

Recent advances in the morphological and functional heterogeneity of the biliary epithelium

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Abstract

This review focuses on the recent advances related to the heterogeneity of different-sized bile ducts with regard to the morphological and phenotypical characteristics, and the differential secretory, apoptotic and proliferative responses of small and large cholangiocytes to gastrointestinal hormones/peptides, neuropeptides and toxins. We describe several *in vivo* and *in vitro* models used for evaluating biliary heterogeneity. Subsequently, we discuss the heterogeneous proliferative and apoptotic responses of small and large cholangiocytes to liver injury and the mechanisms regulating the differentiation of small into large (more differentiated) cholangiocytes. Following a discussion on the heterogeneity of stem/progenitor cells in the biliary epithelium, we outline the heterogeneity of bile ducts in human cholangiopathies. After a summary section, we discuss the future perspectives that will further advance the field of the functional heterogeneity of the biliary epithelium.

Keywords: Bile ducts, cAMP, gastrointestinal hormones, growth factors, neurotransmitters, VEGF

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Introduction

Morphological heterogeneity of the biliary epithelium

The liver is formed by two types of epithelial cells, hepatocytes and cholangiocytes.¹ In rodents, while hepatocytes contribute to 70% of the total liver mass, cholangiocytes account for 3–5% of the total liver population.¹ The liver plays a key role in the homeostasis of the whole body metabolism: in particular, synthesizing many proteins and enzymes, the liver plays a primary role in the regulation of the energetic metabolism, and it also contributes to the detoxification and elimination of a wide variety of both endogenous and exogenous molecules.¹

In addition, the liver also secretes bile^{2,3} that is key for the digestion of fats. The human biliary epithelium originates at the level of the biliary pole of hepatocytes that are responsible for the production of bile.³ Once produced, the bile is secreted into the lumen of the bile canaliculus, and move

through the liver lobule in centrifugal direction. Cholangiocytes modify canalicular bile³ before reaching the duodenum² by a number of reabsorptive/secretory events modulated by various gastrointestinal hormones/peptides, neurotransmitters and bile acids.^{4,5}

The bile canaliculi are narrow spaces of 0.5–2 μ m delimited by simple introflections of the plasma membrane at the biliary poles of adjacent hepatocytes.⁶ The biliary pole of hepatocytes is: (i) characterized by numerous and short microvilli that protrude into the lumen; and (ii) isolated from the vascular pole by occluding junctions.⁶ The canals of Hering originate in the proximity of the portal spaces. The point at which the bile canaliculi continue into the canals of Hering is defined as the ductule-canalicular junction. At this level, bile ducts are in part lined by cholangiocytes as well as hepatocytes.^{7–9} At this point of the biliary tree, partially undifferentiated cells have been identified: the hepatic progenitor cells (HPCs). They constitute a

resident stem cell compartment in the liver, and they are able to differentiate into both hepatocytes and cholangiocytes. The canals of Hering are therefore points of anatomical and physiological connection between bile canaliculi and interlobular bile ducts in the portal space.^{7,9,10} The canals of Hering are lined by a few (three or four) cubic-shaped cholangiocytes.^{7,9,10} The canals of Hering continue into the interlobular ducts, lined by a continuous layer of cylindrical-shaped cholangiocytes.^{7,9,10} The interlobular ducts are characterized by a diameter of 15–100 μm and, in the portal space they draw up alongside the ramifications of the hepatic artery and of the portal vein.^{7,9,10} The interlobular bile ducts then continue into ducts of progressively larger size to form two large intrahepatic ducts (right and left hepatic ducts), which drain respectively into the right and left hepatic lobe of the liver and, at the hilum, they give rise to the extrahepatic bile ducts.^{7,9,10}

In humans, the biliary network is subdivided into two portions: the intrahepatic (IHBDs) and the extrahepatic bile ducts (EHBDs). IHBDs start at the ductular-canalicular junction with the canals of Hering and continue with bile ductules, interlobular, septal area (zonal) and segmental ducts.¹¹ Area (zonal) and segmental ducts are considered large intrahepatic bile ducts, whereas septal ducts represent an intermediate link between the large and the interlobular biliary system.¹¹ This classification is based on the diameter of the ducts: bile ductules (15 μm), interlobular ducts (15–100 μm), septal ducts (100–300 μm), area (zonal) ducts (300–400 μm), segmental ducts (400–800 μm) and hepatic ducts (800 μm) (Table 1).^{12–16} In rat liver, the intrahepatic biliary tree has been divided according to size differences (small, $\leq 15 \mu\text{m}$ in diameter, and large, $\geq 15 \mu\text{m}$ in diameter, bile ducts)^{12,17} (Table 1) and functional heterogeneity (Tables 2 and 3).^{16,17,24–26,43,55,56,57}

Small ductules are lined by 4–5 small cholangiocytes, which are characterized by the presence of a basement membrane and tight junctions between cells and microvilli projecting into the bile duct lumen. Small bile ducts join into interlobular ducts ranging from 20 to 100 μm in cross-sectional diameter. In interlobular bile ducts, cholangiocytes become larger and more columnar in shape and display a primary cilium.^{6,16,17,25} Large and septal IHBDs have several morphological/histological aspects and an

embryological origin in common with EHBDs.¹¹ The EHBDs consist of the left and right hepatic ducts, the common hepatic duct, the gallbladder with the cystic duct, the bile duct (choledochus) and the hepatopancreatic ampulla. The hepatopancreatic ampulla drains into the duodenum via the Papilla of Vater. From a histological point of view, a unique feature that is shared by the large intrahepatic bile ducts and the extrahepatic biliary system is the presence of glands in the duct walls, currently named peribiliary glands (PBGs).^{11,58}

In support of the morphological heterogeneity of the biliary epithelium, electron microscopic studies in rodent liver sections and intrahepatic bile duct units (IBDUs)¹⁷ have demonstrated that small and large cholangiocytes have a multi-lobulated nucleus, numerous vesicles at the sub-apical region, tight junctions, high density of microvilli and lysosomes and only a few mitochondria.⁶ Small cholangiocytes show a high nucleus-to-cytoplasm ratio, whereas large cholangiocytes display a small nucleus and abundant cytoplasm.⁶ The presence of a larger nucleus and a smaller cytoplasm in small, Ca^{2+} -dependent cholangiocytes suggests an undifferentiated nature for these cells that may contain an HPC subpopulation.¹ This view is supported by a number of studies showing that small cholangiocytes are more resistant to liver injury (such as BDL and carbon tetrachloride (CCl_4)),^{20,24} replicate during damage of large cholangiocytes^{20,59} and respond (with changes in secretory and proliferative activities) in response to agonists (e.g. histamine and H1 histamine receptor agonists)^{43,44} that stimulate Ca^{2+} -signalling (see below). On the other hand, the presence of a larger cytoplasmic area in large, cAMP-dependent cholangiocytes is perhaps an index of a more differentiated and mature characteristic for these cells.¹ This may explain in part why large cholangiocytes constitutively express more membrane receptors, transporters and channels (compared to small cholangiocytes) and are able to respond to several agonists such as secretin and somatostatin.¹

Vascularization of the biliary epithelium

The biliary epithelium is nourished by a vascular network of small vessels (peribiliary vascular plexus), which derives from branches of the hepatic artery and flows mainly into the hepatic sinusoids directly (lobular branch) or through branches of the portal vein (prelobular branches).^{60–62} While a well-defined peribiliary vascular plexus is present around large bile ducts,⁶³ the peribiliary vascular plexus gets smaller and less visible around small ductules.⁶⁰

General functional characteristics of cholangiocytes

Secretin is a key player in the regulation of biliary secretion. Secretin interacts with the G protein-coupled secretin receptor (SR), which is expressed only by cholangiocytes in human and rodent liver.^{17,25,64,65} The adenosine 3',5'-monophosphate (cAMP) is the key signalling molecule that is activated by secretin in large cholangiocytes.^{16,17,25,26} After activation of cAMP synthesis by secretin, there is

Table 1 Terminology of the human and rat intrahepatic bile ducts

Human bile ducts (Diameter in μm)	Rat bile ducts (Diameter in μm)
(Large bile ducts)	
Hepatic ducts (>800)	
Segmental ducts (400–800)	
Area (zonal) ducts (300–400)*	
(Small bile ducts)	
Septal bile ducts (100–300)	
Interlobular bile ducts (15–100)	Large bile ducts (>15)
Bile ductules (cholangioles) (<15)	Small bile ducts (<15)

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*Definition of the human and rat biliary epithelium.

phosphorylation of protein kinase A (PKA) leading to the activation of cystic fibrosis transmembrane conductance regulator (CFTR), which in turn induces activation of the $\text{Cl}^-/\text{HCO}_3^-$ anion exchanger 2 (AE2) and bicarbonate secretion into bile.^{2,17,25,26,30,31,33,66} Alterations of cAMP levels (e.g. by somatostatin, and $\alpha 2$ - or $\alpha 1$ -adrenergic receptor agonists) induce changes in secretin-stimulated secretion in large bile ducts.^{4,32,33,39,67} While some of these factors influence secretin-induced ductal secretion by direct up/downregulation of cAMP levels, other factors alter secretin-stimulated cAMP levels and ductal secretion by the activation of Ca^{2+} -dependent PKC isoforms.^{4,32,34,39}

Pathophysiological changes of the biliary epithelium

Cholangiocytes are also the target cells in a number of chronic cholestatic liver diseases (i.e. cholangiopathies)

such as primary sclerosing cholangitis (PSC) and primary biliary cirrhosis (PBC), which are characterized by the co-existence of biliary hyperplasia and/or damage.^{1,68} The development of cholangiopathies is due to the dysregulation of the balance between cholangiocyte proliferation/loss,^{1,69} that is coordinately regulated by a number of stimulatory and/or inhibitory factors such as secretin, somatostatin, gastrin, melatonin and serotonin.^{24,34,35,36,41,45,46,51,69,70} In cholangiopathies, during proliferation/loss of certain portions of the biliary tree there are compensatory/repair mechanisms in other specific segments of the biliary epithelium that help to maintain the homeostasis of the biliary epithelium.^{24,34,36,41,69,70} This likely explains why specific bile ducts secrete stimulatory factors, whereas other bile ducts synthesize inhibitory factors/neuropeptides.^{24,34,35,36,41,45,46,51,69,70}

In animal models, biliary proliferation or loss is achieved by a number of surgical or chemical procedures such as: bile duct ligation (BDL), sympathetic and parasympathetic denervation, acute and chronic CCl_4

Table 2 Expression and function of proteins in small and large bile ducts

Markers	Small ducts	Large ducts	Function	References
γ -GT	Not expressed	Interlobular ducts	Glutathione metabolism	(18)
ALP	Not expressed	Interlobular ducts	Inhibition of secretin-induced cholestasis	(18,19)
Cytochrome P450E1	Not expressed	Expressed by large ducts	Dehalogenation of CCl_4	(20, 21, 18)
Lipase, α -amylase and trypsin	Human septal ducts	Large ducts, and peribiliary glands	Biliary development	(22)
Bcl-2	Human small ductules	Not expressed	Anti-apoptotic	(23)
Secretin receptor	Not expressed. <i>De novo</i> expressed after large duct damage	Expressed by large rodent ducts	Modulation of bicarbonate secretion	(16, 17, 24, 25, 26, 20, 27, 28, 29)
CFTR	Not expressed	Expressed by large ducts	Modulation of Cl^- secretion	(17, 25, 26)
$\text{Cl}^-/\text{HCO}_3^-$ AE2	Not expressed	Human and rodent ducts	Regulation of HCO_3^- secretion	(17, 25, 26, 30, 31)
Somatostatin receptor (SSTR ₂)	Not expressed	Expressed by large rat ducts	Inhibition of growth and secretin-cholestasis	(24, 32)
Gastrin/CCK-B receptors	Unknown	Expressed by rat ducts	Inhibition of growth and secretin-cholestasis	(33, 34, 35)
Melatonin receptor	Not expressed	Expressed by large rat ducts	Inhibition of cholestasis by	(36)
D2 dopamine receptors	Not expressed	Expressed by large rat ducts	Inhibition of secretin-induced cholestasis	(37)
M3 acetylcholine receptors	Undefined	Expressed by large rat ducts	Stimulation of secretin-cholestasis	(38)
$\alpha 1$ adrenergic receptors	Expressed by small rat ducts	Expressed by large rat ducts	Stimulation of secretin-induced cholestasis	(39)
Endothelin receptors (ETA and ETB)	Expressed by small rat ducts	Expressed by large rat ducts	Inhibition of secretin-induced cholestasis	(40)
Serotonin receptors	Undefined	Expressed by large rat ducts	Regulation of biliary growth	(41)
NFAT2 and NFAT4	Expressed by small ducts	Low expression in large ducts	Stimulation of small biliary growth	(42)

ALP: alkaline phosphatase; CFTR: cystic fibrosis transmembrane conductance regulator; $\text{Cl}^-/\text{HCO}_3^-$ AE2: chloride bicarbonate anion exchanger 2; γ -GT: γ -glutamyl transpeptidase. Modified with permission from Ref. 148.

