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Molecular Mechanisms of Bile Duct Development

Yiwei Zong and Ben Z. Stanger*

Department of Medicine, Abramson Family Cancer Research Institute, University of Pennsylvania, School of Medicine, Philadelphia, PA, USA

Abstract

The mammalian biliary system, consisting of the intrahepatic and extrahepatic bile ducts, is responsible for transporting bile from the liver to the intestine. Bile duct dysfunction, as is seen in some congenital biliary diseases such as Alagille syndrome and biliary atresia, can lead to the accumulation of bile in the liver, preventing the excretion of detoxification products and ultimately leading to liver damage. Bile duct formation requires coordinated cell-cell interactions, resulting in the regulation of cell differentiation and morphogenesis. Multiple signaling molecules and transcription factors have been identified as important regulators of bile duct development. This review summarizes recent progress in the field. Insights gained from studies of the molecular mechanisms of bile duct development have the potential to reveal novel mechanisms of differentiation and morphogenesis in addition to potential targets for therapy of bile duct disorders.

Keywords

Development; Cholangiocyte; Biliary; Differentiation; Morphogenesis

Introduction

The liver is an important synthetic and metabolic organ whose roles include producing serum proteins and bile for fat emulsification, modification and secretion of toxins, and serving as the site for prenatal hematopoiesis. This review will outline the important morphogenetic and molecular events that underlie development of the bile ducts of the liver. The liver domain is specified from a bipotential field of foregut endoderm (Zaret and Grompe, 2008), a region that has the capacity to become either the pancreas or the liver. Following inductive signaling from structures including blood vessels, the notochord, and cardiogenic mesoderm, two distinct domains are observed: a pancreatic domain consisting of progenitor cells that express the homeobox transcription factor Pdx1, and a liver domain consisting of cells that express albumin (Figure 1). Further signals from fibroblast growth factors (FGFs) and bone morphogenetic proteins (BMPs) promote emergence of the liver bud as committed hepatic progenitor cells proliferate and delaminate from the gut tube (Lemaigre and Zaret, 2004; Lemaigre, 2009; Zaret, 2002). These liver progenitor cells migrate into the septum transversum mesenchyme, where they interact with endothelial cells that provide additional signals to induce liver development. These progenitor cells (also called hepatoblasts) are bipotential cells that serve as the source for the two major functional

*corresponding author: bstanger@exchange.upenn.edu.

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cell types in the liver: hepatocytes and cholangiocytes. Later in development, these cells are further organized into a lobular three-dimensional structure that subserves normal liver function.

One of the important functions of the liver is to produce and transport bile. Bile contains cholesterol, bile pigments, bile acids, phospholipids and bicarbonate. Bile plays an important role in metazoan biology by emulsifying fats and transporting the products of liver detoxification. After its synthesis in hepatocytes, bile is secreted into the canaliculi, a channel that exists between the apical membranes of hepatocytes. From the canaliculi, bile flows into a separate ductal system. Bile ducts are lined by cholangiocytes (also called biliary epithelial cells, or BECs), which are responsible for transporting bile from the liver to the intestine. Dysfunction of bile ducts can lead to the accumulation of bile in the liver, preventing the excretion of detoxifying products and ultimately leading to liver damage due to bile acid-mediated injury.

The mammalian biliary system is a highly branched tubular network, consisting of intrahepatic (IHBDs) and extrahepatic bile ducts (EHBDs). IHBDs are derived from liver progenitor cells, whereas EHBDs appear to arise directly from the endoderm, through a separate process of branching morphogenesis. Bile duct formation requires coordinated cell-cell interactions, resulting in the regulation of cell differentiation and morphogenesis (Raynaud et al., 2009). Developmental defects of the biliary system lead to a number of diseases and are a major indication for pediatric liver transplantation.

Extrahepatic bile duct development

The extrahepatic biliary system is comprised of the hepatic ducts, cystic duct, common bile duct, and the gallbladder. It arises from a region of ventral foregut proximate to liver and ventral pancreas. Interestingly, although the extrahepatic and intrahepatic biliary systems are ultimately in direct contact, their origins are distinct, as lineage tracing studies have shown that EHBDs share a common origin with ventral pancreas but not the liver. Using a Pdx1-Cre mouse strain, Spence and colleagues (Spence et al., 2009) showed that both the extrahepatic biliary system and the ventral pancreas are derived from Pdx1+ cells, while the liver and IHBDs is not. This suggests that the EHBDs/ventral pancreas and the liver are derived from distinct progenitors (Figure 1).

