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Pathobiology of biliary epithelia☆

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Abstract

Cholangiocytes are epithelial cells that line the intra- and extrahepatic biliary tree. They serve predominantly to mediate the content of luminal biliary fluid, which is controlled via numerous signaling pathways influenced by endogenous (e.g., bile acids, nucleotides, hormones, neurotransmitters) and exogenous (e.g., microbes/microbial products, drugs etc.) molecules. When injured, cholangiocytes undergo apoptosis/lysis, repair and proliferation. They also become senescent, a form of cell cycle arrest, which may prevent propagation of injury and/or malignant transformation. Senescent cholangiocytes can undergo further transformation to a senescence-associated secretory phenotype (SASP), where they begin secreting pro-inflammatory and pro-fibrotic signals that may contribute to disease initiation and progression. These and other concepts related to cholangiocyte pathobiology will be reviewed herein. This article is part of a Special Issue entitled: Cholangiocytes in Health and Disease edited by Jesus Banales, Marco Marzioni, Nicholas LaRusso and Peter Jansen.

Keywords

Apoptosis; Bile; Cholangiocytes; Cholangiopathies; Proliferation; Senescence

1 Introduction

Cholangiocytes are now recognized to play an active role in both homeostatic and pathologic pathways. Disruption of normal cholangiocyte function can lead to the development of one of the “cholangiopathies”, a diverse collection of chronic liver diseases that are generally chronic, progressive, often lack effective treatment and may be life threatening (Table 1) [1,2].

In this review, we provide an overview of normal cholangiocyte physiology and function, with selective attention to cholangiocyte dysfunction. In particular, the focus of this article

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will center on mechanisms of secretion and absorption, as well as apoptosis, proliferation, fibrosis, and senescence.

2 The anatomy of the biliary tree

Cholangiocytes constitute only 3–5% of the total population of nucleated cells in the liver, but have a unique morphology depending on their anatomic location within the biliary tree [3,4]. Distal to the canals of Hering, which are lined by both cholangiocytes and hepatocytes, the biliary tree coalesces into different levels of intrahepatic ducts, which include *small bile ductules/terminal cholangioles* (diameter <15 μm), *interlobular ducts* (15–100 μm), *septal ducts* (100–300 μm), *area ducts* (300–400 μm), *segmental ducts* (400–800 μm) and the right and left *hepatic ducts* (>800 μm) [5]. These converge to form the extrahepatic ducts, which are comprised of the *common hepatic duct, cystic duct* and the *common bile duct* [5,6]. In rodents, small (diameter <15 μm) and large (>15 μm) bile ducts are lined by small and large cholangiocytes, respectively [7–9]. In humans, a corollary is believed to exist, but no clear-cut size distinction between “small”, “medium” and “large” cholangiocytes is apparent. In both species, however, both small and large bile ducts are lined with increasing numbers of cholangiocytes, with smaller ducts circumferentially lined by 4 to 5 cholangiocytes (rodents and humans), and larger ducts lined by 8 to 15 cholangiocytes in rodents and up to 40 cholangiocytes in humans [9–12]. Embryologically, intrahepatic cholangiocytes and hepatocytes are both believed to be derived from hepatoblasts [13], while extrahepatic cholangiocytes originate from the endoderm, much like the pancreas and duodenum [14]. This developmental divergence may explain some of the functional differences between extra- and intrahepatic cholangiocytes.

Together with the portal vein and hepatic artery, the biliary tree forms the portal triad, which defines the basic architectural unit of the liver, the lobule [15]. The lobule consists of rows of hepatocytes lined by sinusoids which drain into a central vein. The arterial supply of the biliary tree is provided by the peribiliary vascular plexus, a network of small branches which emerge from the hepatic artery [16]. Blood then flows into branches of the portal vein, or directly into the hepatic sinusoids [16].

In addition to the classic trio of vascular and biliary structures, the portal triad also consists of adrenergic and cholinergic nerves. The large, medium, and septal intrahepatic bile ducts and the surrounding peribiliary glands appear to be well innervated, in contrast to the interlobular ducts and bile ductules, with some nerves intimately associated with the endothelium [17]. Aminergic (releasing tyrosine hydroxylase), peptidergic (releasing neuropeptide Y, substance P, vasoactive intestinal polypeptide [VIP], calcitonin gene-related peptide or galanin) and cholinergic (releasing acetylcholine) sympathetic and parasympathetic nerve fibers have been observed in close proximity to bile ducts [18]. Neuropeptide Y-positive nerves have also been associated with extrahepatic ducts, and may regulate the flow of bile through autocrine and/or paracrine mechanisms [19,20].

Finally, the portal triad is also associated with lymphatics. The hepatic lymphatic system is extensive, comprising as much as 50% of total lymphatic flow [21]. Lymph flows from the

space of Disse to lymphatic capillaries around the portal triads, then enters the liver parenchyma, closely associating with arterial branches [21].

3 Normal cholangiocyte structure and function

Cholangiocytes are highly polarized structures, with an apical (luminal) and a basolateral plasma membrane [22]. This polarity is established by the zonula occludens, tight junctions located near the apical membrane [22]. The surface of the apical membrane is characterized by microvilli, which increase the available surface area by five-fold, and a primary cilium, which responds to mechanical, osmolar and chemical stimuli and controls critical pathways that maintain cholangiocyte homeostasis [4,22,23]. Intracellularly, the actin cytoskeleton provides directionality of vesicular trafficking and maintains the polarity, distribution and functional activity of plasma membrane proteins [24,25].

Communication with neighboring and distant targets is achieved through a number of mechanisms, including endocytosis and exocytosis. While receptor-mediated endocytosis likely occurs at both the apical and basolateral domains, given the presence of coated pits and vesicles at both surfaces, some data suggest that exocytosis may be specific to the apical membrane [22,24,26,27]. Multivesicular bodies are located at the apical domain, where they have the ability to fuse either with apical lysosomes or the plasma membrane, allowing their contents to undergo degradation or secretion into the bile duct lumen, respectively [27]. While the exact role of the vesicles released in this manner remains somewhat unclear, these small (30–100 nm) extracellular vesicles may play a key role in cell-cell communication by transporting proteins, lipids, messenger RNA (mRNA) and microRNAs (miRNA) to local or distant targets [28]. Communication between neighboring cells is further enhanced by the gap junctions between each cholangiocyte, which allow for direct cytoplasmic communication [29].

There is also functional heterogeneity between small and large cholangiocytes, which give rise to functional differences in secretory, absorptive, apoptotic and proliferative ability. These will be further described below and are summarized in Table 2 [12,30–34].

3.1 Secretory and absorptive functions

Classically, it is believed that large intrahepatic cholangiocytes, rather than small cholangiocytes, are responsible for secreting bile. Large cholangiocytes are thought to produce the majority of bile, while the rest (10–30%) is produced by the hepatocytes in rats and humans respectively [35,36]. However, it has been demonstrated that both small and large cholangiocytes express bile acid transporters (e.g., sodium/taurocholate *co*-transporting polypeptide [*ntcp*], organic anion-transporting polypeptide [*oatp1*] and multidrug resistance protein 2 [*mrp2*]) and aquaporins, suggesting that both may play a substantive role in bile acid transport and production [37,38]. Regardless, it is clear that the apical and basolateral membranes of large cholangiocytes possess specialized functions via the presence of different ion carriers and channels (Fig. 1A and B).

On the apical domain, proteins are tailored to enable both secretory (HCO_3^-, Cl^-, water) and absorptive functions (bile acids, glucose, amino acids, water). Bicarbonate is the main

secretory product of the cholangiocytes and functions to regulate pH for activation of pancreatic enzymes and to facilitate the absorption of lipophilic organic acids. HCO_3^- secretion occurs via the Cl^-/HCO_3^- exchanger (anion exchanger 2 [AE2]) [39]. It is driven by intracellular pH as well as the Cl^- concentration gradient thought to be generated primarily by the excretion of Cl^- through the low conductance cystic fibrosis transmembrane conductance regulator (CFTR) [40]. However, a more recent hypothesis centers around the ability of CFTR to regulate ATP release, which then activates inositol 1,4,5-triphosphate receptor (IP_3R) via apical P2Y nucleotide receptors, thereby causing the release of Ca^{2+} from the endoplasmic reticulum [41]. Cl^- secretion is then thought to be mediated by a Ca^{2+}-dependent Cl^- and a Ca^{2+}- and cAMP-independent high conductance Cl^- channel [42,43]. The apical membrane also contains K^+ channels (a Ca^{2+}-activated small conductance [SK2] K^+ channel and a Ca^{2+}-activated intermediate-conductance K^+ channel-1 [IK-1]) and a Na^+-HCO_3^- cotransporter (mice only) [44,45].

