Endodermal and mesenchymal cross talk: a crossroad for the maturation of foregut organs

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The developmental stages of each foregut organ are intimately linked to the development of the other foregut organs such that the ultimate function of any one foregut organ, such as the metabolic function of the liver, depends on organizational changes associated with the maturation of multiple foregut organs. These changes include: (i) proliferation of the intrahepatic bile ducts and hepatoblasts within the liver coinciding with parenchymal expansion, (ii) elongation of extrahepatic bile ducts, which allows for proper gallbladder (GB) formation, and (iii) duodenal elongation and rotation, which coincides with all of the above to connect the intrahepatic, extrahepatic, and pancreatic ductal systems with the intestine. It is well established that cross talk between endodermal and mesenchymal components of the foregut occurs, particularly regarding the vascularization of developing organs. Furthermore, genetic mutations in mesenchymal and hepatic compartments of the developing foregut result in similar foregut pathologies: hypoplastic liver, absence of GB, biliary atresia (intrahepatic and/or extrahepatic), and failure of gut elongation and rotation. Finally, these shared pathologies can be linked to deficiencies in genes specific to the septum transversum mesenchyme (*Hes1*, *Hlx*, and *Foxf1*) or liver (*Hhex* and *Hnf6*), illustrating the complexity of such cross talk.

