cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

REVIEW

Cellular Interactions and Crosstalk Facilitating Biliary Fibrosis in Cholestasis

Ludovica Ceci,[1] Eugenio Gaudio,[1] and Lindsey Kennedy[2,3]

[1]Department of Anatomical, Histological, Forensic Medicine and Orthopedics Sciences, Sapienza, University of Rome, Italy; [2]Department of Research, Richard L. Roudebush VA Medical Center, Indianapolis, Indiana; and [3]Division of Gastroenterology and Hepatology, Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana

SUMMARY

This comprehensive review highlights important research on biliary fibrosis. We discuss hepatic stellate cells and portal fibroblasts and outline the mechanisms and cellular niches influencing biliary fibrosis in different mouse models.

Biliary fibrosis is seen in cholangiopathies, including primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC). In PBC and PSC, biliary fibrosis is associated with worse outcomes and histologic scores. Within the liver, both hepatic stellate cells (HSCs) and portal fibroblasts (PFs) contribute to biliary fibrosis, but their roles can differ. PFs reside near the bile ducts and may be the first responders to biliary damage, whereas HSCs may be recruited later and initiate bridging fibrosis. Indeed, different models of biliary fibrosis can activate PFs and HSCs to varying degrees. The portal niche can be composed of cholangiocytes, HSCs, PFs, endothelial cells, and various immune cells, and interactions between these cell types drive biliary fibrosis. In this review, we discuss the mechanisms of biliary fibrosis and the roles of PFs and HSCs in this process. We will also evaluate cellular interactions and mechanisms that contribute to biliary fibrosis in different models and highlight future perspectives and potential therapeutics. ***(Cell Mol Gastroenterol Hepatol 2024;17:553–565; https://doi.org/10.1016/j.jcmgh.2024.01.005)***

Keywords: biliary fibrosis; portal fibroblasts; hepatic stellate cells; ductular reaction; bile acids; angiogenesis; immune cells.

Cholangiopathies target the bile ducts, leading to fibroinflammatory responses that initiate biliary fibrosis and eventually bridging fibrosis and cirrhosis.[1] Cholangiopathies are diagnosed in pediatric and adult patients, and the 6 most common cholangiopathies include primary sclerosing cholangitis (PSC), primary biliary cholangitis (PBC), cystic fibrosis–associated liver disease, polycystic liver disease, biliary atresia, and cholangiocarcinoma (CCA).[1] PBC and PSC are the most common cholangiopathies, with prevalence reported as 4 in 100,000 and 35 in 100,00, respectively[2,3]; thus, these diseases will be the focus of this review.

During PBC and PSC, cholangiocytes undergo proliferative, senescent, apoptotic, and/or transdifferentiating events, which modulate the microenvironment through the secretion of growth factors, cholangiokines, senescent-associated secretory factors (SASPs), etc. Ductular reaction (DR) is the presence of atypical bile ducts that lack an apparent lumen, and DR may arise from cholangiocytes, hepatocytes, or hepatic progenitor cells (HPCs).[4] It was proposed that senescent cholangiocytes remain at the bile duct and contribute to initial and sustained peribiliary fibrosis via activation of portal fibroblasts (PFs). Contrarily, reactive cholangiocytes undergo proliferation and recruit and activate hepatic stellate cells (HSCs), leading to bridging fibrosis.[5] Cholangiocyte response to injury is multifaceted, and divergent fates differentially modulate fibrosis.

PBC and PSC injury follows similar steps: (1) persistent biliary damage leads to cholestasis, (2) bile acid (BA) buildup causes hepatocellular damage, (3) consequent inflammation and immune cell infiltration occur, and (4) the complex of biliary and hepatocellular damage and inflammation invoke fibrosis.[6] However, there is nuance in fibrogenesis between PBC and PSC. In both PBC and PSC, biliary fibrosis starts from the portal tract and progresses with

Abbreviations used in this paper: **Angpt-1, angiopoietin-1; APC, antigen presenting cell; ASBT, apical sodium-dependent bile acid transporter; BA, bile acid; BAFF, B cell activating factor; BDL, bile duct ligation; BSEP, bile salt export pump; CCA, cholangiocarcinoma; CCl_4, carbon tetrachloride; CD, cluster of differentiation; CDCA, chenodeoxycholate; CDE, choline-deficient, ethionine-supplemented; CK-19, cytokeratin 19; CTL, cytotoxic T lymphocyte; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; DR, ductular reaction; ECM, extracellular matrix; EMT, epithelial to mesenchymal transition; ET, endothelin; FN-1, fibronectin-1; FXR, farnesoid X receptor; Gli-1, glioma-associated oncogene-1; HPC, hepatic progenitor cell; HR, histamine receptor; HSC, hepatic stellate cell; IL, interleukin; KC, Kupffer cell; LCA, lithocholate; MC, mast cell; MCL1, myeloid cell leukemia 1; MCP-1, monocyte chemoattractant protein-1; MDA, methylene dianiline; MoMΦ, monocyte-derived macrophages; MΦ, macrophages; norUDCA, 24-norursodeoxycholic acid; NTCP, Na^+-taurocholate co-transporting polypeptide; OCA, obeticholic acid; PBC, primary biliary cholangitis; PDGF, platelet-derived growth factor; PF, portal fibroblast; PlGF, placental growth factor; PMC, portal mesenchymal cell; PSC, primary sclerosing cholangitis; SASP, senescence-associated secretory phenotype; SLIT2, split guidance ligand 2; TAA, thioacetamide; TCA, taurocholate; TDCA, taurodeoxycholate; TERT, telomerase reverse transcriptase; TGF-β, transforming growth factor-β; Th, T helper; TNF-α, tumor necrosis factor-α; Treg, regulatory T cell; T_{RM}, tissue-resident memory T cells; UDCA, ursodeoxycholic acid; VEGF, vascular endothelial growth factor.**

Most current article

2352-345X
https://doi.org/10.1016/j.jcmgh.2024.01.005

fibrotic septa formation leading to portal-portal pattern fibrosis. At later stages, fibrosis extends to the lobular area and continues into cirrhosis.[7,8] Furthermore, in PBC, the bile ducts are infiltrated by immune/inflammatory cells that damage the portal tract structure and expand the peribiliary stroma. In PSC, patients are characterized by peribiliary inflammation, concentric fibrosis (onion-like structure) and atrophy, and/or disappearance of small bile ducts. Consequently, in both PSC and PBC, the continuous destruction of intrahepatic bile ducts promotes cholestasis.[6]

Fibrosis stage predicts survival in PBC patients independent of biochemical response.[9] Similarly, fibrosis-related scores predict poorer outcomes for PSC patients.[10] In PBC and PSC, biliary fibrosis is a shared pathway, an important prognostic factor, and precedes bridging fibrosis. Obviously, biliary fibrosis is an important pathologic process in PBC and PSC. In this review we will discuss mechanisms and cellular interactions regulating biliary fibrosis.

Hepatic Stellate Cells and Portal Fibroblasts: A Tale of Two Cells

HSCs and PFs are the main hepatic mesenchymal cells that synthesize extracellular matrix (ECM).[11] Previous research hypothesized that fibrocytes and hepatocytes or cholangiocytes undergoing epithelial to mesenchymal transition (EMT) may contribute to fibrosis; however, in fibrotic livers fibrocytes are only 3%–6% of the collagen type I expressing cells,[12] and neither hepatocytes nor cholangiocytes undergo EMT or contribute to ECM deposition.[13,14] HSCs and PFs comprise >95% of collagen expressing cells in fibrosis models.[7] Indeed, both PFs and HSCs are involved in collagen deposition around injured bile ducts.[15]

During normal states, HSCs and PFs are quiescent and may be transiently activated after acute exposure to pathogens or toxins to maintain homeostasis[16]; however, during PBC and PSC, myofibroblast activation is chronic and persistent.[17] HSCs reside in the space of Disse and during quiescence express a neural phenotype; however, injury such as carbon tetrachloride (CCl_4) treatment causes HSCs to release their vitamin A stores, synthesize and secrete collagen type I, proliferate, and obtain a myofibroblast phenotype.[15,18,19] PFs reside near the portal vein,[20] and in the biliary fibrosis model of bile duct ligation (BDL), activated PFs proliferate, take on a myofibroblast phenotype, and secrete collagen type I, collagen type IV, and procollagen III.[21,22] Similarities and differences in morphology, features, and characteristics of PFs and HSCs during quiescence and activation are summarized in Table 1. One study suggests that early ECM deposition in biliary fibrosis is due to PF activation after 48 hours of BDL, with HSC contribution after 72 hours of BDL.[22] Others have confirmed that PFs are primarily activated in BDL, with partial contribution to HSCs.[23] In addition, portal mesenchymal cells (PMCs) in the 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) feeding model of biliary fibrosis display PF signatures, are highly proliferative, and contribute to ECM remodeling. Interestingly, HSC-derived myofibroblasts were non-proliferative but still increased *Col1a1* expression *in vitro*, suggesting that PF-derived PMCs initiate fibrotic septa formation and later recruit HSCs.[24] BDL surgery and DDC feeding induced PMC proliferation and up-regulation of fibrotic genes that were not seen in HSCs,[25] suggesting that these cells are PFs. In *Mdr2*$^{-/-}$ mice, activated PFs accounted for 25%, 51%, and 54% of myofibroblasts at 4, 8, and 16 weeks of age, respectively, with the other percentage composed of HSCs,[26] signifying that both cell types contribute to biliary fibrosis in *Mdr2*$^{-/-}$ mice. Although newer studies identify the importance of PFs in biliary fibrosis, many studies have revolved around HSCs. We will further discuss mechanisms and cellular interactions identified in models of biliary fibrosis that affect PFs and HSCs.