The apical membrane also allows for reabsorption of Na^+ (through NHE2 and NHE3), glucose (through the Na^+-dependent glucose transporter [SGLT1]), glutamate (through the glumate transporter), bile acids (through the ileal bile acid transporter [iBAT] and apical sodium-dependent bile acid transporter [ASBT]) and water (through aquaporin-1 [AQP-1]) [46–51].

On the basolateral domain, HCO_3^- enters the cell via the Na^+-dependent Cl^-/HCO_3^- exchanger (Na^+:HCO_3^- symporter in rodents), with the Na^+ concentration gradient achieved via the Na^+/H^+ exchanger isoform 1 (NHE1) [46,52]. The membrane potential is determined by K^+ channels (Ca^{2+}-activated small conductance [SK2] K^+ channel and intermediate-conductance Ca^{2+}-activated K^+ channel-1 [IK-1]) and Cl^- is actively transported into the cell via the $Na^+/K^+/2Cl^-$ cotransporter [44,53–55]. A Na^+ gradient created by a Na^+/K^+-ATPase provides energy for the transport of solutes [56].

Reabsorption of substrates released into the biliary lumen is hypothesized to permit the modulation of osmolarity and bile acid concentration in bile [57]. These include the uptake of conjugated bile acids from the apical domain which occurs via the apical sodium-dependent bile acid transporter (ASBT), and the reabsorption of glucose (which likely modulates osmolarity both through the uptake of glucose and Na^+, but also via the influx of water molecules), which occurs via the sodium dependent glucose transporter (SGLT1) [50,58]. Absorption of glutamate via the glutamate transporter allows for the recycling of glutathione, which is critically important as it forms a significant component of hepatocyte, bile-salt independent bile flow, being an osmotically active substance, as well as a major element of hepatic detoxification [59]. Luminal glutathione is metabolized by γ-glutamyltranspeptidase into glutamate and a cysteine and glycine dipeptide, which are then reabsorbed via the glutamate transporter and dipeptide transporter respectively [60,61]. Water is also absorbed via AQP1 and Na^+ via NHE2 and NHE3 [46,47].

Secretion of HCO_3^- and Cl^- into the bile duct lumen is controlled through constitutive endocrine, neuronal and paracrine pathways [62]. Secretion is principally stimulated by secretin, whose receptors are located on large, but not small cholangiocytes [63]. Secretin

binds to a G-protein-coupled receptor and stimulates a cAMP/protein kinase A (PKA)-dependent pathway which in turn activates the apical channels outlined above.

Secretion can also be driven by neuropeptide VIP, neurotransmitter bombesin as well as corticosteroids. Both VIP and bombesin act via cAMP signaling, with bombesin also inducing secretion via cAMP-independent methods such as cGMP, Ca^{2+} and microtubule-independent mechanisms [64,65]. In rat cholangiocytes, exposure to corticosteroids, dexamethasone or budesonide leads to biliary bicarbonate secretion due to upregulation of apical Cl^-/HCO_3^- exchanger and basolateral Na^+/H^+ exchanger (NHE1) [66].

Both parasympathetic and sympathetic stimuli are also important for the regulation of biliary secretion. Acetylcholine release secondary to vagal stimulation potentiates HCO_3^- secretion through binding to M_3 muscarinic receptors on the basolateral membrane. Basal paracrine factors that are released into bile include ATP, which binds to the $P2Y_{12}$ purinergic receptor and activates apical Cl^- secretion (through Ca^{2+}-activated Cl^- channels as outlined above) and basolateral HCO_3^- influx (through NHE1), which in turn potentiates HCO_3^- secretion [67,68]. Rat cholangiocytes and a human cholangiocarcinoma cell line express both α- and β-adrenergic receptors [69]. Stimulation of adrenergic receptors potentiates secretin-induced biliary flow and HCO_3^- secretion through a Ca^{2+}- and PKC-dependent augmentation of the cAMP signaling pathway [69].

Inhibition of ductal secretion is controlled by a number of factors including somatostatin, gastrin, dopaminergic agonists, endothelin and gamma-aminobutyric acid (GABA). Somatostatin acts directly by inhibiting secretin-stimulated bicarbonate and fluid secretion, and through disruption of cholangiocyte fluid absorption through an unknown mechanism [70,71]. Gastrin acts via both gastrin and cholecystokinin-B, inducing expression of PKCα, PKCβ1 and PKCβ2 and inhibiting cAMP production [72]. Rat cholangiocytes express dopaminergic receptors on their basolateral surface [73]. Activation of these receptors leads to an increase in intracellular IP_3 and Ca^{2+}, thereby inhibiting secretin-stimulated secretion [73]. Rat cholangiocytes also express endothelin and GABA receptors, which inhibit secretin-induced cholangiocyte secretion via inhibition of adenylyl cyclase and cAMP levels/Cl^- efflux respectively [74].

Lastly, cholangiocytes express a number of proteins whose local function is unknown. This includes pancreatic and salivary alpha-amylase, pancreatic lipase and trypsin, all of which are primarily expressed in both small and large cholangiocytes, specifically large ducts, septal ducts and peribiliary glands [32]. Conversely, only small cholangiocytes express blood group antigen and Bcl-2, an anti-apoptotic protein, and only large cholangiocytes express cytochrome P4502E1 [30,31,33].

4 The role of primary cilia in homeostasis and pathology

Primary cilia are located on both small and large cholangiocytes, and consist of an axoneme (9 pairs of microtubules around a central core), which emerges from a basal body which is derived from a centriole [23]. Cholangiocyte cilia have critical mechano-, osmo- and chemosensory functions (Fig. 2), and their disruption has been hypothesized to lead to

certain cholangiopathies, such as autosomal dominant polycystic kidney disease (ADPKD), which can lead to cystic liver and kidney disease [23].

Their mechanosensory function is enabled through polycystin-1 (PC-1, a cell surface mechanoreceptor) and polycystin-2 (PC-2, a Ca^{2+} channel) [75]. As cilia are moved by fluid flowing through the bile duct lumen, intracellular Ca^{2+} increases, which then inhibits cAMP levels in cholangiocytes via adenylyl cyclase 6 [75].

Similarly, cholangiocytes can respond to changes in bile osmolality through mediation of intracellular Ca^{2+} via the Transient receptor potential vaniloid 4 (TRPV4) channel, which then leads to changes in intracellular ATP release [76]. TRPV4 is inhibited in settings of hypertonicity and activated by hypotonicity. In the setting of a hypotonic luminal milieu, Ca^{2+} shifts from the extracellular to the intracellular space [76]. Additionally, ATP is released, which then leads to the secretion of HCO_3^-, suggesting that the osmosensory function of cilia is integral in regulating bile formation and composition [76].

Lastly, the chemosensory function of cholangiocyte cilia are thought to stem from the presence of specific, yet ubiquitous receptors. These include somatostatin receptor 3 and a serotonin receptor, which have been found on primary cilia of other cell types such as fibroblasts and chondrocytes [77–79]. Cholangiocytes also contain $P2Y_{12}$, a receptor whose ligands are nucleotides (e.g., ATP and ADP). Activation of $P2Y_{12}$ increases intracellular cAMP levels which can then activate a number of cAMP-dependent pathways such as those involving adenylyl cyclase, PKA and a protein called “exchange protein activated by cAMP” (EPAC) [41,75]. Bile acids can also influence cholangiocyte fluid secretion through TGR5, a bile acid receptor located on cholangiocyte cilia, as well as multiple proteins in the nuclear and apical membranes [80]. Activation of TGR5 by bile acids leads to inhibition of adenylyl cyclase, thereby reducing cAMP-dependent cholangiocyte secretion [81].

The functional implications of the mechano-, osmo- and chemosensory functions of cilia have yet to be completed elucidated. It is hypothesized, however, that bile flow may be pulsatile and may have variable tonicity, to which cilia have the ability to respond by modulating the intracellular and extracellular milieu, including biliary fluid composition [23].

It is known that defects in cilia can cause a number of diseases, such as ADPKD, which is caused by mutations in the genes PKD1 or PKD2 (these encode PC-1 and PC-2 respectively) [23]. Abnormal cilia appear to cause a decrease in Ca^{2+} and an increase in cAMP, which then lead to aberrant cholangiocyte proliferation, functional changes to the surrounding cell-matrix and fluid secretion as well as absorption by cholangiocytes [23]. Together, these changes result in cystogenesis, though the precise mechanism for these pathogenic pathways remains to be completed outlined [23].

