Section I **Pathophysiology of pediatric liver disease**

Chapter 1

Liver development

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Introduction

Liver development requires two linked processes: differentiation of the various hepatic cell types from their embryonic progenitors and the arrangement of those cells into structures that permit the distinctive circulatory, metabolic, and excretory functions of the liver.

Primarily through the use of rodent, fish, and frog model systems, many essential regulators of liver development have been identified. These include extracellular signaling molecules, intracellular signal transduction pathways, and transcription factors. In recent years, transcriptional regulation by microRNA has also been implicated in liver development. In addition, a class of biliary diseases associated with defects in the cholangiocyte cilium has highlighted the importance of this structure in bile duct morphology and cellular polarity.

This chapter describes the stages of liver development in conjunction with their associated molecular pathways. Whenever relevant, links to pediatric liver disease will be indicated. One important insight that has emerged from the study of liver development is that the process is not complete at birth, because bile duct remodeling is ongoing (see below). In addition, it has become clear that many of the molecular pathways that direct liver development are reactivated during the course of liver regeneration. Therefore insights derived from the embryonic and fetal liver may be relevant in the context of liver injury at any age.

Overview of liver development

Following gastrulation, all animal embryos are composed of three germ layers: the ectoderm, mesoderm, and endoderm. In humans, gastrulation occurs at approximately day 16 of gestation (embryonic day 7 in the mouse). The major epithelial cells of the liver – hepatocytes and cholangiocytes – are derived from the endoderm (Figure 1.1). However, these cells represent only about two-thirds of the liver volume. The remaining one-third consists of a variety of cells derived primarily from

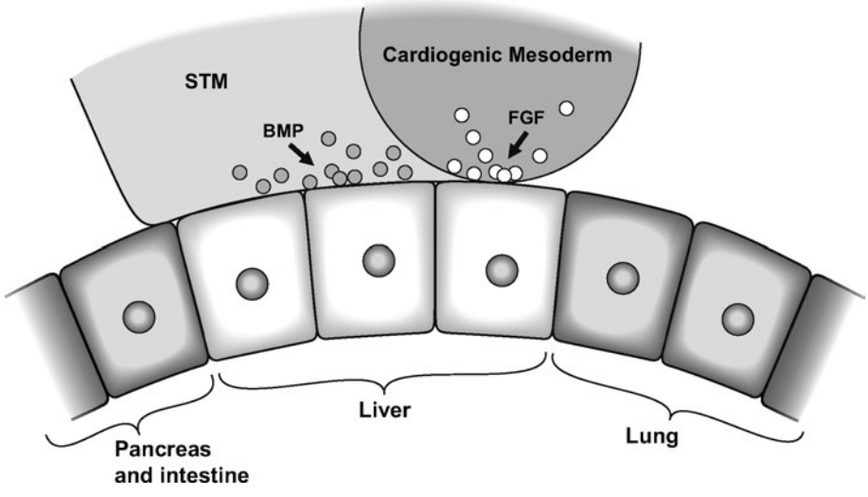

Figure 1.1 Liver specification. Liver specification occurs when the ventral foregut endoderm receives inductive signals from the adjacent cardiogenic mesoderm and the septum transversum mesenchyme (STM). BMP, bone morphogenetic protein; FGF, fibroblast growth factor.

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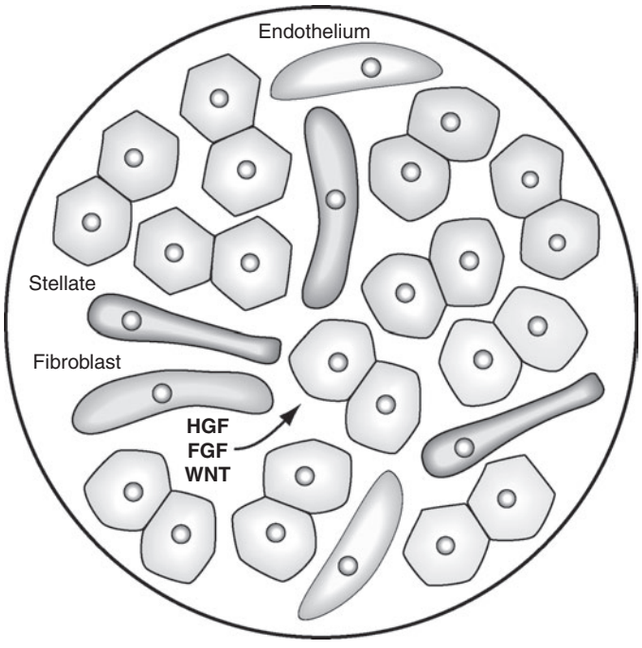

Figure 1.2 The liver bud. Proliferation and expansion of hepatoblasts into the septum transversum mesenchyme requires signals from endothelium and mesenchymal cells, resulting in formation of the liver bud. FGF, fibroblast growth factor; HGF, hepatocyte growth factor.