Table 3 Heterogeneous responses of small and large ducts to neuroendocrine hormones/peptides/growth factors

Factors	Small ducts	Large ducts	Effects	Mechanisms	References
Histamine	H1-H4HRs	H1-H4HRs	Stimulation of small and large biliary growth	H1HR-mediated increase in IP_3/Ca^{2+} /CaMKI in small ducts H2HR-mediated increased in cAMP in large ducts	(43)
H1HR agonists	Expression of H1HR	Expression of H1HR	Increased small biliary proliferation	IP_3/Ca^{2+} /CaMKI	(44)
VEGF	Unknown	VEGF-R2/R3	Stimulation of large biliary growth	IP_3/Ca^{2+} -dependent activation of Src/ERK1/2	(45)
NGF	Unknown	NGF and its TrkA receptors	Enhanced large biliary growth	Enhanced p-AKT and p-ERK1/2	(46)
Glucagon-like peptide 1	Unknown	GLP-1 receptor	Enhanced large biliary growth	PI3K, cAMP/PKA, and CaMKII alpha	(47)
Insulin-like growth factor	Unknown	Response	Protection of large biliary growth	PI3K	(48)
Progesterone	Unknown	PR-B nuclear receptor and PRGMC1/2, mPRalpha	Stimulation of large biliary growth	Autocrine stimulation of biliary growth	(49)
Prolactin	Unknown	Long and short receptors	Enhanced large biliary growth	Autocrine phosphorylation of PKC β -I	(50)
Follicle-stimulating hormone	Unknown	FSH receptor	Stimulation of large biliary growth	Activation of cAMP levels, ERK1/2/Elk-1 phosphorylation	(51)
Testosterone	Unknown	Androgen receptor	Enhanced large biliary growth	Testosterone synthesis	(52)
α - and β -CGRP	Unknown	Response	Stimulation of large biliary growth	Increased cAMP levels, PKA and cAMP levels	(53)
SP	Unknown	NK-1 receptor (NK-1R)	Increased biliary growth	Increased cAMP levels and PKA	(54)

CGRP: calcitonin gene-related peptide; NGF: nerve growth factor; VEGF: vascular endothelial growth factor.

administration, partial hepatectomy, and chronic feeding of bile salts, gamma-aminobutyric acid (GABA) and the toxin, α -naphthylisothiocyanate (ANIT).^{2,24,27,56,26,20,59,66,71,72} The differential effects of these procedures on the secretory, proliferative and apoptotic responses of small and large cholangiocytes are described in the section 'Experimental Models For Studying Cholangiocyte Heterogeneity'.

Since the secretin receptor is only expressed by rodent cholangiocytes,⁶⁵ changes in the functional expression of this receptor may be important for evaluating changes in biliary proliferation/loss and ductal mass.^{24,20,59,66,34,27,72-74} For example, following BDL, partial hepatectomy and taurocholate (TC) or ANIT feeding there is enhanced SR expression and secretin-stimulated cAMP levels and bile and bicarbonate secretion.^{2,66,74} In contrast, damage/loss of bile ducts (e.g. after CCl₄ and GABA treatment) is associated with reduced functional expression of SR.^{20,27} The secretin receptor is also expressed and up-regulated in cholangiocytes in rodent models of polycystic kidney and liver diseases such as in Pkd2 (-/WS25) mice.⁷⁵

Experimental models for studying cholangiocyte heterogeneity

In vivo models

These *in vivo* models include: (i) the cholestatic BDL rodent model^{2,24,26,20,34}; (ii) acute administration of a single dose of CCl₄ or chronic administration of CCl₄^{20,76}; (iii) 70% hepatectomy⁶⁶; (iv) cholinergic (by total vagotomy)⁷² and adrenergic (by a single intraportal administration of 6-hydroxy dopamine (6-OHDA)⁷¹; and (v) chronic treatment with GABA⁵⁹ and chronic feeding with bile acids such as taurocholic (TC) and ursodeoxycholic (UDCA) and tauroursodeoxycholic (TUDCA) acids or ANIT,^{55,56,74,77} which all selectively target different-sized ducts. While the cholestatic BDL model induces the proliferation of large but not small cholangiocytes,^{20,24,59} 70% liver resection has been shown to stimulate the regrowth of both small and large cholangiocytes.^{66,78} Following BDL, cAMP levels and extracellular regulated kinases (ERK) and p70^{s60K} phosphorylation are also increased in large proliferating cholangiocytes.⁷⁹ In support of the notion that cAMP is a key factor for regulation of large cholangiocytes, chronic administration of

forskolin to normal rats enhances the proliferation and secretin-stimulated bile secretion of large bile ducts.⁷³ An interesting model, characterized by the co-existence of biliary proliferation/loss, is represented by the *in vivo* administration of a Vivo Morpholino AANAT (to decrease the biliary expression of arylalkylamine N-acetyltransferase (AANAT), the rate-limiting enzyme for melatonin synthesis from serotonin) in large cholangiocytes. Inhibition of AANAT *in vivo* removes the inhibitory brake of melatonin on biliary growth thereby increasing biliary growth and secretin-stimulated ductal secretion.⁷⁰ Chronic ANIT feeding increased both proliferation and induces damage of small and large cholangiocytes.⁷⁴ This model has important implications for understanding the pathophysiology of cholangiopathies (characterized by the coexistence of both cholangiocyte apoptosis and proliferation).

While the Na⁺-dependent bile acid transporter (ASBT) is predominantly expressed by normal large cholangiocytes,⁵⁵ the chronic administration of TC: (i) increased the functional expression of ASBT in large cholangiocytes and the proliferation and the secretory responses of these cells to secretin^{20,24,55,56,59}; and (ii) induced the *de novo* expression of ASBT and BA transport activity in small cholangiocytes and the *de novo* proliferation of small cholangiocytes.⁵⁶ Other bile acids such as UDCA and TUDCA inhibit large biliary hyperplasia in BDL rats.⁷⁷ We have also shown⁸⁰ that depletion of endogenous bile acid pool (by bile depletion by external bile drainage in BDL rats) reduces biliary hyperplasia and secretin-stimulated ductal secretion and ASBT expression and bile acid transport activity of large bile ducts. Bile acids affect biliary functions by also interacting with cholangiocytes by transporters/membrane receptors such as apical Na⁺-dependent bile acid transporter and TGR5^{5,81,82}. Also, the gallbladder epithelium expresses at the apical pole the bile acid receptor TGR5 (Gpbar-1), a G-protein coupled receptor.

Other models are key for evaluating the damage and apoptotic responses and the repair mechanisms of different sized bile ducts.^{20,27,59,71,72} For example, following the functional damage of the more differentiated, large cAMP-dependent cholangiocytes (e.g. after CCl₄ and GABA treatment) there is the *de novo* proliferation of small, 'progenitor' cholangiocytes, which amplify their Ca²⁺-dependent signalling and also acquire traits of large cholangiocytes to maintain the functional homeostasis of the biliary epithelium.^{20,27,59} The functional damage (loss of secretin response) of large cholangiocytes of BDL rats, accomplished by vagotomy, was prevented by the administration of the adenylyl cyclase activator, forskolin,⁷² or prolonged UDCA or TUDCA feeding by IP₃/Ca²⁺-dependent phosphorylation of PKC α .⁷⁷ The damage and loss of proliferative and secretory functions of large cholangiocytes, by adrenergic denervation of BDL rats, is prevented by the administration of forskolin, β 1- and β 2-adrenergic receptor agonists (clenbuterol or dobutamine)⁷¹ or TC feeding.⁸³ Another model of large biliary damage is represented by the ligation of the hepatic artery in BDL rats.^{84,85} In BDL rats, hepatic artery ligation induced: (i) the disappearance of the peribiliary plexus;

(ii) increased apoptosis and impaired the proliferation and secretin-stimulated choleresis; and (iii) decreased vascular endothelial growth factor (VEGF) secretion in large bile ducts.^{84,85} Hepatic artery ligation-induced effects on large biliary functions were prevented by administration of r-VEGF-A or TC feeding.^{84,85} TC feeding to BDL rats has also been shown to prevent caffeic acid-induced damage of large cholangiocytes by increased biliary VEGF expression.⁸⁶ Also, tumor necrosis factor- α induces large biliary damage with loss of secretin-induced choleresis, an effect that was prevented by TC feeding by a phosphatidylinositol 3-kinase (PI3K)-mediated pathway.⁸⁷

***In vitro* models**

The development and characterization of *in vitro* models (in conjunction with *in vivo* models) has allowed us to better define the proliferative, apoptotic and secretory activities of small and large cholangiocytes or IBDUs isolated from different portions of the biliary epithelium, and immortalized small and large murine lines (Figure 1).^{17,20,25–27,44,56,74,88} These two subpopulations of cholangiocytes and IBDUs were characterized morphologically (via computerized image analysis) (Figure 1),^{25,26} phenotypically (by expression of γ -glutamyl transpeptidase (γ -GT) and cytokeratin-19)^{25,26} and functionally by measuring gene expression for SR, CFTR and Cl[−]/HCO₃[−] AE2 exchanger and secretin-stimulated cAMP levels, Cl[−] efflux and Cl[−]/HCO₃[−] exchanger activity.^{17,25,26}

An important advancement is represented by the development of immortalized small and large murine cholangiocyte cell lines (Figure 1) that originate from small and large ducts, respectively, and have characteristics of freshly isolated small and large cholangiocytes.⁸⁸ Specifically, by the use of these biliary lines a number of studies have elucidated the role of histamine, H1HR agonists, α 1-adrenergic receptor agonists, Ca²⁺-dependent Cl[−] channels, stem cells and granulocyte colony stimulating factors in the regulation of biliary secretion, growth, migration and repair.^{43,44,89–91}

Phenotypical heterogeneity of the biliary epithelium

In situ morphometric analysis in rodent liver sections has shown that: (i) intrahepatic bile ducts are heterogeneous in external diameter; (ii) individual cholangiocytes (lining bile ducts) are heterogeneous in area; and (iii) a linear, significant relationship exists between external bile duct diameter and cholangiocyte areas.^{16,25} We have isolated two distinct subpopulations of small and large cholangiocytes and IBDUs and shown that these two cell types express similar levels of γ -GT and cytokeratin-19, two cholangiocyte-specific markers^{2,66}; and (ii) large (but not small) isolated cholangiocytes express SR, CFTR and Cl[−]/HCO₃[−] AE2 and respond to secretin with increases in cAMP levels, CFTR

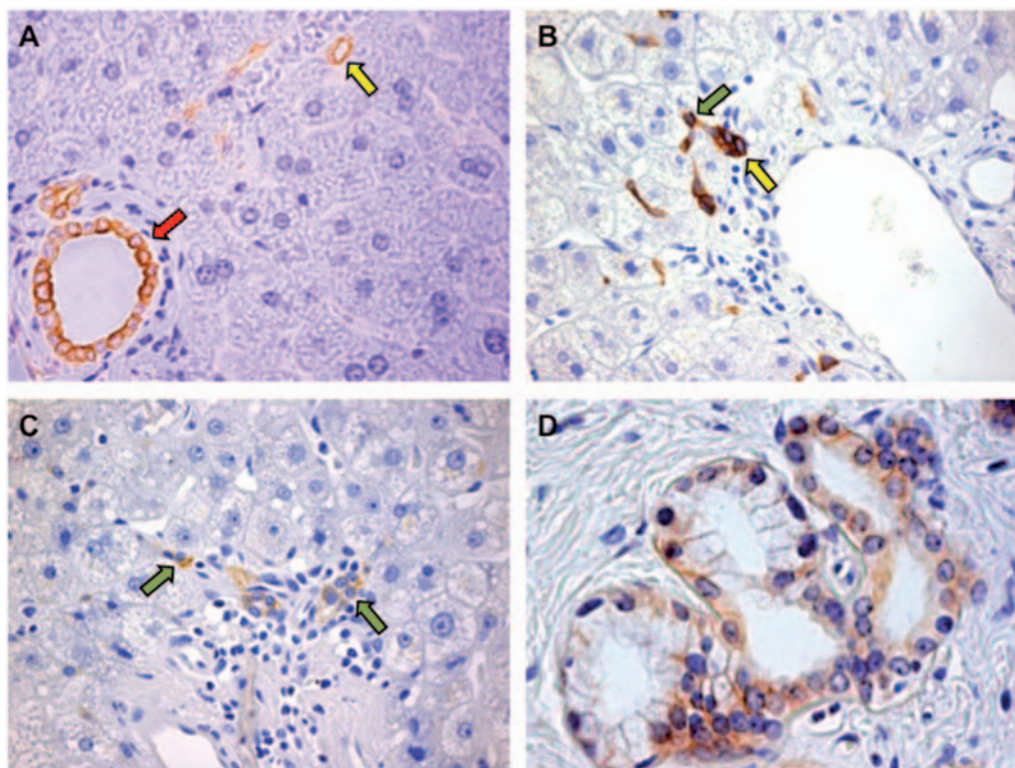


Figure 1 (A) Normal mouse liver. Immunohistochemistry for cytokeratin-19. Cholangiocyte heterogeneity in large (red arrows) or small (yellow arrow) bile ducts. Small ductules are lined by 4–5 small cholangiocytes; in interlobular bile ducts, cholangiocytes are larger and more columnar in shape. (B) Normal human liver. Immunohistochemistry for Cytokeratin-7. Smaller branches of intrahepatic biliary tree are represented by Canal of Hering (green arrow) and bile ductule (yellow arrow). (C) Normal human liver. Immunohistochemistry for EpCAM. Hepatic progenitor cells (EpCAM+) are located within Canal of Hering and bile ductules (green arrow). (D) Normal human liver. Immunohistochemistry for EpCAM. Biliary tree stem/progenitor cells (EpCAM+) are present in peribiliary glands.

activity and $\text{Cl}^-/\text{HCO}_3^-$ AE2 activity and bicarbonate secretion.^{16,17,24,25,26}

Functional heterogeneity of the biliary epithelium

Function of large bile ducts

Various proteins/membrane receptors/channels/transporters differentially expressed in small and large bile ducts are summarized in Tables 2 and 3. In human liver, the hepatic segmental area and septal bile ducts, as well as the PBGs express pancreatic enzymes such as pancreatic lipase, α -amylase and trypsin that may regulate biliary development.^{22,58} In rat liver, the enzymes leucine amino peptidase and cytochrome P4502E1 are only expressed by interlobular ducts.^{18,20,21} While the function of leucine amino peptidase is undefined, cytochrome P4502E1 has been shown to regulate the dehalogenation of the toxin, CCl_4 .^{18,20,21} Both alkaline phosphatase (ALP) and γ -GT are expressed by large rat cholangiocytes.¹⁸ Another study has shown that ALP inhibits: (i) basal and secretin-stimulated bile secretion *in vivo* in BDL rats; and (ii) basal and secretin-induced $\text{Cl}^-/\text{HCO}_3^-$ exchanger AE2 activity in large IBUDs.¹⁹