Ciliary defects have also been implicated in cholangiocarcinoma. Both chemical and mechanical deciliation of normal human and rat cholangiocytes induces proliferation, while mechanical deciliation has the additive effect of increasing anchorage-independent growth, a phenotypic characteristic of tumors, as well as cellular invasion [82,83]. Deciliation also induces mitogen-activated protein kinase (MAPK) and Hedgehog (Hh) signaling, two

pathways involved in the development of cholangiocarcinoma [83]. Overexpression of HDAC6 in normal cholangiocytes leads to deciliation, both of which are seen in cholangiocarcinoma [83]. Restoration of cilia in cholangiocarcinoma cells by HDAC6 suppression results in decreased in proliferation as well as anchorage-dependent growth [83].

5 The role of miRNA in cholangiocyte homeostasis and pathology

MiRNAs are a class of non-coding RNAs that can regulate gene expression in a post-transcriptional manner by binding to complementary sites on mRNA [84]. mRNA that is targeted in this manner is either suppressed or degraded [84]. miRNAs are numerous, and can modify a myriad of processes from development and differentiation to proliferation, and have also been demonstrated to have a role in cholangiopathies (summarized in Table 4) [84,85].

The flow and composition of bile has multiple impacts on cholangiocyte function, from influencing ciliary response pathways to altering proliferation (discussed further below). There is evidence that one of the key regulators of bile acid synthesis and secretion is miR-33a. MiR-33a arises from an intronic region in the gene that encodes for Sterol regulatory element-binding protein (SREBP)-2, and appears to coordinate pathways that control bile acid and cholesterol metabolism [86,87]. MiR-33a can silence ATP-binding cassette transporter A1 (ABCA1) and ABCG1, which are induced by the ligand-activated transcription factor, Liver X Receptor (LXR) and whose activation can lead to cholesterol efflux from peripheral tissues and enterocytes [88]. MiR-33a can also suppress ATP8B1, a canalicular phospholipid flippase which maintains hepatic secretory function by stabilizing membrane polarity, and ABCB11, which encodes the bile salt export protein (BSEP), responsible for canalicular bile export [89]. It has also been demonstrated that CYP7A1, the rate-limiting step of bile acid synthesis, activates SREBP-2, which simultaneously induces miR-33a expression [87]. Activation of miR-33a then leads to a negative feedback loop which inhibits CYP7A1 [87].

MiR-144 also decreases gene expression of ABCA1, and is mediated by two transcription factors of the nuclear receptor family, the aforementioned LXR and the Farnesoid X Receptor (FXR) [90,91]. Both FXR and LXR activation increase levels of miR-144, which leads to suppression of ABCA1 [90,91]. In turn, this leads to decreased plasma HDL and decreased cholesterol efflux from macrophages [90,91].

Additional miRNAs that have been implicated in cholesterol metabolism and bile acid homestasis include miR-122, miR-422a, miR-133. MiR-122 is highly conserved, and is the most abundant liver miRNA (comprising 70% of all miRNA) [92]. It has a myriad of roles in hepatic homeostasis, including control of bile acid synthesis through silencing of CYP7A1 [93]. MiR-422a also silences CYP7A1, thereby providing another pathway to mediate bile acid homeostasis [93].

MiRNAs also appear to coordinate inflammatory cytokine responses both in homeostasis as well as to microbial exposure. For example, one of the key components of T cell

homeostasis is B7-H1, whose translation is suppressed by miR-513 in resting cholangiocytes [94]. IFN-γ stimulation significantly decreases miR-513 expression and inhibition of miR-513 activity leads to B7-H1 protein expression which then triggers apoptosis [94]. Given that higher levels of proinflammatory cytokine responses with molecules such as tumor necrosis factor (TNF)-α and IFN-γ are associated with primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC) and biliary atresia, aberrant B7-H1 expression may play a key role in these pathologies [95–97].

In human cholangiocytes, cytokine signaling in response to microbial exposure can be mediated by miR-98 and *let-7* [98]. These miRNAs repress the translation of the cytokine-inducible Src homology 2-containing protein (CIS), a regulator of cytokine signaling [98]. Stimulation of cholangiocytes with lipopolysaccharide (LPS) or infection with *Cryptosporidium parvum* leads to downregulation of miR-98 and *let-7* and subsequent expression of CIS, which results in increased nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) levels [98].

A number of miRNAs have been associated with cholestasis and specific cholangiopathies. The role of miR-21 in cholestasis and fibrosis has been investigated *in vitro* (murine biliary and human hepatic stellate cell [HSC] lines) and *in vivo* (bile duct ligated [BDL] mice, a model of obstructive cholestasis) [99]. In both settings, inhibition of miR-21 leads to decreased proliferation and fibrosis. In addition, cell lines demonstrate increased apoptosis, while BDL mice have decreased HSC activation and increased expression of the protein, small mothers against decapentaplegic (Smad)-7 [99]. The latter can inhibit both cellular proliferation and fibrosis and is a direct target for miR-21, and thus may be driving the downstream effects of miR-21 [100].

In PBC, miR-506 has been shown to be overexpressed in the intrahepatic bile ducts of patients with PBC as compared to normal and PSC controls [101]. Furthermore, miR-506 can bind specifically to the 3'UTR region of the mRNA for AE2, preventing protein expression [101]. Induction of miR-506 in cultured PBC cholangiocytes leads to decreased AE2 activity, which is abrogated with miR-506 inhibition [101].

MiRNA levels have also been studied in PSC as well as an animal model of PSC, multi-drug resistance gene-2 knockout ($Mdr2^{-/-}$) mice. $Mdr2^{-/-}$ mice have decreased expression of miR-24 at 66 weeks compared to 12 weeks, and a corresponding increase in MEN1 expression with increasing age [102]. Moreover, patients with late stage PSC have increased MEN1 expression compared to controls with early disease [102]. MiR-24 can target a complementary site on MEN1, and it has been shown that increasing MEN1 correlates with increasing expression of fibrosis genes (e.g., fibronectin 1, collagen type 1 α 1, tumor growth factor β 1 and α-smooth muscle actin) [102,103]. Menin, the protein product of MEN1, which itself is a tumor suppressor gene, may thus be responsible for mediating the cholangiocyte response cholangiocytes to injury [102].

MiR-7 (miR-7-a-1 and miR-7-a-2) may also play a substantive role in cholangiocyte proliferation and fibrosis, and susceptibility to injury. It is hypothesized that miR-7 plays a role in modulating the neurogenin-3 (Ngn-3) pathway, which is aberrantly expressed in

tissues of subjects with PSC, as well as experimental models of cholestasis and sclerosing cholangitis [104]. MiR-7 is upregulated in settings of cholangiocyte proliferation and can suppress exedin-3-stimulated proliferation by inhibiting the promitotic effects of Ngn-3 [104]. In addition, Ngn-3 silencing results in virtually absent miR-7 levels despite exposure of normal rat cholangiocytes to exedin-4, compared to cells with intact Ngn-3, demonstrating that miR-7 synthesis is also dependent on Ngn-3 [104].

Investigation of miRNA levels in biliary atresia has demonstrated that miRNAs may influence AKT signaling pathways, which can induce hepatic stem cell activation. The suppression of mRNA targets (e.g., friend of GATA [FOG] and the phosphatase and tensin homolog [PTEN]) by miR-200b and miR-21 respectively, can lead to the activation of the AKT pathway, which then leads to cellular growth and migration. Indeed, higher levels of miR-200b and miR-21 levels are associated with increased fibrosis and biliary atresia respectively [105,106].

Lastly, in an animal model of autosomal recessive polycystic kidney disease (ARPKD), the polycystic kidney (PCK) rat, the majority of miRNAs are downregulated, particularly miR-15a [107]. In cultured normal rat cholangiocytes, suppression of miR-15a leads to cell cycle progression and cyst growth due to increased expression of cell division cycle 25A (Cdc25A) [107].