Ductular Reaction: A Catch-22

DR correlates with fibrosis stage in PBC, and higher DR inversely correlates with ursodeoxycholic acid (UDCA) response in PBC.[27] In PSC, DR is associated with higher expression of ECM-related genes, including fibronectin-1 (FN-1), laminin subunit gamma-2, and collagens.[28] After BDL for 24 hours, DR and portal ECM deposition occur and precede myofibroblast presence that appeared 48 hours after BDL,[21] indicating that DR prompts myofibroblast activation. In turn, prolyl-4-hydroxylase, an enzyme that synthesizes collagen, promotes DR and biliary fibrosis in DDC-fed mice.[29] Cytokeratin-19 (CK-19) is responsible for cell structure and in the liver is a bona fide cholangiocyte marker. Loss of CK-19 reduced DR and biliary fibrosis in DDC-fed mice,[30] indicating cholangiocyte involvement in biliary fibrosis. Cholangiocytes have enhanced transforming growth factor (TGF)-β, which induced transcription of FN-1, and blocking this transcriptional regulation in cholangiocytes blunted biliary fibrosis in *Mdr2*$^{-/-}$ mice.[31,32] Similarly, blocking hepatic TGF-β2 decreased biliary fibrosis in *Mdr2*$^{-/-}$ mice.[33] Other studies have found that *TgfB2*-directed antisense oligonucleotides reduced parenchymal liver fibrosis in CCl_4-treated mice but had limited effect on biliary fibrosis in *Mdr2*$^{-/-}$ mice.[34] Models of liver or biliary fibrosis activate different mesenchymal cells, and thus local cellular niches need to be accounted for when designing fibrosis experiments.

Lineage tracing studies have revealed that DR pattern/morphology varies on the basis of the cell compartment that is injured. Indeed, it has been hypothesized that DR could arise from the biliary system, but also from hepatocytes. One study analyzed *R26*^{tom}*Hnf1b-CreER* mice, which label bile ducts and periportal ductules (but not hepatocytes) with HNF1β, that were subjected to BDL, methylene dianiline (MDA), DDC, or choline-deficient, ethionine-supplemented (CDE) to evaluate DR in acute and chronic models. It was observed that DR arose from HNF1β^{+} biliary comportments in short-term (2–3 weeks) liver injury (BDL, MDA, and DDC); however, CDE feeding for 16 weeks had a small fraction of hepatocytes that co-expressed HNF1β.[35] Another study found that DR invading the parenchyma expressed biliary markers and formed de novo junctions that maintained the canaliculi after CDE feeding. Contrarily, in BDL and DDC models, DR was confined to the portal mesenchyme and

Table 1. List of PF and HSC Features in Quiescence and Activation

Localization in liver	PFs Portal vein	HSCs Space of Disse
Morphology		
Quiescent state	• Flat • Spindle-shaped cells • Dark, oval nucleus • Actively secrete ECM	• Stellate-like shape • Reduced cellular body volume • Spindle-shaped • Voluminous oval or elongated nucleus ○ Often compressed by vitamin A and/or retinoid containing lipid drops placed in the perinuclear cytoplasm • Dilated, rough endoplasmic reticulum • Low number of mitochondria and lysosomes

Activated state	• Spindle-shaped cells
	• Smooth muscle-like features:
	  ○ Contractile apparatus
	  ○ Prominent rough endoplasmic reticulum
	  ○ Golgi apparatus producing collagen
	  ○ Peripheral myofilaments
	  ○ Fibronexus (no lamina)
	  ○ Gap junctions
		• Loss of retinoid/vitamin A lipid droplets
		• Flat-stellate shape
		• Expression of contractile fibers (myofibroblast differentiation – contractility) in cytoplasm
		• Matured rough endoplasmic reticulum associated with a well-formed Golgi apparatus

Functions		
Quiescent state	• Maintain integrity of the biliary tree and portal tract	• Balanced ECM turnover • Low proliferation • Storage of vitamin A and retinoids
Activated state	• Proliferative • Contribute to collagen type I deposition • Regulate cholangiocyte proliferation • Maintain cholangiocyte polarity • Support angiogenesis	• Fibrogenesis • ECM synthesis and degradation • Increased cell proliferation • Contractility • Secretion of cytokines, chemokines, and immunomodulatory signals • Acts as antigen-presenting cells • Role in liver development and regeneration (hepatocyte proliferation) • Support angiogenesis and LSEC capillarization
Markers		
Quiescent state	• Thy-1 • Fibulin-2 • Elastin • Gremlin-1 • ENTPD2 • IL-6	• Vitamin A • Retinoid • Desmin
Activated state	• Thy-1 • Mesothelin • Fibulin-2 • Elastin • Gremlin-1 • Asporin • IL-6 • Collagen 15A1 • Mucin 16 • ENTPD2 • Endoglin • CD73 • CD143 • ACTA2	• Glial fibrillary acidic protein • Neurotrophin receptor p75 • Synemin • Lecithin:retinol acyltransferase • ACTA2 • Reelin • Endoglin • CD73 • CD143
Injury response ECM synthesis	• Primary responders of cholangiocyte damage • Elastic fibers (elastin core surrounded by fibrillin-rich microfibrils) • Collagen XV	• Primary responders of hepatocyte damage • Collagen I and III • Fibronectin • Thrombospondin-1 • Proteoglycans

ACTA2, alpha smooth muscle actin; CD, cluster of differentiation; ENTPD2, ectonucleotidase 2; LSEC, lymphatic sinusoidal endothelial cell; Thy-1, thymocyte differentiation antigen-1.

formed pseudo-ductular structures.[36] Others found that thioacetamide (TAA) and DDC fed mice have a collapse of bile canaliculi that precedes DR; however, during recovery, reduced DR is paralleled with reconstruction of the bile canaliculi.[37] Therefore, DR may have a beneficial role regarding regeneration.

In 2 models of HPC activation, portal HPC expansion was associated with myofibroblast activation and laminin deposition; however, the source of laminin was not evaluated.[38] Others have found that PFs express laminin that supports HPC proliferation in mouse embryonic and neonatal liver.[39] Interestingly, glioma-associated oncogene (Gli1)+ PMCs surrounded intrahepatic bile ducts in BDL and DDC fed mice, but the DR in these models was extended into the parenchyma and surrounded by HSCs. However, the PMCs did not express HSC markers, indicating that they may be PF derived.[25] Interestingly, Gli1 facilitates cholangiocyte maturation and FN1 transcription, leading to ECM deposition *in vitro*.[40] In the dominant-negative TGF-β receptor II (*dnTGFβRII*) model of PBC, cholangiocytes had reduced expression of mature cholangiocyte markers, including secretin and its receptor, and restoration of mature cholangiocyte markers via secretin administration reduced biliary fibrosis and HSC presence.[41] *In vitro*, HPCs differentiated to "mature" biliary-like cells through taurocholate (TCA) stimulation secreted chemokines that drive HSC chemotaxis.[42] The origin and location of the DR are important for demarcating fibrosis patterns. DR in the parenchyma may interact predominantly with HSCs, whereas portal DR may interact with PFs and HSCs.

Biliary Senescence and TGF-β1: Lord of the SASPs

One characteristic of PSC and PBC is the presence of senescent cholangiocytes.[5] *K19-Mdm2$^{flox/flox}$tdTom^{LSL}* mice are a model of tamoxifen-inducible cholangiocyte senescence, and after tamoxifen injection, these mice have elevated collagen deposition surrounding senescent bile ducts. Furthermore, cholangiocytes in these mice release SASPs, specifically TGF-β1, that recruit myofibroblasts.[43] Cholangiocytes from human PSC samples and cholestatic models exhibit telomerase damage, which was not seen in hepatocytes. The telomerase reverse transcriptase (TERT) was epigenetically repressed in senescent cholangiocytes, and overexpression of TERT decreased senescent and TGF-β1–mediated phenotypes of cholangiocytes *in vitro*. Therefore, selective genetic deletion of TERT from cholangiocytes may exacerbate biliary fibrosis.[44] The secretin/secretin receptor axis is enhanced in cholangiocytes in BDL mice, and silencing the secretin/secretin receptor axis reduced biliary senescence, which in turn reduced TGF-β1 levels and biliary fibrosis.[45] This phenotype was also observed in double knockout *SR$^{-/-}$Mdr2$^{-/-}$* mice.[46] Finally, blocking cholangiocyte senescence by p16 Vivo-Morpholino or senolytic (Fisetin) treatment in *Mdr2$^{-/-}$* mice reduced biliary fibrosis.[47] Overall, these studies underline the relationship between biliary senescence, SASPs secretion (eg, TGF-β1), and biliary fibrosis during cholestasis.

