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Normal and Abnormal Development of the Human Intrahepatic Biliary System: A Review

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TERADA, T., KITAMURA, Y. and NAKANUMA, Y. *Normal and Abnormal Development of the Human Intrahepatic Biliary System: A Review.* Tohoku J. Exp. Med., 1997, **181** (1), 19-32 —— Morphology and immunohistochemical features of the developmental process of the human intrahepatic biliary system (IBS) are reviewed. Human IBS arises from the ductal plate, a double-layered cylindrical structure located at the interface between portal mesenchyme and primitive hepatocytes. The ductal plate first appears from primitive hepatocytes (hepatoblasts) around 8 gestational weeks (GW), and its formation proceeds from the hepatic hilum to the periphery. The ductal plate gradually undergoes remodeling from 12 GW; some parts of the ductal plate disappear and other parts migrate into the portal mesenchyme. Around 20 GW, the migrated duct cells transform into immature bile ducts and peribiliary glands. Some immature peribiliary glands transform into pancreatic acinar cells around postnatal 3 months. The immature biliary elements express cytokeratins no. 7, 8, 18 and 19. Several growth factors (TGF-α, HGF) and their receptors (EGFR, MET, ERBB2) were expressed in the primitive IBS cells. Some extracellular matrix proteins including type IV collagen, laminin and tenascin are expressed in the mesenchyme around the primitive IBS. During IBS remodeling, apoptosis and cell proliferation occur with appropriate expression of apoptosis-related proteins (bcl-2, Fas, c-myc, Lewis^y). Some pancreatic digestive enzymes (α-amylase, trypsinogen, lipase), cathepsin B, and matrix metalloproteinases (MMP-1, 2, 3, 9) and their inhibitors (TIMP-1, 2) are expressed in the remodeling IBS cells. Glycoconjugate residues of glycoproteins gradually appear during IBS development. The appropriate expression of these immunophenotypes may play an important role in the normal development of IBS. —— intrahepatic bile duct; ductal plate; remodeling; apoptosis

The normal developmental process of the human intrahepatic biliary system (IBS) has been studied extensively. Knowledge of this process is important for

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the assessment of IBS malformations, including congenital biliary atresia, congenital hepatic fibrosis, Caroli's disease, polycystic disease of the liver and kidneys, and von Meyenburg complex. In this review, the authors describe the present extent of knowledge of the normal development of human IBS with reference to congenital malformations of the IBS.

During the fourth gestational week (GW), the human liver arises as a bud of cells (the hepatic diverticulum) from the foregut. The hepatic diverticulum comprises two parts: a pars cranialis and a pars caudalis. The pars cranialis gives rise to liver precursor cells that grow into the mesenchyme of the septum transversum. The pars caudalis develops into the extrahepatic bile duct and gall bladder. The liver precursor cells grow between small capillaries in the septum transversum. Several theories have been advanced concerning the development of the IBS. One theory maintains that the IBS is derived from ingrowth of the larger hilar duct structures (Hammar 1926). A second theory postulates that the entire IBS is derived from hepatocyte precursor cells, which transform into ductules and ducts to join the extrahepatic biliary bud at the hilum (Desmet 1985, 1991). A third theory combines elements of the first two theories. A fourth theory maintains that the IBS is derived from liver 'stem' cells. Most authors favor the second hypothesis (Desmet 1992a, b), and this theory has been strengthened by routine light microscopic and ultrastructural observations. In addition, recent immunohistochemical studies have reinvestigated the development of the IBS; all support the "hepatocytic" origin of the IBS. These studies made use of immunohistochemical stains for cytokeratins, tissue polypeptide antigen, carcinoembryonic antigen, epithelial membrane antigen and other markers for parenchymal and bile duct cells (Burt et al. 1987; Van Eyken et al. 1988; Shah and Gerber 1989; Desmet et al. 1990; Ruebner et al. 1990; Stosiek et al. 1990). The following review of IBS development is based mainly on the authors' own observations using morphology, histochemistry, lectin-histochemistry and immunohistochemistry.