EVOLUTION AND DEVELOPMENT OF THE FOREGUT ORGANS: MOLECULAR, ANATOMICAL, AND FUNCTIONAL OVERLAP

Over the past decade, much information has been revealed concerning the developmental and pathological processes involving foregut endodermal organs (liver, intestine, pancreas, etc.). The primitive gut segregates into three distinct domains: foregut, midgut, and hindgut, and specification of definitive endodermal (DE) cells in the ventral foregut endoderm occurs at around E8.5 in the mouse. These foregut DE cells eventually give rise to the endodermal components of the liver (extrahepatic and intrahepatic), pancreas, and intestine (1). For example, multipotent DE cells of the hepatic diverticulum (primitive liver bud) undergo epithelial–mesenchymal transition (EMT) and invade the adjacent septum transversum mesenchyme (STM) to form the liver bud at E9.5–10.5 (2). These DE cells contribute to the development, and potentially regeneration, of multiple foregut organs including liver and pancreas (3).
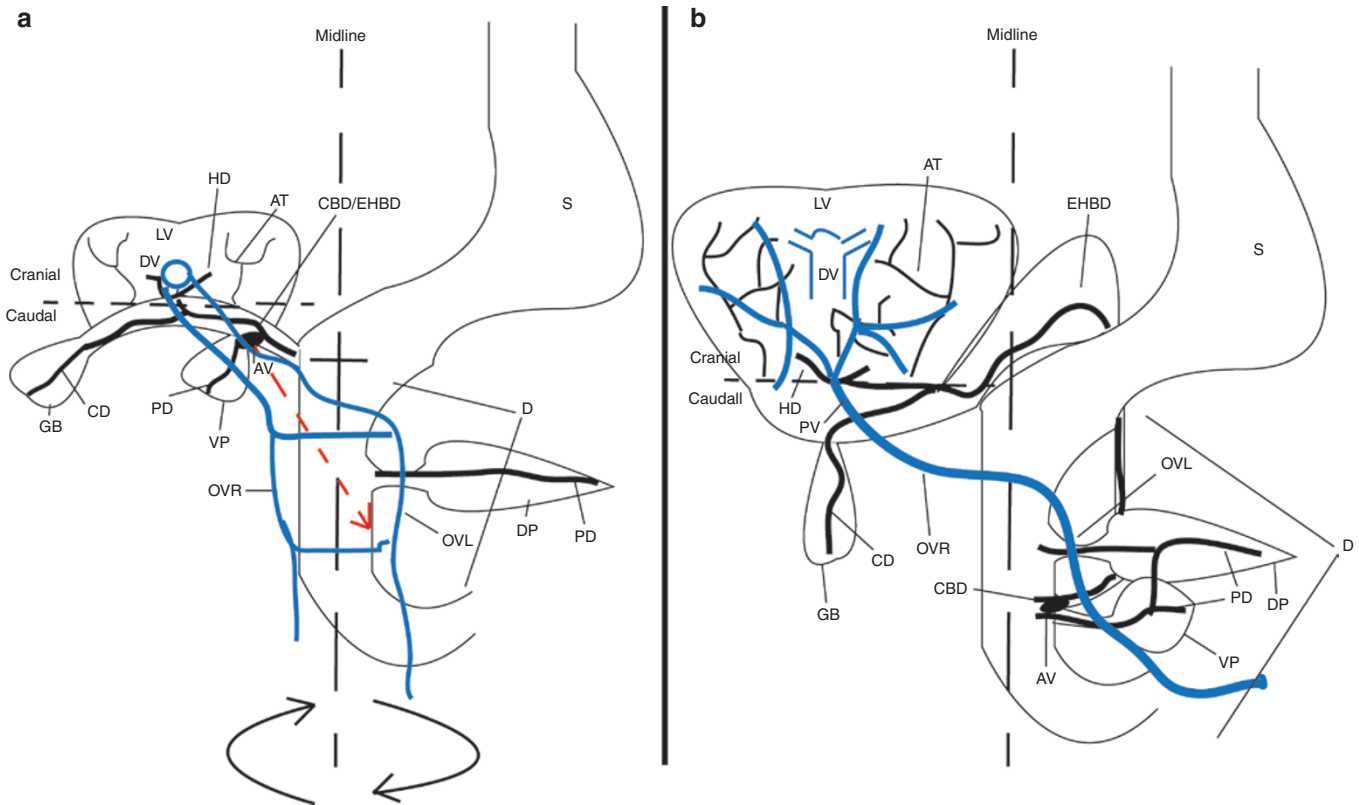
In addition, it is clear that the development and pathology of multiple foregut organs is intimately linked via the extrahepatic biliary duct (EHBD) system as the maturation of multiple foregut organs depends on the proper elongation of the EHBDs (**Figure 1**). It has been postulated that the EHBD serves as a source of progenitor DE cells and that this process is dependent on interaction with surrounding tissue types to support the temporal aspects of foregut maturation (4). For example, mesenchymal tissues such as the lesser ommentum supply the foregut organs with blood and lymph vessels and nerves. This ommenta elongates, along with the EHBD, as it moves from an anterior to a posterior position allowing for the maintenance of blood and bile conduits during duodenal gut rotation. Both the EHBD and mesenchymal tissues are continuous with the endothelium (porta hepatis—transverse fissure) at the level of the liver hilum. The intrahepatic biliary ducts (IHBDs) then develop from the porta hepatis (liver hilum) toward the periphery (E13.5–14.5) before becoming restricted to periportal areas later in gestation (E16–18) (5). Therefore, the communication between multiple cell types might underlie the regionalization, development, and potential specificity of DE organ maturation.

DE, ENDOTHELIAL, AND MESENCHYMAL INTERACTIONS WITHIN THE FOREGUT

DE, mesenchyme, and endothelium interact during development and pathogenesis (has been discussed in depth elsewhere, see ref. 6–8). Endothelial cells (ECs) have been shown to influence IHBD development by communicating, via paracrine mechanisms, with hepatocytes in the region of the portal mesenchyme (9). ECs embedded within the portal mesenchyme attract hepatic cells to periportal areas and release oncostatin M, which is known to induce hepatic maturation. In addition, these developmental cues depend on proper angiogenesis as angiogenic inhibitors prevent the formation of primitive foregut organs (10). Furthermore, angioblasts reside between the mesenchymal component and hepatic components of the liver. In fact, it is well known that the liver is mainly a hematopoietic organ prior to E12.5 (5), replacing the yolk sac as the main site of erythropoiesis by E12 during mouse gestation (11). This shift is induced by the migration of hematopoietic stem cells (angioblasts) into the fetal liver (called definitive erythropoiesis). Erythrocytes derived from the liver have matured from primitive erythrocytes and

