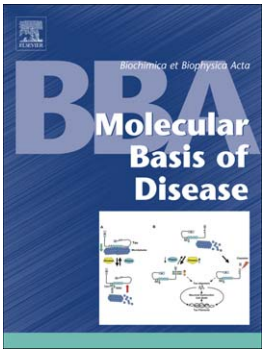

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Repopulating the Biliary Tree from the Peribiliary Glands

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Abstract

The larger ducts of the biliary tree contain numerous tubulo-alveolar adnexal glands that are lined with biliary epithelial cells and connected to the bile duct lumen via small glandular canals. Although these peribiliary glands (PBG) were already described in the 19th century, their exact function and role in the pathophysiology and development of cholangiopathies has not become evident until recently. While secretion of serous and mucinous components into the bile was long considered as the main function of PBG, recent studies have identified PBG as an important source for biliary epithelial cell proliferation and renewal. Activation, dilatation, and proliferation of PBG (or the lack thereof) has been associated with various cholagiopathies. Moreover, PBG have been identified as niches of multipotent stem/progenitor cells with endodermal lineage traits. This has sparked research interest in the role of PBG in the pathogenesis of various cholangiopathies as well as bile duct malignancies. Deeper understanding of the regenerative capacity of the PBG may contribute to the development of novel regenerative therapeutics for previously untreatable hepatobiliary diseases.

Key words:

Peribiliary glands, cholangiopathies, regeneration, bile duct, biliary epithelium, liver.

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1. Introduction

The biliary tree is composed of a three dimensional network of ducts that form a conduit that facilitates bile flow from the hepatocytes of the liver to the intestine. Intrahepatic bile ducts merge to form a common extrahepatic bile duct connecting the liver with the duodenum. The larger intrahepatic bile ducts (IHBDs) and the extrahepatic bile duct (EHBD) contain numerous tubulo-alveolar adnexal glands that are lined with biliary epithelial cells and connected to the bile duct lumen via small glandular canals. Although these peribiliary glands (PBG) were already described by anatomists in the 19th century, their exact function has been poorly studied and their role in the pathophysiology and development of cholangiopathies has long been ignored. However, during the past few years PBG have been a focus of increasing research as they have been identified as niche of multipotent stem/progenitor cells that contribute to the renewal of the luminal biliary epithelium, and play a critical role in the pathophysiology of various cholangiopathies affecting the larger bile ducts.

In this review, the current knowledge of the normal function of PBG, their role in restoration of biliary epithelial lining, and the development of pathologic conditions of the larger IHBDs and EHBD are discussed. To understand the role of the PBG more easily, a short overview of the anatomy of the biliary tree will be given first.

2. Anatomy of the Biliary Tree

2.1 The Biliary Tree and its Epithelial lining

Bile produced by hepatocytes inside the liver is collected in an extensive merging network of ducts through which bile is transported to the duodenum. This three dimensional structure is referred to as the biliary tree. The luminal surface of the biliary tree is lined by a single layer of biliary epithelial cells or cholangiocytes. The biliary tree not only provides a transportation conduit for bile, but also acts as a barrier to protect liver parenchyma and other surrounding structures against harmful endogenous and

exogenous biliary substances. In addition, the biliary epithelium performs absorptive and secretory processes to modify the composition, fluidity and flow of the bile (1,2).

From an embryological point of view, the biliary tree shares a common origin with the liver and ventral pancreas (3). All three originate from a single outpouching (hepatic diverticulum) of the embryological forgut that grows between the layers of the ventral mesentery into the septum transversum of the liver ridge. The latter forms the connective tissue part of the liver, but the hepatocytes and the epithelial cells of the bile ducts originate from the original diverticulum of the forgut. A hollow stalk which connects the developing liver, bile ducts and ventral pancreas to the gut forms later the common bile duct. The gallbladder and cystic duct develop as a secondary outpouching from the hepatic diverticulum. At a later gestational age, intramural glands begin to develop into the cystic duct and bile ducts. This common embryological origin is relevant to understand the presence of multipotent stem/progenitor cells with both hepatobiliary and pancreatic / endocrine lineage traits in both the IHBD and EHBD (4).

The biliary tree can grossly be divided into intrahepatic and extrahepatic bile ducts based on the anatomy and diameter of the ducts (**Figure 1**). The diameter of the ducts becomes progressively larger as bile ducts merge and the biliary tree descents towards the duodenum. The intrahepatic ducts can be subdivided into large and small intrahepatic ducts. The most proximal ends of the smallest branches of the IHBDs originate from the canals of Hering at the ductular-canalicular junction (1,5). The canaliculi proximal from the canals of Hering are small lumens of ~1μm diameter between hepatocytes. The canalicular side of hepatocytes is characterized by numerous, short microvilli that have an important function in the secretion of bile (2). The canals of Hering contain bipotent hepatobiliary stem/progenitor cells, which are perfectly located between hepatocytes and cholangiocytes, and have the potential to differentiate into both cell types. These progenitor cells are activated in pathological conditions that require renewal of the hepatocytes and/or cholangiocytes of the smaller intrahepatic ducts, while their role in normal cell turnover is still debated (6-10). The ductules that emerge from the

canuliculi lead to the interlobular bile ducts, which merge to form the septal ducts. Ductules, interlobular bile ducts, and septal ducts are referred to as small IHBDs. Large IHBDs are area ducts, segmental ducts, and the right and left hepatic duct (5). The proximal end of the extrahepatic common hepatic duct emerges from the liver as the left and right hepatic duct, that confluence together at the hepatic hilum. While the right hepatic duct generally has a relatively short extrahepatic course, the left hepatic duct follows a longer extrahepatic course caudal from segment IV of the liver parenchyma (**Figure 1**). The common hepatic duct conjoins with the cystic duct from the gallbladder and continues as the common bile duct, which eventually merges with the pancreatic duct before draining into the duodenum lumen via the Papilla of Vater. Hence, the EHBDs consist of the right and left hepatic duct, common hepatic duct, the cystic duct with the gallbladder, and the common bile duct. This division is important because the cholangiocytes of EHBD and large IHBD differ from those of the small IHBD in morphology and function (11-14). Moreover, the complex heterogeneity of different sized cholangiocytes results in different targets for cholangiopathies as will be discussed in paragraph 4.

