Development of the Human Intrahepatic Biliary System

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Abstract

In the development of the human biliary system, the extrahepatic bile ducts (EHBD) develop from the embryonic hepatic diverticulum, while the intrahepatic bile ducts (IHBD) originate within the liver from the ductal plate. The ductal plate is a flat muralium of primitive biliary epithelium that develops in the mesenchyme along the branches of theportal vein, by a process which requires a delicate balance between cell proliferation and death. The ductalplate is thus remodelled into the adult system of tubular anastomosing bile ducts and this process is called ductal plate remodelling.

Computerised three-dimensional reconstruction of the developing ductalplate has shown that the ductalplate remodellingprocess starts at theporta hepatis around 11 weeks of gestation andprogresses towards the periphery of the liver. The extrahepatic biliary system is in direct luminal continuity with the developing intrahepatic biliary system throughout gestation and does not have a "solid stage" as suggested previously.

The ductalplate remodelling is controlled by many biochemical and molecular factors, some of which have been identified and studied. It has been suggested that abnormalities in the development of the IHBD could lead to a spectrum of diseases called ductal plate malformation. Biliary atresia is one of the conditions in this spectrum. Currently, we are studying the IHBD in biliary atresia in comparison to the normal developing IHBD, the results of which are presented in this review. Both morphologically and biochemically the IHBD in biliary atresia resembles the primitive foetal ductal plate suggesting a disruption in ductal plate remodelling.

Ann Acad Med Singapore 1999; **28:105-8**

Key words: Bile ducts, Development, Ductal plate, Emb ryology, Liver

Introduction

The development of the human biliary system has been studied extensively. This knowledge is essential to the understanding of the pathogenesis of a spectrum of diseases termed "ductal plate malformation" (DPM). DPM was first coined by Desmet[1] to encompass a spectrum of congenital conditions like biliary atresia, paucity of intrahepatic ducts, autosomal recessive polycystic disease, congenital hepatic fibrosis and others. In a very recent review he subscribed to the view that almost all categories of congenital diseases of the intrahepatic bile ducts (IHBD) result from failure of the ductal plate to mature.[2]

This review addresses the current state of knowledge about the morphological, biochemical and biological aspects in the development of the human biliary system.

Morphology

In the development of the human biliary system, the extrahepatic and intrahepatic systems differ in their origin. The extrahepatic bile ducts (EHBD) and the liver arise from the embryonic hepatic diverticulum which is a ventral projection from the developing foregut.[3,4] The authors have recently used computer generated three-dimensional (3-D) reconstructions to study the development of the human biliary system.[5] Figure 1 shows a 3-D view of the EHBD. We did not find the presence of any "solid stage" in the development of the EHBD in any of our specimens (5.5 to 16 weeks gestation). This confirms earlier reports that the EHBD has a continuous and patent lumen through its entire length during its development.[6]

The IHBD that link the bile canaliculi and the EHBD, arise from the ductal plate, a sheath of primitive biliary epithelium that develops in the mesenchyme along the portal vein branches (Fig. 2). Starting from about 11 weeks of gestation the ductal plate at the porta hepatis starts to undergo remodelling. In this process discontinuous luminal spaces appear in the ductal plate which initially lies close to the liver edge. The ductal plate gets separated from the liver edge by proliferating mesenchymal cells. Certain portions of the ductal plate are

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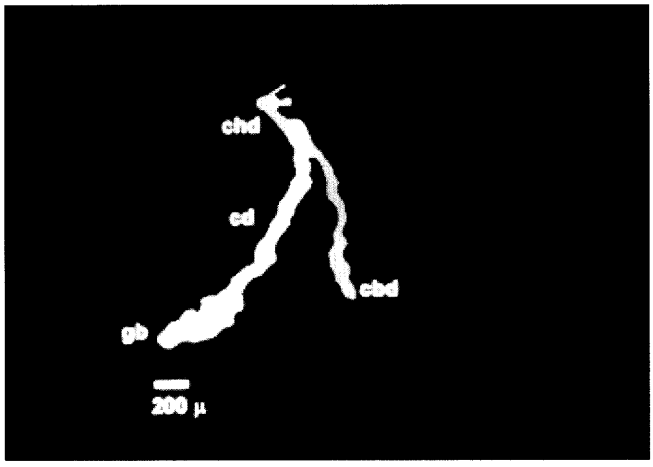

Fig 1. 3-D reconstruction of the EHBD of a 11 week foetus.
cbd: common bile duct; cd. cystic duct, chd common hepatic duct; gb: gall bladder.

