Involvement of cholangiocyte proliferation in biliary fibrosis

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Abstract

Cholangiocytes are the epithelial cells that line the biliary tree. In the adult liver, they are a mitotically dormant cell population, unless ductular reaction is triggered by injury. The ability of cholangiocytes to proliferate is important in many different human pathological liver conditions that target this cell type, which are termed cholangiopathies (i.e. primary biliary cirrhosis, primary sclerosing cholangitis and biliary atresia). In our article, we provide background information on the morphological and functional heterogeneity of cholangiocytes, summarize what is currently known about their proliferative processes, and briefly describe the diseases that target these cells. In addition, we address recent findings that suggest cholangiocyte involvement in epithelial-to-mesenchymal transformation and liver fibrosis, and propose directions for future studies.

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Key words: Biliary epithelium; Cholangiopathies; Cholestasis; Integrins; Liver fibrosis; Proliferation

INTRODUCTION

Cholangiocytes are epithelial cells that line the biliary system and make up 3%-5% of the liver cell population. The biliary system is a tree-like, three-dimensional network of ducts that range in size from small (< 15 µm) to large bile (≥ 15 µm) ducts in animal models. These ducts are lined by cholangiocytes that also vary in size, dependent upon the size of the bile duct. For example, large bile ducts are lined by large cholangiocytes and small bile duct by small cholangiocytes. The major functions of the biliary system are to deliver bile from the liver to the gallbladder to the duodenum and the modification of
bile of canalicular origin\[^{5,7,8}\]. Cholangiocytes modify bile\[^{9-11}\] through a series of re-absorptive and secretory processes involving water, ions, and solutes, which are heavily regulated by gastrointestinal hormones, such as secretin\[^{11,13}\]. Secretin receptors (SR) are present only on cholangiocytes in the liver\[^{14}\]. Large but not small cholangiocytes express SR and are responsive to secretin in the normal rodent liver\[^{1}\]. Small cholangiocytes de novo express secretin receptor during pathological conditions where large functionally active cholangiocytes are damaged\[^{12,15}\]. In large cholangiocytes, secretin increases cyclic adenosine 3′, 5′-monophosphate (cAMP) levels\[^{6,9-19}\] and induces the activation of the Cl-channel (cystic fibrosis transmembrane conductance regulator, CFTR), which leads to the activation of the Cl-/HCO₃⁻ anion exchanger 2 (AE2) and the secretion of bicarbonate in bile\[^{3}\]. In addition to their normal biliary function, cholangiocytes have also been found to detoxify xenobiotics\[^{9-12}\] and they also provide one of the first lines of defense against microbes in the biliary system\[^{7,28}\]. Our knowledge of the factors that control cholangiocyte function has greatly increased in recent years, due to an increased interest in liver diseases, such as biliary cancer, biliary fibrosis and cholestatic liver disease\[^{9-11}\].

Cholangiocytes are the target of many diseases, referred to as cholangiopathies, with a high impact in crosstalk between mesenchymal cells and cholangiocytes, beta 2 (TGF-β2) (PDGF-BB), endothelin-1 (ET1), connective tissue growth factor (CTGF), and transforming growth factor-beta 2 (TGFβ2)\[^{26,29}\].

### CHOLANGIOCYTE PROLIFERATION

In animal models of cholestasis and biliary tract injury, cholangiocyte proliferation is coordinately regulated by a number of neuroendocrine factors, such as hormones, neuropeptides and neurotransmitters, which have been recently reviewed\[^{3}\]. Proliferating cholangiocytes display neuroendocrine phenotypes, and as such, secrete and respond to a number of hormones, neuropeptides and neurotransmitters\[^{8}\]. The capacity of cholangiocytes to proliferate is evident under specific experimental conditions in animal models as well as in human pathological conditions\[^{9,30,31}\]. Four types of “ductular reaction” have been described in animal models\[^{9,30,31}\]. Cholangiocyte proliferation is described as “ductular reaction”, a term coined by Popper to identify the expanded population of epithelial cells at the interface of the biliary tree, which refers to proliferation of pre-existing ductules, progenitor cell activation, and the appearance of intermediate hepatocytes\[^{9,30,31}\]. The ability of cholangiocytes to proliferate is important in many different human pathological conditions. Type I or “typical” cholangiocyte proliferation results in an increased number of intrahepatic bile ducts (hyperplasia), which re mains confined to portal spaces\[^{31}\]. The proliferating cholangiocytes form a well-differentiated three-dimensional network of tubular structures with a well-defined lumen, which originates from pre-existing bile ducts located within portal areas\[^{31}\]. In humans, this type is observed in acute obstructive cholestasis, extrahepatic biliary atresia\[^{8,31,32}\], and in early phases of chronic cholestatic liver diseases (in association with “atypical” proliferation)\[^{9,32}\]. In the rat, “typical” cholangiocyte proliferation occurs after bile duct ligation (BDL), partial hepatectomy, chronic a-naphthylisothiocyanate (ANIT) feeding, chronic L-proline treatment and prolonged oral administration of certain bile acids\[^{9,31}\].