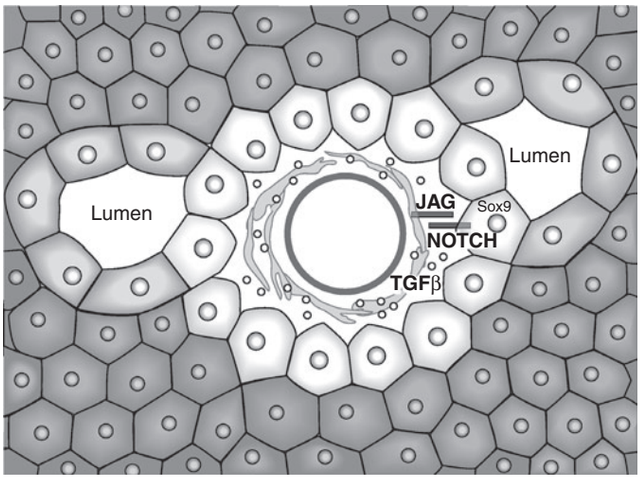

Figure 1.3 The ductal plate. In response to transforming growth factor-β (TGFβ), hepatoblasts in a ring surrounding the portal vein differentiate to form the ductal plate. In a process dependent on JAGGED/NOTCH signaling and regulated by SOX9, the periportal ductal plate cells induce cholangiocyte differentiation in more peripheral hepatoblasts, resulting in ductules.

the mesoderm, including vascular cell types: Kupffer cells, stellate cells, fibroblasts, and leukocytes. Therefore, liver development requires the coordinated integration of these cells from distinct embryonic layers into a single whole.

The first stage of liver development is *specification*, during which endoderm cells adjacent to the cardiogenic mesoderm begin to differentiate into hepatoblasts, as indicated by the expression of proteins such as albumin and α-fetoprotein (Figure 1.1). This is followed by *liver bud formation* and *expansion*: the hepatoblasts proliferate and penetrate the endoderm basement membrane to form the liver bud (Figure 1.2). In humans, this occurs at approximately day 25 (E9 in the mouse). The liver bud then expands in size, intercalating into the adjacent septum transversum mesenchyme (STM), in the process surrounding angioblasts that will ultimately give rise to the portal veins. Other mesenchymal cells are integrated into the liver at this stage and will differentiate into fibroblasts and stellate cells [1].

During the *epithelial differentiation* stage, hepatoblasts mature into hepatocytes or differentiate into cholangiocytes. The differentiation of cholangiocytes occurs in a distinctive spatial pattern, first indicated by the expression of cytokeratin 19 in a sheath of cells surrounding each portal vein branch; this structure is called the *ductal plate* (Figure 1.3). At one or two points along the circumference of the ductal plate, some adjacent hepatoblasts are also induced to differentiate into cholangiocytes, forming a tubular lumen between these cells and the first layer of ductal plate cells. These structures develop into the bile ducts, while the remainder of the ductal plate is lost, most likely via transdifferentiation into periportal hepatocytes [2]. Several congenital diseases are associated with defects in the development and maturation of the ductal plate; collectively, these are referred to as *ductal plate malformations* (see below and Chapter 41). The hepatic artery is the last component of the *portal triad* (portal vein, bile duct(s), and hepatic artery) to appear, and it is dependent on preceding bile duct development [3]. Although the process of hepatic arteriogenesis is not fully characterized, it is likely to involve signaling between the bile ductules and angioblasts in the periportal zone.

Liver specification

Importance of signals from adjacent tissues

In the mouse embryo, the endoderm at E8 comprises approximately 500 cells. Of these, only a few cells in the ventral foregut endoderm will be specified as hepatoblasts (Figure 1.1). This is the combinatorial effect of signals transmitted by the adjacent cardiac mesoderm (CM) and STM, both of which are required for the initial stages of liver development [4–6]. Initially, the endoderm in contact with the CM is stimulated by fibroblast growth factor (FGF) released by the CM. The dose of FGF received is critical, because exposure to higher levels of FGF results in the differentiation of the endoderm to lung rather than liver, and lower levels of FGF result in pancreatic differentiation [7]. Further development of the embryo places the ventral foregut endoderm in contact with the STM, from which the endoderm receives secreted bone morphogenic proteins (BMPs). Both FGF and BMP signals are required for liver specification [4–6].