Morphology

At 6 GW, no biliary elements are present in the liver (Fig. 1A). At 8 GW, a double-layered cylindrical structure (the ductal plate) consisting of flattened cuboidal epithelia appears at the interface between portal mesenchyme and primitive hepatocytes (ductal plate stage) (Fig. 1B). This ductal plate seems to arise from primitive hepatocytes (hepatoblasts). At 12 GW, the ductal plate gradually undergoes remodeling (remodeling stage); some cells of the ductal plate disappear, while other cells migrate into portal mesenchyme (Fig. 1C). At 20 GW, the migrated ductal cells transform into immature bile ducts and into peribiliary glands at the hilum (remodeled stage) (Fig. 1D). The immature bile ducts and peribiliary glands become mature at the end of gestation and early postnatal stage. Some immature peribiliary glands transform into pancreatic

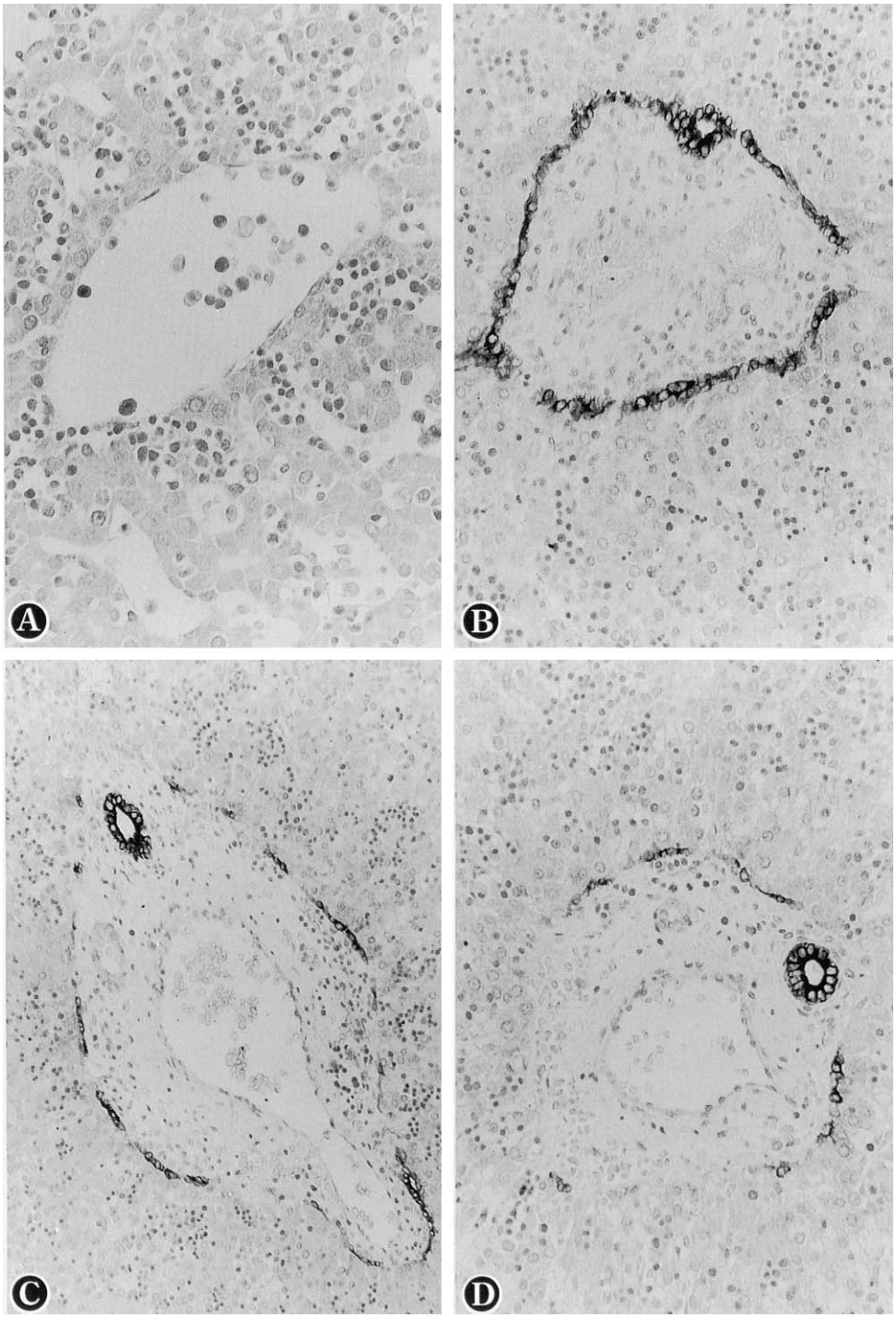

Fig. 1. Normal developmental profile of the intrahepatic biliary system. A: At 6 gestational weeks (GW), no biliary elements are present around the future portal vein. Immunostian for cytokeratin (CK) 19 using AE1 antibodies, ×200. B: At 8 GW, the ductal plate appears at the interface between primitive hepatocytes and portal mesenchyme. The ductal plate is positive for CK 19. Immunostain for CK 19 using AE1 antibody, ×150 C: At 12 GW, the ductal plate shows remodeling. Some parts of the ductal plate disappear, whereas other parts are incorporated into or migrate into the mesenchyme. Immunostain for CK 19 using AE1 antibody, ×130 D: At 20 GW, migrating ductal plate cells transform into immature bile ducts in the portal tract. Some remnants of the ductal plate are still present. Immunostain for CK 19 using AE1 antibody, ×120.

acinar cells positive for pancreatic digestive enzymes around postnatal three months (Terada and Nakanuma 1993a), and about 4% of adult human liver contains tiny clusters of pancreactic acinar cells (Terada et al. 1990). The process of this IBS development proceeds from the hepatic hilum to the hepatic periphery. A schematic diagram of IBS development is depicted in Fig. 2.

