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Functional Anatomy of Normal Bile Ducts

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Abstract

The biliary tree is a complex network of conduits that begins with the canals of Hering and progressively merges into a system of interlobular, septal, and major ducts which then coalesce to form the extrahepatic bile ducts, which finally deliver bile to the gallbladder and to the intestine. The biliary epithelium shows a morphological heterogeneity that is strictly associated with a variety of functions performed at the different levels of the biliary tree. In addition to funneling bile into the intestine, cholangiocytes (the epithelial cells lining the bile ducts) are actively involved in bile production by performing both absorptive and secretory functions. More recently, other important biological properties restricted to cholangiocytes lining the smaller bile ducts have been outlined, with regard to their plasticity (i.e., the ability to undergo limited phenotypic changes), reactivity (i.e., the ability to participate in the inflammatory reaction to liver damage), and ability to behave as liver progenitor cells. Functional interactions with other branching systems, such as nerve and vascular structures, are crucial in the modulation of the different cholangiocyte functions.

Keywords

cholangiocytes; normal liver; bile secretion; peribiliary vascular plexus; ductular reaction

MORPHOLOGICAL AND FUNCTIONAL CHARACTERISTICS OF THE BILIARY EPITHELIUM IN NORMAL LIVER

Cholangiocytes are the epithelial cells that line the biliary tree, a complex network of conduits within the liver that begins with the canals of Hering and progressively merges into a system of interlobular, septal, and major ducts; these structures then coalesce to form the extrahepatic bile ducts, which finally deliver bile to the gallbladder and to the intestine (Fig. 1A). Bile ducts run in parallel with a branch of the portal vein and with one or two branches of the hepatic artery, giving rise to a close anatomic association classically represented in the liver microarchitecture by the portal triad (Fig. 1B). The nomenclature of biliary structures in human liver still refers to the classification originally proposed by Ludwig (1987). According to the ductal diameter, the intrahepatic bile duct system can be categorized into: *small bile ductules* (also called *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 *hepatic* ducts ($>800 \mu\text{m}$). In contrast with the distal, larger branches of the biliary tree that have been well defined in terms of morphology and structure, the microanatomic arrangement of terminal cholangioles and canals of Hering is less characterized and has been recently reviewed (Roskams et al., 2004). The *canal of Hering* is a channel located at the ductular–hepatocellular junction, lined in part by hepatocytes and in part by cholangiocytes, which represents the physiologic link of the biliary tree with the hepatocyte canalicular system extended within the lobule. It is important to note that cells with an intermediate pheno-type between hepatocyte and cholangiocyte are not present in the normal liver. Although in original histological descriptions canals of Hering were thought to be recognizable only at the electron microscopic level, it is currently believed that they can be identified also in routine histological analysis of liver tissue samples when performed with extreme care (Crawford et al., 1998). Canals of Hering are in direct continuity with the terminal cholangioles, which represent the first tubular structure entirely lined by cholangiocytes: they link the canals of Hering on one side to the interlobular ducts on the other. These are the finest ramifications of the biliary tree and traverse the limiting plate between the portal space and the lobule, thus being formed by both an intralobular and an intraportal segment. In the normal liver, some “isolated” cholangiocytes can also be observed as small cell clusters or short cuboidal strings: most likely they represent cross-sections of the canals of Hering and the intralobular segment of the terminal cholangiole.

The small ductules are lined by four to five cholangiocytes, morphologically characterized by a cuboidal shape, with a basement membrane, tight junctions between adjacent cells and microvilli projecting into the bile duct lumen (Benedetti et al., 1996). Recently it has been shown that cholangiocytes also possess primary cilia in their apical cell membrane: these structures possess sensory functions and play an important role in regulating fundamental biological activities of the cholangiocyte, including cell differentiation, proliferation, and secretion. Cholangiocyte cilia contain different proteins (such as polycystins and fibrocystin), which defective function results in biliary dysgenesis (Masyuk et al., 2003a). With the progressive enlargement of the ductal system, cholangiocytes become larger in size and more columnar in shape. Recent studies have shown that some phenotypic differences between cholangiocytes lining small and large bile ducts can be recognized also at the ultra-structural level. Cholangiocytes lining the small ductules show a high nucleus to cytoplasm ratio, in contrast with cholangiocytes of the large ducts possessing a relatively small nucleus and abundant cytoplasm. A rich Golgi apparatus is detectable between the apical pole and the nucleus, whereas rough endoplasmic reticulum is scarce in the smallest ductules, and increased only slightly in the large ducts (Benedetti et al., 1996). Coated pits have been observed on both the apical and basolateral regions, thus suggesting that receptor-mediated endocytosis is an active process performed by cholangiocytes (Ishii et al., 1990).

