Cholangiopathies are diseases involving the intrahepatic biliary tree. They appear to involve chronic inflammation of the bile ducts, which can lead to the development of bile duct cholestasis, proliferation/ductopenia, biliary fibrosis, and malignant transformation. Sustained stimulatory insults to bile duct epithelial cells can induce a ductular reaction, which has a key role in the initiation and progression of cholangiopathies. The epithelial-mesenchymal interaction between reactive cholangiocytes and mesenchymal cells with the inflammatory infiltrates plays a major role in this pathogenesis. Cytokines, chemokines, growth factors and morphogens mediate these interactions in an autocrine or paracrine manner.

Main hepatic myofibroblasts (MFs) in cholangiopathies originate from portal fibroblasts. Hepatic stellate cells and fibrocytes also transform into MFs. Whether cholangiocytes or hepatocytes are a source of MFs via the epithelial-mesenchymal transition (EMT) remains a matter of controversy. Although there have been numerous indirect findings supporting the theory of a cholangiocyte EMT in human tissues, recent studies using lineage tracing methods have demonstrated strong evidence against the EMT. Understanding the pathogenic mechanisms involved with cholangiopathies can allow for better-targeted anti-fibrotic therapies in animal models. Before anti-fibrotic therapies can translate into clinical trials, improved monitoring of the fibrotic progression of cholangiopathies and an accurate assessment regarding the effectiveness of the proposed treatments must be achieved.

Key Words: Cholangiopathies; Epithelial-mesenchymal interaction; Epithelial-mesenchymal transition; Anti-fibrotic therapy

INTRODUCTION

Cholangiopathies are diseases of the intrahepatic biliary tree, in which biliary epithelial cells (BECs) are the primary target in the pathogenesis. Cholangiopathies evolve from chronic inflammation of bile ducts, leading to the development of cholestasis, bile duct proliferation and/or ductopenia. Ultimately, they may progress to biliary fibrosis and malignant transformation of bile ducts. Malignant transformation from chronic inflammation has been encountered in many clinical situations.

The pathogenic mechanisms involved with cholangiopathies remain unknown. BECs may collaboratively work with mesenchymal cells, inflammatory cells and the extracellular matrix (ECM) in the periductal space by secreting inflammatory cytokines, chemo-attractant proteins and/or by sharing cognate receptors with mesenchymal cells. Activated hepatic stellate cells (HSCs), portal fibroblasts (PFs), and fibrocytes of bone marrow origin have been shown to have fibrogenic potentials in cholangiopathies, but their relative contributions remain incompletely understood. The reversibility of hepatic fibrosis even in advanced stage has stimulated research for antifibrotic therapies.

This review summarizes the current findings surrounding potential pathogenic mechanisms involved with cholangiopathies, with a focus on the roles of cholangiocytes. In addition, targeted therapies to reverse cholangiopathies in animal models will be introduced.

PATHOGENESIS OF CHOLANGIOPATHIES

The repair processes of damaged bile ducts involve two distinct pathways, regeneration and fibrosis. During regeneration, injured cells are replaced by the same type cells without permanent structural damage when inflammatory reactions to the biliary tree are transient. However, when chronic inflammation is induced by the derangement of the host’s responses or because of chronic insults to bile ducts, fibrosis develops and connective tissues replace normal parenchymal tissues. Cholangiopathies are a heterogenous group of liver diseases, largely in part due...
to the varying degree of regeneration or fibrosis based on an individual’s intensity and chronicity of the intrahepatic biliary tree insults. These diseases are caused by different kinds of etiologies, such as genetic, immune-mediated, infectious, drug induced, vascular/ischemic disorders and cholangiocarcinomas (Table 1). Despite their heterogeneity, cholangiopathies share a number of basic pathogenic mechanisms and common features such as cholestasis, cholangiocyte proliferation, ductopenia, portal fibrosis and carcinogenesis. The central mechanism for most manifestations involves an inflammatory reaction. Cholangiocyte proliferation can be induced by various stimuli to bile ducts in the early stage of cholangiopathies. As it advances, a decrease in the number of bile ducts ensue in most late stage cholangiopathies. To this end, ductopenia may result primarily from excessive apoptosis that dominates over cholangiocyte proliferation. On the other end of the spectrum, inhibition of apoptosis may lead to cholangiocyte hyperplasia that could facilitate malignant transformation of cholangiocytes. In most cholangiopathies, an extensive fibrotic response takes place in the portal tracts. Biliary fibrosis develops as part of the wound healing response to bile duct injury in chronic cholestatic liver diseases. Because fibrosis is the result of prolonged activation of tissue repair mechanisms, marked liver fibrosis called cirrhosis, is present in the late-stage of cholangiopathies (Fig. 1).