Bile Acids: Chasing the Great White Whale

Bile stasis in PBC and PSC can lead to changes in BA composition and accumulation that perpetuate liver damage.[48] Early studies found that all BAs at physiological concentrations induce HSC proliferation and activation *in vitro*,[49] and taurodeoxycholate (TDCA) and TCA induce PF proliferation and activation in precision cut liver slices from normal rats.[50] Hydrophobic BAs (eg, TCA, TDCA and lithocholate [LCA]) are considered damaging, whereas hydrophilic BAs (eg, UDCA) are considered protective.[51] Supporting this, administration of hydrophilic BAs protects against immune cell infiltration and biliary fibrosis in *Mdr2$^{-/-}$* mice.[52,53] In addition, the hydrophobic BA chenodeoxycholate (CDCA) induces HSC activation and proliferation *in vitro*.[54] These studies suggest that alterations in BA hydrophobicity modulate myofibroblast activation; however, studies have suggested that dampening the overall BA pool may be key.[55] Inhibition of ileal apical sodium-dependent BA transporter (ASBT) reduced biliary BA output and increased fecal BA excretion, and this corresponded with reduced inflammation and biliary fibrosis in *Mdr2$^{-/-}$* mice.[56,57] Similarly, in *dnTGFβRII* mice secretin treatment enhances choleresis, thereby reducing hepatic BA levels, immune cell presence, and fibrosis.[41] Reducing BA levels or hydrophobicity may be important for reversing biliary fibrosis.

Bile salt export pump (BSEP) is the main hepatic BA efflux transporter, but *Bsep$^{-/-}$* mice show a hydrophilic BA composition, and *Mdr2$^{-/-}$* mice crossed with *Bsep$^{-/-}$* mice show reduced immune cell infiltration and biliary fibrosis.[52,53] Na^+-taurocholate co-transporting polypeptide (NTCP) is the major hepatic BA uptake transporter, and inhibition of NTCP reduces biliary damage, inflammation, and fibrosis in DDC fed and BDL mice; however, inhibition of NTCP aggravated inflammation and fibrosis in *Mdr2$^{-/-}$* mice,[58] indicating that targeting BA homeostasis differs between models. Introduction of human *MDR3* (homolog to murine Mdr2), which regulates phospholipid secretion into bile, ameliorates inflammation and biliary fibrosis in *Mdr2$^{-/-}$* mice by reducing toxic BA levels.[59,60] Farnesoid X receptor (FXR) is a nuclear BA receptor that mediates BA synthesis and transporter expression, as well as inflammation. Tropifexor, a non-BA FXR agonist, reduces biliary fibrosis in piglets subjected to BDL for 14 days.[61] A similar non-BA FXR agonist, EDP-305, decreased inflammation and fibrosis in *Mdr2$^{-/-}$* mice.[62] Rat HSCs do not express BSEP,[63] primary human HSCs from fibrotic livers have increased NTCP expression,[64] and the role of FXR on human and mouse HSC activation is controversial.[65,66] Modulation of BA homeostasis may have direct effects on HSC and PF activation but also indirectly influences biliary fibrosis via modulation of inflammation.

BA-based drugs are currently used for PBC and PSC treatment. UDCA and obeticholic acid (OCA, ie, Ocaliva) are first- and second-line treatments, respectively, in PBC[67]; however, only OCA shows beneficial effects on liver enzymes in PSC.[67,68] Cytochrome P450 family 2 subfamily c

polypeptide 70 (*Cyp2c70*) knockout mice lack hydrophilic muricholic acids and display a more human-like BA pool. *Cyp2c70*$^{-/-}$ mice showed spontaneous cholangiopathic features at 8 months of age, and UDCA treatment reduced inflammation and biliary fibrosis in *Cyp2c70*$^{-/-}$ mice.[69] 24-norursodeoxycholic acid (norUDCA) is a side chain-shortened C_{23} UDCA homologue that reduced inflammation and biliary fibrosis in *Mdr2*$^{-/-}$ mice.[70,71] Importantly, norUDCA has shown potential therapeutic benefit in PSC patients.[72] BA-based therapies have important functions in reducing biliary fibrosis.

Endothelial Cells: The Angiogenesis Also Rises

Angiogenesis is the formation of new blood vessels and is noted in PBC and PSC samples.[73,74] In BDL rats, neovascularization is associated with biliary fibrosis.[75] Furthermore, hepatic vascular endothelial growth factor (VEGF) A (*Vegfa*/VEGFA) expression increases during fibrosis progression in CCl_4 treated, BDL, and *Mdr2*$^{-/-}$ mice.[74,76,77] However, neutralizing antibody against VEGFA impairs fibrosis resolution after CCl_4 treatment through enhanced sinusoidal permeability.[78] Importantly, biliary fibrosis in cholangiopathies is associated with portal angiogenesis that may involve vascular endothelial cells as opposed to sinusoidal endothelial cells.[73,74] In line with this, DR in PSC is associated with enhanced fibrogenic gene expression and lies in close vicinity to angiogenic vessels.[28] It was observed that liver blood vessels (portal and central vessels and liver sinusoid) act differently during liver fibrogenesis. Blocking portal angiogenesis alleviates fibrosis, but the inhibition of both central angiogenesis and sinusoidal capillarization promotes liver fibrosis in CCl_4 treated mice.[79] This underlines the strong correlation between portal angiogenesis and liver fibrosis.

HSCs release angiogenic factors, including angiopoietin-1 (Angpt-1) and platelet-derived growth factor (PDGF), that drive angiogenesis.[75,80] Similarly, activated PFs secrete VEGFA, which promotes angiogenesis.[81,82] In turn, VEGFA and Angpt-1 promote HSC activation,[83] indicating a feedback loop between angiogenic and fibrogenic cells. Placental growth factor (PlGF) promotes angiogenesis in CCl_4 mice, and HSCs treated with PlGF *in vitro* have enhanced ECM synthesis.[84] Also, angiotensin enzyme 2 therapy directly blocks HSC activation and biliary fibrosis in *Mdr2*$^{-/-}$ mice.[85] Activated PFs display both fibrogenic and angiogenic gene signatures, specifically split guidance ligand 2 (*Slit2*),[24] and Slit2 drives angiogenesis in DDC fed mice.[86] Portal myofibroblasts also drive angiogenesis through the release of VEGFA-enriched microparticles that signal to nearby endothelial cells.[82] HSCs and PFs interact with endothelial cells to enhance angiogenesis, which, in turn, drives scar formation.

Aside from direct communication between endothelial and fibrogenic cells, angiocrine factors can regulate HSCs and PFs. Endothelin (ET) regulates the synthesis and secretion of TGF-β1 and VEGFA, and cholangiocytes with enhanced ET-A signaling initiate angiogenesis and fibrosis in *Mdr2*$^{-/-}$ mice.[74] Vasohibin-1 (VASH-1) regulates VEGF through a negative feedback loop, and overexpression of VASH-1 reduced VEGF levels, pathologic angiogenesis, and biliary fibrosis in BDL mice.[87] These findings are significant because VEGF promotes pathologic, but not physiological, angiogenesis that is associated with fibrosis.[88] PSC is a risk factor for CCA,[1] and fibroblast-derived VEGFA/C promotes intravasation and early metastasis in CCA[89]; therefore, VEGFA/C may be an important link between PSC and CCA development. It is apparent that angiogenic signaling can influence biliary fibrosis.

Immune Cells: For Whom the Fibrosis Tolls

Innate immunity is one of the first lines of defense against infection and broadly involves 3 cell types: granulocytes (ie, neutrophils, eosinophils, basophils, and mast cells [MCs]), monocytes/macrophages (eg, Kupffer cells [KCs, liver-resident macrophages]), and dendritic cells. If the innate immune response is unable to combat infection, the adaptive immune system is initiated. On first infection, the adaptive immune cells, T and B lymphocytes, perform cell- and antibody-mediated responses, and these signatures are memorized to allow for quicker responses if reinfection occurs.[90] In chronic liver diseases, both innate and adaptive immunity have been identified as drivers of fibrosis.[91] Indeed, initial biliary fibrosis is characterized by portal inflammation (stage 1) but can expand into the periportal area along with inflammatory interface (stage 2). Next, portal-portal fibrosis bridges form with moderate/marked portal inflammation (stage 3), with eventual cirrhosis formation occurring (stage 4).[92,93] Here, we will describe how innate and adaptive immune cells can contribute to biliary fibrosis.

Monocytes and macrophages (MΦ) are phagocytes that maintain homeostasis through the clearance of cell debris and microbes. Hepatic MΦ, consisting of KCs and monocyte-derived macrophages (MoMΦ), exhibit heterogeneity and can contribute to fibrosis progression or resolution.[94] MoMΦ accumulate in PBC and PSC and are associated with DR, and MoMΦ infiltration adjacent to DR predicts advanced cirrhosis in PSC.[95,96] MΦ isolated from human liver fibrosis samples are pro-fibrotic and have overlapping pathways with HSCs, alluding to MΦ induction of HSC activation.[97] In BDL and DDC models, MoMΦ and KCs were pro-inflammatory, but elimination of KCs had no impact on biliary fibrosis.[98] Other research found KCs indispensable in BDL-induced biliary fibrosis.[99] MoMΦ are recruited by cholangiocyte released monocyte chemoattractant protein-1 (MCP-1), and blocking monocyte recruitment attenuated fibrosis in *Mdr2*$^{-/-}$ mice.[100] MCP-1 further promotes pro-inflammatory phenotypes in KCs and recruits MoMΦ in BDL and *Mdr2*$^{-/-}$ mice.[101] HSCs, in turn, imprint pro-inflammatory signatures on MoMΦ.[102] These studies suggest differential roles of infiltrating and resident MΦ on biliary fibrosis.