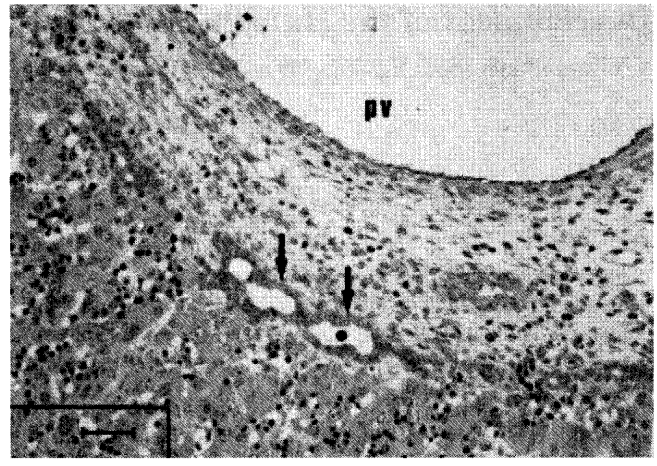

Fig 2. Photomicrograph of a transverse section through the ductal plate in a 11 week foetus. Ductal plate structures (arrow) are seen in the mesenchyme surrounding a portal vein branch (pv) Discontinuous luminal spaces (*) are seen in the ductal plate Bar= 50µ.

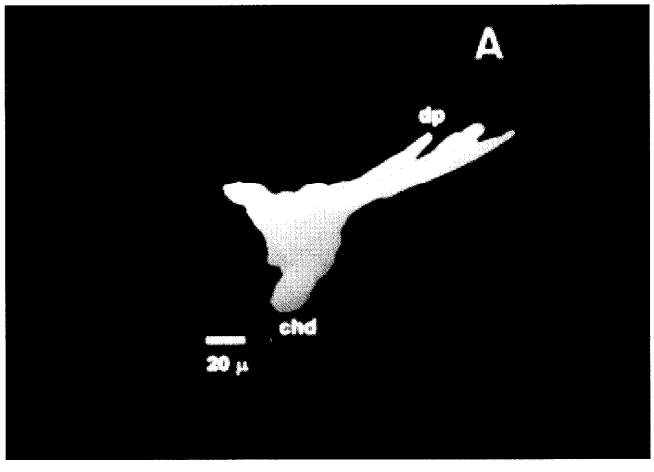

Fig 3A

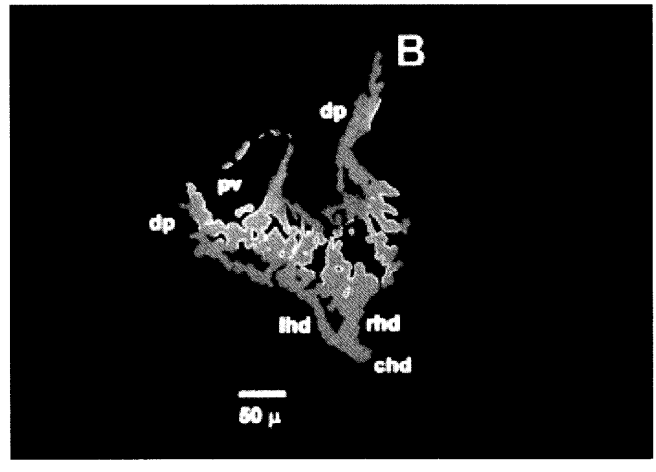

Fig. 3B.

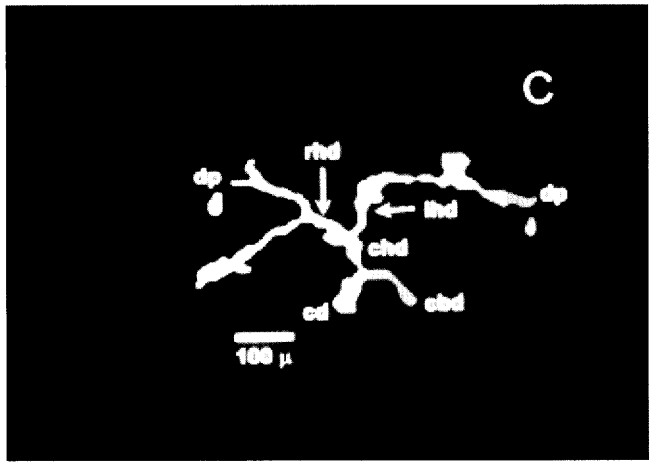

Fig. 3C.