Regulation of secretin-induced large bile duct functions

Several studies have shown that: (i) secretin receptors (SR) are expressed by large but not small cholangiocytes^{24,25,26}; and (ii) secretin stimulates bicarbonate secretion in large bile ducts by interacting with SR, an interaction that leads to enhanced cAMP levels, PKA phosphorylation and opening of CFTR (expressed only in large bile ducts)²⁴ that leads to activation of the $\text{Cl}^-/\text{HCO}_3^-$ exchanger AE2 activity (present only in large bile ducts)^{25,26,31} with subsequent bicarbonate secretion into bile.^{2,4,20,25,26,27,30,34} (Figure 2). In support of the expression of SR in cholangiocytes, an *in vivo* autoradiography study in rodent liver has demonstrated specific binding of labelled secretin to bile duct areas of normal and BDL rats, binding that increased following BDL.⁹² Furthermore, secretin stimulates exocytosis in normal cholangiocytes by SR-mediated increase in cAMP levels.⁹³ A study aimed to evaluate the expression of secretin receptor in human liver samples has provided further evidence for the expression of these receptors in bile ducts but not in hepatocytes.⁶⁴

Also, secretin is a trophic factor for cholangiocytes both *in vivo* and *in vitro*.²⁸ For example, *in vitro* ablation of SR in large cholangiocytes lines and *in vivo* knockout of SR in

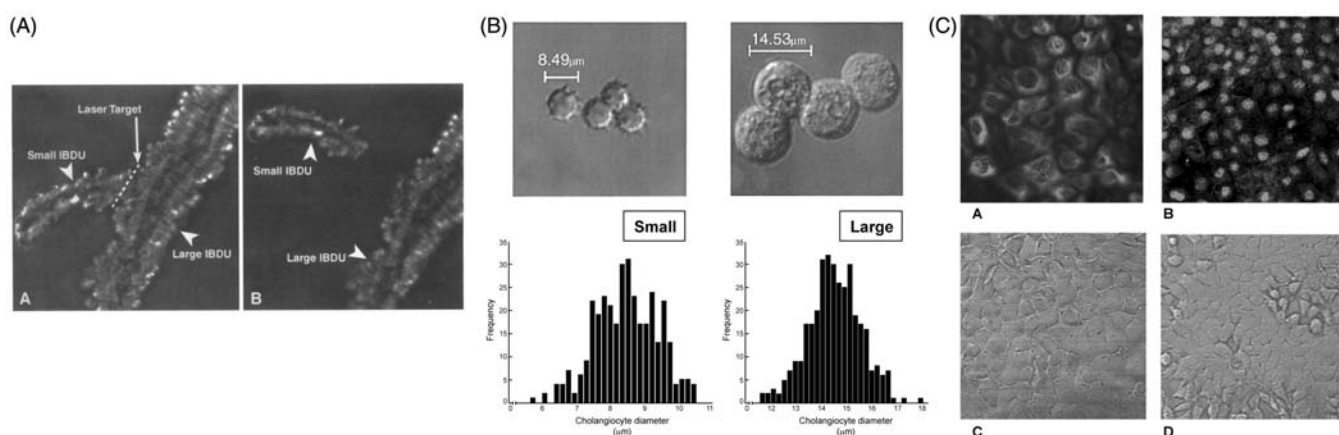


Figure 2 (Left panel) Separation of small from large IBDUs from normal rat liver. A small IBDU is shown branching from a large duct. The small duct was separated from the large duct by a brief exposure of a laser focused on the junction between large and small ducts (arrow) leading to separation. Original magnification $\times 2100$. Modified with permission from Ref. 17. (Centre panel) Frequency distribution of diameters of small and large cholangiocytes purified by counterflow elutriation and immunoaffinity separation from normal rat liver. Note that cells differ in size and morphological appearance (original magnification $\times 625$). Modified with permission from Ref. 20. (Right panel) Evaluation of cholangiocyte characteristics in immortalized small and large cholangiocytes. (A) Biliary features of immortalized small and large cholangiocytes were confirmed by positive CK-19 staining (A) as well as positive SV40 large T antigen immunoreactivity; nuclear staining is observed in the established cell line. (B) Phase contrast image demonstrates the difference of size between (C) small and (D) large cholangiocyte cell lines. Reproduced with permission from Ref. 70

large cholangiocytes reduces biliary proliferation and hyperplasia in BDL mice.²⁸ Also, endothelin-1 (ET-1) decreases secretin-stimulated choleresis *in vivo* and SR gene expression and secretin-stimulated cAMP levels in large cholangiocytes and secretin-induced luminal expansion in IBDUs from BDL rats.⁴⁰ The inhibitory effects of ET-1 were mediated by the activation of ETA receptors.⁸¹ Studies have shown^{33,34,35} that: (i) gastrin-CCK-B receptors are expressed by large cholangiocytes; and (ii) activation of gastrin-CCK-B receptors inhibits SR gene expression and secretin-stimulated cAMP levels and bile secretion and biliary hyperplasia of BDL rats. The inhibitory effects of gastrin on biliary hyperplasia were mediated by translocation of Ca^{2+} -dependent PKC isoforms.^{34,35} A recent study has also shown³⁷ that: (i) D2 (but not D1 and D3) dopaminergic receptors are expressed by large cholangiocytes; and (ii) the D2 dopaminergic agonist, quinlorane, inhibits secretin-stimulated cAMP levels (in purified cholangiocytes), lumen expansion (in isolated IBDUs) and bile secretion in bile fistula rats; the inhibitory effects of quinlorane on secretin-stimulated choleresis were mediated by increased expression of Ca^{2+} -dependent PKC γ and decreased PKA activity.³⁷

Large but not small bile ducts co-express the basolateral receptors for secretin and somatostatin (i.e. SSTR₂ subtype) and respond to these two gastrointestinal hormones with changes in biliary proliferation and ductal secretion (Figure 2)^{17,24,25,26,32}; both subpopulations of small and large cholangiocytes are positive for the biliary marker, cytokeratin-19, CK-19.²⁵ *In vivo*, somatostatin inhibited both basal and secretin-induced choleresis of large bile ducts of BDL rats.³² *In vitro*, somatostatin inhibits secretin-induced cAMP levels and exocytosis of large BDL cholangiocytes.³² We have also shown that somatostatin decreases SR gene expression and secretin-stimulated cAMP levels (a

functional index of biliary proliferation)^{24,66,34} and the proliferation of large cholangiocytes both *in vivo* and *in vitro* by interaction with SSTR₂ receptors,²⁴ a finding that supports the notion that the proliferative compartment of cholangiocytes after BDL is located solely in large cholangiocytes (Figure 2).²⁴

Stimulatory effects of angiogenic factors and other growth factors in the modulation of large bile function

The importance of the peribiliary plexus and its circulating angiogenic factors has been emphasized by a study that showed that, following BDL, the peribiliary plexus undergoes proliferation to support the increased nutritional demands from the proliferating biliary epithelium.⁶⁰ However, the proliferation of the peribiliary plexus only occurs after that of the biliary epithelium suggesting that cholangiocytes secrete angiogenic factors such as VEGF to support the nutritional needs of the proliferating bile ducts.⁶⁰ A study has shown⁴⁵ that large cholangiocytes: (i) express and secrete VEGF-A/C and that the levels of VEGF A/C increase following BDL; (ii) neutralization of VEGF-A/C *in vivo* by anti-VEGF antibodies decreases large bile duct mass; and (iii) r-VEGF-A/C induces large biliary hyperplasia in normal rats by IP₃/Ca²⁺-dependent activation of Src/ERK1/2; further studies are necessary to evaluate the role of VEGF in small biliary functions. Fabris *et al.* have shown that bile ducts are able to generate a VEGF gradient that is key during the migratory stage, when it determines arterial vasculogenesis in their vicinity, whereas angiopoietin-1 signalling from hepatoblasts contributes to the remodeling of the hepatic artery necessary to meet the demands of the proliferating biliary epithelium; however, this study did not determine which cholangiocyte

subpopulation secretes VEGF.⁹⁴ In addition to VEGF, other factors expressed/secreted by cholangiocytes have been shown to regulate large biliary functions. For example, we have shown that: (i) large cholangiocytes secrete NGF and express NGF receptors; and (ii) NGF stimulates biliary proliferation (by both autocrine/paracrine mechanisms) by activation of ERK and PI3-K pathways.⁴⁶

Cholangiocytes from BDL but not normal rats express pancreatic duodenal homeobox-1 (PDX-1), the activation of which by glucagon-like peptide-1 receptor (GLP-1R) triggers cholangiocytes to synthesize IGF-1 and VEGF.⁹⁵ It was not demonstrated which biliary subtypes express *de novo* PDX-1; however, since only large cholangiocytes proliferate after BDL²⁴ these cells are likely the ones to express PDX-1. Also, a recent study has demonstrated⁹⁶ that Hes-1 down-regulation allows PDX-1 to act as a key determinant of large cholangiocyte proliferation in response to BDL. Specifically, an increase in biliary mass, observed after BDL, was significantly reduced in PDX-1(+/-) mice concomitant with reduced Hes-1 expression in proliferating large cholangiocytes.⁹⁶

The activation of M3 ACh receptors, by acetylcholine, enhanced secretin-stimulated biliary secretion by activation of the $\text{Cl}^-/\text{HCO}_3^-$ exchanger AE2 by a Ca^{2+} -dependent, protein kinase C-insensitive pathway that potentiates secretin stimulation of adenylyl cyclase,³⁸ likely AC8. In fact, a recent study supports the concept that AC8 is a key player in the regulation of secretin-induced choleresis in large bile ducts since it is expressed mostly by large cholangiocytes,⁹⁷ the only cell types expressing the secretin receptors.^{17,25,26} Another study has shown that the α -1 adrenergic receptor agonist, phenylephrine, stimulates secretin-stimulated choleresis ductal secretion of BDL rats by Ca^{2+} - and PKC-dependent stimulation of cAMP synthesis. Coordinated regulation of bile secretion by secretin (through cAMP) and α -1 adrenergic receptor activation (through Ca^{2+} /PKC) may be an important compensatory mechanism to sustain ductal bicarbonate secretion in chronic cholestatic liver diseases. Several studies have shown that proliferating cholangiocytes acquire phenotypes of neuroendocrine cells (by expressing neuroendocrine markers such as chromogranin A, glycolipid A2-B4, S-100 protein, and neural cell adhesion molecule)⁹⁸⁻¹⁰¹ and secrete factors that promote (e.g. glucagon-like peptide, insulin-like growth factor I, progesterone, prolactin, follicle-stimulating hormone, testosterone, calcitonin gene related peptide (CGRP), histamine)^{47,49,50-53,102,103} or inhibit the proliferation of cholangiocytes (melatonin, serotonin, and Met-enkephalin)^{41,70,104} (Table 3).

Yes-associated protein (YAP) has been shown to sustain the proliferation of likely large cholangiocytes in the cholestatic BDL rat model.¹⁰⁵ Also, large cholangiocytes express glucagon-like peptide-1 (GLP-1) receptors, whose expression is upregulated after BDL.⁴⁷ Both GLP-1 and exendin-4, a GLP-1R selective agonist, increase the proliferation of large cholangiocytes by activation of PI3K, cAMP/PKA, and Ca^{2+} -dependent CaMKII α pathway.⁴⁷ Also, rodent cholangiocytes express the IGF1 isoform, whose expression increased in large proliferating BDL cholangiocytes.⁴⁸ The locally secreted IGF1 was more potent than the

circulating isoform in protecting cholangiocyte damage induced by hydrophobic bile salts.⁴⁸ The thyroid hormone inhibits the proliferation of large cholangiocytes in BDL rats by phospholipase-C/ IP_3 / Ca^{2+} -dependent downregulation of SRC/ERK1/2 signalling.¹⁰⁶

Progesterone is also an important autocrine/paracrine regulator of large cholangiocyte proliferation.⁴⁹ Cholangiocytes and large cholangiocyte lines (NRIC) express the PR-B nuclear receptor and PRGMC1, PRGMC2, and mPR α .⁴⁹ *In vivo*, progesterone enhances ductal mass of normal rats, whereas the administration of anti-progesterone antibody to BDL decreased large biliary mass.⁴⁹ *In vitro*, (i) cholangiocytes express the biosynthetic pathway for and secrete progesterone; and (ii) inhibition of progesterone steroidogenesis causes a decrease in NRC proliferation.⁴⁹ With regard to other sex hormones, a study has shown that cholangiocytes from female rats express both isoforms (long and short) of prolactin receptors, whose expression increases following BDL in large cholangiocytes.⁵⁰ Cholangiocytes also express and secrete prolactin, which regulate large cholangiocyte proliferation by autocrine pathways. Similarly, another study has demonstrated that: (i) the long receptor isoform of prolactin receptor is present in cholangiocytes; and (ii) the expression of long-form prolactin receptors increased in cholangiocytes during obstructive cholestasis.^{107,108} Cholangiocytes also express FSHR and follicle-stimulating hormone (FSH) and secrete FSH. Both *in vivo* and *in vitro* FSH increased large cholangiocyte proliferation by an autocrine mechanism by cAMP-dependent phosphorylation of ERK1/2 and Elk-1.⁵¹ A recent study by Yang *et al.*⁵² has shown that: (i) cholangiocytes express androgen receptors; and (ii) castration decreases testosterone serum levels and bile duct mass and secretin-stimulated choleresis of large bile ducts from BDL rats. Cholangiocytes express 17 β -hydroxysteroid dehydrogenase 3 (17 β -HSD3, the enzyme regulating testosterone synthesis) and secrete testosterone.⁵² Knock down of 17 β -HSD3 reduces biliary proliferation.⁵² *In vivo* knock-down of α -CGRP decreases large biliary proliferation after BDL. BDL-induced large murine cholangiocyte proliferation was associated with increased serum α -CGRP levels.⁵³ *In vitro*, α - and β -CGRP increased the proliferation of large BDL cholangiocytes, which was associated with activation of cAMP-dependent PKA and cAMP response element binding protein DNA binding.⁵³ In BDL mice lacking the NK-1R gene, there was reduced large biliary proliferation and ductal mass and decreased PKA phosphorylation.⁵⁴ *In vitro*, substance P increased cAMP levels and proliferation of large cholangiocytes.⁵⁴ H3 histamine receptor agonists have been shown to inhibit biliary hyperplasia of BDL rats by downregulation of the cAMP-dependent PKA/ERK1/2/ELK-1 pathway.¹⁰⁹

Inhibitory effects of hormones/peptides and related receptors on large biliary growth

The activation of serotonin 1A and 1B receptors (by administration of serotonin) in large cholangiocytes decreases large bile duct mass in cholestatic BDL rats.⁴¹ Serotonin effects were mediated by enhanced expression $\text{IP}_3/\text{Ca}^{2+}/$

PKC signalling that causes downregulation of cAMP/ERK1/2 signalling.⁴¹ The study has also shown that: (i) large cholangiocytes secrete serotonin; and (ii) inhibition of serotonin secretion increases biliary hyperplasia in BDL rats.⁴¹ A recent study has also shown that cholangiocytes express the neuronal isoform of tryptophan hydroxylase (TPH), synthesize serotonin and deploy serotonin as an autocrine/paracrine signal to regulate regeneration of the biliary epithelium during cholestatic injury induced by BDL.¹¹⁰ Further studies are necessary to determine which biliary cell type is involved in this phenomenon. Moreover, a recent study has demonstrated that repair-related activation of hedgehog signalling promotes cholangiocyte chemokine production.¹¹¹ Specifically, this study has shown that fibroblastic hepatic stellate cells (MF-HSCs) release soluble Hedgehog (Hh) ligands that stimulate cholangiocytes to produce chemokine (C-X-C) motif ligand 16 (Cxcl16) and recruit chemokine (C-X-C) motif ligand 16 (NKT) cells.¹¹¹ Activation of Hh signalling during cholestasis induced by BDL increases the biliary expression of Cxcl16; further studies are warranted to determine the cholangiocyte subpopulation (small vs. large) secrete the specific chemokines.