The Sox17 transcription factor is likely to be a critical determinant of how cells within the Pdx1 domain are assigned to the two different fates of pancreas versus EHBD. At approximately E8.5 of mouse development (total mouse gestation is 18.5 days), cells in the ventral foregut express both Pdx1 and Sox17. Subsequently, the Sox17+/Pdx1+ domain becomes divided into a Sox17+ biliary primordium and a Pdx1+ pancreatic primordium. Inactivation of Sox17 in the ventral foregut causes the loss of EHBDs and leads to the formation of ectopic pancreatic tissue, while misexpression of Sox17 in the Pdx1+ domain suppresses pancreatic development and induces a ductal epithelial fate, suggesting that Sox17 regulates the EHBD/pancreatic cell fate decision.

The appropriate segregation of EHBD and ventral pancreatic lineages is also regulated by Hes1, a transcriptional effector of Notch signaling. Hes1-deficient mice exhibit gallbladder agenesis and ectopic pancreas formation, suggesting that Hes1 is necessary to repress a pancreatic fate within EHBDs (Fukuda et al., 2006; Sumazaki et al., 2004). This phenotype is similar to that of Sox17 conditional knock-out mice, suggesting an interaction between Hes1 and Sox17 during EHBD development. Indeed, inactivation of Sox17 results in a down-regulation of Hes1 expression, while inactivation of Hes1 leads to a paradoxical expansion of the Sox17-domain (Spence et al., 2009; Zaret and Grompe, 2008), suggesting the existence of a Sox17-Hes1 feedback loop.

Other transcription factors required for proper EHBD development include HNF6, HNF1 β , and Hex. Inactivation of HNF6 or HNF1 β results in hypoplasia of the extrahepatic biliary system (Clotman et al., 2002; Coffinier et al., 2002), while disruption of Hex in the foregut converts EHBDs to duodenum-like tissue (Hunter et al., 2007). Misexpression of Sox17 results in an upregulation of HNF6, HNF1 β and Hex expression (Spence et al., 2009), suggesting that these transcription factors are downstream effectors of Sox17. Although the extrahepatic and intrahepatic biliary systems have different origins, they connect to each other to form a continuous biliary network. As formation of the extrahepatic biliary system precedes that of intrahepatic bile ducts, and IHBDs mature in a hilum-to-periphery orientation, one enticing possibility is that extrahepatic biliary cells are involved in the induction of the intrahepatic biliary system.

Intrahepatic bile duct development

IHBDs are located adjacent to portal veins within the liver. Bile drains from the hepatocyte-lined bile canaliculi into IHBDs through a transitional region called the Canal of Hering. Both hepatocytes and cholangiocytes are derived from hepatoblasts. Several steps involving differentiation and morphogenesis are required for normal IHBD development. First, periportal hepatoblasts are induced to differentiate into cholangiocyte precursors, forming ring structures known as “ductal plates” around the portal vein branches. At discrete locations within the ductal plates, tubular structures form which eventually give rise to bile ducts. Subsequently, precursors that fail to be incorporated into the bile ducts regress, leaving mature ducts in place (Figure 2) (Clotman et al., 2002; Lemaigre, 2003; Shiojiri and Katayama, 1987).

It is unclear when periportal hepatoblasts first become committed to the biliary lineage. The biliary marker Sox9 is expressed in a subset of periportal hepatoblasts as early as E11.5 of mouse development, suggesting that biliary specification is initiated prior to this timepoint (Antoniou et al., 2009). Interestingly, the differentiation of biliary epithelial cells is not synchronized but instead occurs in a sequential manner (Antoniou et al., 2009; Zong et al., 2009). Specifically, hepatoblasts on the portal side of the ductal plate differentiate earlier than cells on the parenchymal side. This results initially in a tubular structure lined by cells expressing biliary markers on the portal side and hepatoblast markers on the parenchymal side. This asymmetry resolves with subsequent maturation, as cells on the parenchymal side complete a biliary program and a symmetrical bile duct emerges (Figure 2). In the following section, we will discuss some of the specific signaling molecules that account for this unique form of sequential differentiation.