The secreted glycoproteins of the WNT family also play an essential role in liver development, as indicated by studies in

zebrafish and frog (*Xenopus)* models. At the earliest stages, in the zebrafish the Wnt pathway must be repressed to allow the establishment of the foregut endoderm following gastrulation [8,9]. Later, the Wnt family members Wnt2bb and Wnt2 are required for liver specification and expansion of the liver bud [10]. This example illustrates the general pattern that *regulators often function at multiple stages of organogenesis* (Table 1.1). As a result, genetic defects may affect multiple aspects of liver development.

Transcription factors in liver specification

Members of the Foxa (forkhead box A) family of transcription factors are the earliest known endoderm-specific proteins required for liver specification in the mouse [11], and members of the Gata transcription factor family are required shortly thereafter [12]. Both Foxa and Gata proteins are bound to liver-specific genes such as *Alb* (encoding albumin) in the endoderm *before* these genes are expressed; in fact, Foxa and Gata are bound to liver-specific genes in broad regions of the endoderm that will give rise to intestine and never express *Alb*. Collectively, this indicates that a broad region of the embryonic endoderm is developmentally competent to respond to inductive signals (such as FGF or BMP) by virtue of transcription factor binding to tissue-specific genes. Known as "pioneer factors," the Foxa and Gata proteins have the ability to establish a "pre-pattern" of chromatin modifications that permits the binding of other transcription factors and the activation of cell type-specific gene expression [7,13]. A third transcription factor, hepatocyte nuclear factor-1β (HNF1β), is also required for liver specification; loss of Hnf1β in the zebrafish embryo results in liver bud agenesis through to a failure of the endoderm to respond to FGF [14]. However, it is not known if HNF1β can function as a pioneer factor in binding and releasing repressive chromatin structure.

As might be expected from the essential nature of both the liver itself and its role in fetal hematopoiesis, no human disorders have been linked to defects in liver specification, as these would be expected to result in early fetal loss.

Formation and expansion of the liver bud

Transcription factors in the liver bud

Once they are specified within the endoderm, liver progenitor cells differentiate into hepatoblasts. In order to form the liver bud, the normal cell–cell contacts of the endoderm must be released so that the hepatoblasts can migrate into the adjacent mesenchyme. This also requires disruption of the basement membrane underlying the endoderm layer. Finally, the hepatoblasts must proliferate to rapidly increase the liver mass. Several transcription factors have been linked to these processes. The homeobox protein HHEX is a target of GATA6, and hepatoblasts lacking either GATA6 or HHEX are properly specified but fail to form the liver bud [12,15]. Two other linked transcription factors are required for expansion of the hepatoblasts into the STM. The T-box protein Tbx3 activates expression of the homeobox protein Prox1, and in mouse embryos lacking either Tbx3 or Prox1, the hepatoblasts fail to enter the STM. The likely mechanism for this is a failure to downregulate the cell junction proteins, thus preventing hepatoblasts from separating from each other and adopting migratory properties [16,17].

Inductive signaling in the liver bud

As is the case for hepatic specification, the liver bud stage of liver development depends on intrinsic signals as well as signals from outside the liver primordium. The STM into which the hepatoblasts migrate includes endothelial cells that have not yet been incorporated into blood vessels. In mouse embryos that lack endothelial cells, the hepatoblasts do not enter the STM and no liver bud is formed [18]. The function of the STM endothelium is likely to be mediated in part by the secretion of WNT ligands by the endothelium. These ligands are also secreted by stellate cells in the liver bud, although these are not sufficient to support liver bud development. The requirement for STM-derived signals is also illustrated by the absence of liver bud growth in mice lacking the homeobox protein Hlx, a transcription factor expressed in the STM but not in hepatoblasts [19].

Additional findings confirm the central importance of WNT/β-catenin signaling in liver bud development. β-Catenin is a protein with dual roles: it plays both a structural role as an adapter in bridging the actin cytoskeleton and E-cadherin at the apical junction and a separate regulatory role in transducing WNT signals. The binding of WNT ligands to their receptors on hepatoblasts results in translocation of the transcriptional activator β-catenin to the nucleus and the expression of target genes. Overall, there is a peak of β-catenin activation during liver bud expansion in the mouse, and loss of β-catenin in hepatoblasts leads to severe defects in liver bud growth and differentiation [20]. As in FGF signaling, liver development is sensitive to the magnitude of WNT pathway activity, as artificial β-catenin activation in hepatoblasts results in liver hypoplasia and defective differentiation.

A hypoplastic liver phenotype is also observed in mouse embryos lacking hepatocyte growth factor (HGF), which is released by mesenchymal fibroblasts and binds to the c-Met receptor on hepatoblasts [21]. Furthermore, HGF and Wnt signaling are linked in the mouse, as β-catenin is bound to c-Met at the cell membrane, and the binding of HGF to c-Met results in β-catenin activation [22]. Interestingly, β-catenin also mediates FGF signaling in the liver bud, as FGF released by stellate cells leads to β-catenin activation upon binding to FGF receptors on hepatoblasts [23].