EPITHELIAL-MESENCHYMAL INTERACTIONS IN CHOLANGIOPATHIES

Epithelial-mesenchymal interactions play a major role in the molecular mechanisms involved with chronic cholangiopathies. Sustained signals to cholangiocytes induce cholangiocyte proliferation and lead to the development of reactive cholangiocytes. In the presence of chronic inflammation, the interactions between reactive cholangiocytes, mesenchymal cells, and the inflammatory infiltrates eventually promote biliary fibrosis, and ultimately determine the clinical progression of cholangiopathies (Fig. 2).

1. Cells involved in cholangiopathies

Cholangiocytes and reactive cholangiocytes interact with mesenchymal cells (HSCs, PFs, myofibroblasts [MFs], fibrocytes), endothelial cells, macrophages, and lymphocytes.

1) Cholangiocytes and reactive cholangiocytes

Cholangiocytes, the epithelial cells that line the biliary tree, are heterogenous. Large cholangiocytes are located at the level of interlobular and major bile ducts and they express several

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**Table 1. The Common Causes of Cholangiopathies**

| Immune-mediated diseases | Genetic or inherited diseases |
|--------------------------|------------------------------|
| Primary biliary cirrhosis| Alagille’s syndrome           |
| Primary sclerosing cholangitis | Cystic fibrosis          |
| Graft versus host diseases | Fibropoly cystic diseases* |
| Allograft rejection       | Multidrug resistance-3 deficiency |
| Autoimmune cholangitis    | Idiopathic diseases         |
| Infectious diseases       | Biliary atresia             |
| Bacterial cholangitis     | Sarcoidosis                 |
| Parasitic cholangitis     | Idiopathic adulthood ductopenia |
| Fungal cholangitis        | Malignant diseases          |
| Viral cholangitis         | Cholangiocarcinoma          |
| Drug-induced diseases     | Ischemic diseases           |

*Include autosomal dominant polycystic kidney disease, autosomal recessive polycystic kidney disease, autosomal dominant polycystic liver disease, and Caroli and congenital hepatic fibrosis.

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**Fig. 1.** A putative pathogenic model of cholangiopathies. The initial insult to biliary epithelial cells and the host response may induce an inflammatory reaction. It generally resolves with the resolution of the insulting agent to the biliary tree. However, the persistence of insults to the biliary tree and/or derangement of the host response will lead to chronic inflammation, cholestasis, and bile duct proliferation and ductopenia. Ultimately, chronic cholangiopathies progress to biliary fibrosis and/or malignant transformation.
different ion channels and transporters at the basolateral or apical domain. Smaller bile duct branches, including terminal cholangioles and canals of Hering, can acquire some mesenchymal cell phenotypes in response to the inflammatory reaction during liver damage. These cells have the propensity to have reactivity and plasticity and behave as liver progenitor cells.

Long stimuli to BECs induce ductular reaction. Ductular reaction is characterized as a marked expansion of cholangiocytes or progenitor cell proliferation with dynamic mesenchymal cell interactions. It plays a key role in the initiation and progression of biliary fibrosis. Ductular reactions switch resting cholangiocytes to reactive cholangiocytes. Reactive cholangiocytes are believed to derive from a progenitor cell compartment located in close proximity to terminal cholangioles in the canals of Hering. They appear to play the role of “the pace-maker for portal fibrosis.” These cells secrete proinflammatory, chemotactic cytokines, and growth factors that enable them to recruit inflammatory cells and mesenchymal cells. They activate MFs and stimulate angiogenesis by secreting several cytokines. They express adhesion molecules that control cell-cell and cell-ECM interactions and attenuate differentiated epithelial phenotypes. A number of studies have suggested that reactive cholangiocytes have a major role in the induction of biliary fibrosis.