With regard to the role of endogenous opioids, a recent study has also demonstrated increase in opioid peptide synthesis in bile ducts to limit the excessive growth of large cholangiocytes following BDL by the interaction with the deltaOR expressed by cholangiocytes.^{112,113}

Recent studies have emphasized the importance of melatonin, a chemical synthesized by pineal gland as well as peripheral organs such as the biliary epithelium.³⁶ For example, we have shown that large cholangiocytes express the receptors, MT1 and MT2, and the central clock genes, CLOCK, BMAL1, CRY1, and PER1 that were all upregulated after BDL. *In vivo* administration of melatonin to BDL rats decreased ductal mass, the expression of specific clock genes, cAMP levels, and PKA phosphorylation in large cholangiocytes. In purified cholangiocytes, melatonin reduced biliary proliferation, cAMP levels, and PKA phosphorylation by interaction with MT1 but not MT2. Melatonin may be important for the regression of biliary hyperplasia occurring in specific sized bile ducts in human cholangiopathies. Further studies are needed to evaluate the role of melatonin in the regulation of small biliary functions. Secretin-stimulated choleresis is also regulated by the activation of insulin receptors expressed by large cholangiocytes. In fact, IV infusion of insulin inhibits secretin-stimulated ductal secretion in large bile from BDL rats by activation of PKC α and inhibition of PKA activity.¹¹⁴ M3 (but not M2 and M1) acetylcholine (ACh) receptors are expressed by large IBDUs and cholangiocytes.³⁸ We have recently demonstrated that: (i) large cholangiocytes express α_{2A} -, α_{2B} -, and α_{2C} -adrenergic receptors; and (ii) the α_2 -adrenergic receptor agonist, UK 14,304, decreases secretin-stimulated choleresis by downregulation of cAMP-dependent pathways in BDL rats.⁶⁷ The endogenous cannabinoid neurotransmitter, anandamide, has been shown to inhibit large cholangiocyte hyperplasia of BDL rats by activation of thioredoxin 1/redox factor 1 and AP-

1 activation.¹¹⁵ The role of the endocannabinoid system in small cholangiocytes has not been defined.

Function of small bile ducts

In the classical model of cholestasis (induced by BDL), small (in contrast to large cholangiocytes) do not respond to this pathological perturbation^{20,24} (Figure 2). A number of studies have demonstrated that small cholangiocytes exert their functions by activation of Ca²⁺ (but not cAMP) dependent mechanisms.^{17,19,20,26,27,39,42-44,73} The activation of the Ca²⁺-dependent signalling is associated with the acquisition of the phenotype of large cholangiocytes, when the constitutive ones are damaged in the course of injury^{20,27,59} (Figure 2). Such an event may be important in the reconstitution of the damaged bile ducts.

The role of histamine and histamine receptors in small bile duct function

Among the various factors regulating small biliary functions, a recent study has shown that histamine, both *in vivo* and *in vitro*, stimulates the proliferation of both small (mediated by the H1HR) and large (mediated by H2HRs) cholangiocytes.⁴³ In fact, while H1HR-mediated increased small biliary growth is mediated by activation of IP₃/Ca²⁺/CaMKI signalling, H2HR-mediated increase in cAMP-dependent signalling is key for the proliferation of large cholangiocytes.⁴³ In support of this, another study has shown⁴⁴ that the H1HR agonist, HTMT dimaleate stimulates the proliferation of small bile ducts by activation of IP₃/calmodulin-dependent Protein Kinase (CaMK I)/CREB signalling (Figure 2).

The role of Ca²⁺-dependent signalling in small bile duct function

A number of studies have shown that small cholangiocytes (which do not express CFTR and Cl⁻/HCO₃⁻ exchanger AE2)^{17,25,26} secrete water and electrolytes by the activation of Ca²⁺-dependent pathways. In support of this notion, we have demonstrated that small cholangiocytes synthesize adenosine triphosphate (ATP) and express purinergic (P2) receptors that mediate ductal bile secretion.⁹¹ Furthermore, we have identified TMEM16A channels and Ca²⁺-activated Cl⁻ efflux in small bile ducts that respond to extracellular nucleotides supporting the presence of a non-CFTR Cl⁻ channel. This channel may represent an important compensatory secretory mechanism during damage of larger, cAMP-responsive bile ducts.⁹⁰ There is a secretory gradient in the biliary epithelium with small cholangiocytes secreting water and electrolytes by activation of the IP₃/Ca²⁺/PKC pathway, whereas large cholangiocytes secrete bile by activation of the cAMP/PKA/CFTR/Cl⁻/HCO₃⁻ exchanger.^{24,26,57} Activation of α_1 -adrenergic receptors has been shown to stimulate the growth of small murine cholangiocytes via Ca²⁺-dependent activation of nuclear factor of activated T cells 2 and specificity protein 1.⁴²

Small cholangiocytes are more resistant to toxins, nerve resection and specific diets which suggests that they may be

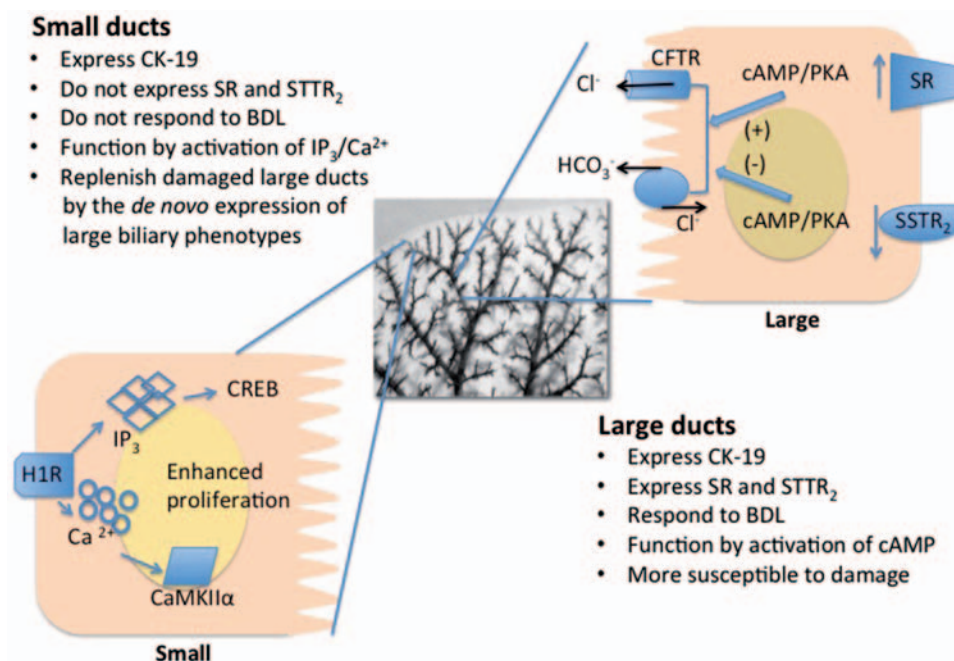


Figure 3 The rat cholangiogram demonstrates that the biliary epithelium is formed by ducts of different diameter, i.e. small and large. The cartoon illustrates that small and large cholangiocytes (lining small and large bile ducts) express the biliary marker, CK-19. Large but not small bile ducts express SR and SSTR₂ and respond to secretin and somatostatin. In large cholangiocytes, secretin interacts with basolateral SR, whose interaction induces an increase in cAMP levels that causes phosphorylation of PKA leading to activation of CFTR, which in turn induces activation of the Cl⁻/HCO₃⁻ AE2 and bicarbonate secretion into bile. Somatostatin decreases basal and secretin-stimulated choleresis by downregulation of cAMP signalling. Small cholangiocytes exert their functions by activation of IP₃/Ca²⁺-dependent signalling. In fact, H1 histamine receptor agonists stimulate the proliferation of cholangiocytes by activation of IP₃/CaMK I/CREB signalling. Following BDL, large but not small cholangiocytes proliferate leading to enhanced large bile duct mass. Following damage of large cholangiocytes (more susceptible to liver injury), small cholangiocytes *de novo* secrete and proliferate acquiring markers of large cholangiocytes to replenish the damaged large bile ducts. Modified with permission from Ref. 12. (A color version of this figure is available in the online journal)

undifferentiated cells or a passive, tubular structure delivering bile from bile canaliculus to larger (more differentiated) hormone responsive ducts, where bile is modified before reaching the small intestine.^{17,24,25} As described above, during the functional damage of large bile ducts (e.g. after acute CCl₄ administration and chronic treatment with GABA or ANIT)^{20,59,27,74} there is *de novo* proliferation of small cholangiocytes which (in addition to amplify their Ca²⁺-dependent signalling) acquire phenotypes of large cholangiocytes (SR and response to secretin)^{20,27,59,74} (Figure 3) and differentiate into larger cholangiocytes (Alpini, 2012, unpublished observations). The reason why small and large cholangiocytes differentially respond to CCl₄ (with changes in apoptotic, proliferative and secretory activities) may be due to the heterogeneous expression of cytochrome P450E1 (the enzyme that inhibits CCl₄ hepatotoxicity) that we have shown to be expressed by large but not small cholangiocytes.²⁰ In addition, small human and rodent cholangiocytes express high levels of the anti-apoptotic proteins (e.g. annexin-V and bcl-2).^{23,116}

Heterogeneity of stem/progenitor cells in the biliary epithelium

A stem cell compartment is present at the level of the smaller branches of intrahepatic biliary tree in adult liver.^{7,8,117} The development of bile duct regeneration in different disease conditions is depicted in Figure 4. Hepatic stem/progenitor cells (HPCs or HpSCs) or oval cells (in rodents) are

bipotent stem cells located in the canals of Hering, which are able to differentiate towards into hepatocytes and cholangiocytes^{8,118} (Figure 4). In adult human livers, HPCs are quiescent stem cells with a low proliferating rate. Even when the liver responds to injury, cell loss and mass are normally restored through the replication of hepatocytes and large cholangiocytes, since they have a near infinite capacity for self-renewal. So, HPCs represent a reserve compartment that is activated only when the mature epithelial cells of the liver are continuously damaged or inhibited in their replication or in cases of severe cell loss. In these conditions, resident HPCs are activated and expand from the periportal to the pericentral zone-giving rise to reactive ductules. Reactive ductules (or ductular reaction) are strands of HPCs representing a trans-amplifying sub-population with a highly variable phenotypical profile.^{8,118} However, recent studies suggest that the normal adult human liver contains a large number of liver progenitor cells that may contribute to liver homeostasis. A study has recently shown that liver progenitor cells residing in bile ducts are the predominant source of new hepatocytes in mouse liver homeostasis and afford near complete turnover of hepatocyte mass within six months.^{119,120} Another study has shown that liver progenitor cells give rise to hepatocytes after 70% partial hepatectomy and CCl₄ intoxication, both of which are experimental models believed to trigger hepatocyte regeneration only by self duplication.¹²¹ These findings are in controversy with the recent study¹²² where

