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Development and Differentiation of Bile Ducts in the Mammalian Liver

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KEY WORDS hepatocytes; biliary epithelial cells; fetal liver; cell lineage; cytokeratin; basal lamina

ABSTRACT The development and differentiation of bile ducts in the human and rodent liver are reviewed. The liver primordium develops as a ventral diverticulum in the anterior intestinal portal region, which consists of endodermal and mesodermal components. The endodermal cells differentiate into hepatocytes and all epithelial cells of the bile ducts in the adult liver. The gallbladder and extrahepatic bile ducts also start to develop from hepatic endodermal cells and hepatoblasts just after liver primordium formation. The gallbladder and cystic duct do not develop through hepatic development in the rat. Intrahepatic bile ducts are formed from periportal hepatoblasts forming the "ductal plate" and expressing alpha-fetoprotein, and albumin and bile duct-specific cytokeratin and develop independently of extrahepatic bile duct formation. The first sign of intrahepatic bile duct differentiation is the increased expression of bile duct–specific cytokeratin and large lumina formation in periportal hepatoblasts, and then deposition of basal laminar components occurs on the basal side. Their development takes place discontinuously along portal veins at the early stage of development, and they then become confluent through development. Periportal connective tissue, glucocorticoid hormones, and basal laminar components may play important roles in the differentiation of bile ducts. *Microsc. Res. Tech. 39:328–335, 1997.* © 1997 Wiley-Liss, Inc.

INTRODUCTION

In the mammalian liver, mature hepatocytes and epithelial cells of intrahepatic bile ducts, extrahepatic bile ducts (the hepatic ducts, the common bile duct, and the cystic duct), and the gallbladder constitute the hepatobiliary system as an endodermal component. Bile ducts function as ducts for bile secretion into the duodenum and are involved in the physiology of the organ. Some animals such as rats and whales lack a gallbladder and cystic duct.

The embryological origin of intrahepatic bile ducts has been advocated, and two theories were proposed in the 1970s. One theory was that periportal hepatocytes differentiate into intrahepatic biliary epithelial cells (Bloom, 1926; Horstmann, 1939; Elias, 1955; Du Bois, 1963; Wilson et al., 1963; Wood, 1965; Picardi et al., 1968a,b; Enzan et al., 1974). The other theory was that extrahepatic bile ducts invade the liver parenchyma along the portal veins and form an arborizing intrahepatic biliary tree (Hammar, 1926; Koga, 1971). Recently, liver stem cells, which are hypothesized to exist between terminal bile ducts and hepatic cords (bile ductules) and are thought to serve as reservoir cells in case of massive hepatic necrosis, have been extensively studied (Marceau et al., 1989; Sell, 1990; Fausto et al., 1993; Grisham et al., 1993; Thorgeirsson et al., 1993; Yang et al., 1993a). The study of bile duct development is currently a matter of great interest. In these studies, various cell-lineage markers have been developed, such as hepatocyte-specific or biliary epithelial cell–specific cytokeratins and epitopes that are recognized by monoclonal antibodies. These markers allow us to reveal cell lineage during liver development. In this review, I summarize the development and differentiation of bile ducts in mammalian livers from recent papers published on this topic and discuss the mechanisms of bile duct differentiation.

FORMATION OF LIVER PRIMORDIUM AND DEVELOPMENT OF EXTRAHEPATIC BILE DUCTS

In the mouse, rat, and human embryos, the liver primordium develops as a diverticulum in the ventral region of the anterior intestinal portal at 9.0 days, 10.5 days, and 3 weeks of gestation, respectively (Du Bois, 1963; Shiojiri, 1979; Shiojiri et al., 1991). When development proceeds, the liver primordium becomes two portions in mouse and human embryos: cranial and caudal (Fig. 1). Hepatic cords, which extend from the cranial portion and consist of small basophilic hepatoblasts, invade the adjacent septum transversum mesenchyme and the omphalomesenteric veins. The cranial diverticulum develops into the liver parenchyma and hepatic ducts. It has been reported that the extrahepatic bile duct starts to develop from the caudal portion with or just after liver primordium formation (Du Bois, 1963; Shiojiri, 1979). However, reexamination of mouse liver development has shown that the boundary between the cranial and caudal portions is not histologically clear and that the caudal portion can extend hepatic cords (Shiojiri and Katayama, 1987). Histo-