MCs release histamine and other mediators after their activation. In PSC livers, MCs reside in fibrotic areas and correlate with portal fibrosis.[103,104] Interestingly, HSCs

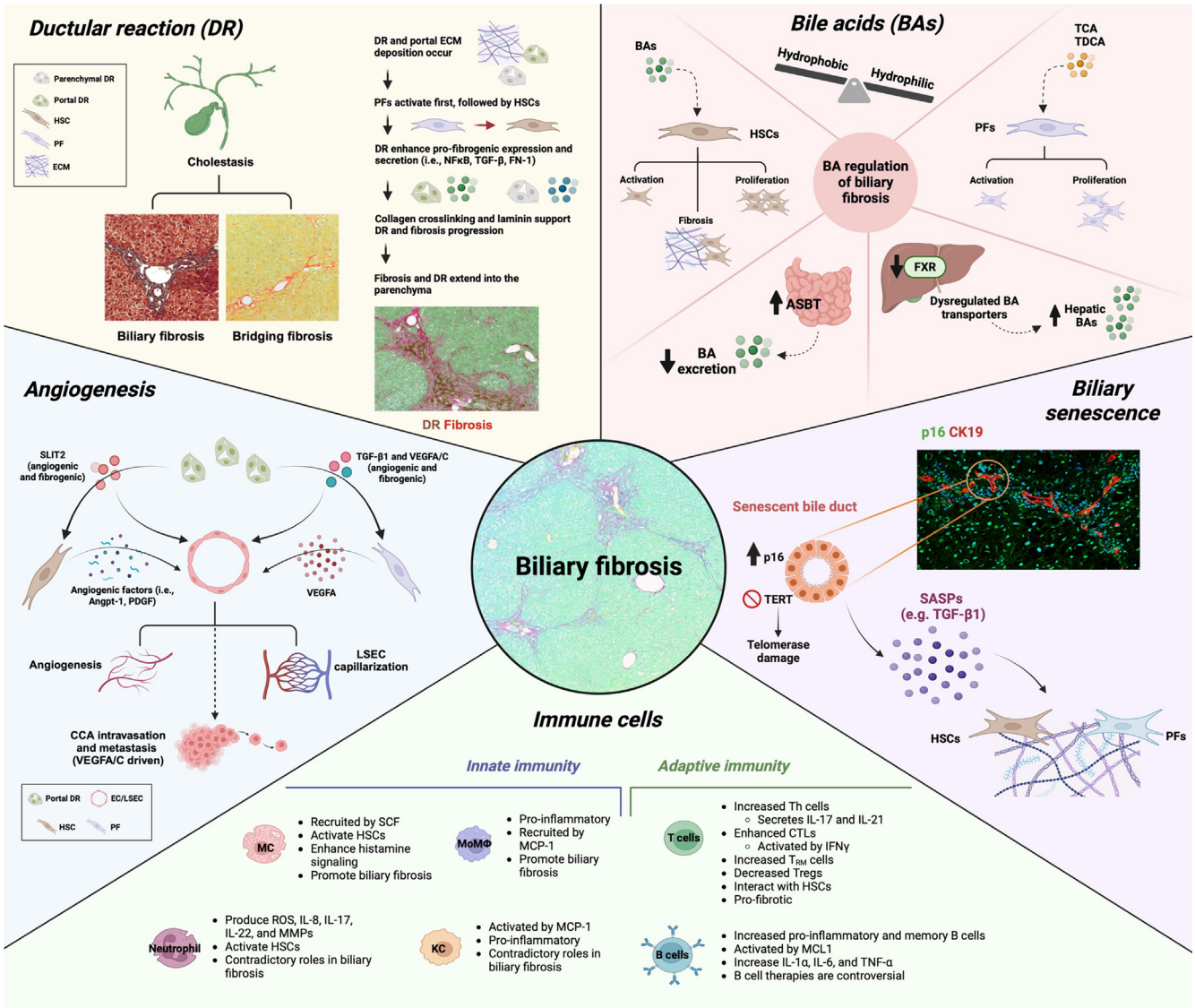

Figure 1. Graphical representation of cellular crosstalk supporting biliary fibrosis. DR can occur at the portal and parenchymal area, differentially facilitating biliary and bridging fibrosis. Different factors secreted from DR cells can contribute to fibrosis progression. Cholestasis leads to hepatic BA buildup, and various changes in BA excretion/secretion, transport, homeostasis, and interactions with PFs and HSCs can lead to biliary fibrosis. Senescent cholangiocytes have increased p16 expression, temolerase damage, and SASPs secretion, including TGF-β1, which stimulate activation of both PFs and HSCs during cholestasis. Angiogenesis is closely associated with biliary fibrosis, and different angiocrine signals between PFs, HSCs, and endothelial cells/LSECs can give rise to these pathologies. Last, immune cells infiltrate the liver during cholestasis and cholangiopathies, and the different immune subtypes have varying roles on PFs, HSCs, and biliary fibrosis. BA, bile acid; DR, ductular reaction; HSC, hepatic stellate cell; LSEC, lymphatic sinusoidal endothelial cell; PF, portal fibroblast; SASP, senescence-associated secretory phenotype; TGF-β, transforming growth factor β. Image made with BioRender.com.

produce stem cell factor, a MC chemoattractant, that induces MC homing *in vitro*.[105] One study found that *in vitro* co-culture of MCs with HSCs reduced HSC fibrogenesis[106]; however, the HSC:MC ratio was 1:2.25, which is not appropriate for physiological standards. Selectively inhibiting MC-dependent H2 histamine receptor (HR) signaling reduces biliary fibrosis induced by MC injection,[107] and liver-specific down-regulation of H2HR reduces hepatic MC infiltration and biliary fibrosis in *Mdr2*$^{-/-}$ mice.[108] Similarly, H1HR and H2HR antagonists diminish portal MC presence, as well as biliary fibrosis in *Mdr2*$^{-/-}$ mice.[109] Last, when MC recruitment or activation is blocked, HSC number and biliary fibrosis are repressed in *Mdr2*$^{-/-}$ mice and BDL models.[110–112] Targeting MCs or MC-derived mediators, such as histamine, may be useful for regression of biliary fibrosis.

Neutrophils infiltrate the liver in acute infection or injury and act in a protective manner, but chronic liver disease leads to aberrant neutrophil signaling that may perturb damage.[113] Human PSC samples have increased neutrophils in the portal region,[114] and neutrophils are recruited early (3 hours after BDL).[115] In DDC fed and CCl_4 treated mice,

Table 2. Potential Therapeutic Agents for PBC and PSC

Drug class	Drug name/agent	Target/mode of action	Experimental model	Reference(s)
Anti-neoplastic	Bortezomib	Proteosome inhibition	*Mdr2*$^{-/-}$ mice	32
Senolytic	Sec 5-27	SR antagonist	*Mdr2*$^{-/-}$ mice	45
	Fisetin	Anti-oxidation	*Mdr2*$^{-/-}$ mice	47
Antifibrotic	FAP-specific inhibitor (FAPi) (S)-N-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)-quinoline-4-carboxamide	Fibroblast activation protein (FAP)	CCl_4 and *Mdr2*$^{-/-}$ mice	34
Bile acid–based	Tetrahydroxylated bile acids (THBAs)	Less toxic bile acid pool	*Mdr2*$^{-/-}$ mice	52,53
	SC-435	Ileal ASBT inhibition	*Cyp2c70*$^{-/-}$ and *Mdr2*$^{-/-}$ mice	55,57
	A4250	Ileal ASBT inhibition	*Mdr2*$^{-/-}$ mice	56
	Myrcludex B	NTCP inhibition	DDC, BDL, and *Mdr2*$^{-/-}$ mice	58
	Synthetic human *ABCB4* gene therapy	Restore ABCB4/MDR3	*Mdr2*$^{-/-}$ mice	59,60
	Tropifexor	FXR agonist	BDL piglets	61
	EDP-305	FXR agonist	*Mdr2*$^{-/-}$ mice	62
	Nor-UDCA	Anti-cholestatic and anti-inflammatory	*Mdr2*$^{-/-}$ mice	70,71
Anti-angiogenic	Ambrisentan	ET-A antagonist	*Mdr2*$^{-/-}$ mice	74
	Sorafenib	Kinase inhibitor	BDL rats	75
	MCR84	VEGF-neutralizing antibody	BDL mice	78
	αPlGF	PlGF-neutralizing antibody	CCl_4 mice	84
	AdVASH1 (synthetic human *VASH1* gene therapy)	Restore vasohibin-1	BDL rats	87
Immunomodulatory	Cenicriviroc	CCR2/5 antagonist	BV6 injected mice	100
	Cromolyn sodium	Mast cell stabilizer	*Mdr2*$^{-/-}$ mice and BDL rats	110,112
	Mepyramine and ranitidine	H1 and H2 histamine receptor antagonists	*Mdr2*$^{-/-}$ mice	109
	SCH-527123	CXCR1/2 inhibitor	DDC mice	116
	S63845	MCL1 inhibitor	*Mdr2*$^{-/-}$ mice	129
	Anti-CD20 antibody	B cell depletion	*dnTGFβRII* mice	134,135
	Anti-IFNγ antibody	IFNγ neutralization	*Mdr2*$^{-/-}$ mice	143
	4-OI	Itaconate derivative	DDC and *Mdr2*$^{-/-}$ mice	145

CD20, cluster of differentiation 20; CXCR1/2, C-X-C chemokine receptor type 1/2; Cyp2c70, cytochrome P450 family 2 subfamily c polypeptide 70; FAP, fibroblast activation protein; IFNγ, interferon γ; Mdr2/MDR3, multidrug resistance 2/3; SR, secretin receptor; THBA, tetrahydroxylated bile acid; VASH1, vasohibin 1.