Type II or “atypical” proliferation is characterized by irregular proliferation of intrahepatic bile ducts not only confined to portal areas, but also sprouting into periportal and parenchymal regions\[^{9,31,32}\]. This implies that the newly formed bile ducts are functionally ineffective\[^{31}\]. In humans, this type is observed in PSC, PBC, after massive hepatic necrosis, focal nodular hyperplasia, chronic cholestatic diseases, alcoholic liver diseases, and a long-standing hepatic biliary obstruction\[^{9,31,32}\]. Type II refers to ductular metaplasia of liver cell plates, predominantly observed in chronic cholestatic conditions like PBC\[^{9,31,32}\]. It is thought that the “atypical” proliferation arises from both proliferation of pre-existing ductules and expansion of the hepatic progenitor cell compartment\[^{31}\].

Type III ductular proliferation, sometimes called “ductular hyperplasia” or “oval cell” in the past\[^{39}\] consists in the massive proliferation of ductular hepatocytes (derived from oval cells) or progenitor cells in the liver, with submassive hepatic necrosis\[^{9,31}\]. It involves activation and proliferation of hepatic progenitor cells,
appearing as periportal ductular structures in the case of
submassive hepatocellular necrosis, and representing an
alternative parenchymal regeneration when hepatocellular
regeneratory capacity is insufficient, which is primarily the
case in chronic liver disease [6,31].

Type IV ductular hyperplasia, now called “oval cell”,
occur in early stages of carcinogenesis in rat liver and is
caused by chemicals, like ethionine, 2-acetylaminofluorene,
and furan [9,32,33]. This type of proliferation induces the
formation of disorganized tubular structures with a poorly
defined duct lumen, which randomly sprout into hepatic lobules, creating a distorted hepatic architecture [9,33].

“Oval cells” are cell types activated to proliferate in early
stages of intoxication with carcinogens. The nature of these
cells, that is, whether they are fibroblasts, endothelial
cells, transformed hepatocytes, or biliary ductules, is a subject of debate [8].

One of the functional characteristics of proliferating
cholangiocytes is that they acquire a neuroendocrine phenotype, especially in “atypical” ductular reaction [9,32,34,35].

Proliferating cholangiocytes, from “atypical” proliferation,
show phenotypical features of neuroendocrine cells like
chromogranin A, S-100 protein glycolipid A2-B4, and a neural cell adhesion molecule [9]. During cholestatic liver
diseases, the cholangiocytes express neuroendocrine phenotypes and respond to a number of hormones
and neuropeptides [34]. For example, these proliferating
cholangiocytes have increased expression and secretion
of serotonin, endogenous opioid peptides and neuro-
phenylalkanes (and their corresponding receptors) [23]. They
also show an enhanced response to hormones and
neuropeptides such as secretin, gastrin, somatostatin,
acetylcholine, and adrenergic and dopaminergic ago-
nists [9,33]. Studies suggest that the formation of a neuroendocrine compartment is crucially instrumental in
the progression of liver disease [9]. Thus, understanding
how we can manage the proliferation of cholangiocytes
is important for the development of treatments for liver
diseases.

CHOLESTATIC LIVER DISEASES AND
BILIARY FIBROSIS

In response to acute liver injury, cholangiocytes pro-
liferate in an effort to regain proper liver function.
Human chronic liver diseases are characterized by repet-
titive liver injury due to chronic infection by viral agents
(hepatitis B and C viruses), toxin/drug exposure (alcohol
consumption), and autoimmune attack (PBC/PSC) [26].

Chronic liver diseases cause a continuous activation
of the wound-healing response that results in an accum-
ulation of extracellular matrix, eventually leading to
cirrhosis and hepatic failure [26]. Thus, cirrhosis can
be defined as an advanced stage of fibrosis involving
the formation of abnormal cell clusters surrounded by
excessive extra-cellular matrix, which also results in
significant changes in vascular framework [26,36-38].

As originally described, liver fibrosis during acute and
chronic cholestasis involves the stepwise process that
includes “ductular reaction”, accompanied by poly-
morphonuclear leukocytes and an increase in matrix
deposition, leading to periportal fibrosis and eventually
biliary cirrhosis [9,39].

APOTOPSIS IN CHOLESTATIC LIVER
DISEASES

Apoptosis is thought to play a major role in cholestatic
liver diseases such as PBC, PSC and biliary atresia [40,41].

In immune-mediated liver diseases, such as PBC, PSC
and autoimmune hepatitis, recent studies have indicated that programmed cell death ligands and circulating
apoptotic markers might serve as diagnostic markers
for these diseases [40,42]. Apoptosis of cholangiocytes
has been observed in a number of animal models of chole-
stasis and biliary injury [12,13,43-45]. A recent study has shown that anti-apoptotic receptor 5 (DR5) monoclonal
antibody induced cholangitis that exhibited the typical
histological appearance of PSC and PBC [46]. These
findings led them to believe the death signal mediated
by TNF-related apoptosis-inducing ligand (TRAIL)
receptor 2/DR5 may be a key regulator of cholestatic
liver injury [46].