6 Pathways of cholangiocyte biology: proliferation, apoptosis, senescence and reactivity

6.1 Proliferative ability

Proliferation of cholangiocytes has been shown to be modulated by gastrointestinal hormones, bile acids, angiogenic factors, steroids, and neuropeptides and neurotransmitters, which has been well-reviewed here [108]. Importantly, by virtue of their higher nucleus to cytoplasm ratio, capacity to proliferate as well as acquire the phenotype of large cholangiocytes, it is hypothesized that small cholangiocytes are less differentiated and can act as hepatic progenitor cells [10,74,109]. Regardless of whether this is true, it is well known that cholangiocyte proliferation can be stimulated by a number of factors which are summarized in Table 3. The gastrointestinal hormones somatostatin and gastrin are known to inhibit cholangiocyte proliferation through the somatostatin receptor subtype 2 (SSTR2) and cholecystokinin-B (CCK-B) receptors respectively [110,111]. In a preclinical study involving PCK rats, the somatostatin analogue octreotide was shown to inhibit cholangiocyte proliferation and liver fibrosis [112]. Gastrin administration can inhibit and even reverse, through apoptotic pathways, cholangiocyte proliferation in bile duct-ligated rats [72,113]. Conversely, glucagon-like peptide 1 (GLP1) increases cholangiocyte proliferation, as does its receptor agonist exendin-4 [114]. Interestingly, exendin-4 has been shown to prevent apoptosis of cholangiocytes [115].

Bile acids may also modulate cholangiocyte proliferation, though the direction of effect is dependent on the exact bile acid in question. *In vitro*, taurocholate and taurolithocholic acid have been shown to stimulate the proliferation of large, but not small, cholangiocytes, as

well as induce secretin receptor gene expression, cAMP levels and Cl^-/HCO_3^- exchanger activity [116]. Not only have the proliferative effects of both bile acids been replicated in rats, it has also been shown that they induce concurrent secretin-induced bicarbonate secretion in these animals [117]. By contrast, ursodeoxycholic acid (UDCA) and tauroursodeoxycholate inhibit overall cholangiocyte proliferation in BDL rats as well as reduce the expression of the cholangiocyte apical bile acid transporter, which in turn is hypothesized to reduce the ability of endogenous bile acids to stimulate proliferative and secretory pathways [118]. The inhibitory effects of UDCA likely extend to hepatic cystogenesis in polycystic liver diseases, which are characterized by biliary cysts that undergo progressive growth and secretion [119]. Chronic administration of UDCA to PCK rats inhibits cyst development and subsequent fibrosis [119]. The anti-proliferative effect of UDCA also occurs *in vitro*, and has been shown to be secondary to a phosphoinositide 3-kinase (PI3K)/AKT/extracellular signal-regulated kinase (ERK) 1/2-dependent mechanism rather than apoptosis [119].

Angiogenic factors have been demonstrated to not only mediate the growth of vascular support structures, but also induce a concurrent proliferation of cholangiocytes. In response to bile duct ligation, cholangiocytes have an increased expression of vascular endothelial growth factor receptor (VEGFR) proteins 2 and 3, as well as increased secretion of VEGF [120]. This autocrine mechanism is mediated via the IP_3-Ca^{2+}-PKCα pathway [120]. Under pathologic circumstances (e.g., ADPKD), VEGF, VEGFR and additional angiogenic factors such as angiopoietin (Ang)-1 and Ang-2 and their receptor, tyrosine kinase (Tie-2) are upregulated [121]. In addition, their proliferative effect on cholangiocytes appears to be potentiated in states of disease [121].

Like angiogenic factors, serotonin appears to provide an autocrine pathway for regulation of cholangiocyte proliferation [122]. Cholangiocytes express serotonin receptors and secrete both serotonin and nerve growth factor (NGF), which have an anti-proliferative and stimulatory effect on cholangiocyte proliferation respectively [122,123]. Serotonin appears to act in an autocrine fashion via the IP_3-Ca^{2+}-PKCα pathway by downregulating cAMP-dependent signals, while NGF acts in a paracrine manner through AKT- and ERK 1/2-dependent pathways [122,123].

Both parasympathetic and sympathetic innervation are necessary to maintain homeostatic rates of cholangiocyte proliferation [124,125]. Denervation studies involving total vagotomy (with an associated drop in cholangiocyte cAMP levels) or chemically induced adrenergic inhibition leads to reduced cholangiocyte proliferation, increased apoptosis and reduces secretin-induced choleresis [124,125]. Furthermore, knockout of alpha-calcitonin gene related peptide (α-CGRP), a neuropeptide involved in the innervation of sensory nerves, leads to a decrease in proliferation following bile duct-ligation, which is rescued by the administration of α-CGRP [126].

Cholangiocytes constitutively express all histamine receptor subtypes, but the histamine H3 receptor (HH3R) has been shown to be particularly upregulated in bile duct-ligated rats [127]. This is associated with diminished proliferation in the absence of increased apoptosis [127].

As certain cholangiopathies, such as PBC, predominantly affect a particular sex, there has been an interest in evaluating the role of sex hormones in bile duct proliferation. Cholangiocytes express both estrogen receptor-alpha and -beta (ER-α and ER-β), and undergo an upregulation of ER-β following bile duct-ligation [128]. In turn, addition of 17-β-estradiol has been shown to induce proliferation through the Src-Shc-ERK1/2 pathway [129]. Inhibition of estrogen signaling in both female and male rats through ovariectomy and tamoxifen respectively leads to decreased cholangiocyte proliferation and an increase in apoptosis [128,130].

Another hormone that increases cholangiocyte proliferation is gonadotropin-releasing hormone (GnRH) [131]. Both normal cholangiocytes, as well as cholangiocytes under cholestatic conditions (e.g., BDL rats) express GnRH receptors [131]. In both *in vivo* and *in vitro* experiments, treatment with GnRH causes biliary proliferation through an autocrine pathway, whereas knockdown or pharmacologic inhibition of GnRH abrogates this effect [131].

Lastly, matrix metalloproteinases (MMPs) may play a role in biliary proliferation, particularly in the setting of polycystic liver diseases [132]. These proteases are responsible for remodeling the extracellular matrix and are upregulated in cholangiocytes from humans with polycystic liver disease and PCK rats [132]. The proliferative ability of MMPs is mediated by interleukin (IL)-6 and IL-8, and can be inhibited by pharmacologic means, leading to suppression of cyst formation and subsequent fibrogenesis [132].

6.2 Apoptotic pathways

It is known that cholangiocytes apoptose under both normal and pathologic conditions. During ductal development, for example, apoptosis is a normal phenomenon that allows for the regression of the ductal plate [133]. Apoptosis is also the mechanism by which aberrant ductal proliferation is reversed in the setting of transient biliary obstruction [134]. Apoptosis has also been implicated in cholestatic liver diseases such as PBC, PSC and biliary atresia [135,136]. Circulating markers of apoptosis have been found in patients with PBC and PSC [135]. Furthermore, human cholangiocytes from patients with PSC or PBC express higher levels of TNF-related apoptosis-inducing ligand (TRAIL) and demonstrate greater apoptosis [137]. Apoptosis has also been demonstrated in rodent models of cholestatic liver disease [137–139].

6.3 Senescent ability

In response to cellular injury, cells that fail to undergo repair may potentially follow a pathway to either apoptosis or to cellular senescence (cell cycle arrest); it has been demonstrated that senescent cholangiocytes accumulate in a number of chronic liver diseases, especially PSC, which may be one of the mechanisms that leads to fibrosis [140,141]. Senescent cholangiocytes are irreversibly arrested at the G_1 phase of the cell cycle and do not respond to various external stimuli even though they remain metabolically active [142]. Once cellular senescence is established, cells become resistant to apoptosis and thus the senescent phenotype may be the mechanism by which cells inhibit propagation or neoplastic transformation of damaged cells [143].

Two major tumor suppressor pathways control cellular senescence. These involve activation of the cyclin-dependent kinase inhibitors (CDKN) $p21^{CIP1}$ and $P16^{INK4A}$, which are mediated by the p53 and the retinoblastoma (pRB) proteins respectively [143]. In *in vitro* and *in vivo* experiments, overexpression of constitutively active neuroblastoma RAS viral oncogene homolog (NRAS) has been shown to promote cyclin-dependent kinase inhibitor 2A (CDKN2A)/$p16^{INK4a}$ expression and cholangiocyte senescence in an ETS proto-oncogene 1 (ETS1)-dependent manner [144]. NRAS/MAPK promotes ETS1/2-dependent transcription of p16 and insult-induced senescence [144].