DR-associated neutrophils increase, and loss of neutrophils mitigates liver fibrosis.[116] Early *in vitro* studies found that neutrophil production of reactive oxygen species promoted collagen synthesis in HSCs.[117] In addition, neutrophils are a main source of interleukin 17 (IL-17) and IL-22, which show pro-fibrogenic effects on HSCs.[118,119] However, depletion of neutrophils after CCl_4 administration impairs fibrosis regression.[120] Also, neutrophils promote fibrosis resolution through matrix metalloproteinase secretion in BDL rats.[121] Others have found that neutrophil attenuation had no effect on biliary fibrosis in BDL rats.[122] Neutrophil involvement in biliary fibrosis is nuanced and may rely on the timing of infiltration and exposure to acute versus chronic damage.

In the spleen or lymph node, B cells, while in contact with cluster differentiation (CD)4 T helper (Th) cells, recognize antigen that causes them to differentiate to germinal center B cells or plasmablasts that can give rise to memory B cells or plasma cells, respectively. Plasma cells secrete antibodies in response to antigen, and B cells can produce cytokines through interaction with other immune cells.[123] PBC and PSC patients have increased numbers of B cells and plasma cells in the portal region,[124–127] and memory B cells are found in the fibrotic septa in PBC and PSC.[128] *Mdr2*$^{-/-}$ mice have enhanced portal infiltration of pro-inflammatory B cells, which is attributed to increased expression of myeloid cell leukemia 1 (MCL1) in cholangiocytes. When *Mdr2*$^{-/-}$ mice were treated with S63845, an MCL1 inhibitor, hepatic B cell number decreased along with biliary fibrosis.[129] Pro-inflammatory B cells increase in CCl_4 treated mice, and activated HSCs augmented B cell activity. Conversely, anti-B cell activating factor (BAFF) treatment had no effect on fibrosis in *Mdr2*$^{-/-}$ mice; however, total depletion of B cells using anti-CD20 antibody treatment reduced hepatic fibrosis in *Mdr2*$^{-/-}$ mice.[130] Genetic loss of B cells in *dnTGFβRII* mice led to enhanced pro-inflammatory markers, including IL-6 and tumor necrosis factor (TNF)-α,[131] that are known to activate HSCs.[132] Similarly, anti-CD20 antibody depletion of B cells in *dnTGFβRII* mice increased inflammation and IL-1α levels,[133] which are known to promote HSC proliferation.[132]

Others found that anti-CD20 antibody reduced hepatic inflammation in *dnTGFβRII* mice.[134,135] B cell modulation of biliary fibrosis is controversial and requires further investigation.

Naive T cells survey lymphoid organs and, after contact with an antigen presenting cell (APC), travel to the lymph nodes to (1) undergo clonal expansion, (2) become effector T cells (ie, Th cells, cytotoxic T lymphocytes [CTLs], regulatory T cells [Tregs]), and (3) traffic to the infected organ. Infection can produce memory T cells that recognize antigen on reinfection to become effector T cells.[136] T cells can promote HSC activation,[137] and HSCs cause apoptosis of T cells in fibrosis.[138] Interestingly, HSCs counteract APC initiated naive T-cell activation.[139] PBC patients have an increased number of Th cells and tissue-resident memory T cells (T_{RM}), whereas Tregs are decreased.[126,140,141] Additionally, PSC patients have increased Th cells and CTLs near the bile ducts,[142] and enhanced interferon γ signaling in PSC and *Mdr2*$^{-/-}$ mice enhances CTL cytotoxicity and biliary fibrosis.[143] Th cell-derived IL-21, but not IL-17, enhanced fibrosis and CTL activity in *dnTGFβRII* mice.[144] In *Mdr2*$^{-/-}$ and DDC fed mice, intrahepatic T_{RM} cells expanded, and inhibiting T_{RM} cell activity reduced biliary fibrosis.[145] Nor-UDCA attenuates CTL activity and biliary fibrosis in *Mdr2*$^{-/-}$ mice.[70] Interestingly, loss of γδ T cells, a primitive T-cell subset, blocks liver fibrosis by inducing apoptosis in HSCs and inhibiting Th cell activity.[146] T cell heterogeneity is complex, and understanding how different T cells interact with PFs and HSCs will be essential for defining immunomodulation of biliary fibrosis.

Conclusion

In relation to biliary fibrosis, HSCs have historically been studied; however, research has identified PFs as an important contributor. Numerous studies have identified that PFs are the first fibrogenic cells to activate in cholestatic damage, thereby initiating biliary fibrosis, with recruitment of HSCs coming later. Indeed, the recruitment of HSCs later may be important in initiating bridging fibrosis. It is also key to understand that variations in damage initiation between BDL, DDC, *Mdr2*$^{-/-}$, and other models differentially impact PFs and HSCs. Discernable differences in fibrosis between models should be taken into consideration when designing experiments and analyzing samples. Additionally, variations in DR and its localization (eg, portal or parenchymal) can variably impact PFs and HSCs. This area of study can be further complicated when considering the different cellular sources that can give rise to DR and the role of senescent cholangiocytes and secreted SASPs on the activation/modulation of PFs and HSCs.

The buildup of BAs initiates both biliary and hepatocellular damage, which may variably induce fibrosis. The role of BA accumulation has been well-studied in HSCs; however, more work is needed in PFs. Unfortunately, BA receptor and transporter expression are controversial or unknown in HSCs and PFs. Portal angiogenesis is a pathologic feature known to promote fibrosis, and better demarcation of angiogenesis and how it interacts with HSCs and PFs is needed. Last, the portal niche can be inundated with infiltrating immune cells that show diversity. This complex immune response shapes cellular interactions, but the plethora of immune subsets makes teasing out mechanisms difficult.

Unsurprisingly, complex cellular interactions and diverse cell populations in cholangiopathies have varying impacts on biliary fibrosis (Figure 1). Studies thus far have identified potential therapeutics for PBC and PSC (Table 2); however, additional research will uncover novel therapeutic avenues. Although biliary fibrosis is multifarious, appreciating the nuance will be beneficial for furthering this area of study.

References

1. Lazaridis KN, LaRusso NF. The cholangiopathies. Mayo Clin Proc 2015;90:791–800.
2. Toy E, Balasubramanian S, Selmi C, et al. The prevalence, incidence and natural history of primary sclerosing cholangitis in an ethnically diverse population. BMC Gastroenterol 2011;11:83.
3. Boberg KM. The clinical burden of biliary disease: a global perspective. In: Hirschfield G, Adams D, Liaskou E, eds. Biliary disease: from science to clinic. New York: Springer International Publishing, 2017:1–15.
4. Roskams TA, Theise ND, Balabaud C, et al. Nomenclature of the finer branches of the biliary tree: canals, ductules, and ductular reactions in human livers. Hepatology 2004;39:1739–1745.
5. Guicciardi ME, Trussoni CE, LaRusso NF, et al. The spectrum of reactive cholangiocytes in primary sclerosing cholangitis. Hepatology 2020;71:741–748.
6. Pinzani M, Luong TV. Pathogenesis of biliary fibrosis. Biochim Biophys Acta Mol Basis Dis 2018;1864(Pt B):1279–1283.
7. Iwaisako K, Jiang C, Zhang M, et al. Origin of myofibroblasts in the fibrotic liver in mice. Proc Natl Acad Sci U S A 2014;111:E3297–E3305.
8. Kinnman N, Housset C. Peribiliary myofibroblasts in biliary type liver fibrosis. Front Biosci 2002;7:d496–d503.
9. Murillo Perez CF, Hirschfield GM, Corpechot C, et al. Fibrosis stage is an independent predictor of outcome in primary biliary cholangitis despite biochemical treatment response. Aliment Pharmacol Ther 2019;50:1127–1136.
10. Oyama A, Takaki A, Adachi T, et al. Oxidative stress-related markers as prognostic factors for patients with primary sclerosing cholangitis in Japan. Hepatol Int 2023;17:1215–1224.
11. Fabris L, Spirli C, Cadamuro M, et al. Emerging concepts in biliary repair and fibrosis. Am J Physiol Gastrointest Liver Physiol 2017;313:G102–G116.
12. Kisseleva T, Uchinami H, Feirt N, et al. Bone marrow-derived fibrocytes participate in pathogenesis of liver fibrosis. J Hepatol 2006;45:429–438.
13. Scholten D, Osterreicher CH, Scholten A, et al. Genetic labeling does not detect epithelial-to-mesenchymal transition of cholangiocytes in liver fibrosis in mice. Gastroenterology 2010;139:987–998.
14. Taura K, Miura K, Iwaisako K, et al. Hepatocytes do not undergo epithelial-mesenchymal transition in liver fibrosis in mice. Hepatology 2010;51:1027–1036.

