deficiency.16 Overexpression of H19 in THP-1 macrophages enhanced the Rho-GTPase CDC42 and RhoA expression.86 H19 was
also found to induce elevated expression of epithelial cell adhesion molecule and decreased expression of hepatic zinc finger E-box binding homeobox 1 in BDL mice; knockdown of epithelial cell adhesion molecule or overexpression of zinc finger E-box binding homeobox 1 alleviated H19-induced fibrosis in these animals.\textsuperscript{84}

In summary, recent studies suggest that targeting cholangiocyte-derived H19 is a promising therapeutic strategy for cholestatic liver injury because H19 can contribute to cholangiocyte proliferation, HSC activation, liver inflammation, EMT of hepatocytes, and disruption of bile acid homeostasis (Figure 2). Nonetheless, it is essential to delve into the mechanisms and pathways implicated in the elevated expression of H19 during cholestasis, as well as identify the miRNAs that may be regulated by H19.

**MEG3**

MEG3 is a matrilineal expression imprinted gene found by Miyoshi et al\textsuperscript{85} in 2000, which is located on human chromosome 14 q32.3, approximately 1.6 kb in length. MEG3 is the human homolog of gene trap locus 2 (Gtl2) in mice. MEG3 does not encode any protein, which functions at the RNA level. Moreover, MEG3 is expressed in many normal tissues, and there are 12 subtypes in MEG3 family, which is also the first lncRNA to be found to act as a tumor suppressor.\textsuperscript{90}

It was found that MEG3 can contribute to hepatic insulin resistance in high-fat diet or ob/ob mice.\textsuperscript{91} The expression of MEG3 was down-regulated in patients with liver fibrosis and in mice with carbon tetrachloride--induced liver fibrosis. MEG3 gradually decreased along with the progression of liver fibrosis, and this effect was due to MEG3 methylation.\textsuperscript{92} Overexpression of MEG3 in LX-2 cells reduced cell proliferation, promoted cell apoptosis, and increased Col1A1 expression.\textsuperscript{93} MEG3 is gradually decreased along with the progression of liver fibrosis. MEG3 may induce increased alanine transaminase expression and AST levels and disrupt bile acid homeostasis. Elevation of MEG3 in hepatocellular carcinoma cells was verified to act as a guide RNA scaffold to enhance polyphosphatidylactone-binding protein 1 recruitment to SHP mRNA, leading to SHP decay, which could be the reason that MEG3 induced cholestatic liver damage.\textsuperscript{93} In the future, additional studies should be conducted to verify whether MEG3 can be considered as a viable therapeutic target for cholestatic liver disease.

**LIN00663**

LIN00663 was found to be up-regulated in activated HSCs (LX-2). LINCO0663 can sponge miR-3916 and contribute to the activation, EMT, and migration of cultured LX-2 cells. Overexpression of LIN00663 aggravated cholestatic liver fibrosis in BDL mice.\textsuperscript{94} Furthermore, it was found that forkhead box A1 up-regulated LIN00663 by interacting with the promoter of LIN00663.\textsuperscript{95} LIN00663 regulated splicing factor 2–fibroblast alternative splicing by splicing hsa-miR-3916.\textsuperscript{94} The alternative spliced form of fibroblast, EDA-fibroblast, may induce fibroblast proliferation and differentiation, EMT, extracellular matrix production, and tissue fibrosis.\textsuperscript{95} Therefore, the forkhead box A1/LINC00663/hsa-miR-3916/splicing factor 2–fibroblast axis may represent a potential target for the treatment of cholestatic liver disease.

**Conclusions and Prospects**

miRNAs and IncRNAs have been verified to be closely related to the development of cholestatic liver diseases.\textsuperscript{96} miRNAs are potential new biologic indicators for diagnosis, treatment, and prognosis estimation of cholestatic liver diseases.\textsuperscript{96} The recent advancements in miRNA and IncRNA research have enhanced the understanding of the significance of these ncRNAs in the progression of cholestatic liver diseases. However, certain issues remain to be further clarified. For example, the diversity of miRNAs and IncRNAs in the exosomes derived from different cells as well as their special functions during cholestatic liver diseases are important aspects that scientists urgently need to clarify. IncRNAs research is a relatively new focus in human chronic liver diseases. As the identification and evaluation of ncRNAs continue to advance, the potential discovery of additional IncRNAs involved in cholestatic liver diseases and their impacts on disease progression becomes more feasible. The gradually emerging insights into the regulatory effects of these ncRNAs on liver function have started to influence research, particularly in the exploration of effective drugs. Further studies are essential to elucidate the roles of these ncRNAs and develop miRNA/IncRNA-based therapies for cholestatic liver diseases.

**Author Contributions**

J.X. and Y.W. conceptualized the study, provided resources, supervised and administered the project, and acquired funding; L.H., B.L., H.W., C.D., B.Z., J.W., and R.C. reviewed and modified the manuscript; Y.Z., Y.L., W.H., J.X., F.M., and Y.W. reviewed and edited the manuscript; Y.L., W.H., and Y.W. prepared tables and figures; Y.Z., Y.L., and Y.W. wrote the manuscript; and J.X. and Y.W. finalized the manuscript. All authors read and gave approval for publication.

**Disclosure Statement**

None declared.

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