Figure 1. Development of the foregut organs. The development and pathology of multiple foregut organs is intimately linked via the extrahepatic biliary duct (EHBD) system. The maturation of multiple foregut organs is associated with proper elongation of the EHBDs. (**a**) View from left side of wild-type mouse embryo before E12.5; the midgut (duodenum), ampulla of vater, and ventral pancreas remains adjacent to the liver hilum, vascular remodeling begins with arterialization, and EHBDs/gallbladders (GBs) have not elongated. (**b**) View from left side of wild-type mouse embryo after E12.5, the midgut rotates and descends to align the ventral pancreas and ampulla of vater with the dorsal pancreas (gut rotation), the EHBDs/GBs elongate to maintain continuity, and the liver expands in size as vascular remodeling increases with the invading portal tract. Disturbances in the development of the liver and/or septum transversum mesenchyme can lead to abnormal EHBD elongation (atresia) and abnormal gut rotation. The failure of foregut portions of gut to expand leaving the midgut at the level of the liver hilum may be related to defects in EHBD, intrahepatic biliary duct, or both (biliary atresia). The failure of caudal portion of EHBD to proliferate cranially with the portal mesenchyme and caudally to elongate EHBD and allow duodenal rotation may be a developmental hallmark of biliary disease. These events occur around 12th wk in humans and at about E12 in mice and gives right to partitioning of the hepatic ducts and artery/veins. AT, artery/arterialization; AV, ampulla of vater; CBD, common bile duct; CD, common duct; D, duodenum; DP, dorsal pancreas; DV, ductus venosus; GB, gallbladder; HD, hepatic duct; LV, liver; OVL, left umbilical vein; OVR, right umbilical vein; PD, pancreatic duct; S, stomach; VP, ventral pancreas.

resemble those found in the bone marrow. The functional switch of the liver to a metabolic organ after E12.5 illustrates the potential for gene expression within one resident population of cells to influence development of the other resident/transient populations within the fetal liver microenvironment. The early liver reaches its peak volume of hematopoietic output from E13 to E14, accounting for about 70% of total hematopoiesis. By E15, this output begins to decrease and by E17.5, this has been reduced to about 30% of the total hematopoietic activity. By late gestation, the erythroid cells are near the end of their peak replicative phases, and hepatoblasts are reaching their peak of replication. These events are hallmarks of proper endodermal–mesenchymal cross talk. Such interaction among the functional components of the foregut organs is not surprising given their intimate functional and pathological relationships. The cross talk between cell types that reside in adjacent populations (endodermal–mesnchymal–endothelial cross talk) often results in distinct migratory and maturational (differentiation) cues that stimulate EMT within endodermal

populations. These temporal aspects of foregut development also align with distinct but overlapping gene regulatory mechanisms (signaling cascades).