While cell–cell adhesion by hepatoblasts must be decreased to permit expansion of the liver bud into the STM, at later stages hepatoblasts must adhere to each other as a normal aspect of their epithelial nature. The signaling protein transforming growth factor-β (TGFβ) functions in part to promote

Table 1.1 Major regulatory factors in liver development

Human gene or gene family	Protein type	Function
Hepatic specification		
FGF family	Secreted proteins	Specification of endoderm to hepatoblast fate
BMP family	Secreted proteins of the TGFβ superfamily	Specification of endoderm to hepatoblast fate
FOXA family	Forkhead box transcription factors	Specification of endoderm to hepatoblast fate
GATA4 and *GATA6*	Zinc-finger transcription factors	Specification of endoderm to hepatoblast fate
HNF1B	Homeodomain transcription factor β	Specification of endoderm to hepatoblast fate
WNT	Secreted proteins	Specification of endoderm to hepatoblast fate
Liver bud formation and growth		
HHEX	Homeodomain transcription factor	Liver bud formation
PROX1	Prospero-type homeodomain transcription factor	Liver bud expansion into the STM
TBX3	T-box transcription factor	Liver bud expansion into STM
ONECUT family (HNF6 and OC1)	Onecut transcription factors	Stage-specific effects on liver growth
WNT	Secreted signaling proteins	Stage-specific effects on liver growth
CTNNB1	β-Catenin; nuclear effector of WNT signaling	Effector of WNT signaling
HGF	Secreted signaling protein	Fetal liver growth
MET	Hepatocyte growth factor receptor	Fetal liver growth
Ductal plate formation and morphogenesis		
TGFB	Secreted signaling proteins of the TGFβ superfamily	Ductal plate specification
ONECUT family	Onecut transcription factors	Establishment of TGFβ gradient
JAG	Membrane-bound signaling proteins	Biliary differentiation and morphogenesis
NOTCH	Membrane-bound receptors for JAG ligands	Biliary differentiation and morphogenesis
SOX9	SRY-related HMG-box transcription factor	Biliary differentiation and morphogenesis
miR-30 family	MicroRNA	Biliary morphogenesis
Hepatocyte and cholangiocyte differentiation		
HNF4A	Orphan nuclear receptor HNF4α	Hepatocyte differentiation
HNF1A	Homeodomain transcription factor HNF1α	Hepatocyte differentiation
HNF1B	Homeodomain transcription factor HNF1β	Bile duct morphogenesis
FOXA family	Forkhead box transcription factors	Bile acid metabolism, bile duct growth
ONECUT family	Onecut transcription factors	Cholangiocyte and hepatocyte differentiation
NR5A2	Liver receptor homologue 1 (LRH1), a nuclear receptor family transcription factor	Multiple hepatocyte metabolic pathways
OSM	Secreted signaling protein	Hepatoctye differentiation
Extrahepatic bile duct development		
SOX17	SRY-related HMG-box transcription factor	EHBD formation
HES1	Basic helix-loop-helix (bHLH) transcription factor	EHBD formation
HHEX	Homeodomain transcription factor	EHBD differentiation
HNF6	Onecut transcription factor	EHBD differentiation, gallbladder formation
HNF1B	Homeodomain transcription factor HNF1β	EHBD differentiation

EHBD, extrahepatic bile duct; STM, septum transversum mesenchyme; TGF, transforming growth factor

expression of the adhesion proteins E-cadherin and β_1-integrin in the liver bud. Cell–cell adhesion and liver bud growth are defective in mouse embryos lacking Smad2 and Smad3, two proteins essential for TGFβ signal transduction [24].

The fetal liver is also highly populated with hematopoietic cells, and beginning at approximately 6 weeks of human gestation it is the major source of blood cells. The hematopoietic cells and the fetal liver cells are mutually dependent. Absence of the liver bud results in death from anemia [11]. Conversely, the developing hepatocytes express the receptor for oncostatin M, a signaling molecule that is released by the hematopoietic cells, and loss of this receptor leads to incomplete hepatocyte differentiation [25].

Overall, hepatoblasts receive a variety of signals from the surrounding mesenchyme that promote the migration, proliferation, and cell–cell interactions necessary for establishment of the liver bud. The STM cells differentiate into non-epithelial cell types of the liver, such as stellate cells and perivascular mesenchymal cells [26].