Cholangiocytes lining the ductules are generally called small cholangiocytes and cells become progressively larger in size as they descent to form the interlobular ducts and septal ducts (1). Large cholangiocytes populate the large IHBD and EHBD. Cholangiocytes lining the canals of Hering and ductules are cuboidal in shape and the more ‘downstream’ the cholangiocytes are located the more of a columnar-shape they obtain (15-17). In addition, small cholangiocytes have a high nuclear/cytoplasmic ratio, whereas more distally, the larger cholangiocytes show more cytoplasm in relation to their nucleus (16). Besides differences in morphology, proteins/channels/membrane receptors are differentially expressed in small and large cholangiocytes (18,19). Large cholangiocytes are more responsive to hormones and constitutively express more membrane receptors and channels (20). In addition, the small cholangiocytes secrete water and electrolytes by Ca^{2}+- dependent mechanisms, whereas the large

cholangiocytes, which express CFTR and AE2, secrete biliary fluids by activation of a cAMP-dependent pathway (18,20-22). Large cAMP-dependent cholangiocytes are more susceptible for liver and bile duct injury as has been shown by *in vivo* models (23-25). Large, but not small ducts, respond with proliferation following bile duct ligation (BDL) (23). Furthermore, after CCL_4 administration to a mouse model, the normally quiescent small cholangiocytes acquired *de novo* proliferative and secretive capacity and replenished the damaged large cholangiocytes (24,25). This indicates that small cholangiocytes are able to compensate the loss of large cholangiocytes by differentiation into large cholangiocyte phenotypes. All together, the differences in morphology and function may imply that small cholangiocytes are primitive cells whereas the secretive and hormone responsive large cholangiocytes are more differentiated cells.

The apical side of cholangiocytes is covered with numerous microvilli, which increase the surface area of the epithelium and thereby enhance its absorptive and secretory function (26) (**Figure 2**). In addition, cholangiocytes contain in their apical membrane primary cilia that sense and transmit signals from the cell exterior into the cell interior. The length of the cilia varies along the bile duct; cilia of the large cholangiocytes are approximately 2 times longer than those in the small bile ducts (27). These cilia sense bile flow rates, osmolarity and molecular biliary components and, subsequently, provide activation of intracellular pathways. In this way, cell differentiation, proliferation, and secretion can be regulated (28). Cholangiocytes actively contribute to bile production and bile flow by secretion of substances (i.e bicarbonate, chloride and water) into bile (2). In addition, cholangiocytes are believed to modify bile composition by resorption of biliary components such as bile salts, a process known as the cholehepatic shunt(29).

At regular intervals small indentations or pits can identified in the luminal biliary epithelial lining of the larger IHBDs and EHBD (**Figure 2**). These pits represent the ostia of the PBG lining the bile ducts. PBG are present along both large IHBDs and EHBDs, including the cystic duct, but they are not found along the small intrahepatic interlobular ducts and ductules, or the gallbladder (1,30,31). The epithelial lining of the small canals

running through the bile duct wall and draining the PBG is in continuity with the luminal biliary epithelial lining of the bile ducts. The acini of PBG contain epithelial cells of various maturational stages and lineages, as will be discussed in more detail below.

2.2 Non-epithelial Components of the Bile Ducts

Below the single layer of epithelial cells covering the central lumen, the large IHBD and EHBD consist of a hypocellular fibrous band: the duct wall, which provides most of its thickness and tensile strength. The ductal wall contains few myofibroblasts, nerves, blood vessels, lymphatics, and the intramural PBG. The IHBDs are fixed in a loose, fibrous connective tissue of the portal tract, whereas EHBD have a similar fixation in the hepatoduodenal ligament. The periductal tissue, which encircles the duct wall, comprises of loose connective tissue with nerve bundles, lymphatics, blood vessels, isolated longitudinal and circular bundles of smooth muscle cells, and the extramural PBG (**Figure 3**). The amount of periductal tissue corresponds with the diameter of the bile ducts and the amount of smooth muscle cells increases as the bile duct approaches the Papilla of Vater (1).

2.3 Vasculature of the Bile Ducts

A network of fine vessels, called the peribiliary vascular plexus (PVP) nourishes the epithelium and bile duct wall of the EHBD and larger IHBD. Blood supply to the PVP is provided by small branches of the hepatic artery as well the gastroduodenal artery. Blood from the PVP ultimately drains into the sinusoids of the liver or small veins along the EHBD that drain into the portal vein (32-34). A more expanded and well-defined PVP is found surrounding the larger bile ducts, whereas a less visible vascular network encircles the smaller bile ducts (35). The PVP expands as cholangiocytes proliferate due to manipulation or stress of the bile duct. In this situation, cholangiocytes produce vascular endothelial growth factor (VEGF) to initiate accompanying PVP proliferation (36). In BDL experiments, proliferation of the PVP in response to VEGF production

occurred only around the large cholangiocytes (32,35). This supports the observation that small and large cholangiocytes respond differently to injury.

3. Peribiliary glands

3.1 Historical perspective

First descriptions of the PBG can be found in publications by Kiernan and the German anatomists Theise and Von Luschka, published halfway the 19th centrury (37-39). In his anatomy textbook published in 1862, Von Lushka reported “Drüsen” or glands with a grape vine-like structure in the bile duct wall (37-39). In those early years, mainly morphological descriptions of the PBG were presented and they were referred to as parietal sacculi, diverticula, or glands (of Luschka) (40). Opinions concerning their function varied, as some suggested that the glands serve merely as mucus secreting structures, while others suggested that they could retain and concentrate bile, acting as miniscule gall bladders (**Table 1**). In 1925, Burden published an extensive histologic description of the anatomy of the bile duct including the PBG which closely resembles modern observations and current insight in the structure of the PBG (41). Burden discriminated simple sacculi and tubes near the bile duct lumen and more numerous serous and mucous glands in the outer wall of the bile duct. One of the first papers that associated PBG with renewal of the luminal biliary epithelium was published by Hou in 1961 (42). In a study in guinea pigs, Hou showed that the bile duct epithelium completely regenerates within 3-14 days after mechanical or chemical injury by the migration of epithelial cells from proliferating glands (crypts). A few years later, these observations were confirmed by Cohen, who demonstrated that the PBG of rat bile ducts serve as centers of cell division and epithelial renewal (43). This author pointed at an analogy with the regenerative capacity of the crypts of intestinal epithelium, which is particularly interesting given the development of the bile ducts as an outpouching from the embryological forgut, giving the bile ducts and small intestine a shared embryological