Signals regulating biliary differentiation

TGF β signaling

The TGF β pathway was the first pathway shown to regulate liver cell differentiation *in vivo*. Prior to ductal plate formation, a gradient of Activin/TGF β signaling – with high levels in the periportal region and low levels in the parenchymal region – is generated (Clotman et al., 2005; Clotman and Lemaigre, 2006). This gradient plays an important role in biliary differentiation, as blocking TGF β signaling *in vivo* with anti-TGF β antibodies represses biliary differentiation, while incubation of liver explants with TGF β promotes ectopic biliary differentiation (Clotman et al., 2005). Consistent with the notion that TGF β directly promotes biliary differentiation, TGF β 1, 2, and 3 are all able to induce the expression of biliary markers and repress the expression of hepatocyte markers (HNF4 α , albumin, apolipoprotein A, and transthyretin) in *in vitro* cultured hepatoblasts (Antoniou et al., 2009). Furthermore, deletion of one copy of Smad2 and Smad3 – mediators of transcription induced by TGF β signaling, results in a disruption of hepatic architecture (Weinstein et al.,

2001). Thus, TGF β signaling appears to directly regulate biliary differentiation in a spatially constrained manner.

What controls the shape of the TGF β gradient? One determinant appears to be the transcription factors HNF6 and OC-2., because in the absence of these factors the TGF β gradient is disrupted and TGF β signaling is detected throughout the parenchyma (Clotman, 2005). In parallel, regulation of TGF β expression also contributes to the formation of this signaling gradient. While TGF β 1 is expressed widely in the liver, both TGF β 2 and TGF β 3 are expressed predominantly in the periportal region (Antoniou et al., 2009; Clotman and Lemaigre, 2006). Finally, a third mechanism that is used to generate the TGF β signaling gradient depends on microRNA expression. Rogler and coworkers have demonstrated that microRNA-23b is dominantly expressed in parenchymal region but not in periportal region and that this microRNA-23b is able to down-regulate TGF β signaling by targeting Smads mRNAs (Rogler et al., 2009). Knockdown of miR-23b promotes the expression of biliary markers in a fetal liver stem cell line (HBC-3), while ectopic expression of miR-23b represses the biliary differentiation of HBC-3 cell line.

Notch signaling

Notch signaling controls cell fate determination in a number of tissues. Mutations in the Notch signaling ligand Jag1 or receptor Notch2 are responsible for Alagille syndrome (AGS) (Li et al., 1997; McDaniell et al., 2006; Oda et al., 1997), a bile duct paucity condition discussed in detail below. Mice doubly heterozygous for Jag1 and Notch2 mutations recapitulate the AGS phenotype including bile duct paucity (McCright et al., 2002). Activating Notch signaling in isolated liver progenitor cells results in the upregulation of biliary markers *in vitro* (Tanimizu and Miyajima, 2004). Conditional deletion of RBPJ, an essential component of canonical Notch signaling, leads to a reduced number of biliary epithelial cells at E16.5, confirming a role of Notch signaling in biliary fate specification (Zong et al., 2009). Moreover, misexpression of constitutively active forms of either Notch1 or Notch2 is sufficient to induce ectopic biliary cell differentiation and tubule formation (Tchorz et al., 2009; Zong et al., 2009).

Interestingly, inactivation of Hes1 or Notch2 in mice does not disrupt biliary differentiation (Cheng et al., 2007; Geisler et al., 2008; Kodama et al., 2004; Lozier et al., 2008), but rather leads to defective morphogenesis. One possible explanation for this result is functional redundancy with compensation by one or more of the multiple Notch signaling components that are expressed in the embryonic liver (Kodama et al., 2004; Loomes et al., 2002; Nijjar et al., 2001). Another possible explanation for intact biliary differentiation in gene deletion studies utilizing conditional Notch2 mutants is that the gene was deleted relatively late in liver development, possibly after biliary fate determination had already occurred (Geisler et al., 2008; Hunter et al., 2007; Lozier et al., 2008; Zong et al., 2009).