2) Mesenchymal cells

HSCs are the main resident mesenchymal cell in normal liver. During the quiescent state, HSCs are located in the subendothelial space of Disse and store vitamin A. HSCs are highly responsive to stimuli such as oxidative stress and proinflammatory cytokines released during inflammation. During an activated state, HSCs lose their stored retinoids and transform into a MF-like cell. Besides HSCs, PFs and cells of bone marrow origin have recently been shown to have fibrogenic potential.

PFs are located in close vicinity to the interlobular bile ducts in the portal space. Signals derived from reactive cholangiocytes induce proliferation. Transdifferentiation of PFs into portal MFs and PFs can regulate proliferation of BECs. The contribution of each MF precursor in the different etiologies in chronic liver diseases remains controversial. A recent study suggested that the origins of main MFs are different in various liver diseases. In a CCl₄ injury model, HSCs are the predominant source of MFs, whereas PFs are predominant in biliary fibrosis. Also, one study showed that HSCs do not undergo myofibroblastic differentiation in biliary fibrosis in two cholestatic injury rat models involving arterial liver ischemia and bile duct ligation (BDL).

Bone marrow-derived fibrocytes can also be transformed into liver MFs. However, the proportion is around 5% to 10% of all type I collagen-expressing cells and they disappear after the early phase in BDL rats. As a result, the clinical significance of fibrocytes may be minor.

It has been suggested that cholangiocytes or hepatocytes might transform into mesenchymal cells via epithelial-mesenchymal transition (EMT). Whether EMT may contribute to the generation of liver MFs is still a matter of controversy and requires further study.
cytokine secretion, and regulation of angiogenesis and immune responses. They express α-smooth muscle antibody (α-SMA) and have biologic properties of motility and contractility. In cholangiopathies, MFs are localized mainly around the portal space and crosstalk with reactive cholangiocytes by sharing several agonists and receptor systems.22

3) Endothelial cells and macrophages

Endothelial cells regulate vascular remodeling associated with factors able to induce angiogenesis. In cholangiopathies, a brisk angiogenesis takes place in close vicinity to the damaged bile ducts. Endothelial cells have the ability to evoke angiogenesis and interact with mesenchymal cells or can transition into mesenchymal cells. In primary biliary cirrhosis (PBC), an increased number of vascular structures in the inflamed portal tracts together with upregulation of proangiogenic factors have been observed.23

Kupffer cells, the most common resident macrophages in the liver, are actively involved in the initiation of fibrogenesis by producing inflammatory mediators. Kupffer cells are also involved in the resolution of liver cirrhosis by their ability to degrade ECM components and secrete several matrix metalloproteinases (MMPs).24,25 In PBC, liver-infiltrating macrophages enhance the proinflammatory activity of cholangiocytes in response to toll like receptor stimulation.26 On the other hand, after restoring bile flow in BDL animal models, macrophages appear to clear apoptotic cholangiocytes in portal tracts, and secrete several MMPs, remodeling the fibrous septa and reversing biliary fibrosis.27

4) ECM

The ECM consists of different structural components, including collagens, fibronectin and proteoglycans and is a reservoir for multiple growth factors, cytokines, and MMPs. The ECM provides multiple functions; providing tensile strength and resilience, modulating diffusion and vascular flow, regulating cell movement and signaling, in addition to serving as ligands and receptors.28 It modulates the interactions between epithelial cells and the stromal microenvironment and signals derived from the ECM regulate surrounding cells.

2. Signals regulating epithelial-mesenchymal interactions in cholangiopathies

Various cytokines, growth factors and morphogenic signals induce inflammatory cells to infiltrate into periductular spaces and activates immunity, angiogenesis, cellular proliferation, and ECM deposition.22 Proinflammatory and chemotactic cytokines such as interleukin (IL)-1, IL-6, IL-8, tumor necrotic factor (TNF-α), interferon (IFN)-γ, nitric oxide (NO), stromal cell-derived factor-1 (SDF-1), and monocyte chemotactic protein-1 (MCP-1), growth factors such as transforming growth factor-β (TGF-β), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), connective tissue growth factor (CTGF), and angiopoietin-1, -2 are secreted by cholangiocytes, mesenchymal cells, inflammatory cells and endothelial cells. Also wingless/β-catenin (Wnt/β-catenin) signaling, Hedgehog (Hh) and Notch ligands are released from HSCs, reactive cholangiocytes and MFs. Cognate receptors are also expressed on these cells.22