origin. During the last two decades, the morphology and anatomy of PBG along the intrahepatic and extrahepatic bile ducts was further characterized by the meticulous work performed by Nakanuma and his co-workers (1,30,44,45). The exact function of the PBG, however, remained a subject of debate (**Table 1**). Until a few years ago, a mucinous and serous secretory function of the PBG was most prominently accepted in literature as the primary function of PBG, although sparse hints were made that PBG could be a source of stem/progenitor cells for biliary epithelial regeneration (30). The first study that demonstrated the presence of hepatobiliary stem/progenitor cells in the EHBD of mice was reported by Irie et al in 2007(46). These investigators, however, did not explicitly link the presence of stem/progenitor cells to the PBG. Presence of hepatobiliary stem cells in the PBG was subsequently suggested in clinical studies of human bile ducts(47,48). A first extensive description of the phenotype and proliferative potential of PBG stem/progenitor cells was provided by Cardinale et al (48). In addition to the *in vitro* proliferation and differentiation of EHBD-derived stem/progenitor cells into hepatocytes and cholangiocytes, these investigators also provided evidence for their potential to differentiate into C-peptide secreting neo islet-like cells (4,48-53). These publications have sparked further and more detailed research of the PBG as niches of multipotent stem/progenitor cells and identified the large IHBD and EHBD as attractive sources of and targets for regenerative medicine programs for disease of the liver, bile duct and pancreas, including diabetes. Moreover, the PBG have been receiving increasing attention in recent years for their putative role in the pathophysiology of various cholangiopathies as well as bile duct cancer, as will be discussed below.

3.2 Architecture of the PBG

The PBG are tubulo-alveolar glands composed of acini of serous and mucinous epithelial cells (cholangiocytes) of various maturational stages, as well as lower numbers (1-10%) of stem/progenitor cells that display variable endodermal lineage traits (48,51,54). Serous acini in the PBG outnumber the mucous ones. The glandular

epithelial cells are organized in circular or lobular structures that are supported by a hypocellular fibrous matrix that can be distinguished from the fibrous stroma and periductal connective tissue of the bile duct wall. PBG communicate with the bile duct lumen through their own small canals lined with cholangiocytes that are arranged in continuity with the luminal epithelial lining. PBG can be found along the entire large IHBD and EHBD. The highest density of PBG are seen at the branching points of the biliary tree with the greatest number of PBG at the hepato-pancreatic common duct and along the cystic duct (48,51,53,54). The gallbladder, however, does not contain PBG (31). In general, EHBD contain a higher density of PBG than IHBD (**Figure 1, 4**). The intrahepatic septal bile ducts show occasionally small PBG, whereas no PBG can be found in and around the interlobular ducts (1,51) (**Figure 1**).

PBG can be subdivided in intra- and extramural glands, defined by their exact location and distance of the PBG to the lumen. Intramural glands are located close to the luminal epithelium in the fibrous duct wall and have a direct connection with the lumen via short, small canals. They have also been called periluminal PBG (47). The extramural glands are located more distant from the bile duct lumen at the fibromuscular junction connecting the fibrous bile duct wall with the more loose connective tissue surrounding the bile ducts (**Figure 3**). The extramural PBG have also been called deep PBG (47) and they are connected with the central bile duct lumen via more tortuous canals running transverse through the bile duct wall. Apart from their connections with the bile duct lumen, some PBG, especially at the level of the common bile duct, display narrow tubular connections with neighboring PBG, resulting in channels that run parallel to the central bile duct lumen (30,31). In this way, they form a tubular network that interconnects different duct segments and provides a bypass along the bile duct lumen (**Figure 5**). In general, intramural glands are relatively simple tubular mucinous glands, whereas extramural glands have a more complex tubulo-alveolar structure with a mixture of serous and mucinous acini (1,31). From the luminal side of the bile duct, the ostia of the

PBG canals appear as ‘pits’ in the epithelial surface creating a link between the bile duct lumen and the base of the PBG (**Figure 2**).

The PBG are surrounded by small blood vessels that are part of the PVP and originate from hepatic artery branches (55,56). This fine peribiliary vascular network is particularly concentrated around the extramural glands (**Figure 3**). Apart from the delivery of oxygen and nutrients, the PVP may deliver blood-borne substances that act as stimuli for secretion or absorption by the PBG (30). In addition, the extramural glands are richly surrounded by nerves and ultrastructurally, glandular epithelial cells make contact with unmyelinated nerve fibres or axonal buttons through cytoplasmic processes that penetrate the basement membrane of the PBG (30). Although the anatomical relationship between PBG, vasculature and nerve system have been described in great detail, the exact role of blood-borne substances and nervous innervation in controlling exocrine and endocrine function, as well as proliferation, of PBG remains rather speculative and this is an area that deserves further research.

3.3 Secretory Function of the PBG

PBG of large IHBD and EHBD are assumed to participate in the bile composition-modifying function of the biliary tree through active secretion and absorption of biliary substances, such as water, sodium, bicarbonate and chloride (30). In addition, the glands contribute to the defense mechanisms of the bile ducts against toxic and detergent effects of biliary components by the production and secretion of mucinous glycoproteins. Moreover, serous acinar cells of the PBG have been shown to produce enzymes such as lipase, alpha1-antitrypsin, chymotrypsin, and stain positive for IgA, lactoferrin, lysozyme, and lectin, suggesting an active role in local and mucosal immunity of the biliary tree. To this end, PBG may contribute to the local sterility of the biliary tree. For a comprehensive review of the secretory function of PBG epithelial cells the reader is referred to Nakanuma et al. 1994.