This morphological heterogeneity is strictly associated with a variety of different functions performed by cholangiocytes at different levels of the biliary tree (Fig. 2). In addition to funneling bile into the intestine, cholangiocytes are actively involved in bile production by performing both absorptive and secretory functions as well as in regenerative/repairative processes. In humans, around 40% of the total bile production is of ductal origin. Secretory functions are mainly performed by cholangiocytes lining the interlobular, septal, and major ducts, as they express the appropriate ion transport systems and hormone receptors in polarized domains of the plasma membrane. Other biological properties appear to be restricted to the smaller bile duct branches (terminal cholangioles and canals of Hering), such as *plasticity* (i.e., the ability to undergo limited phenotypic changes), *reactivity* (i.e., the ability to participate in the inflammatory reaction to liver damage), and the ability to *behave as liver progenitor cells*, which are variably elicited only after liver injury (Sell, 2001). In particular, given the strong capacity of mature hepatocytes to proliferate, cholangiocyte ability to behave as liver progenitor cells becomes evident only when hepatocellular

proliferation is hampered as a result of severe liver damage, as that induced by several toxins or drugs, or occurring under certain conditions, i.e., viral hepatitis or non alcoholic steatohepatitis (Sell, 2001). This functional and morphological specialization of biliary tree domains has important clinical relevance, because cholangiopathies are mostly characterized by focal rather than diffuse patho-biological processes. As a consequence, the clinical presentation of cholangiopathies may vary depending on the site of injury affecting the different segment of the biliary tree: for instance, the interlobular bile ducts are selectively damaged in primary biliary cirrhosis (PBC) and in “small-duct” primary sclerosing cholangitis (PSC), whereas extrahepatic and the main intrahepatic bile ducts are affected in the classic form of PSC (“large-duct” PSC). Also cholangiocarcinomas arising from the intrahepatic portion of the biliary tree show distinct clinical and epidemiological features from those originating from the extrahepatic bile ducts. We will discuss the role of biliary epithelium in bile production and focus on the functional interactions with other branching structures of the liver, such as nerves and vessels, as they are involved in the regulation of cholangiocyte function during liver repair after hepatic damage.