Because Notch signaling requires cell-cell contacts, the most likely sources of Notch ligand during ductal plate induction are the endothelial cells comprising the portal veins, or the closely associated portal mesenchyme. Indeed, Jag1 is first expressed by portal (but not central) endothelial cells, and its expression domain later expands to include mesenchymal cells as well as biliary precursor cells (Flynn et al., 2004; Kodama et al., 2004; Loomes et al., 2002; Louis et al., 1999; McCright et al., 2002; Tanimizu and Miyajima, 2004; Zong et al., 2009). This expression pattern likely underlies the two-step process by which bile ducts develop. Endothelial/mesenchymal expression of Jag1 is likely to initiate Notch signaling in the apposing hepatoblasts, triggering their biliary (Kodama et al., 2004; Loomes et al., 2002; McCright et al., 2002; Tanimizu and Miyajima, 2004). These cells, which comprise the “first layer” of the ductal plate, express Jag1 and induce the adjacent hepatoblasts to adopt a biliary program (Figure 2; Zong et al., 2009). Ensuing tubulogenesis, which accompanies

induction of a second layer, occurs in discrete positions within the ductal plate. The factors that determine the position of tubule formation are unclear at present.

Wnt/ β -catenin pathway

Several studies have implicated Wnt signaling in biliary fate determination. Expression of β -catenin, a key component of the canonical Wnt pathway, is temporally regulated during hepatoblast differentiation, reaching its highest level at E12.5 followed by a decrease in signaling over the next several days and a complete loss after E16.5 (Micsenyi et al., 2004). Significantly, deletion of β -catenin in the liver is associated with a paucity of primitive bile ducts and defects in hepatoblast proliferation and survival (Tan et al., 2008). Conversely, constitutive activation of the Wnt/ β -catenin pathway by deletion of APC promotes biliary differentiation and suppresses hepatocyte differentiation (Decaens et al., 2008). Furthermore, Wnt-3A induces ectopic biliary differentiation in ex vivo liver culture (Hussain et al., 2004). Thus, it appears that Wnt/ β -catenin signals – like TGF β and Notch signals – seem to exert a positive influence on biliary differentiation. The possibility of an epistatic relationship between these pathways remains to be addressed.

Regulation of biliary morphogenesis

Coordinating differentiation and tubulogenesis by transient asymmetry—

Biliary morphogenesis is the process by which biliary epithelial cells establish polarity and cell contacts, organizing into functional duct structures. Lumen formation is a critical step in tubulogenesis. The conventional view regarding tubulogenesis had been that cholangiocyte differentiation preceded lumen formation in the ductal plates. However recent studies suggest that differentiation and morphogenesis are closely coordinated. Nascent bile ducts pass through a previously undescribed intermediated stage characterized by the asymmetric expression of biliary and hepatoblasts markers (Antoniou et al., 2009; Zong et al., 2009). Specifically, at this transient stage, biliary markers (CK19, SOX9, OPN, HNF1 β , etc.) are expressed by the cells on the portal side (the first biliary layer) of the lumens, but not the parenchymal side (the second biliary layer). By contrast, HNF4 α , a hepatoblasts marker, is expressed by cells on the parenchymal side, but not the portal side, of the newly formed ductal plate. Bile duct lumens form after the differentiation of the first layer cells but preceding differentiation of the second layer cells, suggesting that biliary differentiation and tubulogenesis are integrated during bile duct development.

The mechanisms underlying asymmetric tubulogenesis seem to involve both Notch and TGF β signaling. Transient asymmetry is a consequence of the sequential differentiation of the two layer biliary epithelial cells in the ductal plate. Both Jag1 and Hes1 exhibit an asymmetric expression pattern, with both proteins initially detected on the portal side of the ductal plate and later expanding to the parenchymal side (Zong et al., 2009). Importantly, Hes1 is expressed in the second layer cells preceding their expression of biliary markers. TGF β signaling is another signal regulating the asymmetric differentiation (Antoniou et al., 2009). TGF β 2, TGF β 3 and TGF β receptor II show asymmetric distribution, with expression localized only to the cells on the parenchymal side.

The transcription factor Sox9 regulates the timing of asymmetric tubule maturation, as the absence of Sox9 results in a disruption of bile duct morphogenesis. In particular, the maturation from asymmetric to symmetric bile ducts is delayed in Sox9 mutants (Antoniou et al., 2009). Sox9 may be a direct target of Notch signaling, as expression of a constitutively active form of Notch1 results in an upregulation of Sox9 expression in vivo, and Notch1 is capable of binding directly to the Sox9 promoter (Zong et al., 2009). Conversely, there is evidence that the Notch target Hes1 lies downstream of Sox9 (Antoniou

et al., 2009; Seymour et al., 2007). One possibility to reconcile these findings is that Sox9 and Notch are part of a cross-regulatory positive feedback loop.