1) Proinflammatory and chemotactic cytokines

Most cholangiopathies are associated with significant amounts of inflammatory infiltrate in the portal spaces. “Reactive” cholangiocytes secrete proinflammatory and chemotactic cytokines such as TNF-α, IL-1, IL-6, IL-8, MCP-1, IFN-γ, and NO that have an effect on the function of inflammatory cells. INF-γ promotes MHC class II expression in human cholangiocytes. MCP-1, released from cholangiocytes, promotes PFs proliferation, myofibroblastic differentiation, and procollagen-1 messenger RNA expression.29

SDF-1 is a cytokine with chemoattractant properties for monocytes, lymphocytes, hematopoietic stem cells, and B cell precursors. In immune-mediated cholangiopathies, such as PBC and primary sclerosing cholangitis (PSC), SDF-1 is selectively upregulated in cholangiocytes and recruits CXC chemokine receptor 4 (CXCR4), SDF-1 receptor-positive infiltrating T lymphocytes around bile ducts. Also, CXCR4, expressed in HSCs, induce HSC activation, proliferation, and production of collagen by administration of SDF-1.30

2) Growth factors

(1) TGF-β

TGF-β is currently considered the most potent fibrogenic cytokine in the liver. TGF-β is known to stimulate HSC activation, PF differentiation into liver MFs, and matrix production. TGF-β production is strongly up-regulated in mainly HSCs, cholangiocytes, and KCs.12

(2) PDGF

PDGF is recognized as the most potent mitogen for HSCs. It stimulates HSC proliferation and migration and induces HSCs transdifferentiation into MFs. PDGF-B subtype has a central role in biliary repair as well as in biliary fibrosis. Following BDL in rats, PDGF is expressed in reactive cholangiocytes and stimulates HSCs chemotaxis toward bile ducts, and conversion of PFs into portal MFs.31

(3) VEGF and angiopoietins

Cholangiocytes, HSCs, and endothelial cells may express VEGF and its cognate receptors. In BDL rodents, both VEGF and its cognate receptors are up-regulated in cholangiocytes and stimulate proliferation.32 VEGF may also contribute to liver fibrosis. It stimulates proliferation of activated HSCs and increases collagen production, migration and chemotaxis of human HSCs. Angiopoietins are a different family of vascular growth factors that act in concert with VEGF to promote the remodeling, matu-
reration, and stabilization of blood vessels.\(^{33}\)
(4) CTGF

Reactive cholangiocytes are the main sources of CTGF in experimental BDL animal models.\(^{34}\) CTGF promotes proliferation and collagen production in HSCs.\(^{35}\) Also, it induces extensive fibrosis in biliary atresia and desmoplastic reactions in cholangio-carcinomas.\(^{36}\)

(5) HGF

In cholangiopathies, HGF has the ability to enhance or prevent fibrosis. HGF is released from MFs, neutrophils and stromal cells and it binds to the Met receptor expressed in the reactive cholangiocytes and HSCs. Complex interactions between the inflammatory cells, stromal cells and cholangiocytes result in a dysmorphic repair response that leads to cirrhosis.\(^{37}\) On the other hand, HGF is a potent growth factor for cholangiocytes and also works as a blockade of biliary EMT. Cholangiocytes treated with HGF have an attenuated transition toward a mesenchymal phenotype. They appear to prevent hepatic MF activation and biliary fibrosis.\(^{38}\)

3) Morphogens

(1) Hh

Hh signaling involved in the development and progression of cancer and also in the repair process in tissue injury. Hh ligands released by MFs activate Hh signaling in reactive cholangiocytes, endothelial cells, and liver progenitor cells.\(^{39}\) In the liver of PBC patients, Hh ligands and Hh target genes are present in bile ductules and stromal cells.\(^{40}\) PDGF-B increases Hh production in HSC, and the Hh would then promote the acquisition of EMT features by reactive cholangiocytes.\(^{41,42}\)