Cytokeratin profile

Human adult hepatocytes express cytokeratin (CK) no. 8 and 18, while human adult IBS cells express CK 7, 8, 18, and 19 (Van Eyken et al. 1990). In embryonic liver before 10 GW, ductal plate cells and primitive hepatocytes express CK 8, 18 and 19. The ductal plate cells continue to express CK 8, 18 and 19 during the development, while primitive hepatocytes stop expressing CK19 after 10 GW and persist to express only CK 8 and 18 during the rest of the development. After 20 GW, expression of CK 7 appears in the primitive bile duct cells. The primitive intrahepatic peribiliary glands, which are seromucous tubuloalveolar glands present around the large intrahepatic bile ducts near the hilum (Terada et al. 1987; Terada and Nakanuma 1988a), also show CK profiles similar to the primitive intrahepatic bile duct cells (Terada and Nakanuma 1993a). The expression of CK 19 is a useful tool for identifying primitive IBS cells, since the primitive hepatocytes do not express this antigen after 10 GW. Other markers for IBS cells are tissue polypeptide antigen, epithelial membrane antigen and carcinoembryonic antigen.

Growth factors

Growth factors play a pivotal role in liver regeneration and development, acting either as potent mitogenic or as mito-inhibitory regulators (Fausto and Mead 1989; Michalopoulos 1990). During IBS development, transforming growth factor α and its receptor (epidermal growth factor receptor) are expressed in the primitive IBS cells (Terada et al. 1994b). Epidermal growth factor is not expressed in the developing IBS cells. Faint expression of hepatocyte growth factor/scatter factor is present in primitive IBS cells in the early stage of human

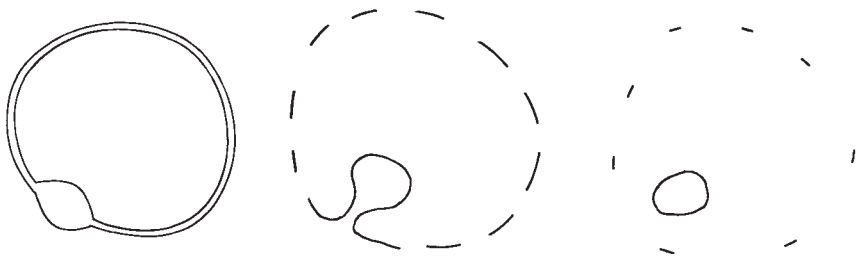

Fig. 2. Schematic representation of the embryonic ductal plate, its remodeling stage and its remodeled stage (immature bile duct). For further explanation, see text and Fig. 1.