15. Geerts A. History, heterogeneity, developmental biology, and functions of quiescent hepatic stellate cells. Semin Liver Dis 2001;21:311–335.
16. Kisseleva T, Cong M, Paik Y, et al. Myofibroblasts revert to an inactive phenotype during regression of liver fibrosis. Proc Natl Acad Sci U S A 2012;109:9448–9453.
17. Karin D, Koyama Y, Brenner D, et al. The characteristics of activated portal fibroblasts/myofibroblasts in liver fibrosis. Differentiation 2016;92:84–92.
18. Lepreux S, Desmouliere A. Human liver myofibroblasts during development and diseases with a focus on portal (myo)fibroblasts. Front Physiol 2015;6:173.
19. Seifert WF, Roholl PJ, Blauw B, et al. Fat-storing cells and myofibroblasts are involved in the initial phase of carbon tetrachloride-induced hepatic fibrosis in BN/BiRij rats. Int J Exp Pathol 1994;75:131–146.
20. Lorena D, Darby IA, Reinhardt DP, et al. Fibrillin-1 expression in normal and fibrotic rat liver and in cultured hepatic fibroblastic cells: modulation by mechanical stress and role in cell adhesion. Lab Invest 2004; 84:203–212.
21. Desmouliere A, Darby I, Costa AM, et al. Extracellular matrix deposition, lysyl oxidase expression, and myofibroblastic differentiation during the initial stages of cholestatic fibrosis in the rat. Lab Invest 1997; 76:765–778.
22. Tuchweber B, Desmouliere A, Bochaton-Piallat ML, et al. Proliferation and phenotypic modulation of portal fibroblasts in the early stages of cholestatic fibrosis in the rat. Lab Invest 1996;74:265–278.
23. Yang W, He H, Wang T, et al. Single-cell transcriptomic analysis reveals a hepatic stellate cell-activation roadmap and myofibroblast origin during liver fibrosis in mice. Hepatology 2021;74:2774–2790.
24. Lei L, Bruneau A, El Mourabit H, et al. Portal fibroblasts with mesenchymal stem cell features form a reservoir of proliferative myofibroblasts in liver fibrosis. Hepatology 2022;76:1360–1375.
25. Gupta V, Gupta I, Park J, et al. Hedgehog signaling demarcates a niche of fibrogenic peribiliary mesenchymal cells. Gastroenterology 2020;159:624–638 e9.
26. Nishio T, Hu R, Koyama Y, et al. Activated hepatic stellate cells and portal fibroblasts contribute to cholestatic liver fibrosis in MDR2 knockout mice. J Hepatol 2019;71:573–585.
27. Overi D, Carpino G, Cristoferi L, et al. Role of ductular reaction and ductular-canalicular junctions in identifying severe primary biliary cholangitis. JHEP Rep 2022;4: 100556.
28. Govaere O, Cockell S, Van Haele M, et al. High-throughput sequencing identifies aetiology-dependent differences in ductular reaction in human chronic liver disease. J Pathol 2019;248:66–76.
29. Zhang J, Lyu Z, Li B, et al. P4HA2 induces hepatic ductular reaction and biliary fibrosis in chronic cholestatic liver diseases. Hepatology 2023;78:10–25.
30. Chen Y, Guldiken N, Spurny M, et al. Loss of keratin 19 favours the development of cholestatic liver disease through decreased ductular reaction. J Pathol 2015; 237:343–354.
31. Aseem SO, Jalan-Sakrikar N, Chi C, et al. Epigenomic evaluation of cholangiocyte transforming growth factor-beta signaling identifies a selective role for histone 3 lysine 9 acetylation in biliary fibrosis. Gastroenterology 2021;160:889–905 e10.
32. Jalan-Sakrikar N, De Assuncao TM, Shi G, et al. Proteasomal degradation of enhancer of zeste homologue 2 in cholangiocytes promotes biliary fibrosis. Hepatology 2019;70:1674–1689.
33. Dropmann A, Dooley S, Dewidar B, et al. TGF-beta2 silencing to target biliary-derived liver diseases. Gut 2020;69:1677–1690.
34. Yang AT, Kim YO, Yan XZ, et al. Fibroblast activation protein activates macrophages and promotes parenchymal liver inflammation and fibrosis. Cell Mol Gastroenterol Hepatol 2023;15:841–867.
35. Jors S, Jeliazkova P, Ringelhan M, et al. Lineage fate of ductular reactions in liver injury and carcinogenesis. J Clin Invest 2015;125:2445–2457.
36. Clerbaux LA, Manco R, Van Hul N, et al. Invasive ductular reaction operates hepatobiliary junctions upon hepatocellular injury in rodents and humans. Am J Pathol 2019;189:1569–1581.
37. Kamimoto K, Nakano Y, Kaneko K, et al. Multidimensional imaging of liver injury repair in mice reveals fundamental role of the ductular reaction. Commun Biol 2020;3:289.
38. Lorenzini S, Bird TG, Boulter L, et al. Characterisation of a stereotypical cellular and extracellular adult liver progenitor cell niche in rodents and diseased human liver. Gut 2010;59:645–654.
39. Tanimizu N, Kikkawa Y, Mitaka T, et al. alpha1- and alpha5-containing laminins regulate the development of bile ducts via beta1 integrin signals. J Biol Chem 2012; 287:28586–28597.
40. Jalan-Sakrikar N, De Assuncao TM, Lu J, et al. Hedgehog signaling overcomes an EZH2-dependent epigenetic barrier to promote cholangiocyte expansion. PLoS One 2016;11:e0168266.
41. Kennedy L, Carpino G, Owen T, et al. Secretin alleviates biliary and liver injury during late-stage primary biliary cholangitis via restoration of secretory processes. J Hepatol 2023;78:99–113.
42. Pozniak KN, Pearen MA, Pereira TN, et al. Taurocholate induces biliary differentiation of liver progenitor cells causing hepatic stellate cell chemotaxis in the ductular reaction: role in pediatric cystic fibrosis liver disease. Am J Pathol 2017;187:2744–2757.
43. Ferreira-Gonzalez S, Lu WY, Raven A, et al. Paracrine cellular senescence exacerbates biliary injury and impairs regeneration. Nat Commun 2018;9:1020.
44. Jalan-Sakrikar N, Anwar A, Yaqoob U, et al. Telomere dysfunction promotes cholangiocyte senescence and biliary fibrosis in primary sclerosing cholangitis. JCI Insight 2023;8.
45. Wu N, Meng F, Zhou T, et al. The secretin/secretin receptor axis modulates ductular reaction and liver fibrosis through changes in transforming growth factor-beta1-mediated biliary senescence. Am J Pathol 2018; 188:2264–2280.

46. Zhou T, Wu N, Meng F, et al. Knockout of secretin receptor reduces biliary damage and liver fibrosis in Mdr2(-/-) mice by diminishing senescence of cholangiocytes. Lab Invest 2018;98:1449–1464.
47. Alsuraih M, O'Hara SP, Woodrum JE, et al. Genetic or pharmacological reduction of cholangiocyte senescence improves inflammation and fibrosis in the Mdr2 (-/-) mouse. JHEP Rep 2021;3:100250.
48. Zeng J, Fan J, Zhou H. Bile acid-mediated signaling in cholestatic liver diseases. Cell Biosci 2023;13:77.
49. Svegliati-Baroni G, Ridolfi F, Hannivoort R, et al. Bile acids induce hepatic stellate cell proliferation via activation of the epidermal growth factor receptor. Gastroenterology 2005;128:1042–1055.
50. Wood MJ, Sjoblom P, Lindenberg S, et al. Effect of slow and ultra-rapid freezing on cell surface antigens of 8-cell mouse embryos. J Exp Zool 1992;262:330–339.
51. Fickert P, Fuchsbichler A, Marschall HU, et al. Lithocholic acid feeding induces segmental bile duct obstruction and destructive cholangitis in mice. Am J Pathol 2006;168:410–422.
52. Wang R, Sheps JA, Liu L, et al. Hydrophilic bile acids prevent liver damage caused by lack of biliary phospholipid in Mdr2(-/-) mice. J Lipid Res 2019;60:85–97.
53. Fuchs CD, Dixon ED, Hendrikx T, et al. Tetrahydroxylated bile acids improve cholestatic liver and bile duct injury in the Mdr2(-/-) mouse model of sclerosing cholangitis via immunomodulatory effects. Hepatol Commun 2022;6:2368–2378.
54. Zimny S, Koob D, Li J, et al. Hydrophobic bile salts induce pro-fibrogenic proliferation of hepatic stellate cells through PI3K p110 alpha signaling. Cells 2022:11.
55. Truong JK, Bennett AL, Klindt C, et al. Ileal bile acid transporter inhibition in Cyp2c70 KO mice ameliorates cholestatic liver injury. J Lipid Res 2022;63:100261.
56. Baghdasaryan A, Fuchs CD, Osterreicher CH, et al. Inhibition of intestinal bile acid absorption improves cholestatic liver and bile duct injury in a mouse model of sclerosing cholangitis. J Hepatol 2016;64:674–681.
57. Miethke AG, Zhang W, Simmons J, et al. Pharmacological inhibition of apical sodium-dependent bile acid transporter changes bile composition and blocks progression of sclerosing cholangitis in multidrug resistance 2 knockout mice. Hepatology 2016;63:512–523.
58. Slijepcevic D, Roscam Abbing RLP, Fuchs CD, et al. Na(+) -taurocholate cotransporting polypeptide inhibition has hepatoprotective effects in cholestasis in mice. Hepatology 2018;68:1057–1069.
59. Aronson SJ, Bakker RS, Shi X, et al. Liver-directed gene therapy results in long-term correction of progressive familial intrahepatic cholestasis type 3 in mice. J Hepatol 2019;71:153–162.
60. Wei G, Cao J, Huang P, et al. Synthetic human ABCB4 mRNA therapy rescues severe liver disease phenotype in a BALB/c.Abcb4(-/-) mouse model of PFIC3. J Hepatol 2021;74:1416–1428.
61. Xiao Y, Wang Y, Liu Y, et al. A nonbile acid farnesoid X receptor agonist tropifexor potently inhibits cholestatic liver injury and fibrosis by modulating the gut-liver axis. Liver Int 2021;41:2117–2131.
62. An P, Wei G, Huang P, Li W, et al. A novel non-bile acid FXR agonist EDP-305 potently suppresses liver injury and fibrosis without worsening of ductular reaction. Liver Int 2020;40:1655–1669.
63. Hannivoort RA, Dunning S, Vander Borght S, et al. Multidrug resistance-associated proteins are crucial for the viability of activated rat hepatic stellate cells. Hepatology 2008;48:624–634.
64. Salhab A, Amer J, Lu Y, et al. Sodium(+)/taurocholate cotransporting polypeptide as target therapy for liver fibrosis. Gut 2022;71:1373–1385.
65. Fickert P, Fuchsbichler A, Moustafa T, et al. Farnesoid X receptor critically determines the fibrotic response in mice but is expressed to a low extent in human hepatic stellate cells and periductal myofibroblasts. Am J Pathol 2009;175:2392–2405.
66. Fiorucci S, Antonelli E, Rizzo G, et al. The nuclear receptor SHP mediates inhibition of hepatic stellate cells by FXR and protects against liver fibrosis. Gastroenterology 2004;127:1497–1512.
67. Carbone M, Mells GF, Pells G, et al. Sex and age are determinants of the clinical phenotype of primary biliary cirrhosis and response to ursodeoxycholic acid. Gastroenterology 2013;144:560–5609 e7, quiz e13–e14.
68. Lindor KD, Kowdley KV, Luketic VA, et al. High-dose ursodeoxycholic acid for the treatment of primary sclerosing cholangitis. Hepatology 2009;50:808–814.
69. de Boer JF, de Vries HD, Palmiotti A, et al. Cholangiopathy and biliary fibrosis in Cyp2c70-deficient mice are fully reversed by ursodeoxycholic acid. Cell Mol Gastroenterol Hepatol 2021;11:1045–1069.
70. Zhu C, Boucheron N, Muller AC, et al. 24-Norursodeoxycholic acid reshapes immunometabolism in CD8(+) T cells and alleviates hepatic inflammation. J Hepatol 2021;75:1164–1176.
71. Halilbasic E, Fiorotto R, Fickert P, et al. Side chain structure determines unique physiologic and therapeutic properties of norursodeoxycholic acid in Mdr2-/- mice. Hepatology 2009;49:1972–1981.
72. Fickert P, Hirschfield GM, Denk G, et al. Norursodeoxycholic acid improves cholestasis in primary sclerosing cholangitis. J Hepatol 2017;67:549–558.
73. Medina J, Sanz-Cameno P, Garcia-Buey L, et al. Evidence of angiogenesis in primary biliary cirrhosis: an immunohistochemical descriptive study. J Hepatol 2005;42:124–131.
74. Owen T, Carpino G, Chen L, et al. Endothelin receptor-A inhibition decreases ductular reaction, liver fibrosis, and angiogenesis in a model of cholangitis. Cell Mol Gastroenterol Hepatol 2023;16:513–540.
75. Thabut D, Routray C, Lomberk G, et al. Complementary vascular and matrix regulatory pathways underlie the beneficial mechanism of action of sorafenib in liver fibrosis. Hepatology 2011;54:573–585.
76. Yoshiji H, Kuriyama S, Yoshii J, et al. Vascular endothelial growth factor and receptor interaction is a prerequisite for murine hepatic fibrogenesis. Gut 2003;52:1347–1354.
77. Tanaka A, Tsuneyama K, Mikami M, et al. Gene expression profiling in whole liver of bile duct ligated