Differentiation of hepatoblasts into hepatocytes and cholangiocytes

The epithelial cell population of the liver comprises hepatocytes and cholangiocytes. Hepatocytes perform the metabolic and synthetic functions of the liver, as well as the excretory function of bile synthesis. Cholangiocytes modify the bile and form the bile ducts that serve as the conduit of bile to the small intestine. At approximately week 9 of human gestation (E13 in the mouse embryo), differentiation of hepatoblasts into cholangiocytes and hepatocytes begins. This process continues through approximately 6 months of age, and thus cholestatic disorders of infancy appear in the context of a developing hepatobiliary tree. Macroscopically, the differentiation begins at the hilum and proceeds toward the periphery. Microscopically, cholangiocyte differentiation is restricted to cells surrounding the portal vein branches, forming a structure known as the ductal plate, which in three dimensions more closely resembles a cylindrical sheath) (Figure 1.3).

Hepatocyte differentiation

In differentiating hepatocytes, TBX3 is a positive regulator of hepatocyte-specific transcription factors, including HNF4α and the CCAAT/enhancer binding protein-α (C/EBPα). Genome-wide studies have shown that HNF4α and C/EBPα are bound to large sets of genes that define the hepatocyte phenotype, including those responsible for glycogen, triglyceride, and protein metabolism [27,28]. In addition, TBX3 represses the cholangiocyte transcription factor HNF6 [16]. Another hepatocyte-specific transcription factor is the POU-homeobox protein HNF1α; deficiency of HNF1α results in phenylketonuria through loss of *PAH* expression (encoding phenylalanine hydroxylase) [29]. Several other key liver-specific proteins, such as albumin, α_1-antitrypsin, and fibrinogen, are also downregulated in the absence of HNF1α [29]. The importance of HNF1α is highlighted by the finding that exogenous expression of *Hnf1A*, *Gata4*, and *Foxa3* in mice is both necessary and sufficient to convert adult fibroblasts to a hepatocyte-like state [30].

In addition to its role in hepatic specification, FOXA2 is important in the mature hepatocyte, where it regulates bile acid metabolism and is bound to many other metabolic genes [31]. FOXA1 and FOXA2 also function in the cross-talk between differentiating hepatocytes and cholangiocytes. Conditional deletion of Foxa1 and Foxa2 at E14.5 in the mouse liver leads to biliary hyperplasia, associated with excess interleukin-6 signaling from hepatocytes to cholangiocytes. Interleukin-6 acts as a growth factor for cholangiocytes, so that under normal circumstances, FOXA-mediated repression of interleukin-6 production by hepatocytes halts the growth of cholangiocytes after sufficient ducts have been formed [32]. Following differentiation, both hepatocytes and cholangiocytes proliferate to achieve the proper liver size, in a process that is controlled by Yap1 in mice, a component of the Hippo signaling pathway [33]. The Hippo/Yap1 pathway controls organ size in different tissues through effects on cell growth and apoptosis.

Other factors that define the hepatocyte cell type include several nuclear hormone receptors whose function is to respond to metabolites and xenobiotics, such as farsenoid X receptor (encoded by *NR1H4*), pregnane X receptor (*NR1I2*), constitutive androstane receptor (*NR1I3*), and liver receptor homologue-1 (*NR5A2*). Each of these genes is expressed as the hepatoblast differentiates into a hepatocyte.

Cholangiocyte differentiation and intrahepatic bile duct morphogenesis

As in earlier stages of liver development, extracellular signals are key determinants of hepatoblast differentiation (Figure 1.4). The portal vein endothelium and/or the portal mesenchyme secrete members of the TGFβ protein family (activin and TGFβ1–3), so that the periportal hepatoblasts are exposed to higher levels than more distant hepatoblasts [34]. This signal induces the differentiation of the periportal hepatoblasts into CK19-positive cholangiocyte precursors, forming the ductal

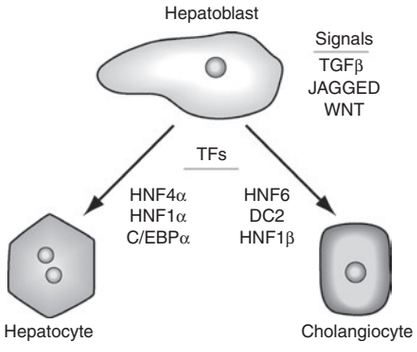

Figure 1.4 Hepatoblast differentiation. The differentiation of hepatoblasts into cholangiocytes and hepatocytes is controlled by inductive signals and transcription factor networks. C/EBP, CCAAT/enhancer binding protein; DC2, dendritic cells type 2; HNF, hepatocyte nuclear factor; TGFβ, transforming growth factor-β.

plate. Antibody-mediated blockade of TGFβ in the E10.5 mouse embryo inhibits cholangiocyte differentiation, and in ex vivo cultures of liver bud tissue exogenous TGFβ is sufficient to induce biliary differentiation [34]. Signaling through WNT and FGF signaling is also important in hepatoblast differentiation, by promoting a cholangiocyte cell fate over the hepatocyte cell fate. Activation of WNT signaling *after* liver bud formation results in a loss of hepatocytes, whereas biliary differentiation is maintained; conversely, loss of WNT pathway function in hepatoblasts leads to biliary hypoplasia.