HEPATIC ENDODERM INDUCTION PATHWAY

Hlx, *Lhx2*, and *N-myc* in the STM

During the migration of hepatic endodermal (HE) cells into the STM, early HE cells undergo a shift in morphology from a columnar epithelium to a stellate-like appearance. It has been speculated that the STM is capable of inducing morphological changes in endodermal cell types via cell nonautonomous molecular signals (FGFs, BMPs, HGF, *Hlx*, and *Lhx2*—see **Figure 2**) that also interact with ECs within the portal mesenchyme. Furthermore, since mutations in homeobox genes expressed in the STM (*Lhx2*, *Hlx*, and *Nmyc*) and HE (*Hhex* and *Prox1*) produce similar hematopoietic pathologies (**Table 1**) (12–42), they may play overlapping roles in fetal liver erythropoiesis. In fact, mutations in genes required for definitive erythropoiesis, such as *Rb* and *C-myb*, show similar
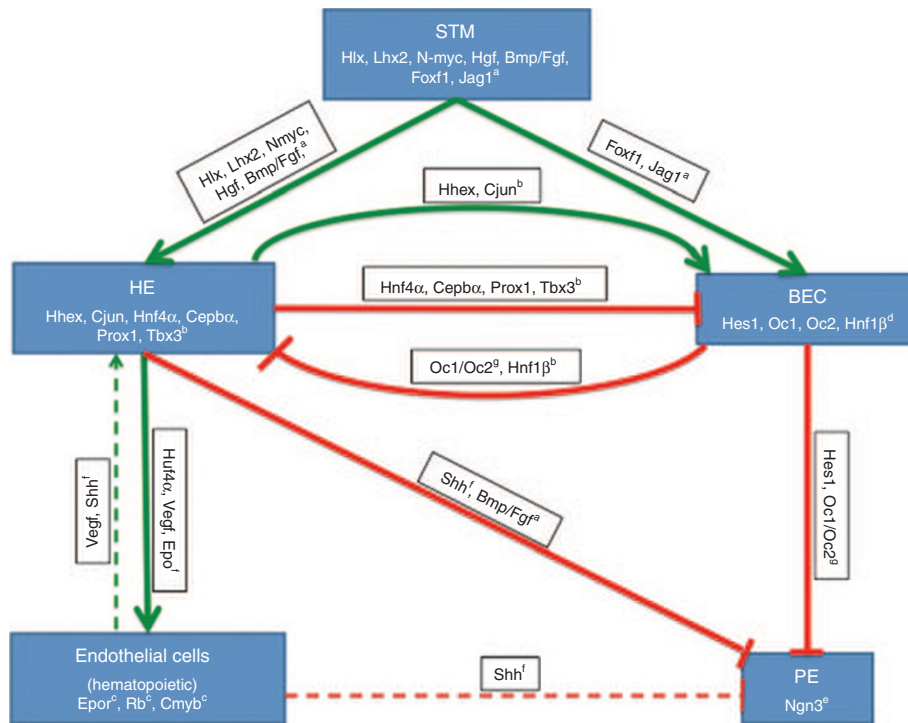
Figure 2. Foregut signaling cascade. The temporal aspects of foregut development can be distinguished by gene regulation within distinct cell types. In general, gene signaling from the septum transversum mesenchyme (STM) can induce the differentiation of distinct cell populations (definitive endoderm, hepatic, hepatocyte/biliary epithelial cell (BEC), and endothelial cell (EC)). In turn, these cell types provide genetic feedback to maintain hemostasis of developing organs systems throughout development. For example, genes from the STM (*Hlx, Lhx2, N-myc*, etc.) stimulate genes from the hepatic compartment (*Hhex, Prox1, Hnf4α, Cebpa*, etc.), which then regulates gene expression with the BEC (*Oc1, Oc2, Hnf1b*, etc.) and EC (*Shh, Vegf, Epo*, etc.) compartments, and suppresses pancreatic (PE) gene regulation. Such communication may define the molecular microenvironmental niche within and between developing foregut organs. Blue boxes indicate genes expressed within that tissue type and white boxes indicate which genes from those tissues positively (green) or negatively (red) influence gene regulation in other tissue types. Superscript letters a–g indicate the tissue types each gene is expressed in: [a]mesenchyme (STM); [b]hepatocyte (HE); [c]endothelium (EC); [d]biliary (BEC); [e]pancreatic (PE); [f]HE/EC, and [g]HE/BEC. HE, hepatic endoderm.

hepatic defects as mutations in genes expressed in cells within the STM and HE (**Table 1**). In summary, all of these findings support an intimate reciprocal developmental relationship (molecular and anatomical/morphological) between STM, EC, and hepatic endoderm in which paracrine signaling between these three cell compartments may regulate a complex signaling mechanism that regulate foregut development.

Hhex, *Prox1*, and *Tbx3* in HE Progenitor Cells

The homeobox gene *Hhex* has important developmental roles in both EC and HE cells. In the liver, *Hhex* is expressed in both endodermal and ECs but not in STM or definitive erythroids (43). *Hhex* has no effect on the development of primitive erythroids, but the absence of *Hhex* leads to a failure of definitive erythropoiesis both *in vitro* (44) and *in vivo* (23) (unpublished data). A role for *Hhex* in foregut organ development has also been observed in Zebrafish (45) and Xenopus (46). Such a role in endodermal development is quite conserved as *Caenorhabditis elegans* has an orthologue for *Hhex* (called *Pha-2*), which when mutated results in abnormal; pharynx development. *Hhex* and its orthologues likely represent a conserved mechanism for endodermal development (13,47,48). Intriguingly, other homeobox genes have been implicated at similar timepoints in HE cells and result in similar defects. Both of the homeobox genes *Hhex* and *Prox1* have been proposed to be involved in liver specification