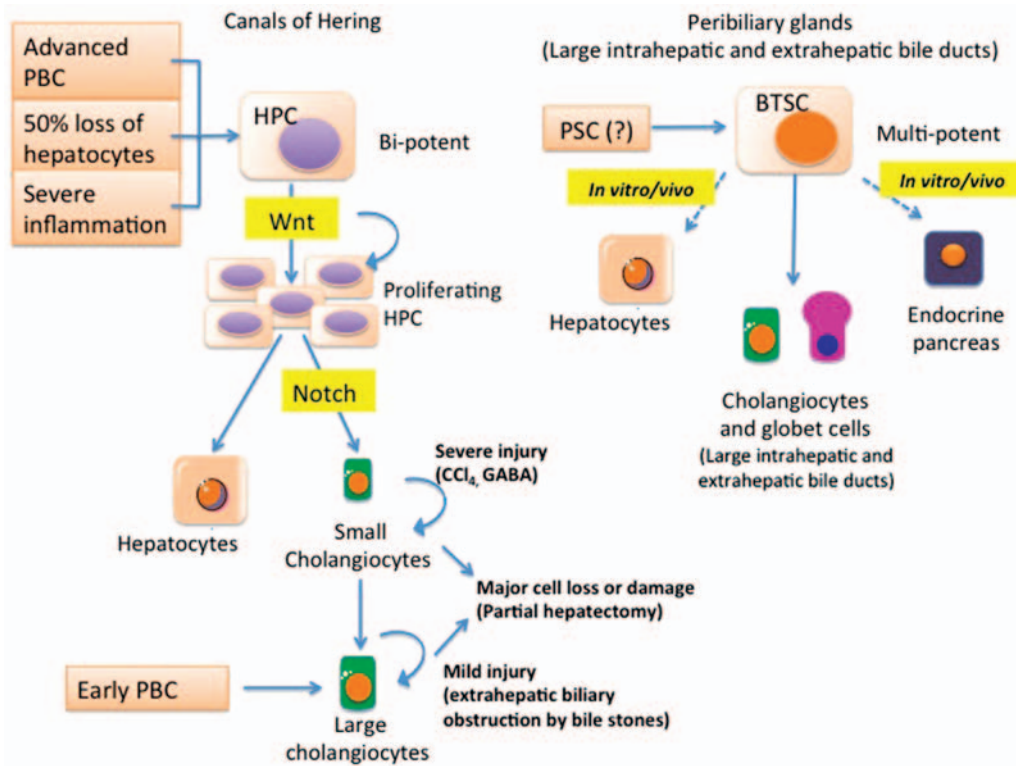


Figure 4 Model of bile duct regeneration in different disease conditions. Stem cell niches are located in the canals of Hering and peribiliary glands. Different events and diseases lead the damage of different cell population. The Wnt signalling plays important role in stem cell expansion. Moreover, bipotent cells differentiate into cholangiocytes after the activation of Notch pathway. During cholangiopathies only large cholangiocytes are the target cells of the damage and they start to proliferate leading to the activation and proliferation of small cholangiocytes. During some conditions such as 'ductopenic diseases', the number of small and large bile ducts is reduced leading the activation of the hepatic progenitor cells. HPC: hepatic stem/progenitor cells; BTSC: biliary tree stem cells; PBC: primary biliary cirrhosis; PSC: primary sclerosing cholangitis. (A color version of this figure is available in the online journal)

the authors generated a hepatocyte fate-tracing model based on timed and specific Cre recombinase expression and marker gene activation in all hepatocytes of adult Rosa26 reporter mice with an adeno-associated viral vector. This study demonstrated that: (i) newly formed hepatocytes derived from preexisting hepatocytes in the normal liver; and (ii) liver progenitor cells contributed minimally to hepatocyte regeneration after acute injury. This study supports the concept that liver progenitor cells contribute only minimally to normal hepatocyte turnover and to the regeneration of acutely lost hepatocytes. In this view, liver progenitor cells provide a backup system for injury states in which the proliferative capabilities of hepatocytes or cholangiocytes are impaired.

Recent evidence suggests that the resident stem/progenitor cell pool participates in the repair of liver damage either through the replacement of apoptotic cells or by driving fundamental repair processes, including fibrosis and angiogenesis. In this context, ductular reaction has been independently correlated with progressive fibrosis in adult and pediatric NASH and in HCV related cirrhosis.^{123,124} Ductular reaction may be considered a main driver of fibrosis. Ductular reaction can modulate hepatic fibrogenesis during liver injury through two possible mechanisms: (i) ductular reaction cells produce agents that are chemotactic for inflammatory cells and may

activate HSCs¹²⁵; and (ii) ductular reaction cells undergo epithelial-mesenchymal transition, contributing to the portal myofibroblastic pool.^{126,127}

The study of well-described stem cell niches in other organs (intestinal, hair-follicle and hematopoietic stem cell compartment) has indicated that Wnt and Notch signalling pathways are important for the regulation of stem-cell proliferation and differentiation towards committed lineages (Figure 4). Recently, the activation of HPCs and the profile of the ductular reaction have been studied in different human pathologies clarifying the role of signals involved in stem cell niche modulation. In human livers, the activation of the Wnt pathway plays a significant role in HPC expansion while the Notch pathway is involved in the fate choice of HPCs towards the cholangiocyte lineage.¹²⁸

The local cellular microenvironment has a key role in achieving a defined progenitor specification and driving the acquirement of divergent cell fates in response to diverse diseases.¹²⁹ In particular, during biliary regeneration, the expression of Jagged 1 (a Notch ligand) by myofibroblasts promoted Notch signalling in HPCs and thus their biliary specification to cholangiocytes. Alternatively, during hepatocyte regeneration, macrophage engulfment of hepatocyte debris induced Wnt3a expression. This resulted in canonical Wnt signalling in nearby HPCs, thus promoting their specification to hepatocytes.¹³⁰

PBGs and biliary tree stem/progenitor cells (BTSCs)

Glands of the biliary tree (PBGs) are tubular-alveolar glands composed of serous and mucinous acini located along extrahepatic and large intrahepatic bile ducts.¹¹ Glandular epithelial cells are reabsorptive with relevance to the mechanisms of concentration and reabsorption of bile constituents from the duct lumina to the surrounding vessels, including the lymphatics. These glands show some secretory activities (secretion of mucinous substances) and are also positive for pancreatic digestive enzymes.⁵⁸

Recently, a stem/progenitor cell niche has been described within PBGs¹³⁰ (Figure 4). PBGs contain cells, which normally proliferate and are responsible for the renewal of the surface epithelium generating mature cells such as cholangiocytes and goblet cells (in the middle of the biliary tree).¹³¹ This niche is composed of cells with classic phenotypic traits of stem/progenitor cells of endodermal origin with respect to transcription factors (SOX9, SOX17, PDX1), surface (EpCAM, LGR5, CD133) and cytoplasmic markers (CK7, CK19), and with evidence for proliferation. BTSCs are also able to express markers of pluripotency such as (OCT4A, SOX2 and NANOG).¹³¹

The study of the organization of cells within glands of the biliary tree has shown that the BTSC within PBGs resembles the organization of the stem cell niche within intestines: (i) the more undifferentiated and transit-amplifying cells are located at the bottom of the glands; (ii) the cells with an intermediate phenotype between progenitor-like cells and mature cells are found in the middle of the glands; and (iii) the fully differentiated cells are in continuum with the surface epithelium. Accordingly, the distribution of proliferating cells followed a similar gradient and proliferating cells are mostly located at the bottom of glands and no or few proliferating cells are present in the surface epithelium.¹³¹

BTSC are easily isolated from fetal and adult extrahepatic biliary tree tissue.^{130,132} Both *in vitro* and *in vivo*, BTSC demonstrated multipotency since they are able to generate mature hepatocytes, cholangiocytes and β -pancreatic cells. With regard to the endocrine pancreatic fate, pre-induced neo-islet structures were implanted into mouse fat pads, and the animals were treated with a toxin to destroy their own pancreatic beta cells. Those mice that received the human neo-islets showed significant resistance to hyperglycemia (a model of diabetes) compared to controls that did not receive cell therapy.^{130,132}

From an embryological point of view, BTSCs could represent the remnant in adults of the common stem/progenitor (PDX1+/SOX17+) for liver, bile duct system and pancreas, which exists at earlier stages of development.¹³³ Indeed, the biliary system shares a common origin with ventral pancreas.¹³⁴ A common stem/progenitor for liver, bile duct system, and pancreas exists at earlier stages of development, when the anterior definitive endoderm is forming the foregut.^{135–137} The extrahepatic biliary tract originates directly from a portion of the ventral endoderm deriving from a pancreatobiliary stem/progenitor expressing PDX1 and SOX17.¹³⁸

In conclusion, beside the well-known niche at the level of Canals of Hering, recent findings indicate an additional set, indeed, a large number of cells with the phenotype of endodermal stem/progenitor cells within PBGs of the large intrahepatic bile ducts both in fetal and adult life implicating a newly identified reservoir of stem cells for the renewal of the large bile duct epithelium.

Heterogeneity in cholangiopathies

Chronic cholestatic disease differentially affects the biliary epithelium, leading to the cholangiocytes proliferation/apoptosis, inflammation and fibrosis of different sized bile ducts.^{139,140} PBC and PSC are the two most common cholangiopathies observed in the biliary epithelium. Although those diseases are all characterized by progressive destruction of bile ducts, leading to ductopenia, progressive liver injury and cirrhosis, they differ in clinical presentation, natural history and complications. PBC, which almost selectively targets small, intrahepatic bile ducts, affects mostly middle-aged women (F/M ratio 9/1).¹⁴⁰ Disease course is often subtle, fatigue being the most common symptom; a diagnostic hallmark is the serum positivity for anti mitochondria antibodies (AMA). Cases resistant to medical therapy (based on the oral administration of UDCA, 15–20 mg/kg BW) may progress towards cirrhosis and liver failure.¹⁴⁰ There is no substantial increase in the risk of liver cancer development in PBC patients; similarly, there is not a significant clinical association with inflammatory bowel diseases (IBD) such as Crohn's diseases or ulcerative colitis.¹⁴⁰

PSC primarily affects large-sized bile ducts¹⁴¹ although 15% of the cases affect small bile ducts.¹⁴² In a percentage ranging from 5 to 15%, PSC may target small but not larger ducts. Experts now believe that what is also known as 'small duct PSC' may be a different disease from PSC. PSC commonly presents with recurrent cholangitis or altered liver function tests, sustained by dominant strictures. In contrast to PBC, PSC is slightly more frequent in males and has no serological hallmark; the diagnosis is confirmed by typical radiological findings at magnetic resonance (MRCP). PSC, but not PBC, is strongly associated with IBD (ulcerative colitis in particular) and to increased risk for liver cancer development. Specifically, PSC patients are at significant risk for cholangiocarcinoma development as compared to the general population (odds ratio = 131).¹⁴⁰ Interestingly, 60% to 80% of the cholangiocarcinoma are found in the perihilar region.¹⁴³ Classical PSC, which affects mostly large ducts, is the major risk factor for cholangiocarcinoma in USA and Europe.¹⁴⁴ Cystic fibrosis may target bile ducts; patients with a liver phenotype show damaged large but not small ducts. Biliary atresia, the most common reason of cholestasis in infants and children, induces necro-inflammatory destruction of large bile ducts but the small bile ducts are also involved as the disease progresses.¹⁴⁵ However, the pathogenesis and molecular mechanisms of biliary atresia is unclear.

Although there are several biological differences between small and large cholangiocytes, it is still unclear how certain diseases may selectively target either one or the other section of the biliary tree. Further information may

come by a different profile of expression of microRNAs (miRNAs). Two sets of microRNAs profiling data show 35 hepatic microRNAs and 17 microRNAs (microRNAs are related to cell proliferation, apoptosis, inflammation, oxidative stress and metabolism) were found differentially expressed in liver tissue and blood mononuclear cells in PBC patients and normal controls, respectively.^{146,147} Also, miR506 was found upregulated in the biliary epithelium of PBC patients, an event associated with decreased $\text{Cl}^-/\text{HCO}_3^-$ AE2 expression.¹⁴⁸

Summary and future perspectives

In this review, we have summarized recent findings about the heterogeneity of different sized (small and large) cholangiocytes regarding: (i) morphological and phenotypic characteristics; (ii) secretory responses to gastrointestinal hormones/peptides, neuroendocrine hormones and bile salts; and (iii) proliferative and apoptosis activities in response to injury, toxins and hormones/peptides. In brief, we have demonstrated that while large cholangiocytes in large ducts function by cAMP-dependent pathways, small cholangiocytes (lining small bile ducts) exert their function by activation of $\text{IP}_3/\text{Ca}^{2+}$ -dependent signalling. We have also described the differentiation of small cholangiocytes into large cholangiocytes following the damage of the latter cells. To replenish the large, damaged bile ducts small cholangiocytes amplify their Ca^{2+} -dependent signalling and also acquire *de novo* markers of large cholangiocytes. We have also discussed the heterogeneity of stem/progenitor cells in the biliary epithelium and the heterogeneous profile of human cholangiopathies. The studies discussed in this review have strong clinical implications since human cholangiopathies are characterized by spotty rather than diffuse pathology.

Further studies are necessary for defining the: (i) precise functions of small cholangiocytes, which may contain a stem/progenitor cell subpopulation; and (ii) signalling mechanisms by which small cholangiocytes replenish the biliary during damage of larger, more senescent cholangiocytes. Further studies are necessary for better evaluating the role of angiogenic and nerve growth factors and innervations in the function of small and large cholangiocytes. Since melatonin has been shown to modulate biliary functions by modulation of clock gene expression, studies should be performed to evaluate the role of circadian rhythm and clock genes in the regulation of biliary diseases. Additional studies are necessary to evaluate the role of melatonin synthesized centrally (e.g. from the pineal gland) and peripherally (biliary epithelium) in the paracrine/autocrine modulation of biliary proliferation/loss. Further studies of microRNAs should be performed on the microRNA profiling in small and large cholangiocytes and its relevance to the differential roles of different sized cholangiocytes in biliary pathophysiology.

Author contributions: YH wrote the first draft of the review article and reviewed the entire manuscript. SG and HF were key in performing in the previous years some of the experiments discussed here. They also reviewed the entire

manuscript. FM wrote some portions of the review and reviewed the entire manuscript. MM, KD, DA, JV, PO, EG and AF wrote some portions of the review and reviewed the entire manuscript. GC wrote some portions of the review and reviewed the manuscript. GA wrote second draft of the review and reviewed the entire manuscript. He also finalized the manuscript.