Two members of the ONECUT family of transcription factors named HNF6 (OC1) and OC2 link transcriptional regulation to TGFβ signaling in the periportal region. In the absence of both HNF6 and OC2, the gradient of TGFβ activity surrounding the portal vein is lost. Both cholangiocyte and hepatocyte differentiation are affected by the loss of the TGFβ gradient. There is a loss of bile duct development in *Hnf6*/*Oc2*-deficient mice, while the parenchymal hepatocytes have a mixed hepatocyte/cholangiocyte phenotype [34,35]. This establishes HNF6 and OC2 as key promoters of cholangiocyte differentiation.

The JAGGED (JAG)/NOTCH signaling pathway is also essential in biliary development. Unlike secreted ligands such as WNT, FGF, and TGFβ, the JAGGED ligands and their NOTCH receptors are integral membrane proteins. Therefore JAGGED/NOTCH signaling requires direct cell–cell contact. In the mouse liver bud, *Jag1* is expressed in the periportal mesenchyme, resulting in Notch activation exclusively in a sheath of cells surrounding the portal vein. These ductal plate cells are identified by the expression of the biliary cytokeratin CK19. In mice lacking Jag1 in the periportal mesenchyme, the initial biliary specification occurs, but the subsequent formation of biliary tubules fails[36], most likely because Notch activity in the initial ductal plate cells results in *Jag1* expression in these same cells – thereby transmitting the signal of Notch activation one cell layer further away from the portal vein (Figure 1.3). At one or two points around the ductal plate circumference, this results in a primitive ductule comprising two layers surrounding a lumen [37]. The remaining portions of the ductal plate do not form bile ducts; instead they become periportal hepatocytes, cells of the canals of Hering linking the canaliculi to the bile ducts, and cholangiocyte-like progenitor cells [2]. Bile duct tubulogenesis is regulated in part by the transcription factor SOX9, as conditional deletion of Sox9 in the fetal mouse liver results in a delay in duct formation. The importance of Notch signaling in controlling biliary differentiation is further supported by studies in the mouse showing that ectopic Notch activity in the liver results in excessive biliary differentiation, while loss of Notch activity leads to a loss of bile ducts [38,39].

In humans, mutations in one allele of *JAG1* or *NOTCH2* results in a syndrome of intrahepatic biliary hypoplasia, facial, cardiovascular, and vertebral anomalies known as Alagille syndrome (Chapter 14) [40]. The multisystem manifestations of JAG/NOTCH haploinsufficency highlight the widespread importance of this pathway and its sensitivity to cellular levels of ligand or receptor.

Both cholangiocyte and hepatocyte differentiation are controlled by networks of transcription factors (Figure 1.4). Conditional deletion of *Hhex* in the mouse liver bud results in abnormal bile ducts and decreased expression of *Hnf6* and the homeodomain transcription factor HNF1β, thereby placing Hhex above these factors in the regulatory hierarchy [41]. Mice in which *Hnf1B* is deleted in the liver bud have a nearly complete lack of mature bile ducts, although the ductal plate forms normally [42]. This implicates HNF1β in the maturation of the ductal plate cells. The function of HNF1β is not strictly limited to cholangiocytes, as the expression of several hepatocyte-specific genes is also disrupted in this mouse model. Loss of *Hnf6* results in significantly decreased HNF1β levels, whereas *Hnf6* expression is normal in livers lacking *HnF1β* [34,35,42]. In zebrafish, forced expression of *hnf1b* rescues biliary defects in larvae in which *hnf6* is knocked down, supporting hnf1b as downstream of hnf6. This implies that the pathway proceeds from HHEX activation of HNF6 to HNF6-mediated activation of HNF1β in cholangiocytes. The importance of HNF1β in bile duct development is supported by the description of an infant with cholestatic jaundice and intrahepatic bile duct paucity associated with a mutation in *HNF1B* [43].