in zebrafish and mice and mutants show similar phenotypes (13,49,50). *Prox1* is not known to be expressed in ECs and the failure of the HE cells to migrate in *Prox1*−/− embryos may be dependent on its interaction with other HE genes (*Hhex* and *Tbx3*). *Prox1* and *Tbx3* may be downstream of *Hhex* as the emergence of the pseudostratified epithelium established at E9.0 within the STM is present in *Tbx3* and *Prox1* mutants but not in *Hhex* mutants (13). Therefore, these HE genes may interact to control EMT of HE cells at the laminin-rich membrane of the liver hilum. Furthermore, *Hhex* expression in endodermal cells and ECs was unchanged at E9.5 in *Tbx3*−/− mice, and *Hlx* expression in STM was also unaffected (27). These observations support the proposal for a mechanism of homeobox gene communication (between HE and STM cells) regulating early hepatic cell fate decision and indicate that hepatic differentiation from DE may be preferentially directed toward biliary epithelial cells (BEC) in the absence of homeobox signaling at E9.5 in both STM and HE cells (**Figure 2**).

CELL FATE CHOICE: BILIARY VS. HEPATOCYTE

Hepatocytes and Erythropoiesis: *Cebpα* and *HNF4α*/ Erythropoietin in Hepatic Cells of the Liver

The maturation of the liver results in distinct populations of differentiated BECs and hepatocytes. The regulation of the hepatocyte/BEC cell fate decision may be in part regulated

Table 1. Gene deficiencies within the STM, HE, and endothelium

Gene deficiency	Expression onset	Tissue	Liver	Death	Hematopoiesis	References
Lhx2	E9	STM	Hypoplasia	E16	Anemia[b]	(20)
Hlx	E9.5	STM	Hypoplasia	E15	Anemia[b]	(14)
N-myc	E9.5	STM	Hypoplasia	E11–12.5	Anemia[b]	(13)
Hgf	E9-10	STM	Hypoplasia	E12.5–15.5	Anemia[b]	(30,32)
Cjun/Cmet	E8.5–9.5	HE	Hypoplasia	E13.5	Anemia[b]	(15,17)
Rb	E8.25–12.5	Liver EC	Hypoplasia	E14.5	Anemia[b]	(21)
C-myb	E8.25-12.5	Liver EC	Hypoplasia	E15	Anemia[b]	(26)
Hhex[a]	E8.25–8.5	HE and EC	Hypoplasia (DPM)	E10.5	Anemia[b]	(9,18–19)
Prox1	E9–9.5	HE	Hypoplasia	E14.5	Anemia[c]	(12,33)
Tbx3	E9	HE	Hypoplasia	E14.5	Anemia[c]	(23)
Cepbα	E16–17	HE	Nonperiportal cysts (BEC/EHBD)	P1	Metabolic defect[c]	(35,38)
Hnf4α[a]	E8.75–9	HE	Hypoplastia	E10.5	Anemia[c]	(22,29)
Epo	E12.5	HE and EC (EPOR)	Hypoplastia	E12.5–13.5	Anemia[b]	(36)
Foxf1	E8.5–9.5	STM	Biliary atresia (no DPM)	E10-12	Anemia[c]	(24,27–28)
Hes1[a]	E8.5–11.5	BEC (EHBD)	Biliary atresia (no DPM)	E18.5	Anemia[c]	(34)
Jag1[a]	E9	STM	Biliary atresia (no DPM)	E10–11	Anemia[b,c]	(16,37)
Hnf6 (Oc1/Oc2)[a]	E8.5–13.5	HE-BEC (EHBD/IHBD)	All of the above defects	Viable	Anemia[c]	(10,25)
Hnf1β[a]	E7.5–13.5	HE-BEC (EHBD/IHBD)	Biliary atresia and arterial defect	E7.5, viable	Anemia[c]	(11)
Bmp	E8.5	STM	Hypoplasia	E9.5	Anemia[c]	(8,31)

[a]Multiple models (null/conditional/targeted). [b]Definitive erythropoiesis defect. [c]Other hematopoietic defect.