3.4 Stem/progenitor Cells of the PBG

Although the PBG were already identified in the 1960's as a bile duct component from which cholangiocytes can proliferate and contribute to the renewal and repair of the luminal epithelial lining of EHBD and large IHBD (42,43), it was not until very recently that PBG were shown to contain multipotent stem cells and committed progenitor cells that can differentiate in more mature lineages with hepatocellular, cholangiocellular or pancreatic characteristics (47,48). While the majority of PBG cells are sero-mucinous epithelial cells, a small number of stem/progenitor cells are present in the PBG, depending on the location of the PBG and according to two axes (49). Firstly, a longitudinal or proximal-to-distal axis can be identified with a high density of primitive stem cells in PBG near the duodenum, while more committed progenitor cells are found closer to the liver and pancreas. Stem cell niches with phenotypic traits of pluripotency (NANOG, OCT4, and SOX2), self-replication (SALL4), and proliferation (Ki-67) are observed near the duodenum, while progressing to the pancreas expression of pancreatic/endoderm markers (SOX17, PDX1, LGR5) and pancreatic endocrine maturation markers (NGN3, MUC6, and insulin) have been identified (57,58). At the level of the intrapancreatic part of the distal common bile duct, the PBG are juxtaposed to the pancreatic duct glands, which line the pancreatic duct system and are considered to be an anatomical counterpart of the PBG (59,60). In the more proximal part of the EHBD, close to the liver hilum, PBG show expression of biliary/endodermal markers (SOX17, SOX9) and as the bile duct becomes intrahepatic, some PBG cells express albumin, indicative for hepatocellular maturation (51). Secondly, a radial axis can be identified with the highest number of cells with primitive stem cell characteristics in the extramural PBG located near the fibromuscular outer layer of the bile ducts and more committed progenitor cell types in the intramural PBG that are located closer to the central bile duct lumen. This differential expression of mature primitive and mature phenotypes according to the localization of the PBG in the bile duct wall has been interpreted as maturational lineage from the deepest PBG to the luminal bile duct epithelium. Differentiation of the

stem cells occurs along small canals connecting the PBG to the lumen of the bile duct. These canals provide migration of primitive cells at the base of the PBG that fully differentiate into mature cells, and thereby enhance repopulation of the biliary epithelium. It has been demonstrated that EpCAM can be used as a transit-lineage marker to identify intermediate cells between the deepest PBG cells and the lumen (51,54). Cells expressing mature markers (e.g. albumin, insulin, CK19, CK7, EA2) gradually appear in transition towards the surface epithelium.

Stem cells have been isolated from the IHBD and EHBD and proved to have the potential to expand *in vitro* and differentiate towards hepatocytes, cholangiocytes, and pancreatic endocrine cells (48). This indicates that stem/progenitor cells inside the PBG have the capability to differentiate towards several mature cells of the same embryologic endoderm origin.

Interestingly, repopulation of the biliary epithelium from the PBG resembles the natural turnover in the intestine, which also originates from the embryologic endoderm. In the intestine, the villi of the mucosa represent a differentiated cell compartment receiving maturing cells from the crypts that are considered to be a stem cell/progenitor compartment (61,62). Stem cells are mainly situated at the base of these crypts and respond to injury and natural mature cell loss with proliferation and differentiation. In parallel with the intestinal crypts, the most primitive PBG cells are located at the base PBG, while transit-amplifying cells populate the intermediate compartment, and mature cells are continuous with the epithelium (62). Similar to the crypts in the intestine, PBG are stimulated to proliferate and repair the epithelium in pathological conditions of luminal epithelial injury or loss (63-65). Another similarity between stem/progenitor cells in the PBG and the intestinal crypts is expression of the intestinal stem cell marker Lgr5 (52,62). Lgr5+ cells have recently been found in intrahepatic stem cells at the level of canals of Hering as well as in the PBG of the EHBD (51,62). These Lgr5+ progenitor cells have been able to produce large numbers of hepatocytes and cholangiocytes *in vitro* when stimulated with the right culture medium and growth factors (61,66). Despite

these similarities, there is also an important difference between bile ducts and intestine: epithelium of the intestine is renewed every 4-5 days, driven by the proliferation of the stem cells in the crypts(67), yet turnover of the epithelium of the large bile ducts is believed to occur at a much lower rate. Therefore, it remains unclear whether PBG derived stem/progenitor cells are involved in the regular renewal of the bile duct epithelium or only in pathological conditions with significant biliary epithelial cell injury and loss.

4. The Role of PBG in Cholangiopathies

PBG cells (both mature epithelial cells and stem/progenitor types) have been identified to contribute to the development of certain pathological conditions of the bile ducts, collectively known as cholangiopathies. PBG are involved in several cholangiopathies of the larger bile ducts whereas diseases that selectively target the small bile ducts (such as primary biliary cholangitis) entail different pathological mechanisms. In some cholangiopathies again a similar response in the PBG compartment occurs as is observed in the intestinal crypts in disease of the intestine. Examples are swelling of and increased mucus secretion in response to irritation and inflammation (i.e. alcoholic cirrhosis, hepatolithiasis and bacterial cholangitis), cellular proliferation in response to substantial injury of the mature epithelial lining (i.e. post-ischemia and post-transplantation), and the development of malignancies from these stem/progenitor cell compartments (i.e. subtypes of intrahepatic and hilar cholangiocarcinoma).

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4.1 Cholangitis and Hepatolithiasis

In patients with cholecystitis and / or bacterial cholangitis marked histological changes of the PBG are observed in addition to the inflammatory infiltrates. Based on necropsy studies, Burden reported already in 1925 a marked proliferation and cystic

dilatation of intramural glands that are filled with mucus (41). The author concluded that inflammation and irritation of the bile duct and its glands is followed by overproduction of mucus. In a more recent study, immunohistochemical detection of cell proliferation (Ki-67) demonstrated that mild bacterial cholangitis is associated with proliferation of mature cholangiocytes aligning the lumen, while additional proliferation of the PBG cells is seen in cases of severe cholangitis (47). Marked proliferation and dilation of intra- and extramural PBG is also frequently found in patients with hepatolithiasis (68). Considerable amounts of proliferating and dilated glands are positive for sialated and sulfated acids and/or neutral mucins, which has been interpreted as increased secretion of large amounts of mucins into the biliary lumen (30). Differences in the expression patterns of endodermal stem/progenitor cell markers in PBG (i.e. increased expression of endodermal S/P cell markers) have been noted in hepatic resection specimens of patients with hepatolithiasis, compared to healthy control livers (obtained at autopsy), supporting the involvement of PBG in this type of cholangiopathy (65). Moreover, multiple proliferating and dilated PBG in the hepatic hilum have been described in patients with alcoholic cirrhosis (69).