Fig. 3. 3-D reconstruction at the porta hepatis of a 7-week (A), 11-week (B) and 13-week old (C) foetus
cbd: common bile duct; cd: cystic duct; chd: common hepatic duct; dp: ductal plate; gb: gall bladder; lhd: left hepatic duct; rhd: right hepatic duct, pv: portal vein.

remodelled into mature tubular bile ducts while the remaining redundant portions are removed. This orderly process of selection and deletion is called ductal plate remodelling.[5,6]

The ductal plate remodelling process starts at the porta hepatis where it is said to occur between 11 and 13 weeks of gestation, and progresses towards the periphery of the liver.[5-7] The porta hepatis therefore is, the crucial region where the EHBD merges into the IHBD. According to some reports remodelling along the more distal portal vein branches may even occur after birth.[8,9] Figure 3 shows the biliary structures at the porta hepatis as gestation progresses. At 7 weeks of gestation (Fig. 3A), early ductal plate structures with luminal spaces were seen at the pbrta hepatis. In three-dimension they were seen as finger-like projections continuous with the common hepatic duct at the porta hepatis. The 11-week-old foetus (Fig. 3B) showed the presence of abundant ductal plate structures in close proximity to the portal vein branches. Right and left hepatic ducts could clearly be seen to be continuous proximally with the ductal

plate structures along the branches of the portal vein. As gestation progressed, the ductal plate attained the tubular configuration of an adult and in the 13-week-old foetus (Fig. 3C) the left and right hepatic ducts and their branches could be seen. In all ages of gestation the developing ductal plate was in luminal continuity with the common hepatic duct at the porta hepatis.

We have applied this technique of computer generated 3-D reconstruction to a liver sample from a biliary atresia patient. At the porta hepatis, the biliary structures strongly resembled the primitive foetal ductal plate (unpublished data). There was no evidence of tubular left and right hepatic ducts or interlobular ducts as is expected at the porta hepatis. The biliary structures surrounded and hugged the portal vein branch exactly like the ductal plate of early foetuses.

Biochemistry/Cell Biology

Cytokeratin (CK) profile of the developing biliary system has been studied extensively. Human adult hepatocytes express CK 8 and 18, while adult IHBD express CK 7, 8, 18 and 19. In embryos, ductal plate cells and primitive hepatocytes express CK 8, 18 and 19. In older foetuses, the hepatocytes express only CK 8 and 18, while the ductal plate continues to express CK 8, 18 and 19. After 20 weeks of gestation, the ductal plate expresses CK 7. The differing CK profile of the hepatocytes and ductal plate cells during foetal life helps to identify the cells immunohistologically.[10] Changes in hepatocyte cytokeratin expression have been shown to occur with intrahepatic cholestasis, and these changes reflect the severity and duration of the cholestasis.[11]

A search of existing literature yields many reports on the factors involved in the ductal plate remodelling process. Growth factors like TGFα and TGFβ1 are reported to be involved in the development of the IHBD.[12,13] The mesenchyme plays a major role in the morphogenesis of epithelial structures by not only providing the necessary mechanical support and shape but also by providing molecular and biochemical signals that are essential to the developing epithelial structures. Laminin and type IV collagen are components of basement membrane and are closely associated with the development of the IHBD. Tenascin, a glycoprotein that plays a role in cell migration and epithelial-mesenchymal interactions, is expressed around the developing IHBD cells.[14] Proteolytic enzymes known as matrix proteinases are known to play a role by degrading extracellular matrix proteins and facilitating cell migration.'" Matrix metalloproteinases are expressed in the ductal plate cells as they migrate into the mesenchyme of the portal tract. Mucins apomucins, glyconjugates residues are also expressed in the developing ductal plate cells and probably play a part in the remodelling.[10]

In an earlier report, we showed that the immunolocalisation pattern of TGFβ1 within the ductal plate epithelial cells changed as gestation progressed.'* The duct al plate epithelium from the very early stages showed positive TGFβ1 immunoreactivity. The intensity and distribution of the peptide within the biliary epithelium was similar to that of the surrounding hepatocytes. As the ductal plate was remodelled into tubular bile ducts, the immunoreactivity was enhanced and polarised towards the apical portion of the epithelial cell. Using computerised image analysis techniques, we have shown that the apical polarisation in the tubular bile ducts was significantly different from the ductal plate epithelium. In biliary atresia, it was seen that significant apical polarisation did not occur, and image analysis confirmed the striking similarity between the distribution of this peptide within the biliary epithelial cells in biliary atresia and the primitive ductal plate in the normal foetuses.