SECRETION OF PROFIBROGENIC
FACTORS BY CHOLANGIOCYTES

Sedlaczek et al [47] demonstrated that during the pro-
gression of biliary fibrosis, proliferating bile duct
epithelial cells are the predominant source of the profibrogenic factor CTGF. Newly formed bile ducts also
express the message for alpha 1 (IV) procollagen, indicat-
ing that proliferating cholangiocytes are a potential
source of hepatic collagen during fibrosis [48]. TGFβ2
expression has been shown to be a specific property
of proliferating bile duct epithelial cells and it has been
postulated that its expression was related to the forma-
tion of specialized periportal connective tissue
during bile duct proliferation [49]. In addition, platelet-
derived growth factor is expressed in proliferating
cholangiocytes during experimental bile fibrosis in rats [50]. Administration of pentoxifylline exerts an
antifibrogenic effect by reducing the PDGF-induced
ERK-dependent signaling and proliferation of extra-
cellular matrix-producing cells [51]. Other studies have
shown that during biliary injury and fibrosis, the
hedgehog pathway activation induces cholangiocyte
production of chemokines that recruit natural killer T
cells to portal tracts [52]. Hedgehog ligands regulate tissue-
remodeling responses during embryogenesis and adult
tissue repair [52,38]. Cholangiocytes produce and respond to hedgehog ligands [18,32]. Hedgehog pathway activation
promotes proliferation and enhances viability of these
cells, which unrestrained, could cause progressive fi
brosis and hepatic architectural distortion\cite{33}. The targeting of the profibrogenic program that is activated in proliferating cholangiocytes and the profibrogenic factors they secrete, might provide an unique opportunity for the development of treatments for biliary fibrosis.

**EPI THEL IAL-TO-MESENCHYMAL TRANSITION (EMT)**

Cholangiocytes normally exist in an highly differentiated state that allows them to modify bile of canalicular origins by a coordinated series of hormone-regulated secretory and absorptive processes\cite{34}. Cholangiocytes proliferate in response cholestasis induced by bile duct ligation and during other pathological conditions such as, partial hepatectomy and CCl\(_4\)-induced liver damage\cite{35}. Evidence suggests that proliferating cholangiocytes have a role in the induction of fibrosis, either directly through epithelial-mesenchymal transition\cite{36}, or indirectly through the activation of hepatic stellate cells\cite{37}. EMT is a complex process that involves cross talk among several signaling pathways that collaborate to affect global, but gradual, changes in cell structure and function\cite{38}. In this dynamic process cells eventually lose their typical epithelial characteristics (proteins that mediate cell-cell, cell-matrix contacts and cytoskeletal organization)\cite{39,40}. These changes cause epithelial cells to dissociate from their neighbors, gradually acquire a motile phenotype, and eventually migrate out of epithelial sheets and into adjacent mesenchyme\cite{21,56}. There are three biological subtypes of EMT, which have been previously reviewed\cite{57,59}. Type 1 is present during implantation, embryogenesis and organ development. Type 1 also generates mesodermal and endodermal mesenchyme that can then undergo mesenchymal-to-epithelial transition (MET) to generate a secondary epithelia that can undergo additional rounds of EMT and MET to form various organs\cite{37,59}. Although these concepts remain to be proven, it is possible that the balance between EMT and MET controls the outcome of chronic liver injury. Type 2 is associated with inflammation\cite{37,59}. When there is injury with inflammation, this type generates fibroblastic cells that eventually cause organ destruction\cite{37,59}. Type 3 is the result of genetic and epigenetic changes in cancer cells with invasion and spread of tumor cells that eventually form metastatic tumors apart from the primary tumor\cite{37}.

EMT is involved in tissues that are being developed or remodeled\cite{37}. The presence of EMT in embryonic development\cite{36,57} and cancer invasion and metastasis\cite{40} is well established, and there is some evidence for EMT in the liver\cite{37}. As a highly regenerative organ, the liver has the ability to restore its mass even in the face of extensive functional cell loss. However in situations of prolonged injury, this method of repair can lead to fibrosis\cite{41}. Recent data suggest the classification at cellular, molecular and tissue level, multiple mechanisms for fibrosis as follows: (1) chronic activation of the wound-healing reaction; (2) oxidative stress and related reactive intermediates; and (3) profibrogenic derivatives of EMT\cite{26}. Liver fibrosis develops from a heterogeneous population of profibrogenic hepatic myofibroblasts (MFs) that may originate from activated hepatic stellate cells and portal fibroblasts, bone marrow derived cells, or possibly cholangiocytes and hepatocytes that have undergone EMT\cite{26}. These myofibroblasts are characterized by increased proliferation, migration, and contractility, and a relative resistance to apoptosis\cite{37}. TGF\(\beta\) plays a major role in the induction of EMT in development, carcinogenesis, and fibrosis, with different isoforms mediating various effects depending the cell type and setting\cite{42}. EMT in response to TGF\(\beta\)-1 and in fibrosis is mediated predominantly via Smad-dependent (mainly Smad3) pathways\cite{57,59}. TGF\(\beta\)-1 has previously been shown to play a critical role in the progression of liver fibrosis\cite{43}. In fact, a recent study demonstrated that blockage of TGF\(\beta\)-1 in proliferating biliary epithelia retards biliary fibrosis in an animal model of liver fibrosis\cite{44}. Interestingly, a recent study demonstrated that EMT contributes to portal tract fibrogenesis during human chronic liver disease, which is characterized by chronic inflammation\cite{45}. In fact, inflammation plays a key role as the convergence point between EMT and the progression of fibrosis in many organ systems, and has been previously reviewed\cite{46}.