This relationship has been recently demonstrated in cholangiocytes of patients with PSC. Cholangiocytes of PSC patients appear to be largely senescent, with active NRAS [144]. The addition of a microbial insult (modelled by LPS) activates a NRAS-dependent pathway that involves CDKN2A/$p16^{INK4a}$ and leads to senescence in normal human cholangiocytes [144]. The CDKN2A/$p16^{INK4a}$ pathway is driven by ETS1, which can bind to the CDKN2A/$p16^{INK4a}$ promoter, demonstrating that it is a direct transcriptional regulator [144]. Increased expression of phosphorylated ETS1 was also demonstrated in human PSC liver samples as well as a murine model of PSC. In addition, it was shown that CDKN2A repression is controlled by a repressive histone 3 lysine 4 trimethylaton (H3K4Me3) mark, which is converted to a permissive mark after exposure with long-term LPS treatment. This then allows ETS1 to bind to CDKN2A, demonstrating that there is epigenetic regulation involved the binding of ETS1.

Another mechanism which may be implicated in the regulation of cholangiocyte senescence includes the forkhead box A2 (FoxA2) [145]. FoxA2 regulates cell differentiation and tissue regeneration, and has been found to be constitutively upregulated in murine liver progenitor cells and small mouse cholangiocytes as compared to large mouse cholangiocytes [145]. Furthermore, FoxA2 was silenced in liver tissues derived from patients with cholestatic liver disease (PSC and PBC). After BDL and $MDR2^{-/-}$ mice (a murine model of PSC) were treated with stem cell therapy involving small mouse cholangiocytes, pathways indicative of fibrosis and hepatic senescence were diminished, while those involving HSC senescence were increased [145]. This suggests that FoxA2 is integral in regulating both fibrosis and divergent senescent pathways [145].

Senescent cells can also transition to a potentially pathologic state which involves hypersecretion of proinflammatory cytokines such as IL-6 and chemokines such as IL-8 [146]. This state is referred to as a senescence-associated secretory phenotype (SASP) [147]. SASP affects neighboring cells and the microenvironment by inducing and reinforcing senescence, activating immune responses, inducing fibrosis and even potentiating tumorigenesis [148]. Escape of cholangiocytes from the senescent state is considered a key mechanism in the development of cholangiocarcinoma, the neoplastic transformation of cholangiocytes [149].

Despite the striking heterogeneity between small and large cholangiocytes, it is less clear whether they have differential responses with regards to senescence and eventual fibrosis. The engraftment of transplanted small mouse cholangiocytes in BDL mice results in increased FoxA2 and decreased fibrosis, demonstrating that small cholangiocytes can induce

recovery to cholestatic injury [145]. And yet, as discussed above, evidence suggests that both small and large cholangiocytes can exhibit senescence in pathologic settings, such as PSC, which classically affects the large bile ducts, and PBC, which affects the small bile ducts [145]. Senescent cholangiocytes have also been discovered in diseases that arise from hepatocellular injury, such as non-alcoholic steatohepatitis and viral hepatitis [141]. Thus, it is possible that in states of disease, the reparative ability of small cholangiocytes is overwhelmed by the presence of senescent, abnormally functioning small cholangiocytes, thereby leading to progressive injury and fibrosis [145].

7 The reactive cholangiocyte

All cholangiopathies react to injury by manifesting varying degrees of ductopenia, bile duct hyperplasia, inflammation, fibrosis and cholestasis [150,151]. Ductopenia occurs as a consequence of disproportionate destruction and proliferation of cholangiocytes [152]. The exact mechanism and pathways by which cholangiocyte death occurs is unknown, but it is postulated to occur either through lysis and/or apoptosis, with apoptosis thought to be the dominant mechanism [153]. After an insult, proliferation is activated in particular cholangiocyte subpopulations in order to compensate for the loss of biliary cells and to sustain adequate secretory and absorptive functions [1,74]. These "*reactive cholangiocytes*" are characterized by an enhanced proinflammatory response, including the secretion of several proinflammatory (e.g., TNF-α, IL-6, and IL-8) and chemotactic cytokines (e.g., monocyte chemotactic protein-1, cytokine-induced neutrophil chemoattractant) and growth factors that enable them to recruit inflammatory and mesenchymal cells that promote biliary remodeling (Fig. 3) [154–157]. These features are a component of an intricate crosstalk between a variety of resident and recruited mesenchymal cells (HSC, portal fibroblasts, myofibroblasts, and fibrocytes), endothelial cells, macrophages, and lymphocytes [158]. Angiogenesis is also stimulated by secretion of VEGF, endothelin-1, platelet-derived growth factor-BB, transforming growth factor-β2 (TGF-β2), and connective tissue growth factor [159]. Reactive cholangiocytes also have decreased expression of epithelial markers and acquire mesenchymal features, which play an important role in modulation of the reparative response. The consequence of all these events is the development of portal fibrosis in cholangiopathies [160].

One of the pathways that mediates repair following injury to cholangiocytes is the Hh signaling pathway [161]. Hh ligands stimulate cholangiocytes to express chemokines that recruit mononuclear cell types with cognate receptors for these chemokines, thereby orchestrating a repair-related mechanism for liver inflammation [162]. In addition, activation of the Hh signaling pathway stimulates the accumulation of ductular-type progenitor cells and myofibroblasts [163]. In BDL models of biliary injury, Hh ligand expression and activity is upregulated; these changes are reversed when the biliary obstruction is relieved [163].

In settings of acute or chronic liver injury, however, cholangiocytes can also actively begin secreting paracrine factors that are hypothesized to permit communication with local cells such as HSC, fibroblasts and inflammatory cells. These include IL-6, IL-8, TNF-α, platelet-derived growth factor, endothelin-1, TGF-β, nitric oxide and monocyte chemotactic

protein-1 [164–170]. Thus, it is likely that cholangiocytes not only have reparative properties, but may also potentiate local injury.

Gut-derived bacterial products, such as LPS, activate the inflammatory cascade in immune cells, modulate the function of liver parenchymal cells, and contribute to chronic liver diseases [171,172]. Recently, it was showed that the expression of NLR pyrin domain-containing protein 3 (Nlrp3) inflammasome is increased in reactive cholangiocytes of both *in vitro* and *in vivo* models of a PSC, leading to the secretion of IL-18 and alteration of the epithelial barrier function of cholangiocytes with significantly decreased the expression of zonulin-1 and e-cadherin [173].

In culture, it has been demonstrated that LPS induces activation of NRAS through Toll-like receptor 4 via NF-κB in human cholangiocytes [140]. Growth factor receptor-bound protein (Grb2), a downstream mediator of epidermal growth factor receptor (EGFR), is essential for this activation. Stimulation of the NRAS/MAPK pathway and NF-kB activation promotes a robust proinflammatory response with IL-6 expression and the consequent activation of cholangiocyte proliferation [174].

8 Conclusion

In summary, cholangiocytes are heterogeneous epithelial cells capable of highly specialized functions. Small and large cholangiocytes possess unique cellular ultrastructure and polarized membrane proteins that allow different responses to homeostatic stimuli as well as to injury. Far from being passive structures, however, cholangiocytes can sense and respond to endogenous molecules (bile acids, oxysterols, nucleotides) as well as exogenous, microbial-derived metabolites including LPS present in bile. In response, cholangiocytes can release autocrine and paracrine signals to maintain homeostasis or to adapt a proinflammatory phenotype. Additionally, cholangiocytes have been shown to be capable of undergoing senescence, which provides protection against neoplastic development, but can also contribute to disease due to the development of an aberrant phenotype such as SASP. Understanding the pathophysiology underpinning differentiation into a proliferative, reactive, senescent or inflammatory phenotype, may lead to the development of novel therapeutics and enhance biomarker discovery.