Although the exact triggers and signaling pathways that lead to proliferation and hypersecretion of the PBG are largely unknown, it seems plausible that chronic irritation due to chemical substances, bacterial infection, or inflammatory infiltrates is involved.

4.2 Primary Sclerosing Cholangitis

Primary sclerosing cholangitis (PSC) is chronic inflammatory disease of the EHBD and large IHBDs, characterized by progressive injury and obliterative concentric periductal fibrosis, leading to biliary strictures and ultimately progression to end-stage liver disease. Terasaki et al. were the first to describe the involvement of PBG in PSC in 1997. These investigators observed marked proliferation of the PBG in association with lymphoplasmacytic infiltration and fibrosis (70,71). More recently, Carpino et al. provided evidence for activation of the PBG stem/progenitors cells in patients with PSC, which

was suggested to contribute to biliary fibrosis and is accompanied by an enhanced expression of Hedgehog pathway components (64). The investigators hypothesized a 'PSC sequence' in which inflammatory cells lead to PBG hyperplasia, which in turn stimulates myofibroblast activation, with bile duct wall fibrosis as a consequence. Although this study suggests a key role for PBG in the progression of bile duct fibrosis in PSC, it needs to be determined whether the chronic activation of PBG stem/progenitors cells contributes to the high risk of cholangiocarcinoma associated with PSC (72).

4.3 Post-ischemic and Post-transplant Cholangiopathy

Biliary complications are a major cause of morbidity and graft failure after liver transplantation. Non-anastomotic biliary strictures (NAS; also known as ischemic-type biliary lesions or ITBL) are the most frequent type of biliary complication, with a reported incidence varying between 1% and 30% (73,74). It is generally agreed that ischemia and bile salt toxicity are major risk factors for the development of bile duct epithelial injury during transplantation, which subsequently leads to periductal fibrosis and narrowing (75). Biliary epithelial cells are known to be very sensitive to ischemia and relatively short periods of ischemia result in a rapid and prolonged depletion of the intracellular adenosine triphosphate (76). As a consequence, biliary epithelial cells lose their intercellular connections and detach from the basement membrane, resulting in sloughing of the epithelial lining and denudation of the bile duct luminal surface. Three independent clinical studies have recently shown that major epithelial cell loss of the EHBD can be observed in more than 80% of all donors livers at the time of transplantation. Yet, NAS only develops in a minority of these patients, suggesting that inadequate proliferation and regeneration of the bile duct epithelium, rather than the degree of initial injury, are important determinants in the pathogenesis of post-transplant NAS. Immunohistochemical analysis of EHBD of patients that underwent re-transplantation of the liver because of severe NAS indeed revealed active proliferation of epithelial cells in the PBG of the affected bile ducts (47). Moreover, ischemic injury of the

extramural PBG and PVP detected in the EHBD of donor livers at the time of transplantation is strongly associated with the development of NAS after transplantation (63). These findings suggest that insufficient regeneration due to loss of PBG or impaired blood supply may explain the development of NAS.

Warm ischemia as occurs in donation after circulatory death (DCD) donors has been identified as a critical risk factor for the development of NAS after DCD liver transplantation. The incidence of NAS after transplantation of livers from adult DCD donors is around 3-fold higher, compared to transplantation of livers from brain dead donors, who do not suffer warm ischemia at the time of organ procurement. Interestingly, transplantation of livers from pediatric DCD donors does not result in a higher incidence of NAS after transplantation, compared to transplantation of livers from pediatric brain dead donors (77). The relatively low incidence of NAS after transplantation of pediatric DCD livers could be explained by an age dependent difference in the regenerative capacity of the biliary epithelium. In this context, it is interesting that the highest density of PBG per unit surface area of the bile duct wall has been found in young children. During childhood, the density of PBG decreases with the increasing diameter of the bile duct wall, reaching a plateau in adults (78).

Collectively, these observations suggest that PBG are critically involved in the proliferation and regeneration of the biliary epithelium of donor bile ducts after transplantation. Adequate preservation of the PBG has, therefore, become an important target in the development of better preservation methods of donor livers, such as machine perfusion (79). Indeed, preliminary evidence suggests that machine perfusion of donors liver provides better protection and preservation of the bile ducts of donor livers, including the PBG and PVP (80,81).

4.4 Cholangiocarcinoma

Cholanciocarcinoma is a malignancy arising from epithelial cells of the biliary tree. Recent studies have suggested that biliary malignancies may not only develop from the

mature luminal epithelia lining, but also from PBG cells, including their(82) stem/progenitor cells (82). Interestingly, all above described cholangiopathies that are associated with marked proliferation of the PBG (i.e. PSC, hepatolithiasis) are well-known risk factors for the development of biliary malignancies. Moreover, bile duct cancer occurs most frequently in parts of the biliary tree that have a high density of PBG, such as the cystic duct, the hepatic hilum and the periampular region (58,82). These findings support a putative role for PBG and their endoderm-derived stem/progenitor cells in the pathogenesis of bile duct cancer. Although the number of publications on the presumed role of stem/progenitor cells is rapidly increasing, formal evidence that stem/progenitor cells from the PBG directly contribute to development of cholangiocarcinoma in humans is still lacking (83).

5. Conclusion and Future Directions

In the past decades great progress has been made in understanding the physiology and pathophysiology of the biliary tree. Although the presence of periductal tubulo-alveolar glands that are connected with the lumen of large IHBD and the EHBD via small canals was already noted in the 19th century, the exact function of these glands remained largely unknown until more recently. While PBG were initially believed to have only a secretory function adding serous and mucinous component to the bile, more recent studies have identified PBG as an important source for biliary epithelial cell proliferation and renewal. Although the exact stimulatory signaling pathways are still unknown, increased sero-mucinous secretion by and dilatation of PBG has been noted in various cholangiopathies, such as bacterial cholangitis and bile stone diseases. It is, however, still not fully determined whether this is a secondary, protective response of PBG to inflammation and biliary injury, or that this indicates an active contribution of PBG to the pathogenesis of these cholangiopathies.