EMT has been implicated as a key mechanism in the pathogenesis of liver fibrosis. In study of human samples from several types of liver diseases, Diaz and colleagues present convincing histological data revealing that EMT occurs in human liver fibrosis, particularly in disease associated with prominent bile ductular proliferation, such as biliary atresia and PBC\cite{58}. They observed significant colocalization between cytokeratin (CK-19, a cholangiocyte-specific epithelial marker) and other markers of EMT (i.e. vimentin, Snail, and fibroblast-specific protein 1) in biliary atresia and PBC. Robertson et al\cite{57} have also demonstrated that biliary EMT occurs during post-transplantation recurrence of PBC. The study found that in livers affected by early recurrent PBC, there were indications that biliary EMT was occurring which was associated by cholangiocyte expression of S100A4 (a key marker of early fibroblast lineage), vimentin and pSMAD 2/3 with the data indicating that this process was driven by TGF-\(\beta\)-1. S100A4 expression appears to occur prior to the onset of the appearance of other features of recurrent PBC, which suggests that EMT may be an initiating event, and may potentially explain the loss of bile duct epithelia during the course of the disease\cite{57}. Rygiel and colleagues have also clearly demonstrated that EMT occurs during portal tract fibrosis\cite{43}. Their work shows that cholangiocytes forming the small and medium sized bile ducts and responding with ductular reaction undergo
EMT during chronic liver disease, which results in the formation of invasive fibroblasts\cite{69-71}. Similar findings have been demonstrated in liver cells from rodents, and humans can undergo EMT\cite{68}. This study found that both hepatic stellate cells and hepatic epithelial progenitor cells coexpress epithelial and mesenchymal markers indicating that EMT occurs in adult livers\cite{68}. This recent evidence indicates that EMT probably plays a critical role in the process of portal fibrosis during chronic liver diseases.

INTEGRINS AND BILIARY FIBROSIS

Integrins are a family of heterodimeric transmembrane glycoproteins composed of α and β chain protein subunits that act as cell surface receptors\cite{69-71}. Integrins are a large family of 24 heterodimers formed from eight β subunits and α subunits that have been identified. Integrins play a role in communicating messages between the cell and the environment via extracellular matrix interactions\cite{69-71}. The binding to extracellular matrix to integrins results cytoplasmic signals in the integrin-expressing cell contributing to cell growth, differentiation, invasion, metastasis, and survival\cite{65-72,74}. Integrins also play a key role in how cells sense to mechanical stimuli in the environment\cite{65-72,74}. Integrins have been shown to interact with cell surface ligands, growth factors, pathogens, soluble proteases and transmembrane proteins\cite{75,76}. The loss of integrin-mediated contacts, usually leads to apoptosis, a process called anoikis\cite{77,78}.

Two recent studies have demonstrated that targeting αvβ6 integrin expressed by proliferating biliary epithelia might provide a novel antifibrotic therapy\cite{65,78}. Patsenker et al demonstrated that αvβ6 integrin is strongly upregulated in proliferating biliary epithelium in rodent (BDL, thioacetamide, Mdr2 (Abcb4)−/− mice) and human models (chronic hepatitis C\cite{69,79}) and that it drives fibrogenesis via adhesion to fibronectin and stimulates auto/paracrine TGF-β1 activation\cite{79}. Most importantly, they demonstrated in vivo that a single dose of a small molecule αvβ6 integrin inhibitor induced antifibrogenic and profibrolytic genes, reduced activated cholangiocyte proliferation, and adhesion to fibronectin\cite{65,78}. In addition, increased vascularization has a key role in the development of biliary fibrosis, as supported by fibrosis that has been limited in animal models where angiogenesis has been inhibited\cite{14}.

REVERSAL OF BILIARY FIBROSIS

Although we have made impressive progress in our understanding of the pathogenesis of liver fibrosis in the past two decades, translation of this knowledge into antifibrotic therapies has ground to a halt short of clinical trials\cite{37}. The reduction of fibrosis within cirrhotic liver tissue would lead to a reduction of portal hypertension and consequent clinical complications, thus improving overall liver function, which would extend the complication-free patient survival time and reduce the need for liver transplants\cite{29}. Studies have suggested the reversibility of liver fibrosis, but the mechanisms for such a reversal are poorly understood\cite{79}. In BDL rats, Popov and colleagues introduced macrophages to damaged biliary epithelium via Roux-en-Y bilio-jejunal anastomosis. After engulfing apoptotic cholangiocytes, macrophages upregulate matrix metalloproteinases and become fibrolytic effector cells\cite{79}. This suggests a link between apoptotic epithelial cells, macrophages, and the reversal of fibrosis.