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Abbreviations

α-CGRP	alpha-calcitonin gene related peptide
Ang	angiopoietin
AE2	anion exchanger 2
ADP	adenosine diphosphate

ADPKD	autosomal dominant polycystic kidney disease
ATP	adenosine triphosphate
ASBT	sodium-dependent bile acid transporter
AQP	aquaporin
Bcl-2	B-cell lymphoma 2
BDL	bile duct ligation
BSEP	bile salt exporter protein
cAMP	cyclic adenosine monophosphate
CCK-B	cholecystokinin-B
Cdc25A	cell division cycle 25A
CDKN	cyclin-dependent kinase inhibitors
CFTR	cystic fibrosis transmembrane conductance regulator
cGMP	cyclic guanine monophosphate
CIS	cytokine-inducible Src homology 2-containing protein
EGFR	epidermal growth factor receptor
EPAC	exchange protein activated by cAMP
ER	estrogen receptor
ERK1/2	extracellular signal-regulated kinase
ETS1	ETS proto-oncogene 1
FOG2	friend of GATA
FoxA2	forkhead box A2
GABA	gamma-aminobutyric acid
GLP1	glucagon-like peptide 1
Hh	hedgehog signaling pathway
H3K4Me3	histone 3 lysine 4 trimethylation
HH3R	histamine H3 receptor
HSC	hepatic stellate cell
iBAT	ileal bile acid transporter
IFN	interferon

IK-1	intermediate-conductance K^+ channel
Grb2	growth factor receptor-bound protein
IL	interleukin
IP_3	inositol 1,4,5-triphosphate
IP_3R	inositol 1,4,5-triphosphate receptor
LPS	lipopolysaccharide
MAPK	mitogen-activated protein kinase
$Mdr2^{-/-}$	multidrug resistance 2 knockout
miRNA	micro-ribonucleic acid
mRNA	messenger ribonucleic acid
mrp2	multidrug resistance protein 2
NF-κB	nuclear factor kappa-light-chain enhancer of activated B cells NGF, nerve growth factor
NHE	Na^+/H^+ exchanger
Ngn-3	neurogenin-3
Nlrp3	NLR pyrin domain-containing protein 3
NRAS	neuroblastoma RAS viral oncogene homolog
ntcp	sodium/taurocholate *co*-transporting polypeptide
oatp1	organic anion-transporting polypeptide
PBC	primary biliary cholangitis
PC	polycystin
PCK	polycystic kidney
PI3K	phosphoinositide 3-kinase
PKA	protein kinase A
PKC	protein kinase C
pRB	retinoblastoma protein
PSC	primary sclerosing cholangitis
PTEN	phosphatase and tensin homolog
SASP	senescence-associated secretory phenotype

SK2	small conductance K^+ channel
SGLT1	sodium-dependent glucose cotransporters
Smad	small mothers against decapentaplegic
SSTR2	somatostatin receptor subtype 2
TGF	transforming growth factor
TNF	tumor necrosis factor
TRAIL	(TNF)-related apoptosis-inducing ligand
TRPV4	transient receptor potential vaniloid 4
VEGR	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
VIP	vasoactive polypeptide

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Highlights

- Cholangiocytes are heterogeneous epithelial cells whose highly specialized functions is largely dependent on their size (e.g., small vs. large cholangiocytes).
- Small and large cholangiocytes possess unique cellular ultrastructure and polarized membrane proteins that allow different responses in both homeostatic conditions as well as in injury.
- Upon injury, reactive cholangiocytes develop a neuroendocrine phenotype and are capable of autocrine and paracrine signaling to maintain homeostasis or adopt proinflammatory responses. These responses can also be mediated by microRNA in both healthy and diseased states.
- Cholangiocytes can also undergo senescence (cell cycle arrest), thus avoiding neoplastic transformation, however this status can also potential disease development via hypersecretion of proinflammatory cytokine and chemokines (termed senescence-associated secretory phenotype [SASP]).

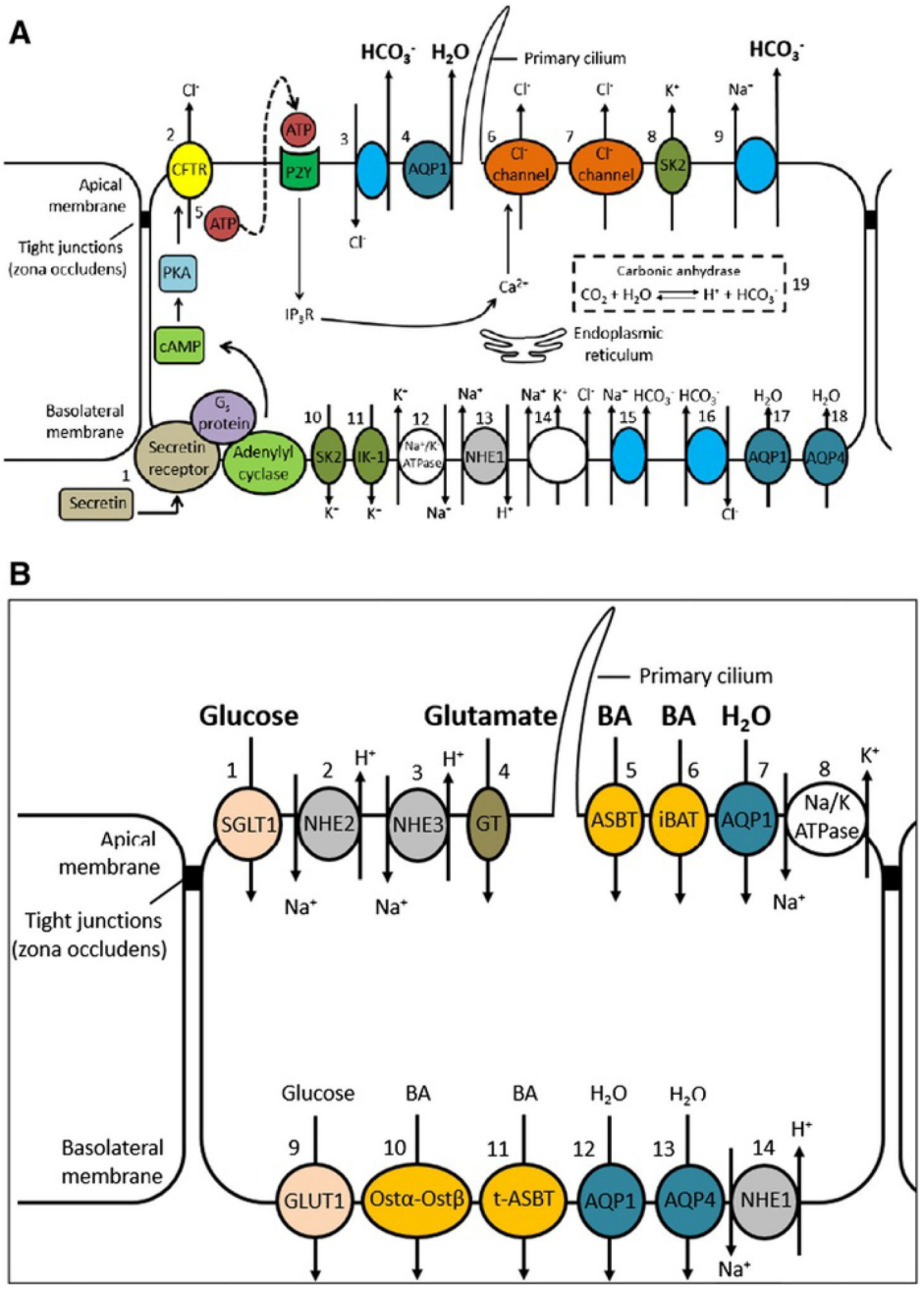

Fig. 1.

A. Cholangiocyte secretory functions. The most widely accepted model of ductal bile formation involves [1] secretin activation of the cAMP-PKA signaling pathway, which then leads to [2] activation of CFTR, causing extrusion of Cl^-. This then stimulates the [3] Cl^-/HCO_3^- exchanger which secretes HCO_3^- and creates an osmotic gradient that leads to the [4] passive movement of water across AQP1. More recently, an alternative hypothesis suggests that [5] activation of CFTR leads to the release of ATP, which then phosphorylates IP_3R on the endoplasmic reticulum. This causes the release of Cl^- via the [6] Ca^{2+}-dependent Cl^- channel, which creates a gradient leading to [3] activation of the Cl^-/HCO_3^-

exchanger. Ca^{2+} is also transported via a [7] Ca^{2+}- and cAMP-independent Cl^- channel. Finally, the apical membrane also contains [8] K^+ channels (SK2 and IK-1) and a [9] Na^+-HCO_3^- cotransporter (mice). On the basolateral domain, there are two types of K^+ channels: [10] SK2 and [11] IK-1. There is also [12] a Na^+/K^- ATPase, [13] a Na^+/H^+ exchanger (NHE1), [14] a $Na^+/K^+/Cl^-$ cotransporter, [15] a Na^+/HCO_3^+ cotransporter (rats), [16] a Na^+-dependent $Cl^-/HCO3^-$ exchanger (humans), and water channels, [17] AQP1 and [18] AQP4. Within the cell, [19] carbonic anhydrase allows for the production of HCO_3^-.
Abbreviations: ATP, adenosine triphosphate; AQP, aquaporin; cAMP, cyclic adenosine monophosphate; CFTR, cystic fibrosis transmembrane conductance regulator; IK-1, intermediate-conductance K+ channel; PKA, protein kinase A; NHE, Na^+/H^+ exchanger; SK2, small conductance K^+ channel.
B. Cholangiocyte absorptive functions. Cholangiocytes have a number of transporters that allow the absorption of a variety of molecules [1]. Glucose is absorbed through SGLT1, Na^+ is absorbed through [2] NHE2 and [3] NHE3, glutamate is absorbed through a glumate transporter, bile acids are absorbed through [5] ASBT and [6] iBAT and [7] water is absorbed through AQP1. On the basolateral membrane, [8] glucose is transported via GLUT1, bile is transported via [9] Ostα-Ostβ and [10] t-ASBT, water is transported via [11] AQP1 and [12] AQP4. Lastly, the basolateral membrane contains a [13] Na^+/H^+ exchanger (NHE3). Abbreviations: ASBT, apical sodium-dependent bile acid transporter; AQP, aquaporin; BA, bile acids; GLUT, glucose transporter; GT, glutamate transporter; iBAT, ileal bile acid transporter; NHE, Na^+/H^+ exchanger; OST, organic solute transporter alpha; SGLT, Na^+-Glucose cotransporter.