BEC, biliary epithelial cell; DPM, ductal plate malformation; EC, endothelial cell; EHBD, extrahepatic biliary duct; EPOR, erythropoietin receptor; HE, hepatic endoderm; IHBD, intrahepatic biliary duct; STM, septum transversum mesenchyme.

by distinct expression of *Hnf4α*/*Cebpα* and *Hnf6*/*Hnf1β*. For example, *Tbx3* expression has been shown to increase *Hnf4α*/*Cebpα*, whereas expression of *Hnf6* decreases *Hnf4α*/*Cebpα*. Therefore, *Tbx3* and *Hnf6* may work together on *Hnf4α*/*Cebpα* expression to calibrate the balance of hepatocyte vs. BEC differentiation from hepatoblasts. It has been established that *Cebpα* and *Prox1* also represses BEC differentiation (42,50), and the regulation of *Hnf4α* has also been implicated in multiple aspects of liver development (though it is not required for hepatic specification). *Hnf4α* may be critical to reinduce a proper epithelial morphology on the endodermal cells after migration through the STM and to reduce EMT by stimulating high levels of cell adhesion genes (33). In addition to its potential role in regulating epithelial morphology in HE cells, HNF4α has also been shown to control erythropoietin (EPO)-stimulated induction of definitive erythropoiesis in the late stages of embryonic hematopoiesis (51). EPO is a protein released by hepatocytes (52) that signals via its receptor (EPOR) on primitive erythrocytes within the hematopoietic liver to induce definitive erythropoiesis. EPO directly stimulates the proliferation and survival of definitive erythrocytes in the fetal liver (51). However, it is not required for primitive erythropoiesis within the yolk sac (site for erythropoiesis before E11.5) or liver (site for erythropoiesis after E11.5). EPO activity is controlled by retinoid X receptor in the yolk sac and by both *Hnf4α* and retinoid X receptor in the fetal liver.

Therefore, definitive erythropoiesis in the fetal liver is intimately tied to hepatocyte differentiation and may explain why defective definitive erythropoiesis is commonly observed when HE genes are mutated (**Table 1**). Interestingly, BEC have also been shown to communicate with the endothelium at periportal areas during embryonic through adult liver development.

Biliary (Intrahepatic and Extrahepatic) Induction Pathway

Hnf6 and *Hnf1β* are intimately linked to the biliary developmental program in HE cells (*Hes1*/*Notch2*/*Sox9*) in response to mesenchymal signals (*Foxf1* and *Jag1*—see **Figure 2**). *Foxf1* is expressed in the STM around foregut organs, *Hes1* is expressed within the epithelial cells of those foregut organs and *Jag1* is expressed in cells within the portal mesenchyme that reside adjacent to BECs. The importance of STM vasculature has been shown in other foregut organs such as the pancreas (53), and in genetic mutations, in the endothelium (9,54–56). Furthermore, perturbation of the communication between *Jag1* within the portal mesenchyme and *Notch2* within HE cells has been implicated in diseases such as Alagille syndrome (57,58). Therefore, *Jag1* within the portal STM can influence BEC differentiation and both *Hnf6* and *Hnf1β* expression within BEC can influence the portal STM development (14,15,59,60) (**Table 1**). Further evidence for interaction between the portal STM, endothelium, and hepatic endoderm during biliary and hepatocyte differentiation comes from the observation that deficiencies

in mesenchymal (*Jag1*), BEC (*Hnf6*), and hepatocyte (*Hhex*) genes in the portal tract (where these three tissues types interact) all result in abnormal development. Finally, it is clear that *Hhex* may be upstream of many biliary and hepatocyte genes that bind *Ecadherin*, and therefore, *Hhex* may stimulate the induction of EMT in hepatic signals via communication with STM and ECs. Thus, all three cell types (STM, hepatic, and EC) share a interdependence for paracrine signaling from each other, and perturbation of genes within this signaling cascade results in functional defects within foregut organs.