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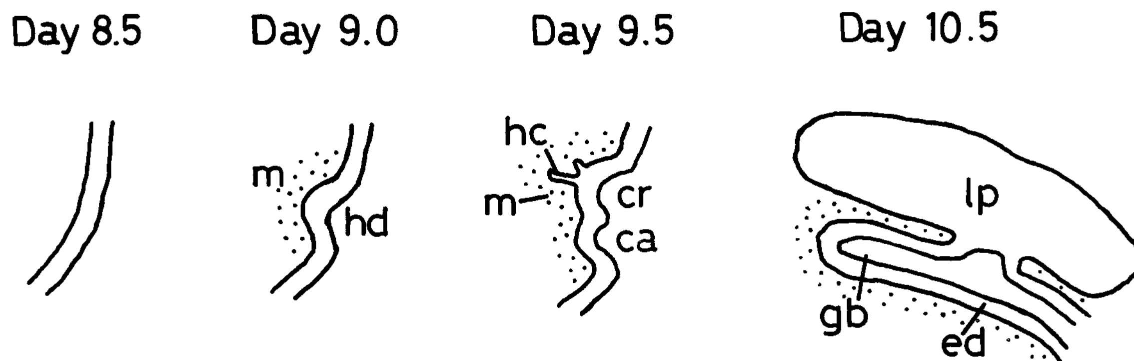

Fig. 1. Schematic drawing of the development of the mouse liver primordium. Mid-sagittal sections of the liver primordia in the ventral region of the anterior intestinal portal are drawn. The hepatic diverticulum (hd) is formed at 9.0 days of gestation and is divided into cranial (cr) and caudal (ca) portions at 9.5 days. Hepatic cords (hc) from the cranial portion then invade the septum transversum mesenchyme (m). ed, extrahepatic bile duct; gb, gallbladder; lp, liver parenchyma.

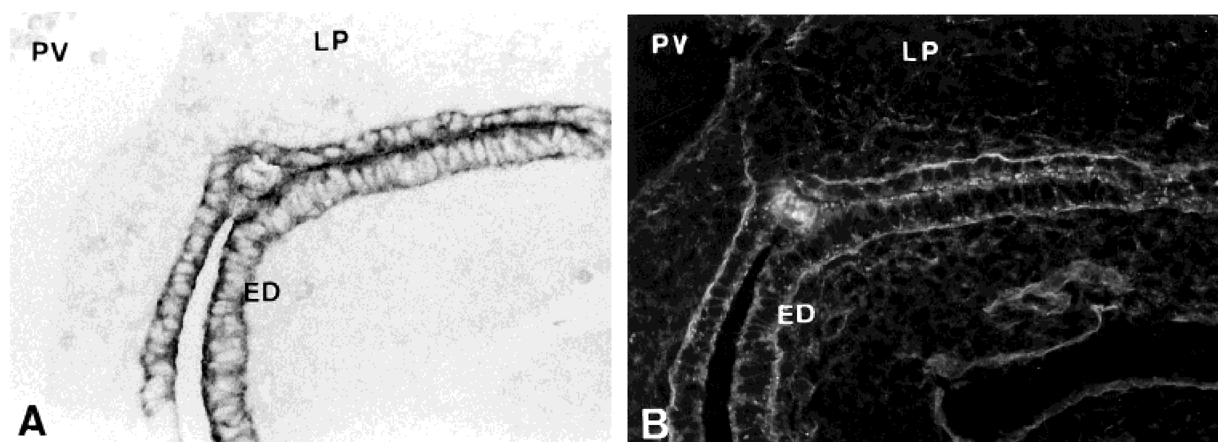

Fig. 2. DBA-binding sites in the extrahepatic bile duct in the 13.5-day mouse fetus. **A:** DBA-binding sites. These binding sites are exclusively expressed in the extrahepatic bile duct (ED) but not in the liver parenchyma (LP). Peroxidase-labeled DBA staining. **B:** Laminin immunofluorescence of a section consecutive to A. The extrahepatic bile duct is laminin positive. PV, portal vein. ×300.