rats: VEGF-A expression is up-regulated in hepatocytes adjacent to the portal tracts. J Gastroenterol Hepatol 2007;22:1993–2000.
78. Yang L, Kwon J, Popov Y, et al. Vascular endothelial growth factor promotes fibrosis resolution and repair in mice. Gastroenterology 2014;146:1339–1350 e1.
79. Lin Y, Dong MQ, Liu ZM, et al. A strategy of vascular-targeted therapy for liver fibrosis. Hepatology 2022; 76:660–675.
80. Semela D, Das A, Langer D, et al. Platelet-derived growth factor signaling through ephrin-b2 regulates hepatic vascular structure and function. Gastroenterology 2008;135:671–679.
81. Loeuillard E, El Mourabit H, Lei L, et al. Endoplasmic reticulum stress induces inverse regulations of major functions in portal myofibroblasts during liver fibrosis progression. Biochim Biophys Acta Mol Basis Dis 2018; 1864:3688–3696.
82. Lemoinne S, Cadoret A, Rautou PE, et al. Portal myofibroblasts promote vascular remodeling underlying cirrhosis formation through the release of microparticles. Hepatology 2015;61:1041–1055.
83. Novo E, Cannito S, Zamara E, et al. Proangiogenic cytokines as hypoxia-dependent factors stimulating migration of human hepatic stellate cells. Am J Pathol 2007;170:1942–1953.
84. Van Steenkiste C, Ribera J, Geerts A, et al. Inhibition of placental growth factor activity reduces the severity of fibrosis, inflammation, and portal hypertension in cirrhotic mice. Hepatology 2011;53:1629–1640.
85. Rajapaksha IG, Gunarathne LS, Asadi K, et al. Liver-targeted angiotensin converting enzyme 2 therapy inhibits chronic biliary fibrosis in multiple drug-resistant gene 2-knockout mice. Hepatol Commun 2019; 3:1656–1673.
86. Coll M, Arino S, Martinez-Sanchez C, et al. Ductular reaction promotes intrahepatic angiogenesis through Slit2-Roundabout 1 signaling. Hepatology 2022; 75:353–368.
87. Coch L, Mejias M, Berzigotti A, et al. Disruption of negative feedback loop between vasohibin-1 and vascular endothelial growth factor decreases portal pressure, angiogenesis, and fibrosis in cirrhotic rats. Hepatology 2014;60:633–647.
88. Calderone V, Gallego J, Fernandez-Miranda G, et al. Sequential functions of CPEB1 and CPEB4 regulate pathologic expression of vascular endothelial growth factor and angiogenesis in chronic liver disease. Gastroenterology 2016;150:982–997 e30.
89. Cadamuro M, Brivio S, Mertens J, et al. Platelet-derived growth factor-D enables liver myofibroblasts to promote tumor lymphangiogenesis in cholangiocarcinoma. J Hepatol 2019;70:700–709.
90. The innate and adaptive immune systems. 2006. Available from: https://www.ncbi.nlm.nih.gov/books/NBK279396.
91. Tilg H, Adolph TE, Tacke F. Therapeutic modulation of the liver immune microenvironment. Hepatology 2023; 78:1581–1601.
92. Lewis J. Pathological patterns of biliary disease. Clin Liver Dis (Hoboken) 2017;10:107–110.
93. Nakanuma Y, Zen Y, Harada K, et al. Application of a new histological staging and grading system for primary biliary cirrhosis to liver biopsy specimens: interobserver agreement. Pathol Int 2010;60:167–174.
94. Wen Y, Lambrecht J, Ju C, et al. Hepatic macrophages in liver homeostasis and diseases-diversity, plasticity and therapeutic opportunities. Cell Mol Immunol 2021; 18:45–56.
95. Guillot A, Winkler M, Silva Afonso M, et al. Mapping the hepatic immune landscape identifies monocytic macrophages as key drivers of steatohepatitis and cholangiopathy progression. Hepatology 2023;78:150–166.
96. Chen YY, Arndtz K, Webb G, et al. Intrahepatic macrophage populations in the pathophysiology of primary sclerosing cholangitis. JHEP Rep 2019;1:369–376.
97. Cheng S, Zou Y, Zhang M, et al. Single-cell RNA sequencing reveals the heterogeneity and intercellular communication of hepatic stellate cells and macrophages during liver fibrosis. MedComm (2020) 2023; 4:e378.
98. De Muynck K, Vanderborght B, De Ponti FF, et al. Kupffer cells contested as early drivers in the pathogenesis of primary sclerosing cholangitis. Am J Pathol 2023;193:366–379.
99. Cai SY, Ge M, Mennone A, et al. Inflammasome is activated in the liver of cholestatic patients and aggravates hepatic injury in bile duct-ligated mouse. Cell Mol Gastroenterol Hepatol 2020;9:679–688.
100. Guicciardi ME, Trussoni CE, Krishnan A, et al. Macrophages contribute to the pathogenesis of sclerosing cholangitis in mice. J Hepatol 2018;69:676–686.
101. Li X, Liu R, Wang Y, et al. Cholangiocyte-derived exosomal lncRNA H19 promotes macrophage activation and hepatic inflammation under cholestatic conditions. Cells 2020;9.
102. Bonnardel J, T'Jonck W, Gaublomme D, et al. Stellate cells, hepatocytes, and endothelial cells imprint the Kupffer cell identity on monocytes colonizing the liver macrophage niche. Immunity 2019;51:638–654 e9.
103. Ishii M, Iwai M, Harada Y, et al. A role of mast cells for hepatic fibrosis in primary sclerosing cholangitis. Hepatol Res 2005;31:127–131.
104. Tsuneyama K, Kono N, Yamashiro M, et al. Aberrant expression of stem cell factor on biliary epithelial cells and peribiliary infiltration of c-kit-expressing mast cells in hepatolithiasis and primary sclerosing cholangitis: a possible contribution to bile duct fibrosis. J Pathol 1999; 189:609–614.
105. Gaca MD, Pickering JA, Arthur MJ, et al. Human and rat hepatic stellate cells produce stem cell factor: a possible mechanism for mast cell recruitment in liver fibrosis. J Hepatol 1999;30:850–858.
106. Amiot L, Vu N, Drenou B, et al. The anti-fibrotic role of mast cells in the liver is mediated by HLA-G and interaction with hepatic stellate cells. Cytokine 2019;117:50–58.
107. Zhou T, Meadows V, Kundu D, et al. Mast cells selectively target large cholangiocytes during biliary injury via