(2) Wnt/\(\beta\)-catenin

In cholangiopathies, activated Wnt/\(\beta\)-catenin pathways in-

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Table 2. The Studies on the Epithelial-to-Mesenchymal Transition of Cholangiocytes

| Study materials | Methods | EMT associated genes | EMT evidences (or for against) | Year, references |
|-----------------|---------|----------------------|-------------------------------|-----------------|
| **For EMT**     |         |                      |                               |                 |
| BDL rodent      | IHC, QRT-PCR, coculture | Hh, \(\alpha\)-SMA, collagen \(\alpha\)1, FN | Hh modulates epithelial-mesenchymal transition | 2007\(^{39}\) |
| Cholangiocyte    |         |                      | Interaction in cholangiopathy | 2008\(^{41}\) |
| PBC liver tissue | IHC, QRT-PCR, microarray, migration assay | Hh, S100A4, Gli2, vimentin | BECs of PBC and BDL show ductular reaction and EMT via Hh pathway | 2008\(^{40}\) |
| BDL rat         | IHC, QRT-PCR, immunocytochemistry | Hh, Gli1,2,3, S100A4, vimentin, N-cadherin, Snail | BECs of BA show ductular reaction and EMT | 2011\(^{42}\) |
| BA liver tissue | BDL rat | S100A4, vimentin, pSMAD 2/3 | TGF-\(\beta\)-1-mediated EMT has a role in the formation of hepatolobiosis | 2010\(^{50}\) |
| BA liver tissue | IHC     |                      | EM1 occurs in human liver fibrosis | 2008\(^{48}\) |
| BA liver tissue | IHC     |                      | E-cadherin, \(\alpha\)-catenin, \(\alpha\)-SMA, vimentin, S100A4, TGF-\(\beta\)-1, pSMAD 2/3 | 2008\(^{48}\) |
| Recurrent PBC liver tissue | IHC | S100A4, vimentin, pSMAD 2/3 | EMT of cholangiocytes may be an initiating event of PBC recurrence | 2007\(^{51}\) |
| BDL rat         | IF, IHC, WB, RT-PCR | \(\alpha\)-SMA, CK-19, S100A4 | HGF ameliorates biliary fibrosis in part by \(\alpha\)-SMA, CK-19, S100A4 | 2006\(^{48}\) |
| BDL rat         | IF, IHC, WB, RT-PCR | Collagen I/III, hsp47, TGF-\(\beta\) | EMT of cholangiocytes | 2008\(^{52}\) |
| Primary human BECs | IF, Invasion assay, In situ hybridization, IHC | S100A4, vimentin, MMP2, \(\alpha\)-SMA, pSMAD 2/3, TGF-\(\beta\)| EMT of cholangiocytes may induce biliary fibrogenesis by TGF-\(\beta\)-1 or infiltrating T cells | 2008\(^{47}\) |
| Hepatotitis liver tissue | IF, IHC, QRT-PCR, WB | S100A4, vimentin, \(\alpha\)-SMA, procollagen 1\(\alpha\)2 | EMT of cholangiocytes induced with poly(I:LC) contributes to the sclerosing cholangiopathy of BA | 2009\(^{52}\) |
| **Against EMT** |         |                      |                               |                 |
| BDL rat         | Cell fate labeling, QRT-PCR, IF, IHC | \(\alpha\)-SMA, desmin, FSP-1, collagen \(\alpha\)1 | EMT of cholangiocytes identified by genetic labeling does not contribute to hepatic fibrosis in mice. | 2010\(^{55}\) |
| BDL rat         | Cell fate labeling, QRT-PCR, IF, IHC | S100A4, vimentin, \(\alpha\)-SMA, procollagen 1\(\alpha\)2 | Cholangiocytes do not undergo EMT in murine models of biliary fibrosis. | 2011\(^{53}\) |

EMT, epithelial-mesenchymal transition; BDL, bile duct ligation; IHC, immunohistochemical staining; QRT-PCR, quantitative reverse transcription polymerase chain reaction; Hh, Hedgehog; SMA, smooth muscle antibody; FN, fibronectin; PBC, primary biliary cirrhosis; Bec, biliary epithelial cell; IF, immunofluorescence; Western blot, hsp47, heat shock protein 47; BECs, human biliary epithelial cells; CLD, chronic liver diseases; CK, cytokeratin; K19\(^{47}\), cholangiocyte-expressed yellow fluorescent protein (YFP); FSP-1, fibroblast-specific protein-1; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; poly(I:LC), polyinosinic-polycytidylic acid, a synthetic analogue of viral dsRNA.
duce cholangiocyte proliferation and biliary differentiation. Wnt pathway is involved in HSC activation and the transdifferentiation of HSCs into MFs.\textsuperscript{41}

(3) Notch

Notch signaling pathways have a role in regulating cell fate determination and in the maintenance of organ phenotypes. Four transmembrane receptors and 5 ligands are involved in this pathway. Notch pathway interacts with Wnt, Hh, and TGF-β. Reactive cholangiocytes express Jagged-1, 2 and Notch 2. Jagged-1 mutation induces Alagille’s syndrome.\textsuperscript{44} The roles of Notch pathway in cholangiopathies have not been explored.