chemical study with peroxidase-labeled *Dolichos biflorus* agglutinin (DBA) suggests that liver parenchymal cells, which are derived from the cranial portion, can differentiate into extrahepatic bile duct cells (Shiojiri and Katayama, 1988). In the rat embryo, the liver primordium does not divide into two portions but develops into the liver parenchyma, the hepatic ducts, and the common bile duct (Elias, 1955; Shiojiri et al., 1991). The gallbladder and the cystic duct are not formed through development. The liver primordium of the rat embryo may correspond to the cranial portion of the mouse and human liver primordia. We also demonstrated that, in the rat embryo, mRNA of alphafetoprotein (AFP) is expressed in the common bile duct cells and hepatic duct cells (Shiojiri et al., 1991). These results support the idea that hepatoblasts (expressing AFP mRNA and AFP) differentiate into extrahepatic bile duct cells during normal development of the rat liver (Elias, 1955) and suggest that, also in mouse and human embryos, the cells from the cranial portion of the hepatic diverticulum may contribute to extrahepatic bile duct formation (Fig. 5).

Two stages in extrahepatic bile duct differentiation proved to be recognizable from developmental studies with cytokeratins as cell-lineage markers in fetal rat liver development (Shiojiri et al., 1991). Young extrahepatic bile duct cells are positively stained by monoclonal antibodies against human cytokeratins (CK) 8, 18, and 19, and near birth CK 7 is expressed. Extrahepatic bile duct cells are also strongly stained with commercially available polyclonal antibodies against calf keratin and anti-human cytokeratin and by peroxidase-labeled DBA through fetal development (Fig. 2) (Shiojiri and Katayama, 1988; Van Eyken et al., 1988a; Shiojiri et al., 1991).

Peribiliary glands that localize in extrahepatic bile ducts and larger intrahepatic bile ducts near the hilum develop from epithelial cells of the bile ducts and their precursors by invasion into the connective tissue (Terada and Nakanuma, 1993). Their development occurs at approximately 7–10 weeks of gestation in the human embryo (Spitz and Petropoulos, 1979; Nakanuma et al., 1994).

The brush cells, which may function as a baroreceptor or chemoreceptor in the common bile duct of the rat, first appear at 4 weeks after birth and substantially increase at 8–16 weeks (Iseki, 1991). Glucagon-positive cells and CCK cells are first detected in the extrahe-

patic biliary tract at approximately 14 days of gestation, and they remain the dominant endocrine cell type in the duct system during the fetal period (Park and Bendayan, 1993). Insulin and pancreatic polypeptide cells are initially observed in the common hepatic duct on days 16 and 18 of gestation, respectively.

DEVELOPMENT AND DIFFERENTIATION OF INTRAHEPATIC BILE DUCTS

Intrahepatic bile ducts start to differentiate from periportal hepatoblasts, which surround large lumina and express AFP and albumin, and their mRNAs, at 13.5 days and at 15.5 days of gestation in the mouse and rat, respectively (Figs. 3, 5) (Shiojiri, 1979, 1981, 1984b; Shiojiri et al., 1991). The precursor of the bile duct in the human fetus is called the “ductal plate,” which consists of double layers of epithelial cells around portal veins and appears at approximately 5–9 weeks of gestation (Du Bois, 1963). Van Eyken et al. (1988a,b) showed that, in human and rat fetuses, the cells of the ductal plates or pearl-like structures originate from peiportal hepatoblasts expressing CKs 8 and 18 and become heavily positive for both cytokeratins.

The first sign of bile duct differentiation from hepatoblasts is also the increased expression of bile duct–specific cytokeratin, which is detectable with polyclonal antibodies raised against calf keratin in addition to the strong expression of CKs 8 and 18. The basal lamina containing laminin and basal laminar components that are reactive with some lectins (e.g., peanut agglutinin; PNA) then appears on the basal side (Fig. 3, Table 1) (Shiojiri and Mizuno, 1983; Shiojiri and Katayama, 1987; Shiojiri and Nagai, 1992; Shiojiri, 1994). The next step in bile duct differentiation is the expression of CK 19, BD1 antigen, DBA-binding sites, and increased expression of A6 antigen in the fetal rat and mouse liver (Table 1) (Shiojiri and Katayama, 1988; Carthew et al., 1989; Gall and Bhathal, 1989; Shiojiri et al., 1991; Engelhardt et al., 1993; Yang et al., 1993b). CK 7 is expressed in the biliary epithelial cells in the late gestational stage (Shiojiri et al., 1991). Differentiated bile ductule cells produce type IV collagen in addition to laminin (Baloch et al., 1992). It has been reported in the human fetus that expressions of carbohydrate chain structures and several mucin core proteins change during intrahepatic bile duct development and maturation (Terada and Nakanuma, 1994a; Sasaki et al., 1995). Most of the “oval cells” that rapidly proliferate in the early stages of chemical hepatocarcinogenesis retain the ability to differentiate into hepatocytes, biliary epithelial cells, and small intestinal cells (Tatematsu et al., 1985; Fausto et al., 1987). These cells resemble epithelial cells of the bile ducts and their precursors appearing after the late gestational stage in their ability to express CK 7 (Shiojiri et al., 1991).