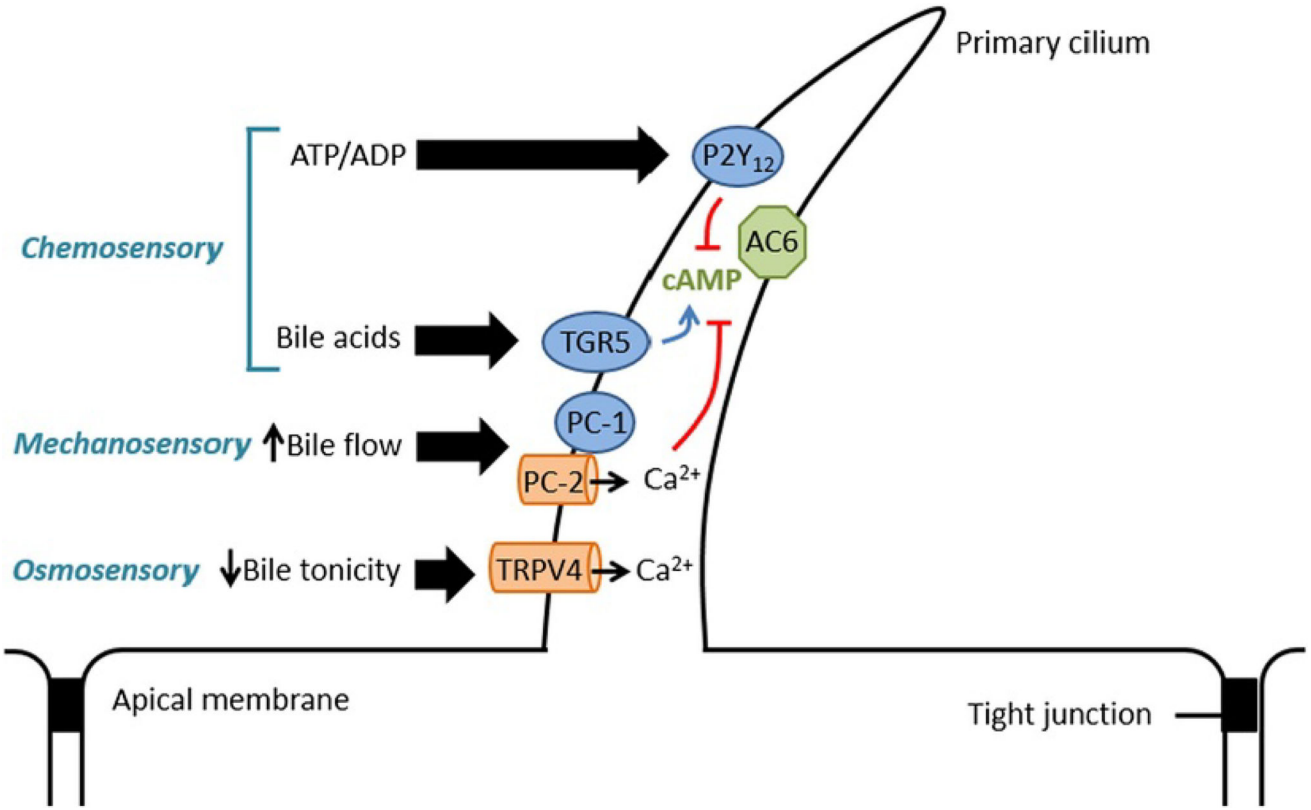

Fig. 2.

Functions of primary cholangiocyte cilium. Cholangiocyte cilia have mechano-, chemo- and osmosensory functions. This figure depicts the ciliary pathways involved in inhibiting cholangiocyte secretion, but the inverse relationships also allow for cholangiocyte secretion (e.g., decreased bile flow, increased bile tonicity etc.). 1) Chemosensory: ATP/ADP and bile acids can lead to inhibition of cholangiocyte secretion through the $P2Y_{12}$ and TGR5 receptors respectively. The activation of $P2Y_{12}$ leads to the inhibition of AC6 which causes decreased cAMP production, thereby causing a reduction in the efflux of Cl^-, which in turn leads to decreased HCO_3^- secretion. Activation of TGR5 by bile acids leads to an increase in cAMP-mediated cholangiocyte secretion. 2) Mechanosensory: An increase in bile flow activates the PC-1/PC-2 complex, which is comprised of a mechanoreceptor (PC-1) and a Ca^{2+} channel (PC-2). This leads to an increase in intracellular Ca^{2+} and inhibition of cAMP-induced biliary secretion. 3) Osmosensory: A decreased in bile tonicity leads to an influx of Ca^{2+}, which again causes a decrease in cAMP-induced biliary secretion. Abbreviations: AC6, adenylyl cyclase 6; ADP, adenosine diphosphate; ATP, adenosine riphosphate; cAMP, cyclic adenosine monophosphate; PC-1, polycystin-1 ; PC-2, polycystin-2; TRPV4, transient receptor potential channel vanilloid subfamily 4.

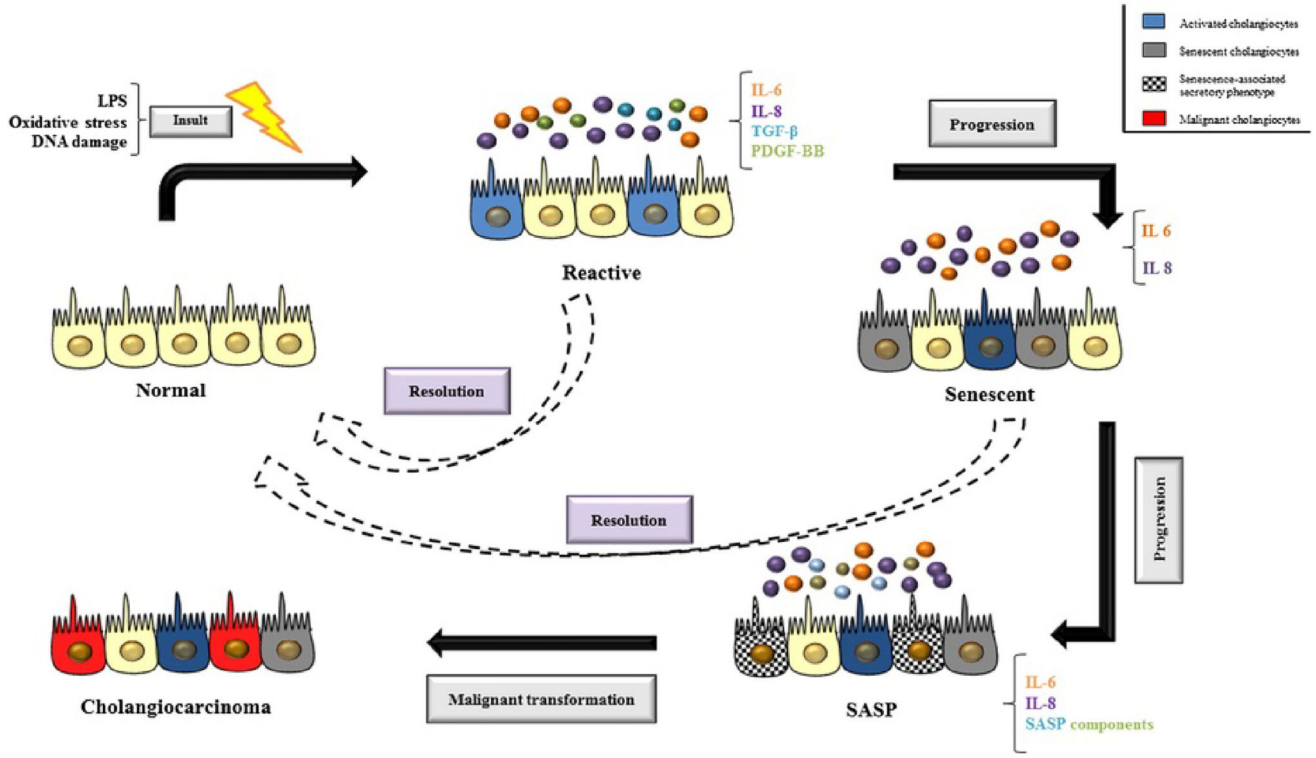

Fig. 3.