FUTURE DIRECTIONS

In order to develop clinical treatments, we need to learn more about how cholangiocytes interact with other cell types, and the role that EMT contributes to biliary fibrosis. The studies presented in this review raise the important question of the relationship between cholangiocytes and myofibroblasts as to whether cholangiocytes may be an additional source of fibroblasts during chronic liver injury. Also, important will be to determine how cholangiocytes contribute to soluble factors and to the activation of myofibroblasts and to the deposition of extracellular matrix. Understanding the interactions and contributions of these cell types to the process of biliary fibrosis will be essential for determining whether different mechanisms of fibrosis occur in the various cholangiopathies, which, in turn, will aid in designing disease-specific therapies.

REFERENCES

1. Glasier SS, Gaudio E, Miller T, Alvaro D, Alpini G. Cholangiocyte proliferation and liver fibrosis. Expert Rev Mol Med 2009; 11: e7
2. Racanelli V, Rehermann B. The liver as an immunological organ. Hepatology 2006; 43: S54-S62
3. Alpini G, McGill JM, Larusso NF. The pathobiology of biliary epithelia. Hepatology 2002; 35: 1256-1268
4. Alpini G, Roberts S, Kuntz SM, Ueno Y, Guha B, Podila PV, LéSage G, LaRusso NF. Morphological, molecular, and functional heterogeneity of cholangiocytes from normal rat liver. Gastroenterology 1996; 110: 1636-1643
5. Roskams TA, Theise ND, Balabaud C, Bhagat G, Bhatkal PS, Bioulac-Sage P, Brunt EM, Crosby HA, Desmet V, Finegold MJ, Geller SA, Gouw AS, Hydroglou P, Kiselys AS, Kojiro M, Lefkowitz RH, Nakanuma Y, Olynynk JK, Park YN, Portmann B, Saxena R, Scheuer PJ, Strain AJ, Thung SN, Wanless IR, West AB. Nomenclature of the finer branches of the biliary tree: canals, ductules, and ductular reactions in human livers. Hepatology 2004; 39: 1739-1745
6. Desmet VJ, The amazing universe of hepatic microstructure. Hepatology 2009; 50: 333-344
7. Lazaridis KN, Strazzabosco M, Larusso NF. The cholangiopathies: disorders of biliary epithelia. Gastroenterology 2004; 127: 1565-1577
8. Alpini G, Ulrich CD 2nd, Phillips JO, Pham LD, Miller LJ, LaRusso NF. Upregulation of secretin receptor gene
expression in rat cholangiocytes after bile duct ligation. Am J Physiol 1994; 266: G922-G928

9 Alvaro D, Mancino MG, Glaser S, Gaudio E, Marzioni M, Francis H, Alpini G. Proliferating cholangiocytes: a neuroendocrine compartment in the diseased liver. Gastroenterology 2007; 132: 415-423

10 Glaser SS, Gaudio E, Rao A, Pierce LM, Onori P, Franchitto A, Francis HL, Dostal DE, Ueno Y, Marucci L, Alpini GD. Morphological and functional heterogeneity of the mouse intrahepatic biliary epithelium. Lab Invest 2009; 89: 456-469

11 Kanno N, LeSage G, Glaser S, Alvaro D, Alpini G. Functional heterogeneity of the intrahepatic biliary epithelium. Hepatology 2000; 31: 555-561

12 LeSage GD, Glaser SS, Marucci L, Benedetti A, Phinizy JL, Rogers R, Caligiuri A, Papa E, Tretjak Z, Jezquel AM, Alpini GD. Acute carbon tetrachloride feeding induces damage of large but small cholangiocytes from BDL rat liver. Am J Physiol 1999; 276: G1289-G1301

13 Tietz PS, Chen XM, Gong AY, Huiebert RC, Masyuk A, Masyuk T, Splinter PL, LaRusso NF. Experimental models to study cholangiocyte biology. World J Gastroenterol 2002; 8: 1-4

14 Alpini G, Glaser SS, Ueno Y, Rogers R, Phinizy JL, Francis H, Baiocchi L, Holcomb LA, Caligiuri A, Glaser ZZ. Bile acid feeding induces cholangiocyte proliferation and secretion: evidence for bile acid-regulated ductal secretion. Gastroenterology 1999; 116: 179-186

15 LeSage GD, Benedetti A, Glaser S, Marucci L, Tretjak Z, Caligiuri A, Rogers R, Phinizy JL, Baiocchi L, Francis H, Lasater J, Ugili L, Alpini G. Acute carbon tetrachloride feeding selectively damages large, but not small, cholangiocytes from normal mice. Hepatology 2000; 31: 307-319

16 Kato A, Gores GJ, LaRusso NF. Secretin stimulates exocytosis in isolated bile duct epithelial cells by a cyclic AMP-mediated mechanism. J Biol Chem 1992; 267: 15223-15229