During bile duct development, the duct precursors are discontinuous along portal veins and later connect with one another and also with the extrahepatic bile ducts (Wilson et al., 1963; Shiojiri and Katayama, 1987). Studies with DBA and calf keratin antibodies have indicated that cells of extrahepatic bile ducts exhibit histochemical characteristics different from precursors of intrahepatic bile duct cells during development (Fig. 2), suggesting that extrahepatic bile duct cells do not invade the liver parenchyma (Fig. 5) (Shiojiri and Katayama, 1988; Van Eyken et al., 1988a).

Intrahepatic bile duct differentiation and development from hepatocytes may continue for 1–2 weeks after birth in the mouse and rat liver. Periportal hepatocytes are positively stained by antibodies against calf keratin and human cytokeratin, which react exclusively with the bile duct cells in the adult liver (Van Eyken et al., 1988a; Shiojiri et al., 1991; Shiojiri, 1994). Adult hepatocytes can form bile ductlike structures in vitro (Block et al., 1996; Nishikawa et al., 1996).

We demonstrated that, before the appearance of intrahepatic bile duct precursors, all hepatoblasts are positive for AFP, albumin, and CKs 8 and 18 but not for markers of mature hepatocytes. These appear near the late gestational stage (at 15.5 days in the mouse and at 17.5 days in the rat; Table 1) (Shiojiri, 1981, 1984b; Shiojiri et al., 1991). In the human fetus, hepatoblasts have been reported to express transiently the bile duct-type cytokeratin, CK 19 (Shah and Gerber, 1989; Stosiek et al., 1990; Haruna et al., 1996). It was also shown that, in mouse and rat fetuses, hepatoblasts are transiently positive for bile duct markers (A6 antigen, CK 52, and reactivity to polyclonal antibodies against calf keratin; Fig. 3, Table 1) (Germain et al., 1988; Engelhardt et al., 1993; Shiojiri, 1994). These results suggest that each hepatoblast is bipotent concerning mature hepatocyte and bile duct cell lineages and that each possesses the characteristics of bile duct cells. It has also been shown experimentally that young fetal hepatoblasts retain the potency to differentiate into bile duct cells and mature hepatocytes (Shiojiri, 1984a; Shiojiri et al., 1991). When young fetal liver fragments that had not yet developed bile ducts were transplanted for 2 months into the testes of syngeneic animals, mature-type hepatocytes and bile duct cells differentiated from hepatoblasts. In the avian embryo, we also demonstrated that immature hepatoblasts of the quail embryo could differentiate into biliary epithelial cells and mature hepatocytes by transplanting embryonic liver fragments onto the chorioallantoic membrane of the chick embryo (Shiojiri and Mizuno, 1987). However, strictly speaking, these results do not demonstrate the bipotentiality of each hepatoblast. Clonal cultures such as those on soft agar or marking of hepatoblasts with a retrovector are necessary for the demonstration of the bipotentiality of each hepatoblast and hepatocyte (Ferry et al., 1991; Bralet et al., 1994).