Terada and Nakanuma[16] found that ductal plate remodelling involved a balance between cell proliferation and death and suggested that deregulation of cell death or apoptosis may lead to DPM.[10] Apoptosis or programmed cell death is as much a part of organogenesis as cell proliferation and differentiation. Certain viruses can inhibit apoptosis, while chemical or physical injuries to the cell can induce apoptosis. Thus insults due to viruses, drugs, chemical or physical injuries during organogenesis can interfere with the process of apoptosis and tip the delicate balance between cell proliferation and death leading to developmental malformations.[17] In our more recent work we therefore studied the cell proliferation (using anti-PCNA antibody in tissue sections) and death (using TUNEL in tissue sections) pattern during ductal plate remodelling and compared it to biliary atresia. We found that the mesenchymal cell proliferationincreased during the remodelling, and continued to remain high even after the remodelling was completed, while hepatocyte proliferation remained constant through the remodelling. We also found that the frequency of apoptosis was high during remodelling. This shows that ductal plate remodelling was achieved by delicately balanced cell proliferation and death. However proliferation and apoptosis of both mesenchymal cells and hepatocytes were lower in the paediatric controls livers than the foetal livers, showing that in the postnatal period these cells have a slow turnover. In our study we compared two paediatric conditions characterised by obstructive jaundice and ductular proliferation, namely biliary atresia and choledochal cyst with bile flow obstruction. We found a marked difference in the frequency of apoptosis between the two conditions. In the liver of choledochal cyst patients, apoptosis among the biliary cells, mesenchymal cells, and hepatocytes was very high, while in biliary atresia, it was conspicuously low (Fig. 4). Other

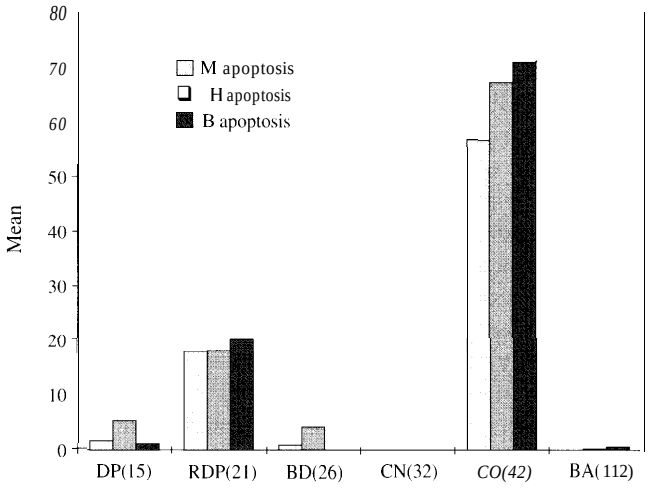

Fig. 4. Graph shows the frequency of apoptosis among the mesenchymal cells (M apoptosis), hepatocytes (H apoptosis) and biliary epithelial cells (B apoptosis), in the different categories studied. Biliary structures in the foetal period were classified into ductal plate (DP), remodelling ductal plate (RDP) and mature bile ducts (BD). Biliary structures in the paediatric livers were classified according to the diagnosis, namely: choledochal cyst without bile flow obstruction (CN), choledochal cyst with bile flow obstruction (CO) and biliary atresia (BA).

conditions of cholestasis have also been reported to have an increased frequency of apoptosis.[18,19] This could be the result of an apoptotic response to cholestatic injury to the cells, as it has been shown that toxic bile salts induce apoptosis in primary hepatocyte culture.[20] It has also been suggested that apoptosis is a common pathway of cell death in liver disease.[21] Inbiliary atresia, the apoptotic response of all the three cell types resembled the ductal plate before the onset of remodelling, suggesting a diminution in the apoptotic process, in spite of increased mesenchymal cell and to a smaller extent hepatocyte proliferation. There is evidence to suggest that TGFβ1 induces apoptosis in liver disease,[18] and our research has shown that in biliary atresia, both apoptosis and TGFβ1 immunolocalisation resemble the primitive ductal plate. This could mean the abnormal biliary structures seen in biliary atresia are primitive ductal plate structures that failed to undergo remodelling effectively.

Future research in this field should be aimed at unravelling the molecular and biochemical events involved in the development of the IHBD, and to apply this knowledge to understand the pathogenesis of the spectrum of disease called DPM. The culmination of the research will lie in finding a way to anticipate and intercept the onset of DPM.

Acknowledgements

The authors would like to thank Dr Peter Mack and the staff of the Experimental Surgery Laboratory for the use of their facilities. The authors also would like to thank the Department of Clinical Research, Ministry of Health, Singapore and the National Medical Research Council for funding this project.

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