The role of microRNA

As detailed above, numerous transcription factors and signaling pathways have been implicated in hepatobiliary development. Recently, investigators have examined more novel mechanisms involved in development, including the role of microRNAs in liver development. MicroRNAs are short (approximately 22 nucleotides) non-coding RNAs that function as post-transcriptional repressors by RNA-induced silencing. Members of the miR-30 family are expressed in ductal plate and juvenile bile ducts, and inhibition of miR-30a in the zebrafish larva results in functional and structural biliary defects [44]. In cultured hepatoblasts, miR-30 regulated the TGFβ receptor ligand activin. Therefore, miR-30 may function in biliary development partly by regulating TGFβ signaling. The miR-23b cluster is predominantly expressed in hepatoblasts outside the portal area, and like miR-30 it can regulate TGFβ signaling, in this case by targeting ASMAD proteins downstream of the TGFβ receptor. Two other miRNAs, miR-495 and miR-218, may also participate in hepatocyte differentiation by repressing HNF6 and OC2. The study of microRNA in liver development is a young field and it is likely that more regulatory pathways involving microRNAs will be discovered in the future.

Diseases of ductal plate malformation

Defects in ductal plate development result in a class of cholangiopathy known as ductal plate malformations (Chapter 41). Ductal plate malformations are characterized by the retention of ductal plate-like structures in the postnatal and adult liver, often associated with biliary cysts and/or

progressive fibrosis. While the *Hnf6*$^{-/-}$ mouse shows evidence of ductal plate malformation with cysts, no human equivalent disease has been related to *HNF6* mutations. However, haploinsufficiency for HNF1β causes bile duct paucity and ductal plate malformation in humans [45]. In addition, several congenital disorders are linked by ductal plate malformation and defects of the cholangiocyte cilium. These include autosomal recessive polycystic kidney disease, congenital hepatic fibrosis, Caroli disease, Meckel syndrome, Joubert syndrome, and others. In these diseases, cholangiocyte differentiation and initial formation of the primitive periportal ductules proceed normally, but they fail to develop mature bile ducts and the ductal plate structure is retained. This is associated with a failure to establish normal cholangiocyte apical–basal polarity. A unifying feature of these diseases is structural or functional defects of the cholangiocyte cilium. The ductal plate malformations (and their associated genes) illustrate the central importance of cholangiocyte polarity, with a basal region resting on the extracellular matrix and an apical region with a functional cilium (see Chapter 41).

Extrahepatic biliary system development

The extrahepatic bile duct system comprises the left and right hepatic ducts, the common hepatic duct, the cystic duct, the gallbladder, and the common bile duct. The cholangiocytes of the extrahepatic ducts are larger than their intrahepatic counterparts but are otherwise very similar morphologically and as measured by gene expression. The transcription factors HHEX, HNF6, and HNF1β regulate both intra- and extrahepatic bile duct development. In *Hhex*-deficient mice, the extrahepatic bile duct epithelium is converted to an intestinal morphology, whereas loss of *Hnf6* results in gallbladder agenesis and abnormal extrahepatic bile duct differentiation [35,41]. While the gallbladder is present in HNF1β-deficient mice, its epithelium is dysplastic [42].

Despite having these regulators in common, the intra- and extrahepatic systems have distinct embryologic origins. While the intrahepatic ducts are derived from liver bud hepatoblasts, as described above, the extrahepatic bile duct system originates from a distinct foregut region distinguished by the presence of the transcription factors PDX1 and SOX17. This region also gives rise to the ventral pancreas [46]. The Notch target gene *Hes1* in the mouse is activated by Sox17 and then completes a negative feedback loop by repressing *Sox17* expression in the pancreatic precursors. Through an unknown mechanism, *Sox17* and *Hes1* expression are maintained exclusively in the bile duct precursors. Loss of *Hes1* in mice results in extrahepatic bile duct agenesis and ectopic pancreatic tissue in the biliary domain [46,47]. Conditional deletion of *Sox17* results in replacement of the extrahepatic biliary structures with ectopic pancreas tissue. If *Sox17* is ectopically expressed in the mouse, Hhex, HNF6, and HNF1β are upregulated and the ventral pancreas is replaced by bile duct tissue [46]. Collectively, these results depict a pathway in which SOX17 promotes biliary differentiation through several biliary transcription factors, while a negative feedback loop involving HES1 prevents biliary differentiation in the pancreatic zone.

It is not known how continuity is assured between the intra- and extrahepatic biliary systems, given their distinct embryonic origins.

Maintaining the developed liver

As summarized above, hepatoblasts are the precursors for the hepatocytes and cholangiocytes that are present at birth. This differentiation is apparently complete in the sense that no cells remain in a hepatoblast state (as determined by the expression of markers such as CK19 and α-fetoprotein) beyond the neonatal period. This raises the question as to what cells are utilized to derive cholangiocytes and hepatocytes and permit growth and liver regeneration after injury.