MECHANISMS OF BILE DUCT DIFFERENTIATION

The concept that periportal connective tissue induces ductal formation from the adjacent hepatoblasts (Du Bois, 1963; Wilson et al., 1963; Wood, 1965; Picardi et al., 1968a,b; Enzan et al., 1974) is generally accepted at present and is based on the observation that periportal connective tissue increases with bile duct differentiation (Fig. 4). In the classical experimental evidence of Doljanski and Roulet (1934), adult hepatocytes are able to differentiate into biliary epithelial cells in coculture with connective tissue, which lends support to this idea. Portal veins and hepatic veins, which are other large veins in the liver, share a common origin from omphalomesenteric veins. No bile ducts are observed near the hepatic veins in the adult animal or throughout fetal

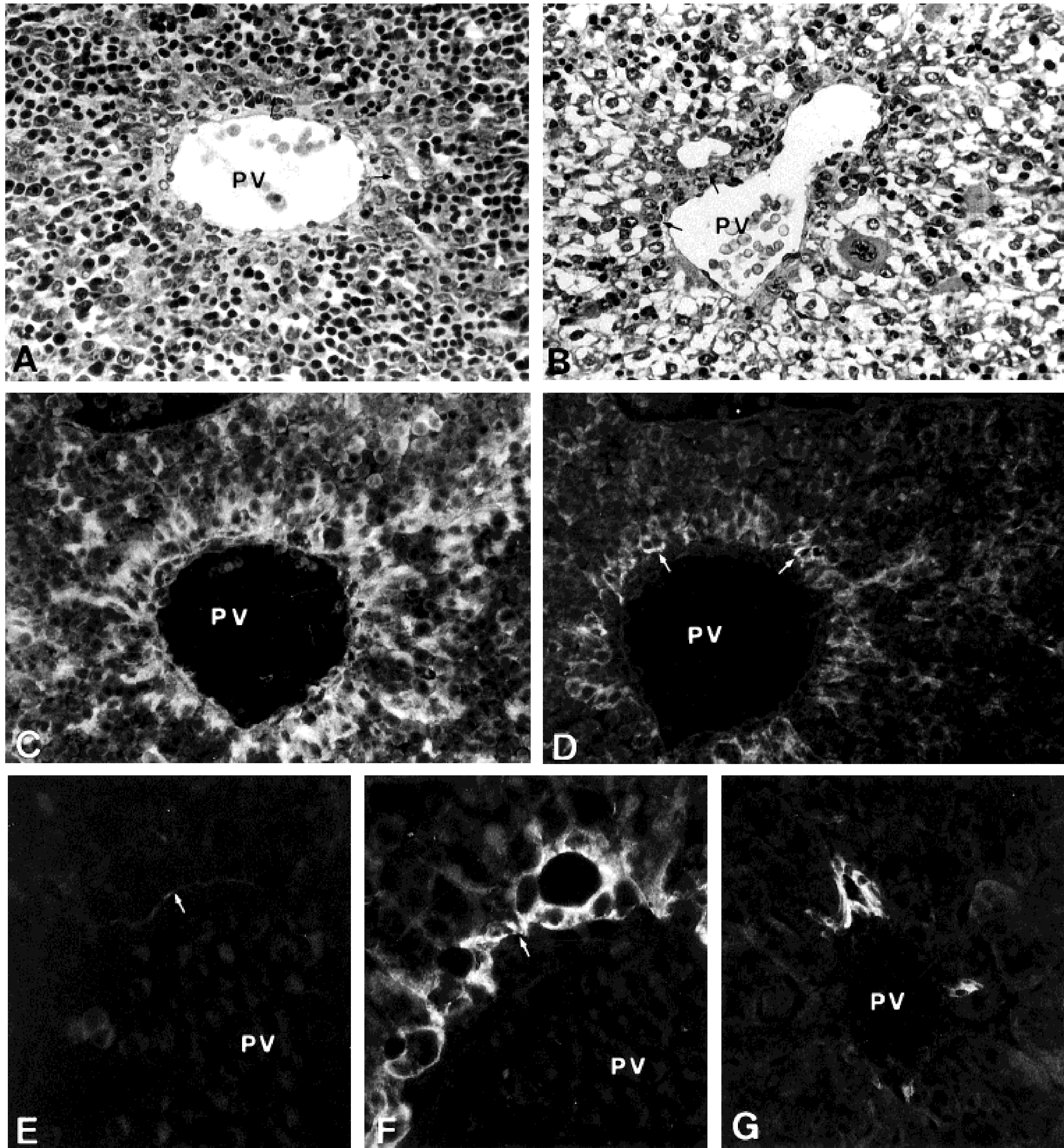

Fig. 3. Intrahepatic bile duct development in the fetal mouse liver. **A:** Precursors of intrahepatic bile ducts (arrows) at 14.5 days of gestation. The hepatoblasts are basophilic and hematopoietic cells are abundant. Hematoxylin-eosin staining. ×300. **B:** Hepatocytes and biliary epithelial cells at 17.5 days. Hepatocytes are larger. Arrows indicate biliary epithelial cells. Hematoxylin-eosin staining. ×300. **C:** AFP immunofluorescence in a 15.5-day liver. **D:** Bile duct–specific cytokeratin immunostaining of a section consecutive to that shown in C. Precursors of intrahepatic bile ducts are more strongly stained (arrows). ×300. **E:** PNA-binding sites in a 17.5-day liver as demonstrated by fluorescein-labeled PNA staining. ×600. **F:** Bile duct–specific cytokeratin staining of a section consecutive to that shown in E. Not all biliary epithelial cells, which strongly react with anti-calf keratin antibodies, are positive for PNA staining. Arrow shows a primitive bile duct structure that is positive for PNA and bile duct–specific cytokeratin. ×600. **G:** Bile duct–specific cytokeratin staining in an adult mouse liver. ×300. PV, portal vein.

development. During early stages of development, the histology of both veins is similar, but as development progresses, the connective tissue becomes abundant near the portal veins as compared with that found near hepatic veins (Shiojiri and Nagai, 1992). These results also suggest the importance of periportal connective tissue in bile duct differentiation. In vivo transplantation studies of fetal liver fragments have indicated that mature hepatocytes differentiate abundantly from immature hepatoblasts when sinusoids are well developed