17 Alpini G, Glaser S, Robertson W, Rodgers RE, Phinizy JL, Lasater J, LeSage GD. Large but not small intrahepatic bile ducts are involved in secretin-regulated ductal secretion. Am J Physiol 1997; 272: G1064-G1074

18 Glaser SS, Gaudio E, Rao A, Pierce LM, Onori P, Franchitto A, Francis HL, Dostal DE, Ueno Y, Marucci L, Alpini GD. Morphological and functional heterogeneity of the mouse intrahepatic biliary epithelium. Lab Invest 2009; 89: 456-469

19 Ueno Y, Alpini G, Yahagi K, Kanno N, Moritoki Y, Fukushima K, Glaser S, LeSage G, Shimosegawa T. Evaluation of differential gene expression by microarray analysis in small and large cholangiocytes isolated from normal mice. Liver Int 2003; 23: 449-459

20 Marzioni M, Fava G, Alvaro D, Alpini G, Benedetti A. Control of cholangiocyte adaptive responses by visceral hormones and neuropeptides. Clin Rev Allergy Immunol 2009; 36: 13-22

21 Greenbaum LE. Hedgehog signaling in biliary fibrosis. J Clin Invest 2008; 118: 3263-3265

22 Strazzabosco M, Fabris L, Spirli C. Pathophysiology of cholangiopathies. J Clin Gastroenterol 2005; 39: S90-S102

23 Alvaro D, Mancino MG. New insights on the molecular and pathophysiological mechanisms of atypical ductular reactions with those seen in experimental liver disease. J Biol Chem 2002; 277: 29655-29663

24 Rygiel KA, Robertson H, Marshall HL, Pekalski M, Zhao L, Booth TA, Jones DE, Burt AD, Kirby JA. Epithelial-mesenchymal transition contributes to portal tract fibrogenesis during human chronic liver disease. Lab Invest 2008; 88: 112-123

25 Marzioni M, Fava G, Benedetti A. Nervous and Neuroendocrine regulation of the pathophysiology of cholestasis and of biliary carcinogenesis. World J Gastroenterol 2006; 12: 3471-3480

26 Parola M, Pinzani M. Hepatic wound repair. Fibrogenesis Tissue Repair 2009; 2: 4

27 Roskams T, van den Oord JJ, De Vos R, Desmet VJ. Neuroendocrine features of reactive bile ductules in cholestatic liver disease. Am J Pathol 1990; 137: 1019-1025

28 Aller MA, Arias JL, García-Domínguez J, Arias JJ, Durán M, Arias J. Experimental obstructive cholestasis: the wound-like inflammatory liver response. Fibrogenesis Tissue Repair 2008; 1: 6

29 Pinzani M. Rombouts K. Liver fibrosis: from the bench to clinical targets. Dig Liver Dis 2004; 36: 231-242

30 Sell S. Comparison of liver progenitor cells in humans atypical ductular reactions with those seen in experimental models of liver injury. Hepatology 1998; 27: 317-331

31 Svegliati-Baroni G, De Minicis M, Marzioni M. Hepatic fibrogenesis in response to chronic liver injury: novel insights on the role of cell-to-cell interaction and transition. Liver Int 2008; 28: 1052-1064

32 Alvaro D, Gigliozzi A, Attili AF. Regulation and deregulation of cholangiocyte proliferation. J Hepatol 2000; 33: 333-340

33 Yang S, Koteish A, Lin H, Huang J, Roskams T, Dawson V, Diehl AM. Oval cells compensate for damage and replicative senescence of mature hepatocytes in mice with fatty liver disease. Hepatology 2004; 39: 403-411

34 Glaser S, DeMorrow S, Francis H, Ueno Y, Gaudio E, Vaculin S, Venter J, Franchitto A, Onori P, Vaculin B, Marzioni M, Wise C, Planthannon M, Savage J, Pierce L, Mancinelli R, Alpini G. Progestosterone stimulates the proliferation of female and male cholangiocytes via autocrine/paracrine mechanisms. Am J Physiol Gastrointest Liver Physiol 2008; 295: G124-G136

35 Roskams T, Cissimian D, De Vos R, Libbrecht L. Neuroregulation of the neuroendocrine compartment of the liver. Anat Rec A Discov Mol Cell Biol 2004; 280: 910-923

36 Friedman SL. Mechanisms of disease: Mechanisms of hepatic fibrosis and therapeutic implications. Nat Clin Pract Gastroenterol Hepatol 2004; 1: 98-105

37 Popov Y, Schuppun D. Targeting liver fibrosis: strategies for development and validation of antifibrotic therapies. Hepatology 2009; 50: 1294-1306

38 Omenetti A, Porrello A, Jung Y, Yang L, Popov Y, Choi SS, Witek RP, Alpini G, Venter J, Vandongen HM, Syn WK, Baroni GS, Benedetti A, Schuppun D, Diehl AM. Hedgehog signaling regulates epithelial-mesenchymal transition during biliary fibrosis in rodents and humans. J Clin Invest 2008; 118: 3331-3342