MOLECULAR INTERDEPENDENCE BETWEEN FOREGUT ENDODERMAL CELL TYPES

In organs derived from foregut endoderm, three main tissue sources must interact during developmental windows in order for normal development to take place: the STM, endothelium, and the DE. It is clear that the genetic mutations specific for any one of these tissue types can result in defects within the other tissues (such as abnormal EHBD elongation and abnormal gut rotation—see **Table 1** and **Figure 1**). The mesenchymal tissues adjacent to the foregut provide an overlapping microenvironment for different foregut-derived endodermal organs. As mentioned earlier, this mesenchymal component provides endothelium as well as connective tissue that act as a conduit to maintain continuity of the bile ducts during gut rotation and EHBD elongation. The failure of these tissues to properly interact can result in defective definitive erythropoiesis (hypoplastic liver, with large sinusoidal spaces, and anemia), failed gut rotation (midgut adjacent to porta hepatis, ectopic tissue, EHBD/IHBD atresia, and ductal plate malformations), and abnormal liver parenchyma (nonperiportal biliary cysts, mesenchymal and vascular disorganization) (**Table 1**). Decreased expression of hepatocyte-related genes during liver development (*Hhex*, *Prox1*, *Tbx3*, *Cebpα*, and *Hnf4α* in endoderm and/or *Hlx*, *Lhx2*, and *Nmyc* in STM—see **Table 1** and **Figure 2**) results in EHBD truncation and liver hypoplasia, defective definitive hematopoiesis (in part via defective regulation of EPO and vascular endothelial growth factor on ECs), and failed gut rotation (absence of gallbladder (GB) and ventral pancreas, and defective migration of HE cells through the STM via EMT and into the liver bud) (**Table 1**). Decreased/absent expression of genes critical to biliary cells (*Hnf6* and *Hnf1β* in endoderm and/or *Foxf1* and *Jag1* in STM—see **Figure 2**) often results in mesenchymal defects paralleled by biliary tract defects (EHBD truncation, IHBD paucity/excessive proliferation, and GB absence/paucity—see **Table 1**). Gene targeting of other genes from both mesenchymal (STM—*Hlx*, *Foxf1*, *Lhx2*, and *Hes1/Jag1*) and endodermal (*Hhex*, *Prox1*, *HNF6/OC1*, *OC2*, *HNF1β*, *HNF4α*, *Cebpα*, and *Tbx3*) compartments produce similar developmental and anatomical pathologies, further supporting the requirement for communication between and within emerging endodermal and mesenchymal (including endothelial) cell populations. Therefore, understanding the molecular interactions between the main foregut cell types (mesenchymal, endothelial, and endodermal) will aid in the diagnosis and treatment of liver diseases as well as a yield a

deeper understanding of the developmental genomic profile associated with foregut maturation, for example, whether genes expressed in the STM may induce biliary vs. hepatocyte differentiation in early HE cells. From an examination of the literature, it seems that the collective anatomical and molecular data support a signaling cascade where STM (*Hlx* and *Lhx2*) communicates with early hepatic cells (*Hhex*, *Prox1*, and *Tbx3*) to drive hepatocyte differentiation (*Hnf4α* and *Cebpα*) and elicit definitive stages of hematopoiesis (EPO and EPOR). In addition, a similar mechanism may exist where signaling from the portal mesenchyme (*Foxf1*, *Hes1*, and *Jag1*) regulates the differentiation of BEC (*Hnf6* and *Hnf1β*) during the development of the EHBD/IHBD. Interestingly, much attention has recently been given to the activity of a mesenchymal cell type called hepatic stellate cells (HSCs—also known as Ito cells). HSCs are derived from the STM (portal mesenshymal cells) of the liver and are active in the fetus and postnatally in the adult (61). HSCs reside between the parenchyme and sinusoidal epithelium and also express many of the mesenchymal genes discussed here that are associated with perturbation of the developing foregut (*Hlx*, *Lhx2*, *Foxfl*, *Jag1*, and *Hes1*). Surprisingly, HSCs are known to release retinoids upon their activation and RXRs are known to control erythropoiesis via EPO modulation. Therefore, it is perhaps not coincidental that EPO is regulated by *Hnf4α* in the liver as retinoid regulation would influence retinoid X receptor activity. Thus, HSCs may be a likely candidate for the mesenchymal component that communicates with endothelial and endodermal compartments of the liver during fetal and postnatal development. Furthermore, HSCs have also been implicated in multiple liver diseases and may represent a conserved cell type between foregut organs whose disturbance may result in pathological manifestations within distinct cell populations. Within the liver, such a relationship may exist between biliary atresia and anemia, where the paucity of EHBD/IHBD development is often observed along with anemia (**Table 1**).