TABLE 1. Histochemical characteristics of hepatoblasts, hepatocytes, epithelial cells of intrahepatic bile ducts, and their precursors in rat and mouse liver development[1]

	Hepato-blasts	Precursors of intrahepatic bile duct cells	Intrahepatic bile duct cells	Hepato-cytes
AFP[2]	++	+ → −	−	++ → −
AFP mRNA[3]	++	+ → −	−	++ → −
Albumin[4]	+	+ → −	−	++
Albumin mRNA[3]	+	+ → −	−	++
ALP[5]	− → +	−	−	+
5′-Nase[5]	− → +	−	−	+
HES$_6$[6]	−	−	−	+
Glycogen[2]	−	−	−	++
CK8[7]	+	++	++	+
CK18[7]	+	++	++	+
CK7[3]	−	− → +	++	−
CK19[3]	−	− → +	++	−
CK39[8]	+	++	++	−
CK52[8]	+	++	++	−
CK55[6]	+	++	++	+
Poly CK[9]	+	++	++	−
GGT[3]	+	++	++	+ → −
PNA[10]	−	− → +	+	−
DBA[11]	−	− → +/−	+/−	−
SBA[10]	−	− → +/−	+/−	−
Laminin[12]	−/+	++	++	−/+
Collagen type IV[13]	−	− → +	+	−
Basal lamina[14]	−	+	+	−
A6[15]	+	+	+	−
BD1[16]	−	−/+	−/+	−
BDS$_7$[8]	−/+	+	+	−

[1]−, negative staining; +, moderate staining; ++, strong staining; −/+, negative cells and positive cells; →, staining changes during development. ALP, alkaline phosphatase activity; GGT, gamma-glutamyltransferase activity; 5′-Nase, 5′-nucleotidase activity; Poly CK, reactivity to anti-calf keratin antibodies; SBA, soybean agglutinin.
[2]Shiojiri (1981) and Shiojiri et al. (1991).
[3]Shiojiri et al. (1991).
[4]Shiojiri (1984b) and Shiojiri et al. (1991).
[5]Shiojiri (1981).
[6]Germain et al. (1988) and Shiojiri et al. (1991).
[7]Shiojiri et al. (1991) and Van Eyken et al. (1988a).
[8]Germain et al. (1988).
[9]Shiojiri (1994).
[10]Shiojiri and Nagai (1992).
[11]Shiojiri and Katayama (1988).
[12]Shiojiri and Katayama (1987).
[13]Baloch et al. (1992).
[14]Shiojiri and Mizuno (1983) and Wood (1965).
[15]Engelhardt et al. (1993).
[16]Yang et al. (1993b).

in the testis (Shiojiri, 1984a). In contrast, bile ducts differentiated abundantly when fetal liver fragments were placed under the newborn skin, where the connective tissue is abundant. These data also agree with the above premise. However, we recently showed that increased expression of bile duct–specific cytokeratin is already seen in hepatoblasts near the smaller portal veins, where the connective tissue is poorly developed (Shiojiri, 1994), suggesting that the connective tissue may not be involved in the stronger expression of cytokeratin (the early phase of bile duct differentiation). The connective tissue may play a role in the stabilization of bile duct differentiation.