BILE DUCTS AND BILE PRODUCTION

Bile production is a complex biological process, in which the function of cholangiocytes is finely tuned to that of hepatocytes. In fact, hepatocytes produce the primary canalicular bile which is then modified by cholangiocytes through a sequence of both secretory and absorptive processes aimed to adjust bile flow and alkalinity to the physiological needs. These processes performed by the biliary epithelium are extensively regulated by a wide range of hormones. The net amount of fluid and bicarbonate secretion is determined by the integration of prosecretory activities, induced by secretin (Strazzabosco, 1997; Lenzen et al., 1997), glucagon (Lenzen et al., 1997), vasoactive intestinal polypeptide (VIP; Cho and Boyer, 1999a), acetylcholine (Alvaro et al., 1997), bombesin (Cho and Boyer, 1999b), with antiseecretory signals, mediated by somatostatin (Gong et al., 2003), insulin (LeSage et al., 2002), gastrin (Glaser et al., 2003), and endothelin 1 (Caligiuri et al., 1998) (Fig. 3A). These different signals crucially interact at the level of adenylyl-cyclase, the transmembrane enzyme regulating the intracellular concentrations of cAMP by conversion of ATP. Seven different adenylyl-cyclase isoforms are expressed by rodent cholangiocytes, each being regulated by a specific mechanism (Spirli et al., 2005). Recently, by investigating one of these isoforms, a new model for regulation of ductal bile secretion involving cholangiocyte cilia has been proposed. Adenylyl-cyclase isoform 6 is expressed by cholangiocyte cilia along with polycystin-1 and polycystin-2: through it cilia function as sensory organelles that detect changes in luminal flow and transmit stimuli into intracellular Ca^{2+} levels and cAMP signalling (Masyuk et al., 2006). Different mechanisms of ductal secretion based on the stimulation by secretin are more classically described. It results in increased cAMP/PKA concentrations, which in turn leads to phosphorylation of Cystic Fibrosis transmembrane conductance regulator (CFTR), thereby stimulating Cl^- and HCO_3^- efflux on one side and inhibiting NHE3-mediated Na^+ absorption on the other (Mennone et al., 2001; Fig. 3B). CFTR is a cAMP-activated slow conductance Cl^- channel expressed on the apical domain of several ion secretory ductal epithelial cells, including cholangiocytes (Cohn et al., 1993; Choi et al., 2001). Cl^- efflux into the ductal lumen is the driving force of a chloride/bicarbonate exchanger that exports HCO_3^- into the bile duct lumen. However, CFTR possesses a permeability to HCO_3^- by its own and is also able to influence other ion carrier activities (Choi et al., 2001). Both CFTR and NHE3 bind to the actin cytoskeleton by means of the ezrin/EBP50 complex, a system allowing the coordinated regulation of CFTR and NHE3 activities by PKA (Fouassier et al., 2001). Additional Cl^- channel are expressed by cholangiocytes and may be relevant for bile formation and cholestasis; among them, apical Ca^{2+} -dependent Cl^- channel is an alternative transport system susceptible to luminal purinergic nucleotide signaling, whose activation could be exploited in the therapy of Cystic

Fibrosis cholangiopathy (Zsemler et al., 2000, 2002). Once ion secretion has been stimulated and an osmotic gradient has been established, water follows the gradient either by paracellular communications or through specific water channels, namely aquaporin 1 and 4 (AQP1 and AQP4; Marinelli et al., 1999, 2000). These transport activities are differently localized in the cholangiocyte: AQP1 is expressed in a subset of subapical vesicles whose insertion into the plasma membrane is stimulated by cAMP; instead, AQP4 is constitutively expressed at the basolateral pole.

The secretory functions of the biliary epithelium are also regulated in a paracrine way by a series of molecules secreted by hepatocytes and contained in the canalicular bile (such as bile salts, glutathione, and purinergic nucleotides), which are delivered to membrane receptors expressed by cholangiocytes. For instance, ATP is released into the bile in micromolar concentrations from both hepatocytes and cholangiocytes: at this level, ATP can bind to the apical P2Y₂ purinergic cholangiocyte receptors, where it mediates multiple secretory effects. ATP can stimulate apical Ca²⁺-activated Cl⁻ channels and basolateral Na⁺/H⁺ exchange (NHE-1), promote Cl⁻ efflux into the bile and HCO₃⁻ basolateral influx. If the cAMP-dependent secretory mechanism fails due to acquired or genetic defects, ductal secretion can be stimulated by exploiting these Ca²⁺-dependent signaling systems. In cholangiocytes, Ca²⁺ signaling occurs as polarized Ca²⁺ waves, starting from the apical pole, where Type III InsP₃ receptors are localized.

The biliary epithelium is also involved in the reabsorption of some biliary constituents, such as glucose and glutathione, and in the chole-hepatic circulation of bile salts. Glutathione is catabolized by the ectoenzyme γ -glutamyltranspeptidase expressed by the apical domain of cholangiocytes. The subsequent uptake of glutamate and cysteinyl-glycine is crucial to avoid depletion of GSH, an essential component of the hepatic detoxification machinery. By mediating the chole-hepatic circulation of bile acids, cholangiocytes take part in the overall regulation of bile secretion. In fact, cholangiocytes are able to take up bile acids from bile by means of an apical, Na⁺-dependent bile acid transporter (ASBT), and then to secrete them into the peribiliary vascular plexus closely adjacent to the bile ducts (see below) by means of two transporters, the t-ASBT, a truncated isoform of ASBT, and the MRP3, a p-glycoprotein. Furthermore, the ability of biliary epithelial cells to take-up bile acids may represent an alternative therapeutic strategy to stimulate ion secretion by cholangiocytes: UDCA stimulates Cl⁻ secretion in cholangiocytes by means of CFTR-mediated ATP release (Spirli et al., 2005). On the other hand, an increased biliary concentration of bile acids in the absence of phospholipids, as in MDR3 deficiency, may alter the epithelial barrier function and cause portal inflammation.