Schematic representation of cholangiocyte plasticity. Endogenous and exogenous insults can activate normal cholangiocytes which are characterized by enhanced secretion of pro-inflammatory cytokines and growth factors that recruit both immune and mesenchymal cells to promote biliary remodeling. After insults, proliferation is also activated as a consequence of cholangiocyte death (lysis and/or apoptosis) and if the cellular injury continues, cholangiocytes can become senescent. Senescent cholangiocytes are under permanent cell cycle arrest and apoptosis resistant. Ongoing cellular injury leads to continuous generation of senescent cells leading to progression into a senescence-associated secretory phenotype. This eventually leads to tissue dysfunction and tumor promotion. The dashed arrows suggest that reactive and senescent cholangiocytes can go through repair mechanisms that are mediated by several pathways, with Hedgehog (Hh) signaling pathway being one of the most important. Abbreviations: IL, interleukin; LPS, lipopolysaccharide; PDGF-BB, platelet-derived growth factor-BB; SASP, senescence-associated secretory phenotype; TGF-β, transforming growth factor β.

Table 1

Classification of cholangiopathies.

Genetic
Alagille's syndrome
Caroli's syndrome
Cystic fibrosis
MDR3 deficiency
Polycystic liver disease (ADPLD, ADPKD, ARPKD)
Immune-mediated
Acute allograft rejection
Chronic allograft rejection
Graft versus host disease
Primary biliary cholangitis
Idiopathic
Biliary atresia
Idiopathic childhood/adulthood ductopenia
IgG4 cholangiopathy
Primary sclerosing cholangitis
Sarcoidosis
Infectious
AIDS cholangiopathy (e.g., viral cholangitis)
Bacterial cholangitis (e.g., *Escherichia coli*, Klebsiella, Enterococcus, Enterobacter, Pseudomonas, anaerobes)
Parasitic cholangitis (e.g., Ascaris lumbricoides, *Opisthorchis viverrini, Clonorchis sinensis, Fasciola hepatica*)
Malignant
Cholangiocarcinoma
Other
Drug-induced (e.g., Floxuridine-induced cholangiopathy, ketamine cholangiopathy)
Vascular/ischemic (e.g., post-liver transplant hepatic artery stenosis, systemic vasculitis)

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; ADPLD, autosomal dominant polycystic liver disease; AIDS, acquired immunodeficiency syndrome; ARPKD, autosomal recessive polycystic kidney disease; IgG4, immunoglobulin G subclass 4; MDR3, multidrug resistance 3.

Table 2

Known differences between small and large cholangiocytes.

Phenotype	Small cholangiocytes	Large cholangiocytes
Location	Smaller bile ducts	Larger bile ducts
Shape	Cuboidal	Columnar
Intracellular structures	High nucleus to cytoplasm ratio	Low nucleus to cytoplasm ratio
	Multilobulated nucleus, few mitochondria, abundant Golgi apparatus, minimal rough endoplasmic reticulum	Multilobulated nucleus, few mitochondria, abundant Golgi apparatus, more rough endoplasmic reticulum
Apical surface	Lysosomes and vesicles localized to the apical membrane, tight junctions between cells, coated pits, microvilli	Lysosomes and vesicles localized to the apical membrane, tight junctions between cells, coated pits, microvilli
Protein expression	Express lipase, α-amylase and trypsin	Express lipase, α-amylase and trypsin
	AE1 absent, AE2 present	AE1 absent, AE2 present
	CFTR absent	CFTR present
	CYP4502E1 absent	CYP4502E1 present
	Blood group antigens present (e.g., A, B, O, Lewis)	Blood group antigens absent
	Bcl-2 present	Bcl-2 absent
Response to injury	Resistant to liver injury	Susceptible to liver injury
	Can proliferate (e.g., behave as liver progenitor cells)	

Abbreviations: AE2, anion exchanger 2; Bcl-2, B-cell lymphoma 2; CFTR, cystic fibrosis transmembrane conductance regulator.

Table 3

Factors that stimulate or inhibit cholangiocyte proliferation.

Class	Known stimulators of proliferation	Known inhibitors of proliferation
Gastrointestinal hormones	Glucagon-like peptide 1 (GLP1)	Somatostatin
	Exendin-4 (GLP1 receptor agonist)	Gastrin
Bile acids	Taurocholate (large cholangiocytes only)	Ursodeoxycholic acid (all cholangiocytes)
	Taurolithocholic acid (large cholangiocytes only)	Tauroursodeoxycholate (all cholangiocytes)
Angiogenic factors	Vascular endothelial growth factor	
	Angiopoietin-1	
	Angiopoietin-2	
Neuropeptides and neurotransmitters	Serotonin Nerve growth factor	
	Parasympathetic innervation	
	Sympathetic innervation	
	Alpha-calcitonin	
	Histamine	
Hormones	17-β-estradiol	
	Gonadotropin releasing hormone (GnRH)	
Other	Matrix metalloproteinases (MMP)	

Table 4

Role of miRNAs in cholangiocyte homeostasis and in cholangiopathies.

Cohort	miRNA	Direction of effect[a]	Affected mRNA	Outcome of miRNA changes
Healthy	miR-33a	–	ABCA1, ABCG1	Maintains cellular cholesterol efflux
		–	ABCB11	Maintains homeostatic BSEP expression for bile acid export into bile canaliculi
		–	ATP8B1	Maintains membrane polarity and secretory function
	miR-144	–	ABCA1	Maintains plasma HDL levels
	miR-122, miR-422a	–	CYP7A1	Maintains homeostatic bile acid synthesis
	miR-98, *let-7*	–	CIS	Suppression of CIS may indirectly suppress NF-κβ
	miR-513	–	B7-H1	Decrease in IFN-γ induced B7-H1 expression (which has dual functions in T cell regulation)
Experimental bliary obstruction (BDL mice)	miR-21	Upregulated	Smad-7	Bile duct proliferation and fibrosis
Experimental sclerosing cholangitis (Mdr2−/− mice)	miR-24	Downregulated	MEN1	Limits expression of fibrosis
Experimental sclerosing cholangitis (BDL and DDC fed mice)	miR-7-a-1, miR-7-a-2	Upregulated	Ngn-3	Inhibits promitotic effect of Ngn-3, thus decreasing cholangiocyte proliferation and collagen deposition
PBC	miR-506	Upregulated	AE2	Decreased AE2 expression leads to impaired biliary secretion
Biliary atresia	miR-200b	Upregulated	FOG2	Increased PI3K/AKT signalling, leading to HSC cell growth and migration with increased fibrosis
	miR-21	Upregulated	PTEN	Increased AKT signalling, leading to HSC cell growth and migration with increased fibrosis
PCK rat	miR-15a	Downregulated	Cdc25A	Cell cycle progression and cyst expansion in normal rat cholangiocytes

Abbreviations: AE2, anion exchanger2; BDL, bile-duct ligation; BSEP, bile salt exporter protein; Cdc25A, cell division cycle 25A; CIS, cytokine-inducible Src homology 2-containing protein; DDC, 3,5;diethoxycabonyl-1,4-dihydrocollidine; FOG2, friend of GATA 2; HDL, high density lipoprotein; IFN, interferon; NF-κβ; nuclear factor kappa-light-chain enhancer of activated B cells; Ngn-3, neurogenin-3; PBC, primary biliary cholangitis; PCK, polycystic rat; PI3K, phosphoinositide 3-kinase; PSC, primary sclerosing cholangitis; PTEN, phosphatase and tensin homolog; Smad; small mothers against decapentaplegic.

[a]No direction of effect is listed for miRNA described in homeostatic circumstances (e.g., “healthy”) as the listed direction of effect is relative to controls. miRNAs are inherently inhibitory; that is, they inhibit their target mRNAs.