Digestive and Liver Disease 42 (2010) 245–252

Contents lists available at ScienceDirect

Digestive and Liver Disease

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

Mini-Symposium

Recent advances in the regulation of cholangiocyte proliferation and function during extrahepatic cholestasis

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ARTICLE INFO

Article history:
Received 8 January 2010
Accepted 8 January 2010
Available online 13 February 2010

Keywords:
Bile duct ligation
Cholangiocyte
Cholestatic liver diseases
Extrahepatic cholestasis
Proliferation

ABSTRACT

Bile duct epithelial cells (i.e., cholangiocytes), which line the intrahepatic biliary epithelium, are the target cells in a number of human cholestatic liver diseases (termed cholangiopathies). Cholangiocyte proliferation and death is present in virtually all human cholangiopathies. A number of recent studies have provided insights into the key mechanisms that regulate the proliferation and function of cholangiocytes during the pathogenesis of cholestatic liver diseases. In our review, we have summarised the most important of these recent studies over the past 3 years with a focus on those performed in the animal model of extrahepatic bile duct ligation. In the first part of the review, we provide relevant background on the biliary ductal system. We then proceed with a general discussion of the factors regulating biliary proliferation performed in the cholestatic animal model of bile duct ligation. Further characterisation of the factors that regulate cholangiocyte proliferation and function will help in elucidating the mechanisms regulating the pathogenesis of biliary tract diseases in humans and in devising new treatment approaches for these devastating diseases.

1. Introduction

The liver, the largest internal organ of the body, is composed of two types of epithelial cells: (i) hepatocytes; and (ii) cholangiocytes [1]. Cholangiocytes line the intrahepatic and extrahepatic bile duct system of the liver [1]. The bile ductules and ducts comprise a branched system of interconnected tubes [1–3], which collect bile secreted at the canalicular membranes of hepatocytes [4], and deliver it to the gallbladder or the duodenum [1,5]. Although cholangiocytes represent a small proportion (3 to 5%) of the cells of the liver [1,5,6], these cells play an important pathophysiological role in the modification of the composition of bile during the transit in the bile ducts, which involves the secretion and absorption of water, electrolytes and other organic solutes from hepatocellular bile [1,5–10]. The modification of bile by cholangiocytes is regulated by a number of gastrointestinal hormones, which has been recently reviewed [11,12]. The regulation of cholangiocyte bicarbonate secretion is regulated by the gastrointestinal hormone secretin [5,12]. Cholangiocytes are the only cell types in the liver that express the secretin receptor (SR) [13], which is of importance to the function of the biliary epithelium in normal and pathological conditions [5,8,14–18]. In large (but not small) cholangiocytes secretin stimulates increases in intracellular cyclic adenosine 3′,5′-monophosphate (cAMP) levels [14,16,19,20] and induces the opening of the Cl^- channel (cystic fibrosis transmembrane conductance regulator, CFTR) [15], which leads to the activation of the Cl^-/HCO_3^- anion exchanger 2 (AE2) [21] and secretion of bicarbonate in bile [5].

Cholangiocytes are the target cells of a number of diseases termed cholangiopathies. This disease class is made up of inherited disorders [Alagille syndrome and cystic fibrosis (CF)], autoim-

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doi:10.1016/j.dld.2010.01.008

mune disorders [primary sclerosing cholangitis (PSC), primary biliary cirrhosis (PBC), autoimmune cholangitis (AIC), allograft rejection, graft-versus-host disease (GVHD)], infections (cholangitis due to bacteria, fungi, parasites or viruses), drug-induced injury, ischaemic injury and diseases of unknown aetiology (biliary atresia and idiopathic vanishing bile duct syndromes) [22]. Cholangiopathies are predominantly characterised by a bile duct-directed inflammatory response that leads to bile duct injury associated with biliary proliferation in the early stage of the disease course [22]. If the biliary injury is chronic there will be increased bile duct loss, biliary fibrosis and the increased incidence of bile duct cancer (i.e., cholangiocarcinoma) [22].

2. Anatomical and morphological features of the biliary tree

The intrahepatic biliary epithelium is divided into extrahepatic and intrahepatic bile ducts [2,3,23,24]. The intrahepatic bile ductal system consists of the portion of (i) bile canaliculi, small spaces localised between two adjacent hepatocytes (0.5–2 mm) forming a three-dimensional network that continues in (ii) bile ductules (canals of Hering), localised at the periphery of the hepatic lobule and characterised by 3–4 cholangiocytes, that form the junction between hepatocytes and cholangiocytes (ductular–canalicular junction) allowing the confluence of the bile in (iii) bile ducts (interlobular bile ducts) localised in the portal space. Interlobular bile ducts progressively continue in larger ducts until the right and left hepatic ducts, that, at the level of the hylus, determine the origin of extrahepatic biliary tree [25–27]. According to Ludwig, the human intrahepatic biliary epithelium is divided into small bile ductules (<15 μm), interlobular ducts (15–10 μm), septal ducts (100–300 μm), segmental ducts (400–800 μm) and hepatic ducts (>800 μm) [24]. The rodent intrahepatic bile duct system has been recently classified into small ducts (<15 μm in external diameter) lined by small cholangiocytes (approximately 8 μm in diameter characterised by high nucleus/cytoplasm ratio) and large bile ducts (>15 μm in diameter characterised by low nucleus/cytoplasm ratio) lined by large cholangiocytes (approximately 15 μm in diameter) [2,16,19,25,28]. These studies have also shown that a significant relationship exists between cholangiocyte area and external bile duct diameter, with small bile ducts lined by small cholangiocytes and large ducts lined by large cholangiocytes [2,16,19]. The latter finding is particularly relevant since it allows for the direct mapping of studies obtained in isolated small and large cholangiocytes to different portions (i.e., small and large) of the intrahepatic biliary epithelium *ex situ* [2,14–19]. In support of the morphological heterogeneity of the biliary epithelium, Masyuk et al. have reconstructed the intrahepatic biliary system that resembles a tree, with the common and hepatic ducts corresponding to the trunk, the intrahepatic bile ducts corresponding to the large branches and the small ductules corresponding to the smallest tree limbs of the tree [29].