Notably, an alternative model of biliary tubulogenesis was proposed in which some cholangiocytes in the first layer of the ductal plate fold up to form a double layer by cell migration (Tanimizu et al., 2009). This model was based on the performance of hepatoblasts in a 3-D cell culture system (Tanimizu et al., 2007; Tanimizu et al., 2009). Time course movie of this culture system showed that hepatoblasts first formed a monolayer; later some cells moved vertically to form the second layer. The formation of the second cell layer was independent of cell proliferation but depended on PI3K/Akt pathway. However, it is important to notice the dramatic difference of environment between the culture system *in vitro* and the liver *in vivo*. For instance, the first layer of cholangiocytes *in vivo* contacts mesenchymal cells on the portal side and hepatoblasts on the parenchyma side, while neither of the contacts exist in the cell culture system. Although the cell migration model still needs to be tested through *in vivo* studies, this cell culture system provided a new tool to dissect the molecular mechanisms of biliary morphogenesis.

Transcriptional network in IHBD development—Two members of the onecut family of transcription factors play an important role in the regulation of IHBD development. Onecut 1 (OC-1; HNF6) was the first transcription factor to be identified as a regulator of biliary development (Clotman et al., 2002). It is highly expressed in biliary precursor cells and is expressed at a much lower level in embryonic hepatoblasts. Loss of OC-1 is associated with defects in both biliary differentiation and morphogenesis, possibly because of the close association between these two processes during bile duct development. Absence of OC-1 causes premature differentiation of biliary epithelial cells, which form cord-like extensions within the liver parenchyma as early as E13.5. In OC-1-null livers, the ductal plates are replaced by abnormal cystic structures which later regress. Deletion of both OC-1 and another onecut transcription factor, onecut 2 (OC-2), results in the emergence of hybrid cells that co-express both hepatocyte and biliary markers. These results suggest that OC-1 and OC-2 act as inhibitors of biliary differentiation and are consistent with their role in shaping the TGF β signaling gradient from the portal region to liver parenchyma (Clotman et al., 2005).

The HNF1 β homeodomain transcription factor is enriched in biliary precursor cells. Deletion of HNF1 β causes a disorganized ductal plate with irregular lumens at E17.5 and postnatal IHBD paucity, a phenotype similar to that observed in HNF6-deficient livers (Coffinier et al., 2002). Expression of HNF1 β is strongly down-regulated in HNF6-null livers, while expression of HNF6 does not change significantly in HNF1 β -null liver, suggesting that HNF1 β is downstream of HNF6 in a transcription factor cascade (Clotman et al., 2002; Coffinier et al., 2002). The HNF6/HNF1 β cascade may also involve C/EBP α (CCAAT/enhancer binding protein α), a basic-leucine zipper transcription factor (Yamasaki et al., 2006). C/EBP α is able to bind to the promoter region of the OC-1 gene and inhibit its expression (Rastegar et al., 2000). Deletion of C/EBP α inhibits hepatocyte maturation and increases the expression of both OC-1 and HNF1 β in the liver (Yamasaki et al., 2006). In wild-type liver, the expression of C/EBP α is suppressed in the periportal hepatoblasts that will ultimately differentiate down a biliary lineage (Rastegar et al., 2000; Yamasaki et al., 2006). However, deletion of C/EBP α alone is not sufficient to induce biliary differentiation (Yamasaki et al., 2006). The repression of C/EBP α in periportal hepatoblasts relies on Sox9, as the absence of Sox9 leads to the persistent expression of C/EBP α in biliary epithelial cells even at E18.5 (Antonioni et al., 2009). An *in vitro* study using FACS sorted DLK+ hepatoblasts showed that Notch signaling is able to decrease the expression of C/EBP α (Tanimizu and Miyajima, 2004).

Another transcription factor, Prox1, exhibits an expression pattern that resembles that of C/EBP α . Prox1 is expressed in hepatoblasts and persisted into hepatocytes but absent from biliary epithelial cells, suggesting that Prox1 may promote a hepatocyte fate and inhibit a biliary fate (Dudas et al., 2004). The exact role of Prox1 in biliary development remains to be defined.