3. Potential role of cholangiocyte EMT in cholangiopathies

Whether or not cholangiocytes transform into mesenchymal cells via EMT is a matter of controversy. EMT describes epithelial cells that adopt structural and functional characteristics of mesenchymal cells: loss of polarity, changes in cell-cell contacts, spindle-like shape, functional mobility changes to surrounding stroma, and production of ECM.\textsuperscript{45} Cholangiocytes are believed to participate in the generation of liver fibrosis by undergoing EMT. Reactive cholangiocytes lose their epithelial characteristics such as E-cadherin, CK-7, or CK-19 and acquire a mesenchymal phenotype as manifested by the expression of fibroblast-specific markers such as the fibroblast specific protein-1 (FSP-1) or vimentin, the ability to migrate and to produce ECM components such as collagen, fibronectin, elastin, and tenascin. The accumulating evidence indicates that EMT probably has a critical role in the process of portal fibrosis during chronic liver diseases (Table 2).\textsuperscript{46,47} Evidence favoring EMT of BEC comes from immunohistochemical staining of tissue in human biliary fibrosis, such as PBC,\textsuperscript{48} biliary atresia,\textsuperscript{49,50} and oriental cholangiohepatitis.\textsuperscript{51} In the livers of human cholangiopathies, co-localization of CK19 (marker of BEC), and vimentin (markers of mesenchymal cell) and increased expression of snail and FSP-1 in proliferative bile ductular cells demonstrate that EMT might occur in biliary fibrosis.\textsuperscript{52} Similar results have been demonstrated in post-transplantation recurrence of PBC. Biliary EMT, indicated as cholangiocyte expression of FSP-1, vimentin and pSMAD 2/3 and which is driven by TGF-β, occurs before the appearance of any other signs of PBC recurrence.\textsuperscript{53} This study suggests that EMT may be an initiating event and could explain the basic pathogenic mechanisms in this disease. The co-localization was particularly marked in small ducts and cells of the ductular reaction, and in diseases like PBC and biliary atresia in which the ductular reaction is most prominent.\textsuperscript{49,55} Another study using tissue sections of BDL induced biliary fibrosis showed BECs not only presenting with co-localization of CK-19 and S100A4, but also with deposition of type I and type III collagen.\textsuperscript{38}

Evidences for cholangiocyte EMT can also be found in cultured cholangiocytes. TGF-β treated BEC in culture undergo EMT and exhibit the acquisition of a MF-like morphology and de novo expression of α-SMA and collagen I.\textsuperscript{39,47} Another experiment revealed that stimulated human BECs with a synthetic analogue of viral dsRNA transformed them into mesenchymal cells, with a resultant increase in the expression of mesenchymal markers and a decrease of epithelial markers. This result suggested that the innate immune response to dsRNA in BECs plays an important role in peribiliary fibrosis via biliary EMT.\textsuperscript{55} Also, the Hh signaling pathway, which is known to be a positive effector of EMT in other tissues, is activated in both cholangiocytes and fibroblastic cells in BDL models and in the livers of PBC patients.\textsuperscript{39-41}

Recently, Chu et al.\textsuperscript{51} reported the strongest evidence against liver epithelial EMT as a source of MFs. They traced the cell fate in three murine models of hepatic fibrosis, in which liver epithelial cells are heritably labeled with yellow fluorescent protein. The result indicated that none of the MFs originated from the genetically marked epithelial cells. This result was consistent with two previous studies.\textsuperscript{54,55} The first study reported evidence against hepatocyte EMT using the robust albumin cre mouse. They demonstrated that hepatocytes do not transform MFs in CCI4-induced hepatic fibrosis.\textsuperscript{54} The second study addressing cholangiocyte EMT used an inducible cytokeratin-19 cre mouse to mark hepatic fibrosis rodents induced with BDL or CCI4 treated. They failed to detect any MFs in the fibrotic liver that originated from cholangiocytes.\textsuperscript{55} Although reactive cholangiocytes express several morphologic and functional markers commonly associated with mesenchymal phenotypes, direct evidence that cholangiocytes are able to transdifferentiate into MFs does not exist.