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REFERENCES

- Alpini G, RT P, LaRusso NF. The pathobiology of biliary epithelia. In: Arias I, Boyer JL, Chrisari F, Fausto N, Schachter D, Shafritz D (eds). *The Liver: Biology & Pathobiology*, (4th ed). Philadelphia, PA: Lippincott Williams & Wilkins, 2001:421-35
- Alpini G, Lenzi R, Sarkozi L, Tavoloni N. Biliary physiology in rats with bile ductular cell hyperplasia. Evidence for a secretory function of proliferated bile ductules. *J Clin Invest* 1988;**81**:569-78
- Nathanson MH, Boyer JL. Mechanisms and regulation of bile secretion. *Hepatology* 1991;**14**:551-66
- Kanno N, LeSage G, Glaser S, Alpini G. Regulation of cholangiocyte bicarbonate secretion. *Am J Physiol Gastrointest Liver Physiol* 2001;**281**:G612-25
- Xia X, Francis H, Glaser S, Alpini G, LeSage G. Bile acid interactions with cholangiocytes. *World J Gastroenterol* 2006;**12**:3553-63
- Benedetti A, Bassotti C, Rapino K, Marucci L, Jezequel AM. A morphometric study of the epithelium lining the rat intrahepatic biliary tree. *J Hepatol* 1996;**24**:335-42
- Roskams TA, Theise ND, Balabaud C, Bhagat G, Bhathal PS, Bioulac-Sage P, Brunt EM, Crawford JM, Crosby HA, Desmet V, Finegold MJ, Geller SA, Gouw AS, Hytioglou P, Knisely AS, Kojiro M, Lefkowitz JH, Nakanuma Y, Olynyk 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**:1739-45
- Gaudio E, Carpino G, Cardinale V, Franchitto A, Onori P, Alvaro D. New insights into liver stem cells. *Dig Liver Dis* 2009;**41**:455-62
- Turner R, Lozoya O, Wang Y, Cardinale V, Gaudio E, Alpini G, Mendel G, Wauthier E, Barbier C, Alvaro D, Reid LM. Human hepatic stem cell and maturational liver lineage biology. *Hepatology* 2011;**53**:1035-45
- Carruthers JS, Steiner JW. Studies on the fine structure of proliferated bile ductules. I. Changes of cytoarchitecture of biliary epithelial cells. *Can Med Assoc J* 1961;**85**:1223-36
- Nakanuma Y, Hosono M, Sanzen T, Sasaki M. Microstructure and development of the normal and pathologic biliary tract in humans, including blood supply. *Microsc Res Tech* 1997;**38**:552-70
- Kanno N, LeSage G, Glaser S, Alvaro D, Alpini G. Functional heterogeneity of the intrahepatic biliary epithelium. *Hepatology* 2000;**31**:555-61
- Schaffner F, Popper H. Electron microscopic studies of normal and proliferated bile ductules. *Am J Pathol* 1961;**38**:393-410
- Ludwig J. New concepts in biliary cirrhosis. *Sem Liv Dis* 1987;**7**:293-301
- Sasaki H, Schaffner F, Popper H. Bile ductules in cholestasis: morphologic evidence for secretion and absorption in man. *Lab Invest* 1967;**16**:84-95

16. Glaser S, Gaudio E, Rao A, Pierce LM, Onori P, Franchitto A, Francis HL, Dostal DE, Venter JK, DeMorrow S, Mancinelli R, Carpino G, Alvaro D, Kopriva SE, Savage JM, Alpini G. Morphological and functional heterogeneity of the mouse intrahepatic biliary epithelium. *Lab Invest* 2009;**89**:456–69
17. Alpini G, Glaser S, Robertson W, Rodgers RE, Phinizy JL, Lasater J, LeSage G. Large but not small intrahepatic bile ducts are involved in secretin-regulated ductal bile secretion. *Am J Physiol Gastrointest Liver Physiol* 1997;**272**:G1064–74
18. Mathis GA, Walls SA, D'Amico P, Gengo TF, Sirica AE. Enzyme profile of rat bile ductular epithelial cells in reference to the resistance phenotype in hepatocarcinogenesis. *Hepatology* 1989;**9**:477–85
19. Alvaro D, Benedetti A, Marucci L, Delle Monache M, Monterubbianesi R, Di Cosimo E, Perego L, Macarri G, Glaser S, LeSage G, Alpini G. The function of alkaline phosphatase in the liver: regulation of intrahepatic biliary epithelium secretory activities in the rat. *Hepatology* 2000;**32**:174–84
20. LeSage G, Glaser S, Marucci L, Benedetti A, Phinizy JL, Rodgers R, Caligiuri A, Papa E, Tretjak Z, Jezequel AM, Holcomb LA, Alpini G. Acute carbon tetrachloride feeding induces damage of large but not small cholangiocytes from BDL rat liver. *Am J Physiol Gastrointest Liver Physiol* 1999;**276**:G1289–301
21. Lakehal F, Wendum D, Barbu V, Becquemont L, Poupon R, Balladur P, Hannoun L, Ballet F, Beaune PH, Housset C. Phase I and phase II drug-metabolizing enzymes are expressed and heterogeneously distributed in the biliary epithelium. *Hepatology* 1999;**30**:1498–506
22. Terada T, Kono N, Nakanuma Y. Immunohistochemical and immunoelectron microscopic analyses of alpha-amylase isozymes in human intrahepatic biliary epithelium and hepatocytes. *J Histochem Cytochem* 1992;**40**:1627–35
23. Charlotte F, L'Hermine A, Martin N, Geleyn Y, Nollet M, Gaulard P, Zafrani ES. Immunohistochemical detection of bcl-2 protein in normal and pathological human liver. *Am J Pathol* 1994;**144**:460–5
24. Alpini G, Glaser S, Ueno Y, Pham L, Podila PV, Caligiuri A, LeSage G, LaRusso NF. Heterogeneity of the proliferative capacity of rat cholangiocytes after bile duct ligation. *Am J Physiol Gastrointest Liver Physiol* 1998;**274**:G767–75
25. Alpini G, Roberts S, Kuntz SM, Ueno Y, Gubba S, Podila PV, LeSage G, LaRusso NF. Morphological, molecular, and functional heterogeneity of cholangiocytes from normal rat liver. *Gastroenterology* 1996;**110**:1636–43
26. Alpini G, Ulrich C, Roberts S, Phillips JO, Ueno Y, Podila PV, Colegio O, LeSage G, Miller LJ, LaRusso NF. Molecular and functional heterogeneity of cholangiocytes from rat liver after bile duct ligation. *Am J Physiol Gastrointest Liver Physiol* 1997;**272**:G289–97
27. LeSage G, Benedetti A, Glaser S, Marucci L, Tretjak Z, Caligiuri A, Rodgers 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 rat liver. *Hepatology* 1999;**29**:307–19
28. Glaser S, Lam IP, Franchitto A, Gaudio E, Onori P, Chow BK, Wise C, Kopriva S, Venter J, White M, Ueno Y, Dostal D, Carpino G, Mancinelli R, Butler W, Chiasson V, DeMorrow S, Francis H, Alpini G. Knockout of secretin receptor reduces large cholangiocyte hyperplasia in mice with extrahepatic cholestasis induced by bile duct ligation. *Hepatology* 2010;**52**:204–14
29. Marzioni M, Glaser S, Francis H, Phinizy JL, LeSage G, Alpini G. Functional heterogeneity of cholangiocytes. *Semin Liver Dis* 2002;**22**:227–40
30. Banales JM, Arenas F, Rodriguez-Ortigosa CM, Saez E, Uriarte I, Doctor RB, Prieto J, Medina JF. Bicarbonate-rich choleresis induced by secretin in normal rat is taurocholate-dependent and involves AE2 anion exchanger. *Hepatology* 2006;**43**:266–75
31. Martinez-Anso E, Castillo JE, Diez J, Medina JF, Prieto J. Immunohistochemical detection of chloride/bicarbonate anion exchangers in human liver. *Hepatology* 1994;**19**:1400–6
32. Tietz PS, Alpini G, Pham LD, LaRusso NF. Somatostatin inhibits secretin-induced ductal hypercholeresis and exocytosis by cholangiocytes. *Am J Physiol Gastrointest Liver Physiol* 1995;**269**:G110–8
33. Glaser S, Rodgers RE, Phinizy JL, Robertson WE, Lasater J, Caligiuri A, Tretjak Z, LeSage G, Alpini G. Gastrin inhibits secretin-induced ductal secretion by interaction with specific receptors on rat cholangiocytes. *Am J Physiol Gastrointest Liver Physiol* 1997;**273**:G1061–70
34. Glaser S, Benedetti A, Marucci L, Alvaro D, Baiocchi L, Kanno N, Caligiuri A, Phinizy JL, Chowdury U, Papa E, LeSage G, Alpini G. Gastrin inhibits cholangiocyte growth in bile duct-ligated rats by interaction with cholecystokinin-B/Gastrin receptors via D-myo-inositol 1,4,5-triphosphate-, Ca^{2+} -, and protein kinase C alpha-dependent mechanisms. *Hepatology* 2000;**32**:17–250
35. Glaser S, Alvaro D, Ueno Y, Francis H, Marzioni M, Phinizy JL, Baumann B, Mancino MG, Venter J, LeSage G, Alpini G. Gastrin reverses established cholangiocyte proliferation and enhanced secretin-stimulated ductal secretion of BDL rats by activation of apoptosis through increased expression of Ca^{2+} -dependent PKC isoforms. *Liver Int* 2003;**23**:78–88
36. Renzi A, Glaser S, DeMorrow S, Mancinelli R, Meng F, Franchitto A, Venter J, White M, Francis H, Han Y, Alvaro D, Gaudio E, Carpino G, Ueno Y, Onori P, Alpini G. Melatonin inhibits cholangiocyte hyperplasia in cholestatic rats by interaction with MT1 but not MT2 melatonin receptors. *Am J Physiol Gastrointest Liver Physiol* 2011;**301**:G634–43
37. Glaser S, Alvaro D, Roskams T, Phinizy JL, Stoica G, Francis H, Ueno Y, Barbaro B, Marzioni M, Mauldin J, Rashid S, Mancino MG, LeSage G, Alpini G. Dopaminergic inhibition of secretin-stimulated choleresis by increased PKC-gamma expression and decrease of PKA activity. *Am J Physiol Gastrointest Liver Physiol* 2003;**284**:G683–94
38. Alvaro D, Alpini G, Jezequel AM, Bassotti C, Francia C, Fraioli F, Romeo R, Marucci L, LeSage G, Glaser S, Benedetti A. Role and mechanisms of action of acetylcholine in the regulation of rat cholangiocyte secretory functions. *J Clin Invest* 1997;**100**:1349–62
39. LeSage G, Alvaro D, Glaser S, Francis H, Marucci L, Roskams T, Phinizy JL, Marzioni M, Benedetti A, Taffetani S, Barbaro B, Fava G, Ueno Y, Alpini G. Alpha-1 adrenergic receptor agonists modulate ductal secretion of BDL rats via Ca^{2+} - and PKC-dependent stimulation of cAMP. *Hepatology* 2004;**40**:1116–27
40. Caligiuri A, Glaser S, Rodgers RE, Phinizy JL, Robertson W, Papa E, Pinzani M, Alpini G. Endothelin-1 inhibits secretin-stimulated ductal secretion by interacting with ETA receptors on large cholangiocytes. *Am J Physiol Gastrointest Liver Physiol* 1998;**275**:G835–46
41. Marzioni M, Glaser S, Francis H, Marucci L, Benedetti A, Alvaro D, Taffetani S, Ueno Y, Roskams T, Phinizy JL, Venter J, Fava G, LeSage G, Alpini G. Autocrine/paracrine regulation of the growth of the biliary tree by the neuroendocrine hormone serotonin. *Gastroenterology* 2005;**128**:121–37
42. Alpini G, Franchitto A, DeMorrow S, Onori P, Gaudio E, Wise C, Francis H, Venter J, Kopriva S, Mancinelli R, Carpino G, Stagnitti F, Ueno Y, Han Y, Meng F, Glaser S. Activation of alpha(1) -adrenergic receptors stimulate the growth of small mouse cholangiocytes via calcium-dependent activation of nuclear factor of activated T cells 2 and specificity protein 1. *Hepatology* 2011;**53**:628–39
43. Francis HL, DeMorrow S, Franchitto A, Venter JK, Mancinelli RA, White MA, Meng F, Ueno Y, Carpino G, Renteria A, Baker KK, Shine HE, Francis TC, Gaudio E, Alpini G, Onori P. Histamine stimulates the proliferation of small and large cholangiocytes by activation of both $\text{IP}_3/\text{Ca}^{2+}$ and cAMP-dependent signaling mechanisms. *Lab Invest* 2012;**92**:282–94
44. Francis H, Glaser S, DeMorrow S, Gaudio E, Ueno Y, Venter J, Dostal D, Onori P, Franchitto A, Marzioni M, Vaculin S, Vaculin B, Katki K, Stutes M, Savage J, Alpini G. Small mouse cholangiocytes proliferate in response to H1 histamine receptor stimulation by activation of the $\text{IP}_3/\text{CaMK I}/\text{CREB}$ pathway. *Am J Physiol Cell Physiol* 2008;**295**:C499–513
45. Gaudio E, Barbaro B, Alvaro D, Glaser S, Francis H, Ueno Y, Meininger CJ, Franchitto A, Onori P, Marzioni M, Taffetani S, Fava G, Stoica G, Venter J, Reichenbach R, DeMorrow S, Summers R, Alpini G. Vascular endothelial growth factor stimulates rat cholangiocyte proliferation via an autocrine mechanism. *Gastroenterology* 2006;**130**:1270–82
46. Gigliozi A, Alpini G, Baroni GS, Marucci L, Metalli VD, Glaser S, Francis H, Mancino MG, Ueno Y, Barbaro B, Benedetti A, Attili AF, Alvaro D. Nerve growth factor modulates the proliferative capacity of the intrahepatic biliary epithelium in experimental cholestasis. *Gastroenterology* 2004;**127**:1198–209