H2HR-mediated cAMP/pERK1/2 signaling. Hepatol Commun 2022;6:2715–2731.
108. Kennedy L, Meadows V, Kyritsi K, et al. Amelioration of large bile duct damage by histamine-2 receptor vivo-morpholino treatment. Am J Pathol 2020; 190:1018–1029.
109. Kennedy L, Hargrove L, Demieville J, et al. Blocking H1/H2 histamine receptors inhibits damage/fibrosis in Mdr2(-/-) mice and human cholangiocarcinoma tumorigenesis. Hepatology 2018;68:1042–1056.
110. Jones H, Hargrove L, Kennedy L, et al. Inhibition of mast cell-secreted histamine decreases biliary proliferation and fibrosis in primary sclerosing cholangitis Mdr2(-/-) mice. Hepatology 2016;64:1202–1216.
111. Hargrove L, Kennedy L, Demieville J, et al. Bile duct ligation-induced biliary hyperplasia, hepatic injury, and fibrosis are reduced in mast cell-deficient Kit(W-sh) mice. Hepatology 2017;65:1991–2004.
112. Kennedy LL, Hargrove LA, Graf AB, et al. Inhibition of mast cell-derived histamine secretion by cromolyn sodium treatment decreases biliary hyperplasia in cholestatic rodents. Lab Invest 2014;94:1406–1418.
113. Tang J, Yan Z, Feng Q, Yu L, et al. The roles of neutrophils in the pathogenesis of liver diseases. Front Immunol 2021;12:625472.
114. Zimmer CL, von Seth E, Buggert M, et al. A biliary immune landscape map of primary sclerosing cholangitis reveals a dominant network of neutrophils and tissue-resident T cells. Sci Transl Med 2021;13.
115. Saito JM, Maher JJ. Bile duct ligation in rats induces biliary expression of cytokine-induced neutrophil chemoattractant. Gastroenterology 2000;118:1157–1168.
116. Arino S, Aguilar-Bravo B, Coll M, et al. Ductular reaction-associated neutrophils promote biliary epithelium proliferation in chronic liver disease. J Hepatol 2023; 79:1025–1036.
117. Casini A, Ceni E, Salzano R, et al. Neutrophil-derived superoxide anion induces lipid peroxidation and stimulates collagen synthesis in human hepatic stellate cells: role of nitric oxide. Hepatology 1997;25:361–367.
118. Fabre T, Molina MF, Soucy G, et al. Type 3 cytokines IL-17A and IL-22 drive TGF-beta-dependent liver fibrosis. Sci Immunol 2018;3.
119. O'Brien KM, Allen KM, Rockwell CE, et al. IL-17A synergistically enhances bile acid-induced inflammation during obstructive cholestasis. Am J Pathol 2013; 183:1498–1507.
120. Calvente CJ, Tameda M, Johnson CD, et al. Neutrophils contribute to spontaneous resolution of liver inflammation and fibrosis via microRNA-223. J Clin Invest 2019; 129:4091–4109.
121. Harty MW, Muratore CS, Papa EF, et al. Neutrophil depletion blocks early collagen degradation in repairing cholestatic rat livers. Am J Pathol 2010;176:1271–1281.
122. Saito JM, Bostick MK, Campe CB, et al. Infiltrating neutrophils in bile duct-ligated livers do not promote hepatic fibrosis. Hepatol Res 2003;25:180–191.
123. Taylor SA, Assis DN, Mack CL. The contribution of B cells in autoimmune liver diseases. Semin Liver Dis 2019; 39:422–431.
124. Takahashi T, Miura T, Nakamura J, et al. Plasma cells and the chronic nonsuppurative destructive cholangitis of primary biliary cirrhosis. Hepatology 2012; 55:846–855.
125. Li Y, Wang W, Tang L, et al. Chemokine (C-X-C motif) ligand 13 promotes intrahepatic chemokine (C-X-C motif) receptor 5+ lymphocyte homing and aberrant B-cell immune responses in primary biliary cirrhosis. Hepatology 2015;61:1998–2007.
126. Cichoz-Lach H, Grywalska E, Michalak A, et al. Deviations in peripheral blood cell populations are associated with the stage of primary biliary cholangitis and presence of itching. Arch Immunol Ther Exp (Warsz) 2018;66:443–452.
127. Chung BK, Henriksen EKK, Jorgensen KK, et al. Gut and liver B cells of common clonal origin in primary sclerosing cholangitis-inflammatory bowel disease. Hepatol Commun 2018;2:956–967.
128. Chung BK, Ogaard J, Reims HM, et al. Spatial transcriptomics identifies enriched gene expression and cell types in human liver fibrosis. Hepatol Commun 2022; 6:2538–2550.
129. Azad AI, Krishnan A, Troop L, et al. Targeted apoptosis of ductular reactive cells reduces hepatic fibrosis in a mouse model of cholestasis. Hepatology 2020; 72:1013–1028.
130. Thapa M, Tedesco D, Gumber S, et al. Blockade of BAFF reshapes the hepatic B cell receptor repertoire and attenuates autoantibody production in cholestatic liver disease. J Immunol 2020;204:3117–3128.
131. Moritoki Y, Zhang W, Tsuneyama K, et al. B cells suppress the inflammatory response in a mouse model of primary biliary cirrhosis. Gastroenterology 2009; 136:1037–1047.
132. Tsukamoto H. Cytokine regulation of hepatic stellate cells in liver fibrosis. Alcohol Clin Exp Res 1999; 23:911–916.
133. Dhirapong A, Lleo A, Yang GX, et al. B cell depletion therapy exacerbates murine primary biliary cirrhosis. Hepatology 2011;53:527–535.
134. Moritoki Y, Tsuneyama K, Nakamura Y, et al. Anti-drug antibodies against a novel humanized anti-CD20 antibody impair its therapeutic effect on primary biliary cholangitis in human CD20- and FcgammaR-expressing mice. Front Immunol 2018;9:2534.
135. Moritoki Y, Lian ZX, Lindor K, et al. B-cell depletion with anti-CD20 ameliorates autoimmune cholangitis but exacerbates colitis in transforming growth factor-beta receptor II dominant negative mice. Hepatology 2009; 50:1893–1903.
136. Wang Y, Zhang C. The roles of liver-resident lymphocytes in liver diseases. Front Immunol 2019;10:1582.
137. Yang Y, Sheng Y, Wang J, et al. Double-negative T cells regulate hepatic stellate cell activation to promote liver fibrosis progression via NLRP3. Front Immunol 2022;13: 857116.
138. Kobayashi S, Seki S, Kawada N, et al. Apoptosis of T cells in the hepatic fibrotic tissue of the rat: a possible inducing role of hepatic myofibroblast-like cells. Cell Tissue Res 2003;311:353–364.

139. Schildberg FA, Wojtalla A, Siegmund SV, et al. Murine hepatic stellate cells veto CD8 T cell activation by a CD54-dependent mechanism. Hepatology 2011; 54:262–272.
140. Brinkmann H, Cerff R, Salomon M, et al. Cloning and sequence analysis of cDNAs encoding the cytosolic precursors of subunits GapA and GapB of chloroplast glyceraldehyde-3-phosphate dehydrogenase from pea and spinach. Plant Mol Biol 1989;13:81–94.
141. Huang B, Lyu Z, Qian Q, et al. NUDT1 promotes the accumulation and longevity of CD103(+) T(RM) cells in primary biliary cholangitis. J Hepatol 2022;77:1311–1324.
142. Liaskou E, Jeffery LE, Trivedi PJ, et al. Loss of CD28 expression by liver-infiltrating T cells contributes to pathogenesis of primary sclerosing cholangitis. Gastroenterology 2014;147:221–232 e7.
143. Ravichandran G, Neumann K, Berkhout LK, et al. Interferon-gamma-dependent immune responses contribute to the pathogenesis of sclerosing cholangitis in mice. J Hepatol 2019;71:773–782.
144. Chan CW, Chen HW, Wang YW, et al. IL-21, not IL-17A, exacerbates murine primary biliary cholangitis. Clin Exp Immunol 2023.
145. Li Y, Li B, Xiao X, et al. Itaconate inhibits CD103+ TRM cells and alleviates hepatobiliary injury in mouse models of primary sclerosing cholangitis. Hepatology 2023.
146. Liu Q, Yang Q, Wu Z, et al. IL-1beta-activated mTORC2 promotes accumulation of IFN-gamma(+) gammadelta T cells by upregulating CXCR3 to restrict hepatic fibrosis. Cell Death Dis 2022;13:289.

Received November 1, 2023. Accepted January 8, 2024.

Correspondence
Address correspondence to: Lindsey Kennedy, PhD, Department of Medicine, Indiana University School of Medicine, Department of Research, Richard L. Roudebush VA Medical Center, 702 Rotary Circle, Room 007, Indianapolis, Indiana 46202. e-mail: linkenn@iu.edu.

CRediT Authorship Contributions
Ludovica Ceci (Writing – original draft, equal; Visualization, equal)
Eugenio Gaudio (Writing – review and editing, equal; Resources, equal; Funding acquisition, equal)
Lindsey Kennedy (Writing – original draft, lead; Visualization, lead; Resources, lead; Conceptualization, lead)

Conflicts of interest
The authors disclose no conflicts.

Funding
Supported by a Career Development Award-2 to LK (1IK2BX005306) from the United States Department of Veteran's Affairs, Biomedical Laboratory Research and Development Service, and research project grants to EG from Sapienza, University of Rome. Portions of these studies were supported by resources at the Richard L. Roudebush VA Medical Center, Indianapolis, IN. The views expressed in this article are those of the authors and do not necessarily represent the views of the Department of Veterans Affairs.