**ANTI-FIBROTIC TARGET THERAPIES IN BILIARY FIBROSIS**

Treatment goals for cholangiopathies are to eliminate causative factors or to provide anti-fibrotic therapy. In BDL induced biliary fibrosis, restoration of bile flow triggers recruitment of macrophages into scarred portal tracts to remove apoptotic cholangiocytes via phagocytosis. Bile flow also helps to upregulate MMPs to remodel the scar, leading to dissolution of fibrosis.\textsuperscript{56} Elimination of causes is not always possible in clinical situations such as PBC, PSC, or BA and it is not enough to reverse cholangiopathies in advanced cholangiopathies. Recent research has shed light about the pathogenic mechanisms for fibrosis, highlighting the cells and signals related to this dynamic process. Increased knowledge of the disease pathophysiology may provide some insights on how to stop or reverse it. Since the cellular sources of major fibrogenic cells may differ among different etiologies, the relative value of a particular anti-fibrotic therapy also may depend on the underlying disease process. In hepatic fibrosis, HSC/MF apoptosis and macrophage-mediated phagocytosis of apoptotic hepatocytes are vital mechanisms that contributes to the recovery from hepatic fibrosis.\textsuperscript{56,57} Because reactive cholangiocytes have a major role as pacemakers for cholangiopathies, preventing or limiting cholangiocyte prolif-
Mechanisms of antifibrotic effects

Targets
Liver collagen
Number
BDL rat
BDL rat

Proliferation
Liver fibrosis
2010
2002
2002
BDL rat

Number
PPAR

Animal model
Cholangiocyte, TGF-
HSCs
HSCs, PFs
MF
1997
2006
HSCs
HSCs
BDL rat
BDL rat
2006
ProcollagenI

Proliferation

In vitro
In vivo
inhibitor of metalloproteinase; PF, portal fibroblast; PIIINP, propeptide of procollagen type III; CTGF, connective tissue growth factor.

induced cAMP levels, similar to levels found in animals with proliferation, secretin receptor gene expression and secretin-lithocholic acid feeding, there was an increase in cholangiocyte increased secretin induced cAMP response and exchanger activity have varied effects on biliary function, apoptosis and growth. In vitro, they stimulated cholangiocyte proliferation and increased secretin induced cAMP response and exchanger activity in isolated rat cholangiocytes. With taurocholate and tauro-lithocholic acid feeding, there was an increase in cholangiocyte proliferation, secretin receptor gene expression and secretin-induced cAMP levels, similar to levels found in animals with BDL. On the contrary, ursodeoxycholate and taurodeoxycholate have been shown to inhibit cholangiocyte proliferation in BDL cholangiocytes, both in vitro and in vivo. The farnesoid X receptor (FXR) is a key regulator of hepatic bile acid homeostasis, the inflammatory response, and liver regeneration. Recent studies reported mRNA expression of FXR in HSCs suggesting that FXR could represent a therapeutic target for the treatment of liver fibrosis. FXR ligands were reported to repress collagen expression in HSCs. FXR protects against hepatic fibrosis in two mouse models for biliary types of liver fibrosis but does not influence hepatic fibrosis such as CCl4. Atorvastatin, HMG-CoA reductase inhibitors, is also effective for inhibiting HSC activation and fibrosis in the BDL model in the early stage, but therapy lacked significant effects on fibrosis during the later stages. Silymarin, a standardized plant extract containing 60% polyphenole silibinin, is effective for reducing biliary fibrosis based on reduced liver collagen content and serum aminoterminal propeptide of procollagen type III on HSCs and PFs in bile duct occlusion model. Pentoxifylline inhibits HSC proliferation and collagen synthesis in vitro, but only moderate decrease in fibrosis in BDL rats. Pentoxifylline can reduce procollagen I, TGF-
, and CTGF effectively, however, TIMP-1 is also elevated. To use pentoxifylline as a potent anti-fibrogenic tool in chronic liver disease, avoidance of TIMP-1 upregulation is required.