47. Marzioni M, Alpini G, Saccomanno S, Candelaresi C, Venter J, Rychlicki C, Fava G, Francis H, Trozzi L, Glaser S, Benedetti A. Glucagon-like peptide-1 and its receptor agonist exendin-4 modulate cholangiocyte adaptive response to cholestasis. *Gastroenterology* 2007;**133**:244–55
48. Gatto M, Drudi-Metalli V, Torrice A, Alpini G, Cantafora A, Blotta I, Alvaro D. Insulin-like growth factor-1 isoforms in rat hepatocytes and cholangiocytes and their involvement in protection against cholestatic injury. *Lab Invest* 2008;**88**:986–94
49. Glaser S, DeMorrow S, Francis H, Ueno Y, Gaudio E, Vaculin S, Venter J, Franchitto A, Onori P, Vaculin B, Marzioni M, Wise C, Piliathananon M, Savage J, Pierce L, Mancinelli R, Alpini G. Progesterone stimulates the proliferation of female and male cholangiocytes via autocrine/paracrine mechanisms. *Am J Physiol Gastrointest Liver Physiol* 2008;**295**:G124–G36
50. Taffetani S, Glaser S, Francis H, DeMorrow S, Ueno Y, Alvaro D, Marucci L, Marzioni M, Fava G, Venter J, Vaculin S, Vaculin B, Lam IP, Lee VH, Gaudio E, Carpino G, Benedetti A, Alpini G. Prolactin stimulates the proliferation of normal female cholangiocytes by differential regulation of Ca^{2+} -dependent PKC isoforms. *BMC Physiol* 2007;**7**:6
51. Mancinelli R, Onori P, Gaudio E, DeMorrow S, Franchitto A, Francis H, Glaser S, Carpino G, Venter J, Alvaro D, Kopriva S, White M, Kossie A, Savage J, Alpini G. Follicle-stimulating hormone increases cholangiocyte proliferation by an autocrine mechanism via cAMP-dependent phosphorylation of ERK1/2 and Elk-1. *Am J Physiol Gastrointest Liver Physiol* 2009;**297**:G11–26
52. Yang F, Priester S, Onori P, Venter J, Renzi A, Franchitto A, Munshi MK, Wise C, Dostal DE, Marzioni M, Saccomanno S, Ueno Y, Gaudio E, Glaser S. Castration inhibits biliary proliferation induced by bile duct obstruction: novel role for the autocrine trophic effect of testosterone. *Am J Physiol Gastrointest Liver Physiol* 2011;**301**:G981–91
53. Glaser S, Ueno Y, DeMorrow S, Chiasson VL, Katki KA, Venter J, Francis HL, Dickerson IM, DiPette DJ, Supowit SC, Alpini G. Knockout of alpha-calcitonin gene-related peptide reduces cholangiocyte proliferation in bile duct ligated mice. *Lab Invest* 2007;**87**:914–26
54. Glaser S, Gaudio E, Renzi A, Mancinelli R, Ueno Y, Venter J, White M, Kopriva S, Chiasson V, DeMorrow S, Francis H, Meng F, Marzioni M, Franchitto A, Alvaro D, Supowit S, DiPette DJ, Onori P, Alpini G. Knockout of the neurokinin-1 receptor reduces cholangiocyte proliferation in bile duct-ligated mice. *Am J Physiol Gastrointest Liver Physiol* 2011;**301**:G297–305
55. Alpini G, Glaser S, Rodgers R, Phinizz JL, Robertson WE, Lasater J, Caligiuri A, Tretjak Z, LeSage G. Functional expression of the apical Na^{+} -dependent bile acid transporter in large but not small rat cholangiocytes. *Gastroenterology* 1997;**113**:1734–40
56. Alpini G, Ueno Y, Glaser SS, Marzioni M, Phinizz JL, Francis H, LeSage G. Bile acid feeding increased proliferative activity and apical bile acid transporter expression in both small and large rat cholangiocytes. *Hepatology* 2001;**34**:868–76
57. Glaser S, Francis H, DeMorrow S, LeSage G, Fava G, Marzioni M, Venter J, Alpini G. Heterogeneity of the intrahepatic biliary epithelium. *World J Gastroenterol* 2006;**12**:3523–36
58. Terada T, Morita T, Hoso M, Nakanuma Y. Pancreatic enzymes in the epithelium of intrahepatic large bile ducts and in hepatic bile in patients with extrahepatic bile duct obstruction. *J Clin Pathol* 1994;**47**:924–7
59. Mancinelli R, Franchitto A, Gaudio E, Onori P, Glaser S, Francis H, Venter J, DeMorrow S, Carpino G, Kopriva S, White M, Fava G, Alvaro D, Alpini G. After damage of large bile ducts by gamma-aminobutyric acid, small ducts replenish the biliary tree by amplification of calcium-dependent signaling and *de novo* acquisition of large cholangiocyte phenotypes. *Am J Pathol* 2010;**176**:1790–800
60. Gaudio E, Onori P, Pannarale L, Alvaro D. Hepatic microcirculation and peribiliary plexus in experimental biliary cirrhosis: a morphological study. *Gastroenterology* 1996;**111**:1118–24
61. Yamamoto K, Phillips MJ. A hitherto unrecognized bile ductular plexus in normal rat liver. *Hepatology* 1984;**4**:381–5
62. Terada T, Ishida F, Nakanuma Y. Vascular plexus around intrahepatic bile ducts in normal livers and portal hypertension. *J Hepatol* 1989;**8**:139–49
63. Gaudio E, Onori P, Pannarale L, Marinozzi G. Microcirculation of the extrahepatic biliary tree: a scanning electron microscopy study of corrosion casts. *J Anat* 1993;**182**:37–44
64. Korner M, Hayes GM, Rehmann R, Zimmermann A, Scholz A, Wiedenmann B, Miller LJ, Reubi JC. Secretin receptors in the human liver: expression in biliary tract and cholangiocarcinoma, but not in hepatocytes or hepatocellular carcinoma. *J Hepatol* 2006;**45**:825–35
65. 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 Gastrointest Liver Physiol* 1994;**266**:G922–8
66. LeSage G, Glaser S, Gubba S, Robertson WE, Phinizz JL, Lasater J, Rodgers RE, Alpini G. Regrowth of the rat biliary tree after 70% partial hepatectomy is coupled to increased secretin-induced ductal secretion. *Gastroenterology* 1996;**111**:1633–44
67. Francis H, LeSage G, DeMorrow S, Alvaro D, Ueno Y, Venter J, Glaser S, Mancino MG, Marucci L, Benedetti A, Alpini G. The alpha2-adrenergic receptor agonist UK 14,304 inhibits secretin-stimulated ductal secretion by downregulation of the cAMP system in bile duct-ligated rats. *Am J Physiol Cell Physiol* 2007;**293**:C1252–62
68. Lazaridis KN, Strazabosco M, LaRusso NF. The cholangiopathies: disorders of biliary epithelia. *Gastroenterology* 2004;**127**:1565–77
69. 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**:415–31
70. Renzi A, DeMorrow S, Onori P, Carpino G, Mancinelli R, Meng F, Venter J, White M, Franchitto A, Francis H, Han Y, Ueno Y, Dusio G, Jensen KJ, Greene JJ, Glaser S, Gaudio E, Alpini G. Modulation of the biliary expression of arylalkylamine N-acetyltransferase alters the autocrine proliferative responses of cholangiocytes. *Hepatology* 2013;**57**:1130–41
71. Glaser S, Alvaro D, Francis H, Ueno Y, Marucci L, Benedetti A, DeMorrow S, Marzioni M, Mancino MG, Phinizz 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 activation of Akt. *Am J Physiol Gastrointest Liver Physiol* 2006;**290**:G813–26
72. LeSage G, Alvaro D, Benedetti A, Glaser S, Marucci L, Baiocchi L, Eisel W, Caligiuri A, Phinizz JL, Rodgers R, Francis H, Alpini G. Cholinergic system modulates growth, apoptosis, and secretion of cholangiocytes from bile duct-ligated rats. *Gastroenterology* 1999;**117**:191–9
73. Francis H, Glaser S, Ueno Y, LeSage G, Marucci L, Benedetti A, Taffetani S, Marzioni M, Alvaro D, Venter J, Reichenbach R, Fava G, Phinizz JL, Alpini G. cAMP stimulates the secretory and proliferative capacity of the rat intrahepatic biliary epithelium through changes in the PKA/Src/MEK/ERK1/2 pathway. *J Hepatol* 2004;**41**:528–37
74. LeSage G, Glaser S, Ueno Y, Alvaro D, Baiocchi L, Kanno N, Phinizz JL, Francis H, Alpini G. Regression of cholangiocyte proliferation after cessation of ANIT feeding is coupled with increased apoptosis. *Am J Physiol Gastrointest Liver Physiol* 2001;**281**:G182–90
75. Wang X, Ye H, Ward CJ, Chu JY, Masyuk TV, Larusso NF, Harris PC, Chow BK, Torres VE. Insignificant effect of secretin in rodent models of polycystic kidney and liver disease. *Am J Physiol Renal Physiol* 2012;**303**:F1089–98
76. Alpini G, Elias I, Glaser S, Rodgers RE, Phinizz JL, Robertson WE, Francis H, Lasater J, Richards M, LeSage G. Gamma-interferon inhibits secretin-induced cholestasis and cholangiocyte proliferation in a murine model of cirrhosis. *J Hepatol* 1997;**27**:371–80
77. Alpini G, Baiocchi L, Glaser S, Ueno Y, Marzioni M, Francis H, Phinizz JL, Angelico M, LeSage G. Ursodeoxycholate and tauroursodeoxycholate inhibit cholangiocyte growth and secretion of BDL rats through activation of PKC alpha. *Hepatology* 2002;**35**:1041–52
78. LeSage G, Glaser S, Robertson W, Phinizz JL, Alpini G. Partial hepatectomy induces proliferative and secretory events in small cholangiocytes. *Gastroenterology* 1996;**110**:A1250
79. Svegliati-Baroni G, Ridolfi F, Caradonna Z, Alvaro D, Marzioni M, Saccomanno S, Candelaresi C, Trozzi L, Macarri G, Benedetti A, Folli F.