39 Roskams T, Desmet V. Ductular reaction and its diagnostic significance. Semin Diagn Pathol 1998; 15: 259-269

40 Kremer AE, Rust C, Eichhorn P, Beuers U, Holdeneried S. Immune-mediated liver diseases: programmed cell death ligands and circulating apoptotic markers. Expert Rev Mol Diagn 2009; 9: 139-156

41 Erickson N, Mohanty SK, Shivakumar P, Sabla G, Chadbrakty R, Bezerra JA. Temporal-spatial activation of apoptosis and epithelial injury in murine experimental biliary atresia. Hepatology 2008; 47: 1567-1577

42 Berg CP, Stein GM, Koppler H, Gregor M, Wesselborg S, Lauber K. Apoptosis-associated antigens recognized by autoantibodies in patients with the autoimmune liver disease primary biliary cirrhosis. Apoptosis 2008; 13: 63-75

43 Glaser S, Alvaro D, Francis H, Ueno Y, Marucci L, Benedetti A, De Morrow S, Marzioni M, Mancino MG, Phinizy JL, Reichenbach R, Fava G, Summers R, Venter J, Alpini G. Adrenergic receptor agonists prevent bile duct injury induced by adrenergic denervation by increased cAMP levels and
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activation of Akt. Am J Physiol Gastrointest Liver Physiol 2006; 290: G813-G826

Marziones M, LeSage GD, Glaser S, Patel T, Marienfeld C, Ueno Y, Francis H, Alvaro D, Tadlock L, Benedetti A, Marconi L, Delitch L, Phimzy JK, Alpini G. Taurocholate prevents the loss of intrathepatic bile ducts due to vagotomy in bile duct-ligated rats. Am J Physiol Gastrointest Liver Physiol 2003; 284: G387-G382

Gaudio E, Barbaro B, Alvaro D, Glaser S, Francis H, Franchitto A, Onori P, Ueno Y, Marzioni M, Fava G, Venter J, Reichenbach R, Summers R, Alpini G. Administration of r-VEGF-A prevents hepatic artery ligation-induced bile duct damage in bile duct-ligated rats. Am J Physiol Gastrointest Liver Physiol 2006; 291: G307-G317

Takeda K, Kojima Y, Ikejima K, Harada K, Yamashina S, Okumura K, Aoyama T, Frese S, Ikeha D, Haynes NM, Cretney E, Yagita H, Sueyoshi N, Sato N, Nakamura Y, Smyth MJ, Okumura K. Death receptor 5-mediated-apoptosis contributes to cholestatic liver disease. Proc Natl Acad Sci USA 2008; 105: 10989-10990

Sedlacsek N, Jia JD, Bauer M, Herbst R, Huelh M, Hahn EG, Schuppan D. Proliferating bile duct epithelial cells are a major source of connective tissue growth factor in rat biliary fibrosis. Am J Pathol 2001; 158: 1239-1244

Milani S, Herbst H, Schuppan D, Kim KY, Riecken EO, Stein H. Procollogen expression by nonparenchymal rat liver cells in experimental biliary fibrosis. Gastroenterology 1990; 98: 175-184

Milan S, Herbst H, Schuppan D, Stein H, Surrenti C. Transforming growth factors beta 1 and beta 2 are differentially expressed in fibrotic liver disease. Am J Pathol 1991; 138: 1221-1229

Grappone C, Pinzani M, Parola M, Pellegreni G, Caligiuri A, DeFranco R, Marra F, Herbst H, Alpini G, Milani S. Expression of platelet-derived growth factor in newly formed cholangiocytes during experimental biliary fibrosis in rats. J Hepatol 1999; 31: 100-109

Pinzani M, Marra F, Caligiuri A, DeFranco R, Gentilini A, Faili P, Gentilini P. Inhibition by pentoxifylline of extracellular signal-regulated kinase activation by platelet-derived growth factor in hepatic stellate cells. Br J Pharmacol 1996; 119: 1117-1124

Omenetti A, Syn WK, Jung Y, Francis H, Porrello A, Witek RP, Choi SS, Yang L, Mayo MJ, Gershwin ME, Alpini G, Diehl AM. Repair-related activation of hedging signal promotes cholangiocyte chemokine production. Hepatology 2009; 50: 518-527

Omenetti A, Yang L, Li YX, McCall SJ, Jung Y, Sicklick JK, Huang J, Choi S, Suzuki A, Diehl AM. Hedgehog-mediated mesenchymal-epithelial interactions modulate hepatic response to bile duct ligation. Lab Invest 2007; 87: 499-514

Alpini G, Pratt RT, LaRusso NF. The pathobiology of biliary epithelia. The Liver; Biology & Pathobiology, 4E I M Arias, Alpini G 2009; 1117-1124

Diaz R, Kim JW, Hui JJ, Li Z, Swain GP, Fong KS, Csiaszar K, Russo PA, Rand EB, Furth EE, Wells RG. Evidence for the epithelial to mesenchymal transition in biliary atresia fibrosis. Hum Pathol 2008; 39: 102-115