Apart from the secretory function, there is substantial evidence that proliferation of PBG epithelial cells is a critical component of biliary epithelial regeneration and repair after massive injury of the luminal epithelium. Moreover, PBG have recently been identified as niches of multipotent stem/progenitor cells with endodermal lineage traits. This has sparked research interest in the role of PBG in the pathogenesis of various cholangiopathies as well as bile duct malignancies. Stem cells have been isolated from the IHBD and EHBD and proved to have the potential to expand *in vitro* and differentiate towards hepatocellular, cholangiocellular, and pancreatic cell types. Together with the rapid development of new technologies, the emerging knowledge of the endogenous mechanisms of biliary regeneration and the existence of multipotent endodermal stem/progenitor cells in the PBG of large intrahepatic and extrahepatic bile ducts provides a strong driving force in the field of regenerative medicine (49,84). Better understanding of the signaling pathways that determine proliferation and differentiation of both mature and stem/progenitor cells of the PBG is needed to develop novel therapies that modulate or stimulate the endogenous regenerative responses in hepatobiliary diseases. In addition, it seems a matter of time until the rapidly emerging field of regenerative medicine will provide effective new treatment options using exogenous cells or organoids to combat diseases of the liver, biliary tree and pancreas.

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Legends to the Figures

Figure 1. Schematic overview and nomenclature of the intra-and extrahepatic ducts of the biliary tree. The blue dots represent appearance of the extramural peribiliary glands, whereas the red dots represent intramural peribiliary glands. As shown in the figure, intra- and extramural peribiliary glands are present along the extrahepatic bile duct, large intrahepatic bile ducts, and septal ducts. The density of the peribiliary glands is high around bifurcations and sparse around the septal ducts. The septal ducts are classified as small intrahepatic ducts yet their biliary epithelium resembles that of the large intrahepatic bile ducts.

Figure 2. **Scanning electron microscopy of the lumimal biliary epithelium of the extrahepatic bile duct.** Biliary epithelial cells (cholangiocytes) have numerous microvilli at their apical membrane. In between cholangiocytes small ‘pits’ are evident. These pits (white arrows) represent the ostia of the draining canals of peribiliary glands that are nested in the wall of large intrahepatic and extrahepatic bile ducts.

Figure 3. Histological section of human extrahepatic bile duct wall with peribiliary galnds. Panel A: Histological section of bile duct wall and epithelium. Intramural and extramural peribiliary glands are indicated. Glands are connected with the central luminal epithelium via small tubules. Panel B: Schematic reconstruction of the histological cross section presented in Panel A.

Figure 4. Schematic overviews of sides with the highest density of peribiliary glands along the intrahepatic and extrahepatic bile ducts. The highest density of peribiliary glands (PBG; blue dots) is found at the bifurcations of the biliary tree. A proximal-to-distal axis can be identified with a high density of primitive stem cells in PBG near the duodenum, while more committed progenitor cells are found closer to the liver

and pancreas. Stem cell niches with phenotypic traits of, self-replication, and proliferation are observed near the duodenum, while progressing to the pancreas expression of pancreatic/endoderm markers and pancreatic endocrine maturation markers have been identified. Towards the liver hilum, PBG show more expression of biliary/endodermal markers and as the bile duct becomes intrahepatic, some PBG cells express albumin, indicative for hepatocellular maturation. Another niche of bipotent hepatobiliary stem/progenitor cells has been identified intrahepatic at the level of the canals of Hering (red dots).

Figure 5. Schematic overview of the bile duct with adjacent peribiliary glands. Simple intramural and more complex tubulo-alveolar extramural PBG are shown, the latter of which may form complex anastomoses between adjacent glands, providing a bypass system parallel to the central bile duct lumen (indicated with an asterix). To discriminate the intramural PBG from the extramural ones, mural stroma is only partially drawn.

Table 1. Historical overview of putative functions of peribiliary glands

Putative functions of peribiliary glands*
- Production and secretion of mucus to protect the biliary epithelium - Production and secretion of digestive enzymes - Production and secretions of substances that contribute to local immunity of the biliary tract - Productions and secretion of endocrine and paracrine molecules - Small expansion tubes to relieve intraluminal pressure in case of obstruction of the biliary tract - Resorption of bile components - Bypass to obstructed or stenotic parts of the intra- and extrahepatic bile ducts - Site of biliary epithelial regeneration - Niche of multipotent stem/progenitor cells

*) Most of the suggested functions are based on histological observations and some functions have been subsequently disproved. For example, the suggestion that peribiliary glands could be small ‘gallbladders’ that reabsorb and retain bile is revised by the observation that generally no bile is present in these glands.