3. Cholangiocyte proliferation in response to bile duct ligation

A number of studies have defined three types of cholangiocyte proliferation: “typical”, “atypical” and oval cell proliferation [30]. “Typical” cholangiocyte proliferation is a hyperplastic reaction, which induces an increase in the number of intrahepatic bile ducts (with a well-defined lumen) confined to portal areas [5,31]. “Atypical” cholangiocyte proliferation is commonly seen in patients with prolonged cholestatic liver diseases such as PBC or PSC and is characterised by irregular proliferation of intrahepatic bile ducts sprouting into periportal and parenchymal regions and occasionally forming anastomosing cords with adjacent hepatocytes [32,33]. Oval cell proliferation takes place in the early stages of chemically induced hepatocarcinogenesis and is characterised by a disorganised proliferation of biliary structures with a poorly defined lumen [34]. Oval cell proliferation is not discussed in this review.

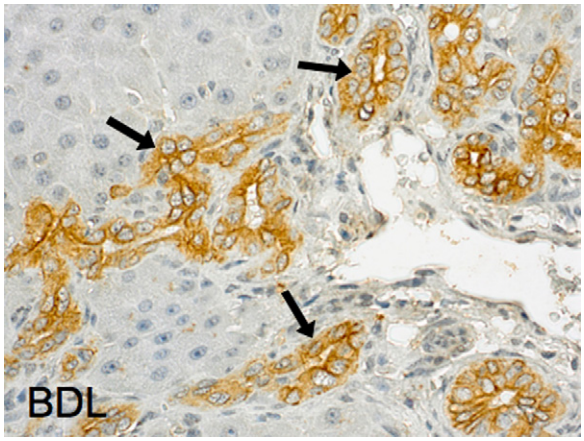

Fig. 1. Immunolocalisation of cytokeratin-19 (CK-19) in proliferating cholangiocytes in the bile duct ligation (BDL) animal model. Original magnification 40×.

In animal models, “typical” cholangiocyte proliferation is achieved by a number of experimental manoeuvres, including BDL (Fig. 1) [5], partial hepatectomy [35], acute carbon tetrachloride (CCl_4) treatment [17,18] and chronic feeding of α-naphthylisothiocyanate (ANIT) [36], or bile salts [37]. In these hyperplastic models, cholangiocyte proliferation is closely associated with increased SR gene expression and secretin-stimulated cAMP levels [5,13,17,18,35–38]. cAMP, which is generated by adenylyl cyclases (AC), plays an important role in the modulation of cholangiocyte function [35,39–42]. A recent study by Strazzabosco et al. demonstrates that differential expression of AC isoforms mediate the secretory functions of small and large cholangiocytes [41]. This study demonstrated that large cholangiocyte responsiveness to secretin was mediated by the expression of AC8 [41]. A number of animal models that mimic cholestatic liver diseases and liver injury have been utilised to expand our knowledge related to the mechanisms of cholangiocyte proliferation [1,12,43]. Of these models of bile duct injury, the BDL model has been the most commonly used. A number of coordinate factors (stimulatory or inhibitory) have been shown to regulate cholangiocyte growth in the cholestatic BDL model. It has been shown that increased biliary pressure is a trigger for the stimulation or inhibition of these putative growth factors [44,45]. A recent study has shown [45] that increased biliary and portal hypertension (induced by the first), represent key proliferative triggers for the growth of bile ducts and hepatocytes. Similar to findings in human cholangiopathies (e.g., PBC and PSC), recent studies in rats have demonstrated that “typical” cholangiocyte proliferation occurs within a limited range of duct sizes [16,18,46]. In rats with BDL, enhanced cholangiocyte proliferative capacity is restricted to large bile ducts [18,46]. In an experimental animal model of bile duct damage, CCl_4 induces loss of large ducts and loss of large duct secretion [17,18,47]. To compensate for the loss of duct function due to this toxin [17,18], small cholangiocytes proliferate and develop *de novo* secretory activity due to *de novo* expression of SR [17,18]. A hallmark of large cholangiocyte proliferation induced by BDL in rats is the increased SR expression and subsequent secretory activity [18,46]. A recent study in mice with BDL demonstrated that similar to rats, the mouse intrahepatic biliary epithelium is morphologically and functionally heterogeneous [16]. These findings are of importance due