BILE DUCTS AND INNERVATION

In recent years an increasing amount of evidence has been produced on the role played by the nervous system in the physiological regulation of some cholangiocyte functions. In the normal liver, extrahepatic bile ducts and peribiliary glands possess well-developed parasympathetic and sympathetic plexuses in their wall; in the portal tract sparse cholinergic and adrenergic nerve fibers are also observed around the bile ducts as well as the portal vein and hepatic artery branches (Terada and Nakanuma, 1989). Given the biological property of cholangiocytes to express the M3 subtype receptor for acetylcholine (Nathanson et al., 1996), parasympathetic innervation may affect a range of cholangiocyte activities, including secretin-induced choleresis, cell proliferation, and apoptosis, regulated by intracellular cAMP levels (LeSage et al., 1999). However, these effects seem to be relevant only in the setting of cholestasis: vagotomy induces impairment of cholangiocyte proliferation, activation of apoptotic cell death and marked reduction in ductal secretion in association with decreased cAMP intracellular levels in experimental cholestasis caused by common

bile duct ligation (BDL), but not in normal rats. Similarly, the sympathetic terminals have also a role in hepatic proliferation, as originally seen in the partial hepatectomy model (Kiba et al., 1994). In fact, proliferating cholangiocytes express $\beta 1$ and $\beta 2$ adrenergic receptors in BDL rats along with the M3 acetylcholine receptor (Glaser et al., 2006). In this experimental model, liver sympathetic denervation induced by chemical treatment with 6-OHDA results in decreased cAMP levels associated with increased cholangiocyte apoptosis and decreased cholangiocyte proliferation, as seen with vagotomy. In addition, cholangiocytes express serotonin 1A and 1B receptors whose activation by the neuroendocrine hormone serotonin has an inhibitory effect on cholangiocyte growth and choleretic activity in BDL rats (Marzioni et al., 2005). During cholestasis, cholangiocytes are also capable to secrete serotonin, thus mediating an additional autocrine loop, which limits the growth of bile ducts. In contrast with the inhibitory effect exerted by serotonin, in experimental cholestasis other substances secreted by cholangiocytes, such as nerve growth factor, may instead autocrinally stimulate cholangiocyte proliferation, based on their expression of nerve growth factor receptors (Gigliozzi et al., 2004).

It is important to note that, as reported for parasympathetic innervation, these effects are relevant only in proliferating but not in quiescent cholangiocytes: in accordance with this observation it is the fact that graft functions are not altered by the complete denervation performed in the transplanted liver (Kjaer et al., 1994; Colle et al., 2004).

Furthermore, a large number of other regulatory neurotransmitters and neuropeptides, including neuropeptide tyrosine (NPY), substance P, VIP, calcitonin gene related peptide (CGRP), glucagon-like peptide, somatostatin, neurotensin, galanin, serotonin, enkephalin and bombesin (Alvaro et al., 2007), can be variably released by the autonomic nervous fibers and may signal to the biliary tree. Among them, while NPY-positive nerves have been observed in the extrahepatic bile ducts where they take part in the modulation of the bile flow, VIP and CGRP coexist in cholinergic nerves innervating portal vein and hepatic artery branches, but not the bile ducts.

BILE DUCTS AND VASCULARIZATION

In contrast to hepatocytes, the biliary epithelium is specifically nourished by a rich network of capillaries localized in close proximity to the intrahepatic bile ducts (peribiliary vascular plexus, PBP), which is crucial for maintaining integrity and function of the biliary epithelium (Kono and Nakanuma, 1992; Fig. 4C). They originate from the terminal branches of the hepatic artery and deliver blood to the sinusoids into the portal vein (Gaudio et al., 1996). This specific vascular supply, lacking in canals of Hering and terminal cholangioles, accounts for the prevalent involvement of the interlobular bile ducts in case of ischemic injury due to obstruction of hepatic artery branches less than 200 μm in diameter (featuring the “ischemic cholangiopathy”; Deltenre and Valla, 2006). Given the fact that cholangiocytes possess receptors for several cytokines, chemokines, and growth factors, PBP is one of the mechanisms enabling an extensive cross-talk of cholangiocytes with other liver cell types, including hepatocytes, stellate cells, and endothelial cells. In particular, through PBP, many substances reabsorbed by the biliary epithelium can be delivered from bile to hepatocytes, thus representing a direct way of communication between cholangiocytes and hepatocytes.