Hex, a homeobox-containing transcription factor that is essential for the formation of the liver primordium (Bort et al., 2006; Keng et al., 2000; Martinez Barbera et al., 2000) is also required for proper development of the biliary system. Early deletion of Hex results in a loss of expression of HNF4 α and HNF6 in hepatoblasts, therefore disrupting the normal hepatoblasts differentiation. Deletion of Hex somewhat later in development is associated with a loss of HNF1 β expression and results in the formation of abnormal cyst structures in the livers (Hunter et al., 2007).

Tbx3, a member of the T-box family of transcription factors, is able to promote hepatoblast proliferation and hepatocyte differentiation while simultaneously inhibiting biliary differentiation. At E14.5, Tbx3-deficient livers showed a paucity of hepatocyte markers but instead exhibited expression of biliary markers (Ludtke et al., 2009). The authors concluded that Tbx3 controls hepatoblast fate through the regulation of a number of fate-determining transcription factors, including C/EBP α , HNF4 α , HNF1 β , and HNF6 (Ludtke et al., 2009; Suzuki et al., 2008).

The proliferation and expansion of biliary cells is controlled by the forkhead box proteins A1 and A2 (Foxa1 and Foxa2). These transcription factors are required for liver specification as no liver bud is formed in the endoderm deficient of Foxa1 and Foxa2 (Lee et al., 2005). Liver-specific ablation of Foxa1 and Foxa2 causes bile duct hyperplasia and fibrosis due to the upregulation of IL-6, a proliferative signal for cholangiocytes (Li et al., 2009). Expression of Foxa1 is upregulated in HNF6 null livers, probably due to the upregulation of TGF β signaling (Plumb-Rudewicz et al., 2004).

Finally, Sall4 – a zinc finger transcription factor – also plays a role in the hepatocyte vs. cholangiocyte cell fate decision. Sall4 is expressed in fetal hepatoblasts but not adult hepatocytes. In-vitro studies have demonstrated that overexpression of Sall4 promotes biliary differentiation at the expense of hepatocyte maturation (Oikawa et al., 2009).

MicroRNA in bile duct development

MicroRNAs (miRNA) are single-stranded, small non-coding RNAs of 21 to 23 nucleotides. They down-regulate the expression of target genes through complementarily binding with 3' UTR of target mRNA, leading to mRNA degradation or translation repression. miRNAs have been shown of importance in varieties of biological process. Recent discoveries have started to dissect the role of miRNA in bile duct development and liver function. A genome-wide study identified a set of 38 miRNA whose expression in liver change significantly along the process of liver development (from late gestation to the perinatal period) (Hand et al., 2009a). Among the 38 miRNAs, miR-30a and miR-30c are found to be expressed specifically in cholangiocytes. Additionally, removing miR-30a in zebrafish caused defects in bile duct formation, suggesting that miR-30a is required for biliary development. On the other hand, global loss of miRNAs by conditional deletion of Dicer in the liver did not lead to significant liver function defects until two month old (Hand et al., 2009b). It is probably due to the fact that the cre-mediated knock-out of Dicer occurred only after biliary development, therefore not disrupt bile duct formation.

Diseases of the Biliary System

Alagille syndrome

Alagille syndrome is an autosomal-dominant congenital disorder which affects the development of multiple organs including the liver, heart, eye, and skeleton. A cardinal feature of this disease is bile duct paucity, which causes bile accumulation in the liver. Mutations in *Jag1* are responsible for over 90% of Alagille syndrome cases, and the mutations in *Notch2* accounts for a smaller fraction of cases (Li et al., 1997; McDaniell et al., 2006; Oda et al., 1997). Interestingly, liver-specific inactivation of *Notch2* but not *Notch1* in mice disrupts intrahepatic bile duct development in mice, consistent the identification of *Notch2* as the dominant receptor responsible for bile duct development (Geisler et al., 2008).