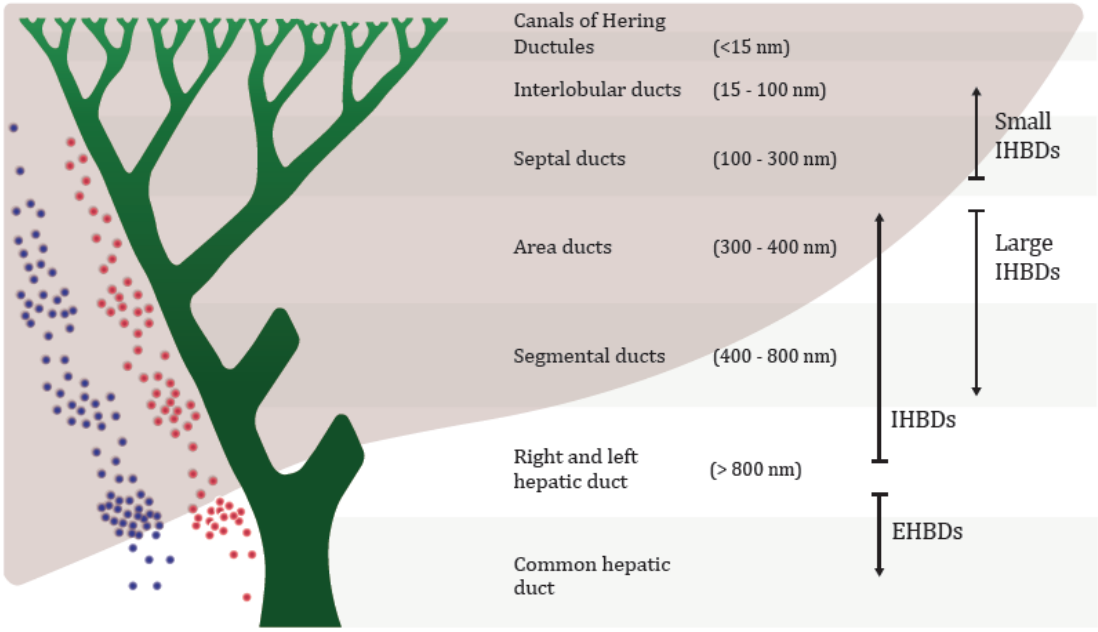

Figure 1

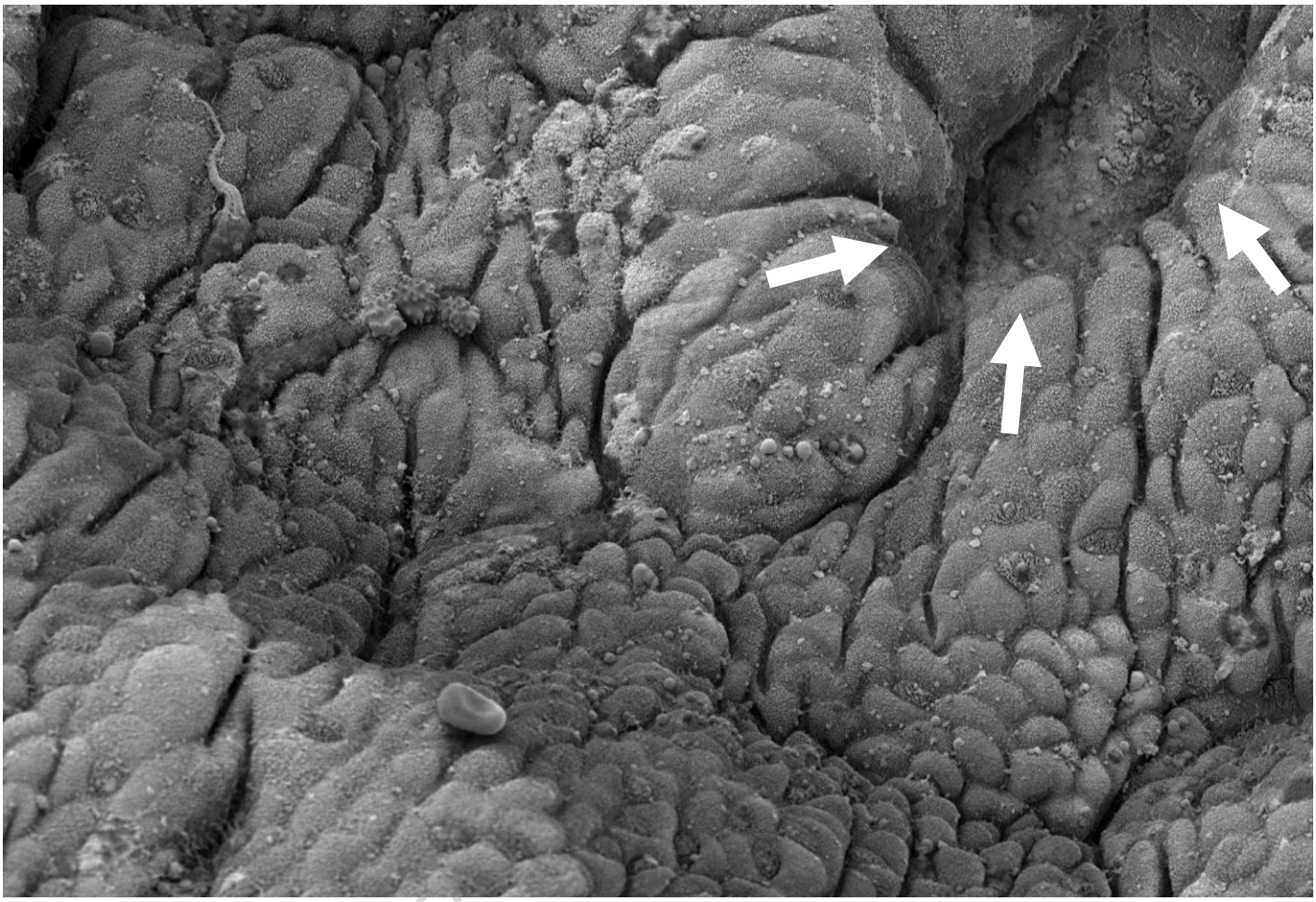

Figure 2

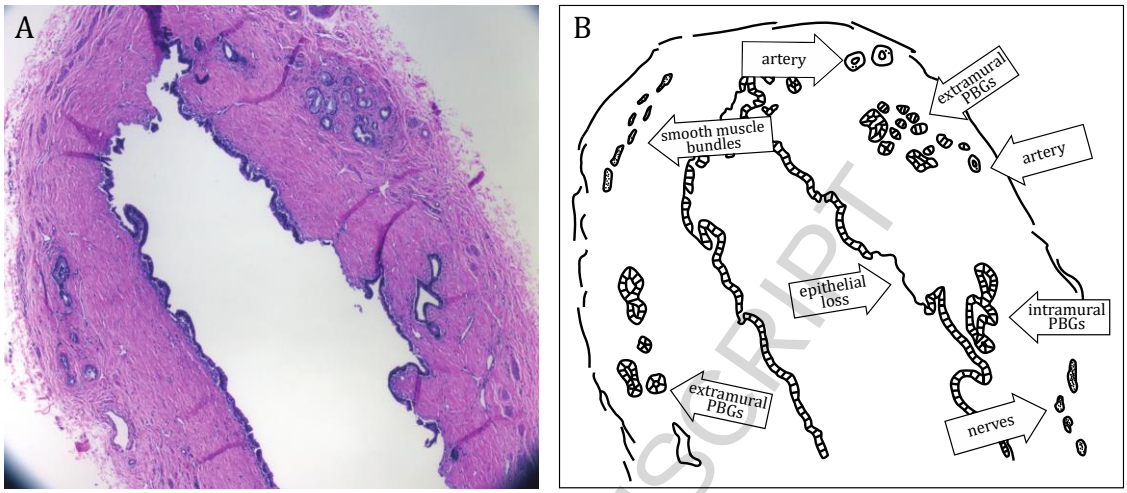

Figure 3

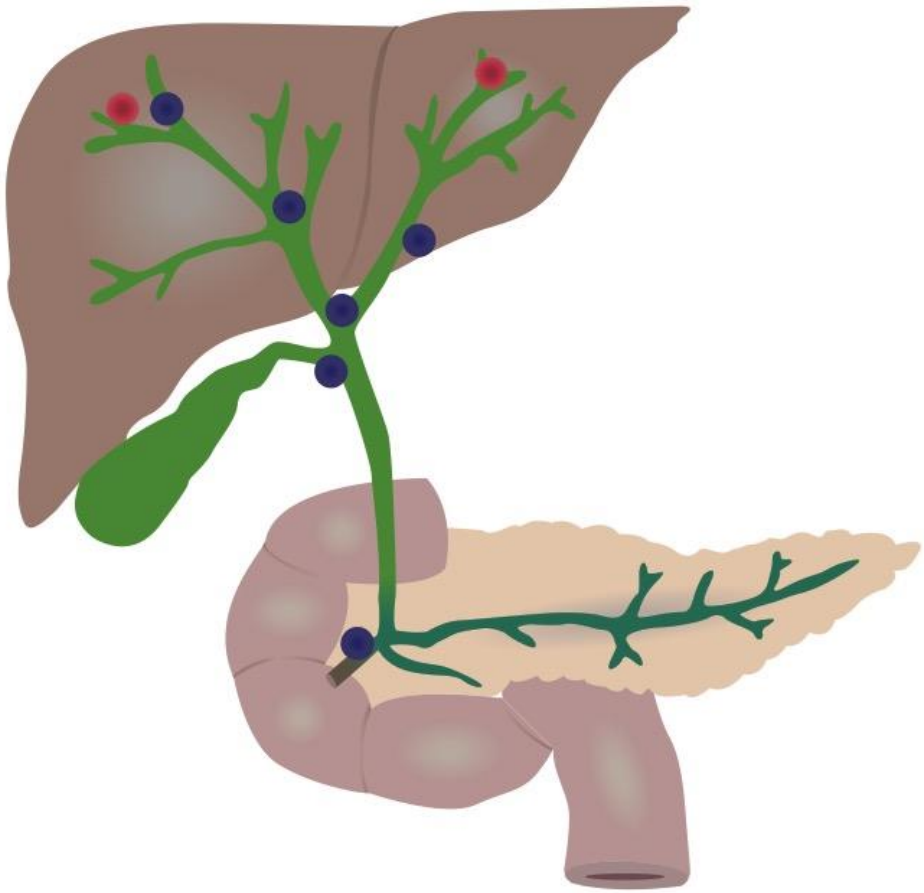

Figure 4

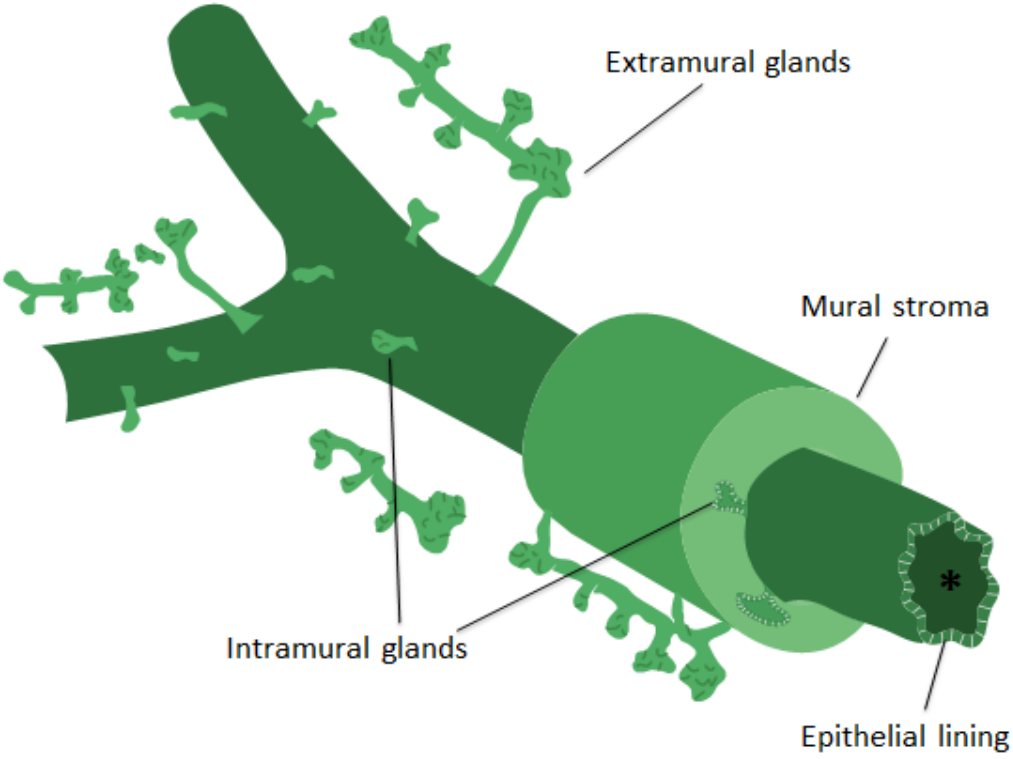

Figure 5

Highlights

- Peribiliary glands (PBG) are reservoirs of multipotent stem cell/progenitor cells in the wall of large intrahepatic and extrahepatic bile ducts
- PBG have been described since the 19th century, but extensive characterisation with regard to their function and specific morphology has only recently been achieved.
- PBG are activated upon damage of the biliary epithelial lining (cholangiocytes) of the larger bile ducts.
- After proliferation and maturation of cholangiocytes derived from the PBG, they migrate via small canals connecting the PBG with the luminal epithelium.