IBS development (Terada et al. unpublished data). MET protein, a receptor of hepatocyte growth factor, is expressed in developing IBS cells before 30 GW, and ERBB2 protein, a product of the c-erb-B-2 protooncogene and a receptor for an unidentified growth factor, is expressed in primitive IBS cells before 20 GW (Terada et al unpublished data). Transforming growth factor $\beta 1$ is also expressed in primitive IBS cells (Tan et al. 1995). These growth factors and their receptors may play an important role in the cell proliferation and morphogenesis of the IBS.

Epithelial-mesenchymal interactions

The extracellular matrix has a central role, not only in structural support, but in cell proliferation, migration and differentiation (Madri and Basson 1992). During the development of human IBS cells, primitive IBS cells are closely associated with the portal mesenchyme, leading some authors to postulate that epithelial-mesenchymal interactions may play an important role in the morphogenesis of IBS (Desmet 1992a; Shah and Gerber 1990; Terada and Nakanuma 1994b). For example, laminin, a component of basement membrane, is closely associated with the developing IBS cells (Shah and Gerber 1990; Terada and Nakanuma 1994b). In addition, type IV collagen, a component of basement membrane, is located at the interface between primitive IBS cells and portal mesenchyme (Terada and Nakanuma 1994b). Tenascin is a hexameric multidomain glycoprotein, and it plays a role in cell migration and epithelial mesenchymal interactions (Chiquet-Ehrismann et al. 1986). During IBS development, tenascin is expressed around the developing IBS cells. It seems likely that laminin, type IV collagen and tenascin play an important role in the normal morphogenesis of IBS through epithelial-mesenchymal interactions.

Apoptosis and its related proteins

Apoptosis (programmed cell death) has been reported to play an important role in fetal organogenesis (Hurie 1988). Apoptosis is mediated by several proteins both inhibitory and stimulatory. Fas antigen (Watanabe-Fukunaga et al. 1992), c-myc protein (Evan et al. 1992), p53 protein (Shaw et al. 1992) are stimulators of apoptosis, whereas bcl-2 protein (Vaux et al. 1988) is an inhibitor. Lewis^y antigen is expressed in damaged and apoptotic cells (Hiraishi et al. 1993). During human IBS development, apoptosis detected by an in situ nick end labeling method (Gavrieli et al. 1992) is found in ductal plate cells and remodeling and remodeled biliary cells (Terada and Nakanuma 1995a). The proliferative activity of IBS cells, as determined by proliferating cell nuclear antigen immunohistochemistry, is also present in the developing IBS cells. Fas antigen, c-myc protein and Lewis^y antigen are consistently positive in the primitive IBS cells. Bcl-2 protein is negative in the early stage of IBS development, but positive in the late stage. The expression of p53 protein is absent throughout IBS development.

These findings show that balanced cell proliferation and apoptosis are involved in the normal development of the IBS, and suggest that c-myc protein, fas antigen, Lewis^y antigens and bcl-2 protein modulate apoptosis of fetal IBS cells, probably by stimulative (c-myc protein and fas and Lewis^y antigens) or inhibitory (bcl-2 protein) effects.

Pancreatic digestive enzymes

Our recent studies have shown that intrahepatic large bile ducts, septal bile ducts and peribiliary glands express α-amylase isozymes, trypsinogen and pancreatic lipase in normal livers and in various hepatobiliary diseases (Terada and Nakanuma 1991, 1993c, d, e; Terada et al. 1992, 1994a). Normal human extrahepatic peribiliary glands also express these pancreatic digestive enzymes (Terada et al. 1993). During IBS development, pancreatic α-amylase but not trypsinogen or pancreatic lipase is expressed in the ductal plate near the hilum (Terada and Nakanuma 1995b). These pancreatic digestive enzymes are expressed in migrating biliary cells, in immature large bile ducts cells in fetal livers, and in immature and mature large bile duct cells in the postnatal liver. Their expression is weak and diffusely cytoplasmic in fetal livers, whereas in postnatal livers their expression is strong, granular and located in the supranuclear cytoplasm. Expression is not found in developing pheripheral, small bile duct cells. These enzymes are also expressed in primitive hepatocytes during 9-25 GW, but disappear thereafter. These results show that primitive large bile ducts and primitive hepatocytes at the early fetal stage express pancreatic digestive enzymes, and suggest that intrahepatic large bile ducts, hepatocytes and the pancreas may have a common cell lineage.