Importantly, inheriting a single mutant *Jag1* or *Notch2* allele is sufficient to cause Alagille syndrome in humans, suggesting that bile duct development is sensitive to the dosage of Notch signaling activity *in vivo* (Artavanis-Tsakonas, 1997; Li et al., 1997; McDaniell et al., 2006; Oda et al., 1997). However, heterozygosity for a *Jag1* null allele in mice does not result in biliary abnormalities unless combined with a *Notch2* hypomorphic allele (McCright et al., 2002). Furthermore, although liver-specific deletion of *Notch2* results in IHBD paucity (Geisler et al., 2008; Loomes et al., 2007; Lozier et al., 2008), deletion of *Jag1* from endoderm-derived liver epithelial cells results in results in a paradoxical bile duct proliferation (Geisler et al., 2008; Loomes et al., 2007; Lozier et al., 2008). These results suggests fundamental differences between mouse and man with respect to either the dose of Notch signaling required for proper bile duct development. Alternatively, these results may indicate a requirement for additional modifier genes present in some human patients but absent from the mouse strains used in these studies, or differences in the source of ligand.

Patients with *Notch2* mutations may have different disease profile from that of patients with *Jag1* mutations. For example, *Notch2* mutations are associated with more severe renal disease (McDaniell et al., 2006). However, this difference alone cannot account for the wide phenotypic variability of AGS. Fringe genes, which regulate Notch ligand-receptor specificity, may be one source of phenotypic variability in AGS (Ryan et al., 2008). However, other genes – including the signaling molecules and transcription factors described above – are likely to play a role in determining the severity and phenotypic spectrum of AGS.

Biliary atresia

Biliary atresia (BA) is a rare condition unique to newborn infants (Kohsaka et al., 2002; Mieli-Vergani and Vergani, 2009). BA is the most frequent indication for liver transplantation in infants and is characterized by bile duct obliteration and/or obstruction. While EHBDs are more severely affected, BA involves both the extrahepatic and intrahepatic biliary systems. The precise etiology of BA is unknown, but in most cases BA is associated with an intensive inflammatory infiltrate. The presence of inflammation has led to speculation that BA results from an infectious or autoimmune destruction of the bile ducts. For example, infection of newborn mice with Rhesus Rotavirus results in a BA-like disease (Czech-Schmidt et al., 2001; Petersen et al., 1997; Riepenhoff-Talty et al., 1993). This topic has been reviewed extensively elsewhere (Mack, 2007).

Developmental errors have been proposed as an alternative explanation for the pathophysiology of BA, possibly through the leakage of bile and consequent inflammation. In one genetic study that examined 102 cases of biliary atresia, 9 patients harbored Jagged1 missense mutation, suggesting that abnormal Notch signaling may predispose to BA (Kohsaka et al., 2002). This study also found that the mutated *Jag1* protein had less ability

than the wild-type form to repress the expression of interleukin-8 upon stimulation with tumor necrosis factor α . Another study investigated the expression of Notch receptors in biliary atresia livers and found an increase of Notch3 expression in neovessels and mesenchymal cells (Flynn et al., 2004).

The CFC1 gene, which encodes the Cryptic protein, has also been proposed to play an etiologic role in BA. Cryptic acts as a cofactor for Nodal signaling and is implicated in L-R axis development (Bamford et al., 2000). Interestingly, about 10% of biliary atresia patients also have additional congenital defects related to left-right axis abnormalities, suggesting a link between signals that regulate bile duct development and those that regulate body *situs*. In a recent study, a CFC1 polymorphism (Ala145Thr) was identified in 5 out of 10 patients, twice the frequency observed in control patients (Davit-Spraul et al., 2008). However this altered form of Cryptic is not sufficient to induce disease, since BA occurred in only one of two brothers harboring the same amino acid substitution (Jacquemin et al., 2002). Another gene regulating L-R axis development, the Inversin gene (*Inv*), may also involve predispose to BA, as mice with a deletion of the *Inv* gene exhibit BA-like disease (Mazziotti et al., 1999). However anomalies of biliary system were not found in humans with *Inv* mutations (Schon et al., 2002).