Choi SS, Omenetti A, Witek RP, Moylan CA, Syn WK, Jung Y, Yang L, Sudan DL, Sicklick JK, Michielti GA, Rojkind M, Diehl AM. Hedgehog pathway activation and epithelial-to-mesenchymal transitions during myofibroblastic transformation of rat hepatic cells in culture and cirrhosis. Am J Physiol Gastrointest Liver Physiol 2009; 297: GI093-GI106

Choi SS, Diehl AM. Epithelial-to-mesenchymal transitions in the liver. Hepatology 2009; 50: 2007-2013

Demetris AJ, Spech S, Nozaki I, Lunz JG 3rd, Stolz DB, Murase N, Wu T. Small proline-rich proteins (SPRR) function as SH3 domain ligands, increase resistance to injury and are associated with epithelial-mesenchymal transition (EMT) in cholangiocytes. J Hepatol 2008; 48: 276-288

Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2002; 2: 442-454

Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou Y, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Brissen C, Yang J, Weinberg RA. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008; 133: 704-715

Greenbaum LE, Wells RG. The role of stem cells in liver repair and fibrosis. Int J Biochem Cell Biol 2009; Epub ahead of print

Nawshad A, Lagamba D, Polad A, Hay ED. Transforming growth factor-beta signaling during epithelial-mesenchymal transformation: implications for embryogenesis and tumor metastasis. Cells Tissues Organs 2005; 179: 11-23

Willis BC, Borok Z. TGF-beta-induced EMT: mechanisms and implications for fibrotic lung disease. Am J Physiol Lung Cell Mol Physiol 2007; 293: L525-L534

Friedman SL. Hepatic fibrosis -- overview. Toxicology 2008; 254: 120-129

Patsenker E, Popov Y, Stickel F, Jonczyk A, Goodman SL, Schuppan D. Inhibition of integrin alphavbeta5 on cholangiocytes blocks transforming growth factor-beta activation and retards biliary fibrosis progression. Gastroenterology 2008; 135: 660-670

LoPresto-Jova MD, Nieto MA. Inflammation and EMT: an alliance towards organ fibrosis and cancer progression. EMBO Mol Med 2009; 1: 303-304

Robertson HI, Kirby JA, Vip WW, Jones DE, Burt AD. Biliary epithelial-mesenchymal transition in posttransplantation recurrence of primary biliary cirrhosis. Hepatology 2007; 45: 977-981

Sicklick JK, Choi SS, Bustamante M, McCaill SJ, Perez EH, Huang J, Li YX, Rojkind M, Diehl AM. Evidence for epithelial-mesenchymal transitions in adult liver cells. Am J Physiol Gastrointest Liver Physiol 2006; 291: G575-G583

Bazan-Socha S, Bukiej A, Marcinkiewicz C, Musial J. Integrins in pulmonary inflammatory diseases. Curr Pharm Des 2005; 11: 893-901

Thomas GJ, Lewis MP, Whawell SA, Russell A, Sheppard D, Hart IR, Speigt PM, Marshall JF. Expression of the alphavbeta5 integrin promotes migration and invasion in squamous carcinoma cells. J Invest Dermatol 2001; 117: 67-73

Walker J, Menko AS. Integrins in lens development and disease. Exp Eye Res 2009; 88: 216-225

Sheppard D. Integrin-mediated activation of latent transforming growth factor beta. Cancer Metastasis Rev 2005; 24: 395-402

Weinreb PH, Simon KJ, Rayhorn P, Yang WJ, Leone DR, Dolinski BM, Pearse BR, Yokota Y, Kawakatsu H, Atakilit A, Sheppard D, Violette SM. Function-blocking integrin alphavbeta5 monoclonal antibodies: distinct ligand-mimetic and nonligand-mimetic classes. J Biol Chem 2004; 279: 17875-17887

Schuppan D, Ocker M. Integrin-mediated control of cell growth. Hepatology 2003; 38: 289-291

Wipf PJ, Hinz B. Integrins and the activation of latent transforming growth factor beta1 - an intimate relationship. Eur J Cell Biol 2008; 87: 601-615

Sheppard D. In vivo functions of integrins: lessons from null mutations in mice. Matrix Biol 2000; 19: 203-209

Frisch SM, Francis H. Disruption of epithelial cell-matrix interactions induces apoptosis. J Cell Biol 1994; 124: 619-626

Patsenker E, Popov Y, Stickel F, Schneider V, Ledermann M, Sägesser H, Niedobitek G, Goodman SL, Schuppan D. Pharmacological inhibition of integrin alphavbeta3
aggravates experimental liver fibrosis and suppresses hepatic angiogenesis. *Hepatology* 2009; 50: 1501-1511

79 Popov Y, Sverdlov DY, Bhaskar KR, Sharma AK, Millonig G, Patsenker E, Krahenbuhl S, Krahenbuhl L, Schuppan D. Macrophage-mediated phagocytosis of apoptotic cholangiocytes contributes to reversal of experimental biliary fibrosis. *Am J Physiol Gastrointest Liver Physiol* 2010; 298: G323-G234

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