Most reported therapies are effective not in advanced biliary cirrhosis but in biliary fibrosis. In clinical settings, some patients already have advanced to severe biliary cirrhosis. It remains unclear whether or not anti-fibrotic therapies are effective in severe cirrhosis. One study using the CCl4-intoxication model of liver cirrhosis has demonstrated that the remodeling of advanced cirrhosis is limited and the liver remains in a

Table 3. The Anti-Fibrotic Trials in Animal Models of Cholangiopathies

| Agents                        | Targets                  | Mechanisms of antifibrotic effects                                      | Animal model | Year, references |
|-------------------------------|--------------------------|------------------------------------------------------------------------|--------------|------------------|
| αvβ6 integrin inhibitor       | Cholangiocyte, TGF-β     | Proliferation↓, adhesion to ECM↓                                       | BDL rat      | 2007†, 2008†     |
| Sorafenib                     | HSCs                     | Number↓, ECM→                                                          | BDL rat      | 2011‡             |
| HGF gene therapy              | Cholangiocyte, TGF-β     | ASMA↓, collagen I/III↓, hydroxyproline↓, TGF-β↓                        | BDL rat      | 2006‡             |
| Troglitazone                  | PPARγ                    | MF↓, ECM↓                                                              | BDL rat      | 2006§, 2005‡     |
| Ursodeoxycholate, taurodeoxycholate | Cholangiocyte              | Proliferation↓                                                         | BDL rat      | 2002†, 2004‡     |
| FXR agonist                   | HSCs                     | Liver fibrosis↓, collagen↓, TGF-β↓, α-SMA↓, TIMP1, 2↓                  | BDL rat      | 2004§             |
| Atorvastatin                  | HSCs                     | Number ↓, ECM →                                                       | BDL rat      | 2010§             |
| Silymarin                     | HSCs, PFs                | Liver collagen↓, PIIINP↓                                               | BDL rat      | 1997§             |
| Pentoxifylline                | HSCs                     | Procollagen↓, TGF-β↓, CTGF↓, TIMP1↑, liver collagen & fibrosis score & PIIINP↓ | BDL rat      | 2002§             |

TGF-β, transforming growth factor-β; ECM, extracellular matrix; BDL, bile duct ligation; HSC, hepatic stellate cell; HGF, hepatocyte growth factor; PPARγ, peroxisome proliferator activated receptor γ; MF, myofibroblast; FXR, farnesoid X receptor; SMA, smooth muscle antibody; TIMP, tissue inhibitor of metalloproteinase; PF, portal fibroblast; PIIINP, propeptide of procollagen type III; CTGF, connective tissue growth factor.
cirrhotic state. However, the least mature ECM, which forms the micronodules, become degraded, leading to an attenuated macronodular cirrhotic liver. The irreversible fibrosis is extensively cross-linked and relatively rich in ECM molecules. It has relatively hypocellular scars, in which the appropriate cellular mediators are absent. Although anti-fibrotic therapies will be more effective before advanced cirrhosis, this study showed that even in patients with advanced cirrhosis, targeted anti-fibrotic therapies are helpful to reduce the magnitude of cirrhosis.\textsuperscript{20}

**CONCLUSIONS AND FUTURE DIRECTIONS**

The pathogenic mechanisms of cholangiopathies are still largely unknown. An emerging concept is that BECs actively participate in the pathogenesis of cholangiopathies by transformation into a reactive cholangiocytes. BECs have a major role in biliary fibrosis by crosstalk with ECM-producing cells, inflammatory cells, and ECM. BECs also promote fibrosis by secreting proinflammatory and/or chemotactic cytokines and by the expression of adhesion molecules. Whether cholangiocytes work directly as MF via EMT remains a controversy. Also, the contributions of HSCs or PFs in cholangiopathies are still unknown. Many trials showed that biliary fibrosis can be reversed by inhibition of reactive cholangiocytes, completely or partially. However, there still remains no effective treatment based on clinical trials. Before anti-fibrotic therapies can translate into clinical trials, better monitoring for fibrotic progression of cholangiopathies and an accurate assessment regarding effectiveness of proposed treatments must be achieved.

**CONFLICTS OF INTEREST**

No potential conflict of interest relevant to this article was reported.

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