FOREGUT PATHOLOGY: THE OVERLAP OF BILIARY ATRESIA AND ANEMIA

Biliary atresia can be described as the paucity, discontinuity, or complete absence of any portion of the EHBD and IHBD trees. From the mutational models discussed here, the occurrence of biliary atresia can be associated with the following observations: failure of elongation of the EHBD (agenesis of the GB/EHBD/IHBD); small, cystic, or atresia of the ventral pancreas; absence of the extensive intestinal loop during secondary gut rotation (midgut remains adjacent to porta hepatis/liver hilum); proliferation of ducts/cysts near the liver hilum/porta hepatis (ductal plate malformation/polycystic liver disease); and defective hematopoiesis/definitive erythropoiesis (anemia/hypoplasia—large hepatic sinusoidal spaces and loose parenchyma). Biliary atresia is often associated with the continuous proliferation of primitive bile ducts at the level of the porta hepatis as the distal portions of right and left hepatic ducts and common bile duct come from EHBD (62,63). Furthermore, the continuous proliferation/dilation

of primitive EHBDs around the porta hepatis may represent a failed compensatory mechanism (64) as cysts around this area are often associated with regenerative foci and influence the ability of foregut organs to produce or replace new cells (65). Most cases of biliary atresia occur at the level of the porta hepatis where defects are not isolated to the common bile duct, hilum, or IHBD alone. This type of atresia accounts for about 90% of biliary atresia cases (types 3 and 4 on the French classification scale and types 2 and 3 on the US/UK scale). Furthermore, biliary cysts at the level of the porta hepatis (portal/hilar plate) are often linked to changes in portal vein pressure, suggesting that defects in circulation at the portal vein can contribute to morphological changes within foregut development. Whether increased pressure within the portal vein is caused by crowding from excessive proliferation of BECs at the level of the porta hepatis, or vice versa, is unknown. It has been suggested that IHBD development is dependent on the presence and proper proliferation of the EHBD (66,67). Therefore, it appears that failure of caudal portion of EHBD to proliferate cranially with the portal mesenchyme and caudally to elongate EHBD (**Figure 1**) and allow duodenal rotation may be a developmental hallmark of biliary disease. In addition, it is clear from numerous observations that the absence of the GB serves as a hallmark for developmental defects in biliary (and therefore foregut) pathology. The movement of endodermal cell populations via their expansion within the primitive midgut/foregut is critical to liver development and regeneration (EHBD/IHBD) (4,68).

In conclusion, the targeted disruption of multiple genes from each cell compartment within the developing foregut organs (mesenchyme, endothelium, and endoderm) reveals how the overlap in anatomy and function of these tissues is related to their shared interdependence on paracrine relationships. We can now begin to gain a better understanding of the molecular, anatomical, and functional manifestations of foregut endodermal pathology. Such mechanisms may initiate from within any compartment of the organs and affect surrounding cell types. The consequence of disease severity may have a temporal aspect throughout development during which distinct molecular, noncell autonomous signaling cascades regulate proper developmental events, such as hematopoiesis. Therefore, disruptions in hematopoiesis during development may occur via changes in molecular signals within different surrounding cell types.

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