The development of several components of the extracellular matrices (collagen types I and III and lectin-binding sites) in the connective tissue near portal veins and hepatic veins has been histochemically analyzed in the fetal mouse liver with special attention given to preferential bile duct differentiation near the portal veins (Shiojiri and Nagai, 1992). The extracellular matrices such as interstitial-type collagens and some lectin-binding sites are more abundant in the connective tissue near the portal veins than in the tissue near the hepatic veins, suggesting their possible involvement in periportal bile duct differentiation. Similar results have been obtained for type I collagen and fibronectin by Baloch et al. (1992). Tenascin is transiently expressed in the connective tissue near newly formed hilar bile ducts, but it is not found in the peripheral bile ducts in the human fetus (Terada and Nakanuma, 1994b). Shah and Gerber (1990) and Terada and Nakanuma (1994b) reported that laminin and type IV collagen, both of which are components of the basal lamina, are identified near the ductal plate, migrating epithelial cells, and peripheral bile ducts in the human fetus, implying that type IV collagen and laminin may play a role in bile duct development. It has also been suggested that several matrix proteinases may play an important role in migration of primitive biliary cells into the periportal connective tissue during human intrahepatic bile duct development (Terada et al., 1995). Volpes et al. (1993) showed that integrins, a cell-surface receptor for extracellular matrices, could be used as differential cell-lineage markers of primary liver tumors; biliary epithelial cells express integrins different from those of mature hepatocytes. These adhesion molecules are worth studying during development because bile duct cells adhere to the basal lamina and the connective tissue containing the extracellular matrices via such molecules.

In organogenesis of animal embryos, growth factors play important roles in differentiation and in cell proliferation (Cross and Dexter, 1991; Jessell and Melton, 1992; Mason, 1994). Parathyroid hormone-related peptide is immunohistochemically demonstrated in the bile ducts of the human fetus (Roskams and Desmet, 1994). Strong immunoreactivity for TGF-alpha and its receptor has been reported in intrahepatic bile duct cells of various developmental stages (Terada et al., 1994). They may function as a growth and differentiation factor for growing and maturing bile ducts. Terada and Nakanuma (1995) also suggested that balanced cell proliferation and apoptosis are involved in the normal development of intrahepatic bile ducts and hepatocytes in human fetuses by analyzing apoptosis and the expression of apoptosis-related proteins.

To elucidate the mechanisms underlying bile duct differentiation, it is necessary to show what kind of factors induce bile duct differentiation from fetal hepatoblasts and hepatocytes in vitro. However, there are few studies focusing on this issue. We have indicated that, in organ culture of fetal liver fragments, glucocorticoids and basal laminar components promote the expression of bile duct–specific cytokeratin and some lectin-binding sites in hepatoblasts (Shiojiri and Mizuno, 1993). Cell culture studies by Germain et al. (1988) and Blouin et al. (1995) have demonstrated that sodium butylate is a strong stimulator of bile duct cell differentiation, although it may not be an intrinsic factor. Hepatocyte growth factor (HGF)/scatter factor, a mediator of epithelial–mesenchymal interactions (Montesano et al., 1991a,b; Sonnenberg et al., 1993) has been shown to induce lumen formation in cultures of rat liver epithelial cell lines (Johnson et al., 1993) and formation of acinar/ductular structures akin to bile ductules in