Matrix proteinases

Proteolytic enzymes, collectively known as matrix proteinases, are known to play an important role in the cell migration of cancer cells (Liotta et al. 1991) and during organ development (Reponen et al. 1994) by degrading extracellular matrix proteins. Matrix proteinases are classified into matrix metalloproteinases (MMPs), serine proteinase, cysteine proteinases, and aspartic proteinases (Liotta et al. 1991). In cancer or developing tissues, there are inhibitors of these matrix proteinases, such as tissue inhibitors of MMP (TIMP) (Liotta et al. 1991). Primitive IBS cells migrate into or are incorporated into portal mesenchyme from the ductal plate in the remodeling stage of IBS development; however, the molecular mechanism of this migration or incorporation is unclear. The authors have demonstrated that MMP-1 is expressed in the ductal plate and migrating primitive biliary cells (Terada et al. 1995). MMP-2, MMP-3 and MMP-9 are expressed in the ductal plate. TIMP-1 and TIMP-2 are expressed in the ductal plate and migrating biliary cells.

Chymotrypsinogen/chymotrypsin and cathepsin B. which are matrix

proteinases and activators of MMPs, are also expressed in the primitive biliary cells. These data suggest that MMP, chymotrypsinogen/chymotrypsin and cathepsin B play a critical role in biliary cell migration during human IBS development by degrading extracellular matrix proteins, and that MMP inhibitors (TIMP-1 and TIMP-2) and MMP activators (chymotrypsin and cathepsin B) play an important role in biliary cell migration. The coordinated expression of MMP, MMP inhibitors and MMP activators may be necessary for the normal development of human IBS.

Mucin and MUC apomucins

Adult intrahepatic large bile ducts, septal bile ducts and peribiliary glands contain mucins (neutral, carboxylated or sulfated) under normal conditions (Terada et al. 1987) as well as in proliferative conditions (Terada and Nakanuma 1988a, 1992). During fetal IBS development, no mucins detectable by conventional mucin stains are present in primitive bile ducts and peribiliary glands. Neutral, carboxylated and sulfated mucins first appear in the large bile ducts and peribiliary glands at postnatal three months (Terada and Nakanuma 1993a). The mucins gradually increase with maturation of the postnatal IBS.

Mucin is a high molecular weight glycoprotein consisting of core proteins (apomucins) and O-linked carbohydrate side chains. Genes for apomucins have recently been identified, cloned and characterized, and at present it is known that there are at least seven genes (MUC 1–7) for the apomucins (Bobek et al. 1993; Toribara et al. 1993). During human IBS development and maturation, ductal plate and primitive biliary cells express MUC 1 apomucin at their luminal border (Sasaki et al. 1995). In contrast, in the postnatal liver, the biliary cells of intrahepatic large bile ducts do not express MUC 1 apomucin but do express MUC 3 apomucin, whereas those of small bile ducts do not. MUC 2 and MUC 5/6 apomucins are absent in the IBS cells of the fetal as well as postnatal livers. These data suggest that the biliary epithelial cells switch MUC 1 apomucin expression before birth to that of MUC 3 after birth.

Glycoconjugate residues

Carbohydrate side chains of glycoproteins, glycolipid and proteoglycans play an important role in adhesion and recognition among cells and between cells and the extracellular matrix (Lowe et al. 1990). It has been increasingly evident that the composition of the carbohydrate chains changes during fetal development, necroinflammation and malignant transformation (Newgreen et al. 1990; Sell 1990). During human IBS development and maturation, ABH blood group antigens are negative in the ductal plate and migrating biliary cells but are positive in immature bile ducts in the fetal liver and in mature bile ducts in the postnatal liver (Terada and Naknanuma 1994a). Lewis^a, sialyl Lewis^a and Lewis^b antigens are negative in the ductal plate, but positive in migrating biliary