Polycystic liver diseases

Polycystic liver disease (PLD) is a congenital disorder featured by presence of biliary cysts which are not connected to the main biliary network (Gunay-Aygun, 2009). PLD is believed to be caused by defects in the ductal plate morphogenesis during embryonic development (Veigel et al., 2009). Interestingly, PLD is usually associated with a renal anomaly called autosomal dominant polycystic kidney disease (ADPKD), suggesting a certain universal mechanism underlying tubular formation and/or function among different organs. Mutations in the PKD1 or PKD2 genes are responsible for most of the ADPKD cases (Gabow, 1993; Reeders et al., 1985). PKD1 and PKD2 encode integral membrane proteins Polycystin-1 and Polycystin-2, respectively, which form a multi-protein complex with other components that localizes to primary cilia, a microtubule-based structure projecting into the lumens of bile ducts from the apical surfaces of cholangiocytes (Adams et al., 2008; Hughes et al., 1995; Mochizuki et al., 1996). Polycystin-1 and Polycystin-2 regulate intracellular calcium levels, aiding in the ability of primary cilia to function as flow sensors (Everson et al., 2004; Masyuk et al., 2006). Another kind of PLD, called autosomal dominant polycystic liver disease (ADPLD), is not associated with any renal diseases and is associated with mutations in the PRKCSH and SEC63 genes (Davila et al., 2004; Drenth et al., 2003; Li et al., 2003). The proteins encoded by these two genes are components of protein complexes involved in protein glycosylation in the endoplasmic reticulum (Drenth et al., 2005).

Conclusions

Because of the morbidity associated with bile duct dysfunction, significant effort has gone into understanding the mechanisms underlying bile duct development. Several key concepts that govern IHBD development have emerged: 1) IHBDs arise from bipotential precursor cells through local signaling events near branches of the portal vein; 2) IHBD development relies on the activity of several cell-cell signaling molecules (in particular, Notch and TGF β) as well as network of transcription factors to confer spatial differentiation clues; 3) IHBD differentiation and morphogenesis are closely coordinated during bile duct development. EHBD development is less well understood, although embryological origins of EHBDs and IHBDs are distinct. Critical transcription factors that distinguish EHBDs from other ventral foregut structures have been delineated, but the mechanisms by which continuity between the extrahepatic and intrahepatic biliary tree is achieved remain obscure. Likewise, factors that dictate the site of tubulogenesis within the ductal plate remain unknown. Better tools for

imaging bile duct development in organ culture or living embryos will help to address these questions and potentially lead to methods for manipulating bile duct number or function under conditions of bile duct paucity or obstruction.

Abbreviations

IHBD	intrahepatic bile duct
EHBD	extrahepatic bile duct

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Figure 1.
Cell lineage determination of ventral foregut endoderm.

Figure 2.
the development of IHBD

Table 1

Signaling pathways regulating biliary development

Signaling pathway	Functions in the IHBD development
Notch	Promote biliary differentiation and tubulogenesis. (Geisler et al., 2008; Kodama et al., 2004; Lozier et al., 2008; McCright et al., 2002; Tanimizu and Miyajima, 2004; Tchorz et al., 2009; Zong et al., 2009)
TGF β	Promote biliary differentiation. (Clotman et al., 2005; Clotman and Lemaigre, 2006)
Wnt/ β -Catenin	Stimulate hepatoblast proliferation and biliary differentiation. (Decaens et al., 2008; Hussain et al., 2004; Tan et al., 2008a, b)

Table 2

Transcription factors regulating biliary development.

Transcription factors	Functions in IHBD development
C/EBP α	Inhibit biliary differentiation; suppress HNF6 and HNF1 β expression. (Shiojiri et al., 2004; Yamasaki et al., 2006)
FoxA1	Repress cholangiocyte proliferation. (Li et al., 2009)
FoxA2	Repress cholangiocyte proliferation. (Li et al., 2009)
FoxM	Promote biliary differentiation. (Krupczak-Hollis et al., 2004)
Hex	Control biliary differentiation and morphogenesis; regulate the expression of HNF4 α , HNF1 β and HNF6. (Hunter et al., 2007)
Hes1	Promote tubulogenesis and ductal plate remodeling
HNF1 β	Regulate bile duct morphogenesis. (Coffinier et al., 2002)
HNF6	Regulate biliary differentiation and morphogenesis. (Clotman et al., 2002)
OC2	Promote biliary differentiation; regulate biliary morphogenesis. (Margagliotti et al., 2007)
Prox1	Regulate hepatoblast differentiation. (Dudas et al., 2004)
Sox9	Temporally regulate the completion of primitive asymmetric duct. (Antoniou et al., 2009)
Sall4	Promote biliary fate determination and inhibit hepatocyte differentiation. (Oikawa et al., 2009)
Tbx3	Inhibit biliary differentiation and promote hepatocyte differentiation. (Suzuki et al., 2008)