BBA - Molecular Basis of Disease xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

Review

Pathobiology of biliary epithelia☆

Angela C. Cheung, Maria J. Lorenzo Pisarello, Nicholas F. LaRusso*

Division of Gastroenterology and Hepatology, Mayo Clinic Center for Cell Signaling in Gastroenterology, Mayo Clinic, Rochester, MN, United States

ARTICLE INFO

Keywords:
Apoptosis
Bile
Cholangiocytes
Cholangiopathies
Proliferation
Senescence

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.

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 lifethreatening (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 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

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, microribonucleic 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

☆ 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.

* Corresponding author at: Mayo Clinic, 200 First Street SW, Rochester, MN 55905, United States.
E-mail address: larusso.nicholas@mayo.edu (N.F. LaRusso).

http://dx.doi.org/10.1016/j.bbadis.2017.06.024
Received 11 May 2017; Received in revised form 22 June 2017; Accepted 26 June 2017

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.

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