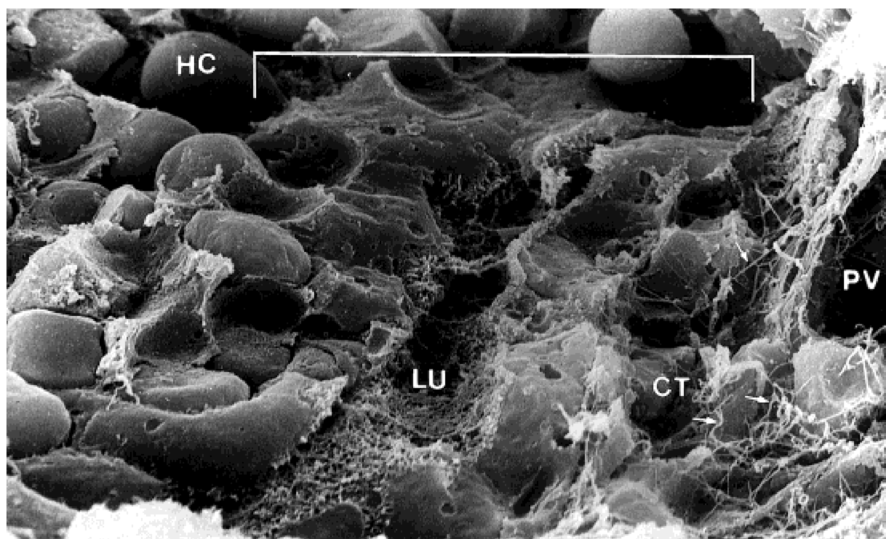

Fig. 4. Scanning electron micrograph of a precursor of the intrahepatic bile duct (square bracket) in a 15.5-day mouse fetus. The precursor possesses a large lumen (LU). Connective tissue (CT) and extracellular matrices (small arrows) are well developed near the portal vein (PV). HC, hematopoietic cells. ×3,000.

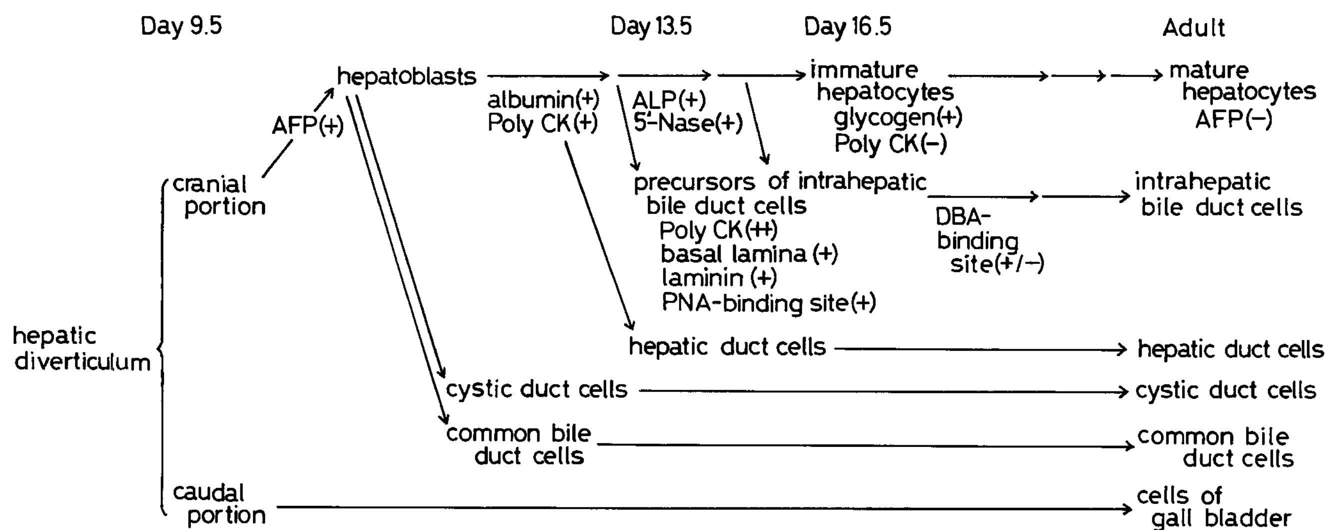

Fig. 5. Schematic diagram of a possible cell lineage in the mouse liver. ALP, alkaline phosphatase activity; Poly CK, reactivity to anti-calf keratin antibodies; 5′-Nase, 5′-nucleotidase activity.

three-dimensional cultures of adult hepatocytes within type I collagen gel (Block et al., 1996). Thus, the role of HGF in bile duct differentiation could be important, especially as a candidate for a bile duct–inducing factor or stabilizing factor from periportal connective tissue during fetal development. Studies with in vitro culture techniques are required to uncover the mechanisms of bile duct differentiation in the future.

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