- Regulation of ERK/JNK/p70S6K in two rat models of liver injury and fibrosis. *J Hepatol* 2003;**39**:528-37
80. Alpini G, Glaser S, Alvaro D, Ueno Y, Marzioni M, Francis H, Baiocchi L, Stati T, Barbaro B, Phinizz J, Mauldin J, LeSage G. Bile acid depletion and repletion regulate cholangiocyte growth and secretion by a phosphatidylinositol 3-kinase-dependent pathway in rats. *Gastroenterology* 2002;**123**:1226-37
 81. Keitel V, Haussinger D. TGR5 in cholangiocytes. *Curr Opin Gastroenterol* 2013;**29**:299-304
 82. Keitel V, Ullmer C, Haussinger D. The membrane-bound bile acid receptor TGR5 (Gpbar-1) is localized in the primary cilium of cholangiocytes. *Biol Chem* 2010;**391**:785-9
 83. Marzioni M, Ueno Y, Glaser S, Francis H, Benedetti A, Alvaro D, Venter J, Fava G, Alpini G. Cytoprotective effects of taurocholic acid feeding on the biliary tree after adrenergic denervation of the liver. *Liver Int* 2007;**27**:558-68
 84. Glaser S, Onori P, Gaudio E, Ueno Y, Pannarale L, Franchitto A, Francis H, Mancinelli R, Carpino G, Venter J, White M, Kopriva S, Vetusch A, Sfera R, Alpini G. Taurocholic acid prevents biliary damage induced by hepatic artery ligation in cholestatic rats. *Dig Liver Dis* 2010;**42**:709-17
 85. 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**:G307-17
 86. Mancinelli R, Onori P, Gaudio E, Franchitto A, Carpino G, Ueno Y, Alvaro D, Annarale LP, DeMorrow S, Francis H. Taurocholate feeding to bile duct ligated rats prevents caffeic acid-induced bile duct damage by changes in cholangiocyte VEGF expression. *Exp Biol Med* 2009;**234**:462-74
 87. Ueno Y, Francis H, Glaser S, DeMorrow S, Venter J, Benedetti A, Fava G, Marzioni M, Alpini G. Taurocholic acid feeding prevents tumor necrosis factor- α -induced damage of cholangiocytes by a PI3K-mediated pathway. *Exp Biol Med* 2007;**232**:942-9
 88. 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**:449-59
 89. Meng F, Francis H, Glaser S, Han Y, DeMorrow S, Stokes A, Staloch D, Venter J, White M, Ueno Y, Reid LM, Alpini G. Role of stem cell factor and granulocyte colony-stimulating factor in remodeling during liver regeneration. *Hepatology* 2012;**55**:209-21
 90. Dutta AK, Khimji AK, Kresge C, Bugde A, Dougherty M, Esser V, Ueno Y, Glaser S, Alpini G, Rockey DC, Feranchak AP. Identification and functional characterization of TMEM16A, a Ca^{2+} -activated Cl^- channel activated by extracellular nucleotides, in biliary epithelium. *J Biol Chem* 2011;**286**:766-76
 91. Woo K, Sathe M, Kresge C, Esser V, Ueno Y, Venter J, Glaser S, Alpini G, Feranchak AP. Adenosine triphosphate release and purinergic (P2) receptor-mediated secretion in small and large mouse cholangiocytes. *Hepatology* 2010;**52**:1819-28
 92. Farouk M, Vigna SR, McVey DC, Meyers WC. Localization and characterization of secretin binding sites expressed by rat bile duct epithelium. *Gastroenterology* 1992;**102**:963-8
 93. 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**:15523-9
 94. Fabris L, Cadamuro M, Libbrecht L, Raynaud P, Spirli C, Fiorotto R, Okolicsanyi L, Lemaigre F, Strazzabosco M, Roskams T. Epithelial expression of angiogenic growth factors modulate arterial vasculogenesis in human liver development. *Hepatology* 2008;**47**:719-28
 95. Marzioni M, Saccomanno S, Candelaresi C, Rychlicki C, Agostinelli L, Shanmukhappa K, Trozzi L, Pierantonelli I, De Minicis S, Benedetti A. Pancreatic duodenal homeobox-1 *de novo* expression drives cholangiocyte neuroendocrine-like transdifferentiation. *J Hepatol* 2010;**53**:663-70
 96. Marzioni M, Saccomanno S, Agostinelli L, Rychlicki C, Minicis SD, Pierantonelli I, Trauner M, Fickert P, Muller T, Shanmukhappa K, Trozzi L, Candelaresi C, Baroni GS, Benedetti A. PDX-1/Hes-1 interactions determine cholangiocyte proliferative response to injury in rodents: possible implications for sclerosing cholangitis. *J Hepatol* [Epub ahead of print] 30 November 2012. DOI: 10.1016/j.jhep.2012.11.033
 97. Strazzabosco M, Fiorotto R, Melero S, Glaser S, Francis H, Spirli C, Alpini G. Differentially expressed adenylyl cyclase isoforms mediate secretory functions in cholangiocyte subpopulation. *Hepatology* 2009;**50**:244-52
 98. 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**:1019-25
 99. Roskams T, Cassiman D, De Vos R, Libbrecht L. Neuroregulation of the neuroendocrine compartment of the liver. *Anat Rec A Discov Mol Cell Evol Biol* 2004;**280**:910-23
 100. Roskams T, Willems M, Campos RV, Drucker DJ, Yap SH, Desmet VJ. Parathyroid hormone-related peptide expression in primary and metastatic liver tumours. *Histopathology* 1993;**23**:519-25
 101. Roskams T, Campos RV, Drucker DJ, Desmet VJ. Reactive human bile ductules express parathyroid hormone-related peptide. *Histopathology* 1993;**23**:11-9
 102. Alvaro D, Metalli VD, Alpini G, Onori P, Franchitto A, Barbaro B, Glaser S, Francis H, Cantafora A, Blotta I, Attili AF, Gaudio E. The intrahepatic biliary epithelium is a target of the growth hormone/insulin-like growth factor 1 axis. *J Hepatol* 2005;**43**:875-83
 103. Francis H, DeMorrow S, Venter J, Onori P, White M, Gaudio E, Francis T, Greene JF Jr, Tran S, Meininger CJ, Alpini G. Inhibition of histidine decarboxylase ablates the autocrine tumorigenic effects of histamine in human cholangiocarcinoma. *Gut* 2012;**61**:753-64
 104. Marzioni M, Alpini G, Saccomanno S, de Minicis S, Glaser S, Francis H, Trozzi L, Venter J, Orlando F, Fava G, Candelaresi C, Macarri G, Benedetti A. Endogenous opioids modulate the growth of the biliary tree in the course of cholestasis. *Gastroenterology* 2006;**130**:1831-47
 105. Bai H, Zhang N, Xu Y, Chen Q, Khan M, Potter JJ, Nayar SK, Cornish T, Alpini G, Bronk S, Pan D, Anders RA. Yes-associated protein regulates the hepatic response after bile duct ligation. *Hepatology* 2012;**56**:1097-107
 106. Fava G, Ueno Y, Glaser S, Francis H, DeMorrow S, Marucci L, Marzioni M, Benedetti A, Venter J, Vaculin B, Vaculin S, Alpini G. Thyroid hormone inhibits biliary growth in bile duct-ligated rats by PLC/IP(3)/ Ca^{2+} -dependent downregulation of SRC/ERK1/2. *Am J Physiol Cell Physiol* 2007;**292**:C1467-75
 107. Bogorad RL, Ostroukhova TY, Orlova AN, Rubtsov PM, Smirnova OV. Prolactin receptors in rat cholangiocytes: regulation of level and isoform ratio is sex independent. *Biochem Biokhimiia* 2006;**71**:178-84
 108. Bogorad RL, Ostroukhova TY, Orlova AN, Rubtsov PM, Smirnova OV. Long isoform of prolactin receptor predominates in rat intrahepatic bile ducts and further increases under obstructive cholestasis. *J Endocrinol* 2006;**188**:345-54
 109. Francis H, Franchitto A, Ueno Y, Glaser S, DeMorrow S, Venter J, Gaudio E, Alvaro D, Fava G, Marzioni M, Vaculin B, Alpini G. H3 histamine receptor agonist inhibits biliary growth of BDL rats by downregulation of the cAMP-dependent PKA/ERK1/2/ELK-1 pathway. *Lab Invest* 2007;**87**:473-87
 110. Omenetti A, Yang L, Gainetdinov RR, Guy CD, Choi SS, Chen W, Caron MG, Diehl AM. Paracrine modulation of cholangiocyte serotonin synthesis orchestrates biliary remodeling in adults. *Am J Physiol Gastrointest Liver Physiol* 2011;**300**:G303-15
 111. 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 hedgehog signaling promotes cholangiocyte chemokine production. *Hepatology* 2009;**50**:518-27
 112. Marzioni M, Svegliati Baroni G, Alpini G, Benedetti A. Endogenous opioid peptides and chronic liver disease: from bedside to bench. *J Hepatol* 2007;**46**:583-6
 113. Nicoll J, Axiotis CA, Bergasa NV. The delta opioid receptor 1 is expressed by proliferating bile ductules in rats with cholestasis:

- implications for the study of liver regeneration and malignant transformation of biliary epithelium. *Med Hypotheses* 2005;**65**:1099–105
114. LeSage G, Marucci L, Alvaro D, Glaser S, Benedetti A, Marzioni M, Patel T, Francis H, Phinizz J, Alpini G. Insulin inhibits secretin-induced ductal secretion by activation of PKC alpha and inhibition of PKA activity. *Hepatology* 2002;**36**:641–51
 115. DeMorrow S, Francis H, Gaudio E, Ueno Y, Venter J, Onori P, Franchitto A, Vaculin B, Vaculin S, Alpini G. Anandamide inhibits cholangiocyte hyperplastic proliferation via activation of thioredoxin 1/redox factor 1 and AP-1 activation. *Am J Physiol Gastrointest Liver Physiol* 2008;**294**:G506–19
 116. Katayanagi K, Van de Water J, Kenny T, Nakanuma Y, Ansari AA, Coppel R, Gershwin ME. Generation of monoclonal antibodies to murine bile duct epithelial cells: identification of annexin V as a new marker of small intrahepatic bile ducts. *Hepatology* 1999;**29**:1019–25
 117. Alison MR, Golding MH, Sarraf CE. Pluripotential liver stem cells: facultative stem cells located in the biliary tree. *Cell Prolif* 1996;**29**:373–402
 118. Zhang L, Theise N, Chua M, Reid LM. The stem cell niche of human livers: symmetry between development and regeneration. *Hepatology* 2008;**48**:1598–607
 119. Schmelzer E, Zhang L, Bruce A, Wauthier E, Ludlow J, Yao HL, Moss N, Melhem A, McClelland R, Turner W, Kulik M, Sherwood S, Tallheden T, Cheng N, Furth ME, Reid LM. Human hepatic stem cells from fetal and postnatal donors. *J Exp Med* 2007;**204**:1973–87
 120. Furuyama K, Kawaguchi Y, Akiyama H, Horiguchi M, Kodama S, Kuhara T, Hosokawa S, Elbahrawy A, Soeda T, Koizumi M, Masui T, Kawaguchi M, Takaori K, Doi R, Nishi E, Kakinoki R, Deng JM, Behringer RR, Nakamura T, Uemoto S. Continuous cell supply from a Sox9-expressing progenitor zone in adult liver, exocrine pancreas and intestine. *Nat Genet* 2011;**43**:34–41
 121. Fausto N. Liver regeneration and repair: hepatocytes, progenitor cells, and stem cells. *Hepatology* 2004;**39**:1477–87
 122. Malato Y, Naqvi S, Schurmann N, Ng R, Wang B, Zape J, Kay MA, Grimm D, Willenbring H. Fate tracing of mature hepatocytes in mouse liver homeostasis and regeneration. *J Clin Invest* 2011;**121**:4850–60
 123. Nobili V, Carpino G, Alisi A, Franchitto A, Alpini G, De Vito R, Onori P, Alvaro D, Gaudio E. Hepatic progenitor cells activation, fibrosis, and adipokines production in pediatric nonalcoholic fatty liver disease. *Hepatology* 2012;**56**:2142–53
 124. Richardson MM, Jonsson JR, Powell EE, Brunt EM, Neuschwander-Tetri BA, Bhathal PS, Dixon JB, Weltman MD, Tilg H, Moschen AR, Purdie DM, Demetris AJ, Clouston AD. Progressive fibrosis in non-alcoholic steatohepatitis: association with altered regeneration and a ductular reaction. *Gastroenterology* 2007;**133**:80–90
 125. Glaser SS, Gaudio E, Miller T, Alvaro D, Alpini G. Cholangiocyte proliferation and liver fibrosis. *Expert Rev Mol Med* 2009;**11**:e7
 126. Omenetti A, Porrello A, Jung Y, Yang L, Popov Y, Choi SS, Witek RP, Alpini G, Venter J, Vandonen HM, Syn WK, Baroni GS, Benedetti A, Schuppan D, Diehl AM. Hedgehog signaling regulates epithelial-mesenchymal transition during biliary fibrosis in rodents and humans. *J Clin Invest* 2008;**118**:3331–42
 127. Wells RG. The epithelial-to-mesenchymal transition in liver fibrosis: here today, gone tomorrow? *Hepatology* 2010;**51**:737–40
 128. Spee B, Carpino G, Schotanus BA, Katoonizadeh A, Vander Borgh T, Gaudio E, Roskams T. Characterisation of the liver progenitor cell niche in liver diseases: potential involvement of Wnt and Notch signalling. *Gut* 2010;**59**:247–57
 129. Boulter L, Govaere O, Bird TG, Radulescu S, Ramachandran P, Pellicoro A, Ridgway RA, Seo SS, Spee B, Van, Rooijen N, Sansom OJ, Iredale JP, Lowell S, Roskams T, Forbes SJ. Macrophage-derived Wnt opposes Notch signaling to specify hepatic progenitor cell fate in chronic liver disease. *Nat Med* 2012;**18**:572–9
 130. Cardinale V, Wang Y, Carpino G, Cui CB, Gatto M, Rossi M, Berloco P, Cantafora A, Wauthier E, Furth M, Inverardi L, Dominguez-Bendala J, Ricordi C, Gerber D, Gaudio E, Alvaro D, Reid LM. Multipotent stem/progenitor cells in human biliary tree give rise to hepatocytes, cholangiocytes and pancreatic islets. *Hepatology* 2011;**54**:2159–72
 131. Carpino G, Cardinale V, Onori P, Carpino G, Cardinale V, Onori P, Franchitto A, Berloco PB, Rossi M, Wang Y, Semeraro R, Anceschi M, Brunelli R, Alvaro D, Reid LM, Gaudio E. Biliary tree stem/progenitor cells in glands of extrahepatic and intrahepatic bile ducts: an anatomical *in situ* study yielding evidence of maturational lineages. *J Anat* 2012;**220**:186–99
 132. Semeraro R, Carpino G, Cardinale V, Onori P, Gentile R, Cantafora A, Franchitto A, Napoli C, Anceschi M, Brunelli R, Bosco D, Torrice A, Reid L, Gaudio E, Alvaro D. Multipotent stem/progenitor cells in the human foetal biliary tree. *J Hepatol* 2012;**57**:987–94
 133. Spence JR, Lange AW, Lin SC, Kaestner KH, Lowy AM, Kim I, Whitsett JA, Wells JM. Sox17 regulates organ lineage segregation of ventral foregut progenitor cells. *Dev Cell* 2009;**17**:62–74
 134. Nakanuma Y. A novel approach to biliary tract pathology based on similarities to pancreatic counterparts: is the biliary tract an incomplete pancreas? *Pathol Int* 2010;**60**:419–29
 135. Lemaigre FP. Mechanisms of liver development: concepts for understanding liver disorders and design of novel therapies. *Gastroenterology* 2009;**137**:62–79
 136. Wandzioch E, Zaret KS. Dynamic signaling network for the specification of embryonic pancreas and liver progenitors. *Science* 2009;**324**:1707–10
 137. Zong Y, Stanger BZ. Molecular mechanisms of bile duct development. *Int J Biochem Cell Biol* 2011;**43**:257–64
 138. Roskams T, Desmet V. Embryology of extra- and intrahepatic bile ducts, the ductal plate. *Anat Rec* 2008;**291**:628–35
 139. Strazzabosco M, Fabris L, Spirli C. Pathophysiology of cholangiopathies. *J Clin Gastroenterol* 2005;**39**:S90–S102
 140. EASL. Clinical Practice Guidelines: management of cholestatic liver diseases. *J Hepatol* 2009;**51**:237–67
 141. Desmet VJ, van Eyken P, Roskams T. Histopathology of vanishing bile duct diseases. *Adv Clin Path* 1998;**2**:87–99
 142. Wee A, Ludwig J. Pericholangitis in chronic ulcerative colitis: primary sclerosing cholangitis of the small bile ducts? *Ann Intern Med* 1985;**102**:581–7
 143. Ahrendt SA, Nakeeb A, Pitt HA. Cholangiocarcinoma. *Clin Liver Dis* 2001;**5**:191–218
 144. Torok N, Gores GJ. Cholangiocarcinoma. *Semin Gastrointest Dis* 2001;**12**:125–32
 145. Arima T, Suita S, Shono T, Shono K, Kinugasa Y. The progressive degeneration of interlobular bile ducts in biliary atresia: an ultrastructural study. *Fukuoka Igaku Zasshi* 1995;**86**:58–64
 146. Padgett KA, Lan RY, Leung PC, Lleo A, Dawson K, Pfeiff J, Mao TK, Coppel RL, Ansari AA, Gershwin ME. Primary biliary cirrhosis is associated with altered hepatic microRNA expression. *J Autoimmun* 2009;**32**:246–53
 147. Qin B, Huang F, Liang Y, Yang Z, Zhong R. Analysis of altered microRNA expression profiles in PBMCs from patients with primary biliary cirrhosis. *J Gastroenterol Hepatol* 2013;**28**:543–50
 148. Banales JM, Saez E, Uriz M, Sarvide S, Urribarri AD, Splinter P, Tietz Bogert PS, Bujanda L, Prieto J, Medina JF, LaRusso NF. Up-regulation of microRNA 506 leads to decreased Cl⁻/HCO₃⁻ anion exchanger 2 expression in biliary epithelium of patients with primary biliary cirrhosis. *Hepatology* 2012;**56**:687–97