In addition to PBP, intrahepatic bile ducts have a close anatomical relationship with other vascular structures, in particular with the hepatic arteries (Fig. 4A,B). It has been shown that among the different portal tract structures, intrahepatic bile ducts have a preferential association with hepatic arterial rather than portal venous vascularization. In fact, hepatic arteries and bile ducts are generally so closely associated that the numerical correlation of

hepatic arterial profiles with bile duct profiles is considered a good index for bile duct preservation or loss; in contrast, the lack of portal vein profiles is not uncommonly observed in the normal portal tract (Crawford et al., 1998). The strong association of biliary epithelium with hepatic artery is a common histological feature, present since the early stages of human liver development, when the hepatic artery branches are formed in close vicinity to ductal plates (Libbrecht et al., 2002), the embryonic structure from which the intrahepatic bile ducts originate. The branching pattern of both systems finely develops in a coordinated manner also in disease conditions, as shown in an experimental rat model of selective cholangiocyte proliferation, where an extensive neovascularization of the arterial bed strictly follows the expansion of the biliary tree induced by α -naphthylisothiocyanate (Masyuk et al., 2003b). Ductular reaction, a common histological response to many forms of liver injury, is characterized by an increased amount of bile ductules at the portal interface associated with an increased number of hepatic arterioles and peribiliary capillaries (Desmet et al., 1995). In mice, inactivation of two transcription factors crucially involved in the development of intrahepatic bile duct epithelium, namely Hnf6 or Hnf1 β (Clotman et al., 2002), resulted in anomalies of the hepatic artery branches, accompanying the abnormal bile ducts (Clotman et al., 2003). A close association of aberrant bile ductal and arterial structures has been similarly described in human developmental cholangiopathies related to ductal plate malformation (DPM), where an enrichment of fine vascular structures surrounds the dysmorphic bile ducts, resulting in a “pollard willow” pattern (Desmet, 1992). Based on these data, it can be argued that a signal originating from the bile duct cells directs arterial vasculogenesis during development and arterial neoangiogenesis in biliary disease conditions. Recently it has been shown that, in cystic cholangiopathies related to DPM, such as the autosomal dominant polycystic kidney disease and Caroli's disease, the cystic epithelium retains an immature phenotype characterized by an up-regulation of different angiogenic growth factors, among which the vascular endothelial growth factor (VEGF): in these developmental diseases, cholangiocytes acquire the capability to secrete VEGF, leading paracrinally to the aberrant vascularization around the cyst to provide its vascular supply (Fabris et al., 2006a). This property is a normal feature of cholangiocyte biology in embryonic development, which is characterized by an highly coordinated and stage-specific reciprocal expression of angiogenic growth factors and their cognate receptors between biliary and arterial structures. Through secretion of VEGF acting on endothelial cells and their precursors, developing bile ducts likely promote both arterial and PBP vasculogenesis (Fabris et al., 2006b). In addition to the paracrine effect on vascular cells, cholangiocytes can also act autocrinally given their capability to express both receptors for VEGF and Tie-2, the receptor for angiopoietins. This feature is unique among epithelial cells: it is normally present during the fetal development, while it is absent in normal, postnatal liver, but can be reactivated in some disease conditions, as seen in ductular reaction (Fig. 5). This finding points to a role for the biliary epithelium in driving angiogenesis also in the course of liver disease, as a sort of protective mechanism against its strong susceptibility to the ischemic injury (Deltenre and Valla, 2006).