cells, and immature and mature bile ducts. Sialyl Lewis^x antigen is positive in the ductal plate, migrating biliary cells and immature bile ducts in the fetal liver, but is negative in the postnatal liver. Lewis^y antigen is negative in the ductal plate and migrating biliary cells, but positive in immature bile ducts in the fetal liver and in mature bile ducts in the postnatal liver. T and Tn antigens are negative in the ductal plate, migrating biliary cells and immature bile ducts, but positive in mature bile ducts. Lewis^x, sialyl Tn and carcinoembryonic antigen are negative throughout the development and maturation. Receptors of *Ulex europaeus* agglutinin I and soy bean agglutinin are absent in the ductal plate, migrating biliary cells and immature bile ducts, but present in mature bile ducts. Receptors of *Concanavalin* A and *Ricknus communis* agglutinin I are negative in the ductal plate, but positive in migrating biliary cells, immature bile ducts and mature bile ducts. The receptor of succinylated wheat germ agglutinin is positive throughout the development and maturation. These data suggest that carbohydrate chain structures change during the development of the IBS, and that new carbohydrate chain structures gradually emerge as the IBS develops and matures.

Development of peribiliary capillary plexus

The IBS is supplied and nourished by a network of capillaries called "peribiliary capillary plexus" (PBP) (Terada et al. 1989a, b; Kono and Nakanuma 1992), and the anatomical relationship between IBS and PBP is important in maintaining the normal functions of the IBS. During the development and maturation of the IBS, no vasculatures are found around the ductal plate, but progenitor vascular cells positive for factor VIII-related antigen, *Ulex europaeus* agglutinin I and succinylated wheat germ agglutinin appear in the mesenchyme of the portal tracts. In the stage of migrating biliary cells (remodeling stage), the progenitor vascular cells transform into capillaries positive for factor VIII-related antigen, *Ulex europaeus* agglutinin I and succinylated wheat germ agglutinin. In the stage of bile duct formation (remodeled stage), capillaries begin to surround the bile ducts and peribiliary glands. The capillaries form premature PBP around 35 GW in large bile ducts, composed of inner and outer layers, and around 6 weeks after birth the capillaries form premature PBP in small bile ducts, consisting of scattered capillaries without layer formation. The premature PBP continues to proliferate postnatally and reaches an adult and mature state at around 15 years. These data suggest that endothelial cells of capillaries of PBP derive from mesenchymal angioblasts at the earliest stage of IBS development, and that the development and maturation of PBP progress parallel to those of the IBS (Terada and Nakamura 1993b).

Ductal plate malformations

Desmet (1992a) postulated that congenital diseases of the IBS can be explained by the failure of the IBS to develop normally and proposed the term

"ductal plate malformations". The "ductal plate malformations" (DPM) include congenital biliary atresia, paucity of IBS, autosomal recessive polycystic disease, congenital hepatic fibrosis, Caroli's disease, von Meyenburg complex, autosomal dominant polycystic disease, and mesenchymal hamartoma. He speculated that these congenital IBS diseases, reviewed below, are attributable to an abnormal development of the IBS, and are characterized by the presence of ductal plate-like biliary elements.

Congenital biliary atresia. This disease does not represent agenesis of the bile ducts, but results from progressive destruction of the bile ducts by a necroinflammatory process of unknown etiology. This disease affects not only part or all of the extrahepatic bile duct but also the IBS. In 20–25% of patients with this disease, the interlobular bile ducts still appear in their primitive embryonic form (DPM) (Raweily et al. 1990; Desmet 1992b). This suggests that the causative factor of atresia starts early in fetal life in these cases, at the time that the IBS is still in its ductal plate configuration. In these cases, the molecular mechanism of the normal IBS development may be impaired. That is, this disease may be caused by abnormalities of several molecular mechanisms for the normal development of bile ducts, including abnormalities in the growth factor/receptor system, in epithelial-mesenchymal interactions, in matrix proteinases, in glycoconjugate side chains, and in PBP development. In contrast, this disease without DPM seems to be caused by necroinflammatory destruction of the extrahepatic bile ducts and IBS. Although the etiology of this destruction is obscure, apoptotic cell death may be responsible for bile duct epithelial death, because the IBS in fetal life shows much more apoptosis than does the adult IBS (Terada and Nakanuma 1995a). Or, the expression of apoptosis-related proteins may be abnormal, leading to accelerated apoptosis of the bile ducts. However, this hypothesis should be investigated using tissue specimens of this disease.

Paucity of intrahepatic bile ducts. Two forms of this disease are recognized: syndromatic (Alagille syndrome) and non-syndromatic. The basic lesion is a destructive form of cholangitis resulting in a reduced number of interlobular bile ducts. This disease may be caused by the failure of the primitive IBS to transform into the mature IBS, or by excessive destruction of IBS cells by apoptosis. Usually, DPM is not found in this disease.