In the uninjured or mildly injured liver, it is clear that mature hepatocytes are the source of new hepatocytes, and a similar process is likely to generate new bile ducts [48]. However, a variety of experimental toxic or cholestatic liver injury models have demonstrated that a non-hepatocyte population can also supply new hepatocytes. Known as oval cells in rodents and liver progenitor cells in humans, their exact nature has not yet been fully defined. They arise from cells in or near the canals of Hering; while several marker genes have been associated with the liver progenitor cell, it is not clear if these reflect a single cell type or distinct multipotent cell populations. A recent advance in the field is the use of genetic tools to perform lineage tracing in a conditional manner, thereby allowing cells to be "marked" *prior* to any injury. The marked cells and their progeny can then be detected over the course of an injury response. This approach has revealed that cells morphologically similar to cholangiocytes and positive for the transcription factor Sox9 are capable of giving rise to hepatocytes and cholangiocytes under some conditions in the mouse. Overall, the epithelial cells of the liver can arise from different cell types depending on the presence of hepatic injury, and even the type and degree of injury [49,50].

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Section I

Pathophysiology of pediatric liver disease

Chapter 2

Functional development of the liver

Frederick J. Suchy

Introduction

The liver attains its highest relative size at about 10% of fetal weight at the ninth week of gestation. Early in gestation the liver is the primary site for hematopoiesis. At 7 weeks of gestation, hematopoietic cells outnumber hepatocytes. Primitive hepatocytes are smaller than mature cells and are deficient in glycogen. As the fetus nears term, hepatocytes predominate and enlarge with expansion of the endoplasmic reticulum and accumulation of glycogen. Hepatic blood flow, plasma protein binding, and intrinsic clearance by the liver (reflected in the maximal enzymatic and transport capacity of the liver) also undergo significant postnatal maturation. These changes correlate with an increased capacity for hepatic metabolism and detoxification. At birth, the liver constitutes about 4% of body weight compared with 2% in the adult. Liver weight doubles by 12 months of age and increases three-fold by 3 years of age.

The functional development of the liver that occurs in concert with growth requires a complicated orchestration of changes in hepatic enzymes and metabolic pathways that result in the mature capacity of the liver to undertake metabolism, biotransformation, and vectorial transport. Greengard has established a paradigm for hepatic development based on a group of several hepatic enzymes studied in the rat and less extensively in humans. In one pattern of hepatic development, enzymatic activity is high in a fetus and falls during postnatal development. Examples would include thymidine kinase and ornithine decarboxylase [1]. The activities of other enzymes are expressed initially during early fetal development and continue to increase progressively after birth. Examples include glutamate dehydrogenase, fructose-1,6-diphosphatase, and aspartate aminotransferase [1]. Another group of enzymes is expressed perinatally and continues to increase progressively after birth. These enzymes include phosphoenolpyruvate carboxykinase (PEPCK) and uridine 5′-diphosphate glucuronyltransferase (UGT). A final pattern of development occurs with enzymes that are expressed significantly after birth and peak at weaning, including alanine aminotransferase and alcohol dehydrogenase.

The stepwise appearance of new groups of enzymes during development may be related causally to sequential changes in the level of circulating hormones [1]. For example, total serum thyroxine and triiodothyronine levels of the human fetus undergo a sudden increase between the ninth and tenth weeks of gestation. Similarly, fetal plasma concentrations of cortisol and cortisone are as high by the third month of gestation as at term. There is also a sudden increase in plasma glucagon at birth, which may influence the expression of the neonatal cluster of enzymes in rat liver. The final steps of biochemical differentiation, including the synthesis of enzymes necessary to process a solid diet, occur just before weaning in the rat. A natural surge in cortisone and thyroxine at this time may be important in mediating this change.

With advances in cellular and molecular biology, mechanisms underlying these developmental changes have been found to be extremely complicated and regulated at transcriptional, translational, and post-translational levels. A complete discussion of this topic is beyond the scope of this review. Only selected examples of developmental changes in the functional capacity of the liver are discussed, particularly those with relevance to understanding susceptibility to liver disease.

Hepatic energy metabolism in the fetus and neonate

There is increasing epidemiological and experimental evidence that perturbations of perinatal energy homeostasis including maternal diabetes, maternal obesity or malnutrition, and intrauterine growth retardation can predispose to obesity and the metabolic syndrome later in life. Therefore, non-alcoholic fatty liver disease (NAFLD), the most common liver disorder in adults and children and a feature of the metabolic syndrome, in part has its origin in the fetus. Recent findings indicate that epigenetic methylation of critical genes involved in metabolism detected in human fetal tissues at birth is strongly associated with childhood total and central body adiposity, factors repeatedly found in patients with NAFLD [2].

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