CONCLUSIONS

Biology of normal cholangiocytes has become a topic of growing interest in the last 15 years, because they have been recognized as the primary cell target of the pathogenetic sequence in a large group of adult and pediatric evolutive liver disorders, collectively termed “cholangiopathies.” These epithelial cells show a complex, multitasked biological behavior, with an heterogeneous profile according to a fine, regional specialization. Originally studied for their relevant contribution to bile secretion, cholangiocytes have recently gained further attention for the functional interactions with nerve and vascular structures, which are involved in the modulation of their plasticity, reactivity and capability to behave as liver

progenitor cells. These latter biological properties are crucial in the induction of repair mechanisms in response to most forms of liver damage.

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Fig. 1.

Normal anatomy of biliary epithelium. **A:** The intrahepatic bile duct epithelium is organized as a three-dimensional branching system of conduits inside the liver, which progressively merge into ducts of increasing size and ultimately deliver bile to the gallbladder and to the duodenum. **B:** In the liver microarchitecture bile ducts (BD) run in parallel between the lobules with a branch of the portal vein (PV) and of the hepatic artery (HA), giving rise to a close anatomic association known as portal triad.

Fig. 2.

Morphological and functional heterogeneity of biliary epithelium. The biliary tree begins with the canals of Hering, located at the ductular-hepatocellular junction, which are lined in part by hepatocytes and in part by cholangiocytes: they constitute the physiologic link of the biliary tree with the hepatocyte canalicular system, and are the site where a facultative progenitor cell compartment resides. Cells lining the intrahepatic biliary tree have different functional and morphological specializations: the terminal cholangioles (size $<15\ \mu\text{m}$) have some biological properties such as plasticity and reactivity; interlobular ($15\text{--}100\ \mu\text{m}$) and large ducts ($100\ \mu\text{m}$ to $800\ \mu\text{m}$) modulates fluidity and alkalinity of the primary hepatocellular bile.

Fig. 3.

Cholangiocyte modifies bile flow and alkalinity by exerting both secretory and absorptive activities. **A:** Cholangiocyte possesses numerous different molecules located on their apical (luminal) and basolateral domain finely regulated by a balance of prosecretory and antiselectory signals induced by a series of hormones, neuropeptides and neurotransmitters interacting at the level of adenylyl-cyclase. **B:** In particular, a relevant role in normal cholangiocyte physiology is played by secretin that increases intracellular cAMP levels and consequently activates CFTR through PKA phosphorylation: the consequent Cl^- efflux into the lumen generates the driving force for HCO_3^- secretion by AE2 exchanger into bile.

Fig. 4.

Preferential association of the bile ducts with hepatic artery branches in normal portal tract and localization of peribiliary vascular plexus (PBP) around the intrahepatic bile duct. **A,B:** In the normal portal tract bile ducts (immunoreactive for cytokeratin-19, CK19, A) are closely associated with one or two branches of hepatic artery (immunoreactive for smooth muscle actin, SMA, B): their numerical correlation is considered a good index to measure bile duct preservation or loss. **C:** Blood is supplied to the bile ducts through a fine system of arterioles and capillaries (recognized by immunoreactivity for CD34, a specific marker of the endothelial phenotype), derived from the hepatic artery, which closely surrounds the

cholangiocytes and drains into venules joining the portal vein system. Serial sections of a liver sample with minimal histological changes (nearly normal liver). Original magnifications: $\times 400$ in A,B, $\times 1,000$ in C.

Fig. 5. Different expression of VEGF, VEGFR-1 and VEGFR-2 on normal bile ducts and reactive ductules. **A–D:** Normal bile ducts (identified by immunoreactivity for CK19, A) are negative for VEGF (B) and for its receptors VEGFR-1 (C) and VEGFR-2 (D); noteworthy, VEGFR-1 is expressed by endothelial cells lining both portal vein and hepatic artery branches (C). **E–H:** In contrast, in ductular reaction cholangiocytes (immunostained by CK19, E) show a strong immunoreactivity for VEGF (F), along with VEGFR-1 (G), and VEGFR-2 (H), which appear to be both up-regulated in disease conditions. Liver samples were obtained from a patient with minimal histological changes (A–D) and from a patient

with extrahepatic bile duct atresia as example of ductular reaction (E–H). Original magnifications: $\times 400$ in A–H.