Autosomal recessive polycystic disease. This disease was previously called "infantile polycystic disease of the liver and kidneys". Four different forms of this disease have been considered, according to the age at presentation: perinatal, neonatal, infantile and juvenile (Blyth and Ockenden 1971). The juvenile form of this disease is called congenital hepatic fibrosis (CHF). Morphologically, this disease is characterized by dilation of the IBS, cyst formation, fibrosis of portal tracts, and numerous bile duct structures resembling the remodeling stage of the fetal IBS (DMP) in addition to polycystic kidneys (medullary sponge kidney) (Nakanuma et al. 1982). This disease may be caused by the failure of the

remodeling ductal plate to transform into immature bile ducts. This failure may be due to abnormalities in the above-described mechanisms in the normal IBS development.

Congenital hepatic fibrosis. CHF is an autosomal-recessive disease; in most cases it is associated with autosomal recessive polycystic disease. Because CHF, polycystic liver diseases, Caroli's disease and von Meyenburg complex occasionally coexist in a single liver, these diseases are collectively termed as "hepatobiliary fibropolycystic disease" (Summerfield et al. 1986). Histologically, CHF is characterized by fibrosis and proliferated ductal structures resembling embryonic ductal plate (DPM) (Jorgensen 1977; Desmet 1992b). It seems likely that the etiology of CHF is the genetic failure of the ductal plate to undergo remodeling by the defect in the above-described normal IBS development.

von Meyenburg complex. This lesion, also called "microhamartoma", is histologicaly characterized by a variable number of bile duct-like structures embedded in hyalinizing stroma. The bile duct structures more or less resemble embryonic ductal plate, suggesting that this lesion is a form of DPM.

Autosomal dominant polycystic disease. This disease is also called "adult type of polycystic liver and kindey disease". Recent studies disclosed the causative genes (PKD-1 and PKD-2) (Kimberling et al. 1988). This disease is histologically characterized by multiple cysts, numerous von-Meyenburg complex and polycystic kidneys. The liver cysts arise from cystic dilation of von Meyenburg complex. Intrahepatic bile ducts may be diffusely dilated (Caroli's disease) (Terada and Nakanuma 1988b), and cholangiography of this disease sometimes resembles that of primary sclerosing cholangitis (Terada and Nakanuma 1995c). Intrahepatic peribiliary glands are also fequently dilated (Kida et al. 1992; Terada and Nakanuma 1990). This disease may be associated with CHF. The presence of von Meyenburg complex and association with Caroli's disease and CHF make this disease a type of DPM, which may be derived from a defect in the normal IBS development.

Caroli's disease. This disease is defined as congenital cystic dilation of the intrahepatic segmental bile ducts. There are two varieties: a pure form in which only dilation of the IBS is present, and a CHF-associated form in which both CHF and dilation of the IBS are recognized in a single liver. The former is rare, whereas the latter is relatively frequent. Cholangitis and hepatolithiasis are occasionally observed during the clinical course. Because of the association with CHF, this condition is recognized as DPM which may be ascribed to abnormal IBS development.

Overview

Histological, histochemical and immunohistochemical features of the normal developmental process of the human IBS were reviewed. The human IBS arises from the ductal plate which is derived from primitive hepatocytes (hepatoblasts).

The ductal plate undergoes remodeling, and finally transforms into mature bile ducts and peribiliary glands. Apoptosis, several proteins and carbohydrate residues are involved in normal IBS development. In addition, congenital IBS abnormalities were briefly reviewed with an emphasis on "ductal plate malformations". These congenital IBS diseases may be derived from the failure of the IBS to undergo the normal developmental process which involves appropriate apoptosis and cell proliferation, and expression of several proteins and glycoprotein residues.

Future directions

The molecular mechanisms of the normal IBS development need to be evaluated more extensively by examining the proteins' expression using Western blot analysis as well as by evaluating the mRNA and DNA using Northern and Southern blot analyses and in situ hybridization. Similar investigations remain to be performed to evaluate the molecular mechanism of congenital IBS disease, as do genetic analyses using modern molecular techniques and transgenic and gene-targeting knock-out mice.

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