Review Article

Biliary tree stem/progenitor cells and perspectives in physiopathology and regenerative medicine

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Abstract: We have recently demonstrated the presence of cells expressing a constellation of endodermal markers in (peri)-biliary glands of extrahepatic bile ducts. These observations *in situ* in human tissues have been completed by the *in vitro* demonstration that a subpopulation of cells [Pdx1+ (pancreatic and duodenal homeobox 1+)/(Sry-related HMG box)17+/EpCAM+ (epithelial cell adhesion molecule+)] isolated from the biliary epithelium have long-term (*in vitro*) persistence and self-renewal, and are able to give rise to a more restricted progeny towards different mature lineages (hepatocytes, cholangiocytes and β-pancreatic cells). The discovery of these cells, named human biliary tree stem/progenitor cells, opens a new scenario impacting many different aspects of hepato-gastroenterology including embryology of liver, biliary epithelium and pancreas, biliary pathophysiology, hepatobiliary cancerogenesis and, finally, regenerative medicine of liver and pancreas. The purpose of this review is to highlight these new aspects of liver and pancreas pathophysiology.

Key Words: Biliary tree stem/progenitor cells; hepatic stem/progenitor cells; embryology; biliary physiology; biliary cancerogenesis; regenerative medicine; liver cirrhosis; diabetes mellitus

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Introduction

We have recently demonstrated the presence of cells expressing a variety of endodermal markers in (peri)-biliary glands (PBGs) of extrahepatic bile ducts (1). The observations *in situ* in human tissues have been completed by the *in vitro* demonstration that (PDX1+/SOX17+/EpCAM+) cells isolated from the biliary epithelium have long-term (*in vitro*) persistence and self-renewal, and are able to give rise to a more restricted progeny towards different mature lineages such as hepatocytes, cholangiocytes and β-pancreatic cells (1). These cells have been termed human biliary tree stem/progenitor cells (hBTSCs) (1-3). hBTSCs are located deeper inside the glands of the entire biliary epithelium, express typical markers of primitive endoderm and bilio-pancreatic progenitors, which are lost (together with accumulation of PAS positivity) when these cells progress and differentiate from deep inside the gland to the lumen, recapitulating the organization of maturational lineages in the intestine (2).

Perspectives in liver development

Beside the well known niche at the level of the canals of Hering, our results indicate an additional niche formed by a large number of cells with the phenotypes of endodermal stem/progenitor cells within PBGs of the large intrahepatic and extrahepatic bile ducts in fetal and adult life, suggesting a newly identified reservoir of stem cells for the renewal of large bile ducts (1-3). In the fetal life, these niches are closely related to the ductal plate, which has been recently demonstrated to generate the liver parenchyma during embryogenesis (4). This relationship is supported by the observation in fetal tissues, where progenitors in large bile ducts were continuous with ductal plates that, in turn, transits into the canals of Hering (5). Moreover, this is in agreement with the hilum to periphery model of bile duct morphogenesis recently proposed by Lemaigre group (6). hBTSCs are endowed with more primitive phenotypes in comparison with human hepatic stem cells (hHpSCs) located in ductal plate (fetal liver) or canals of Hering (adult liver) suggesting the possible existence of a hierarchy (1-3). Based on the theory of the branching tissues and, on the new insights on the biliary tree development from the hilum towards the periphery (6), we hypothesized that hBTSCs give rise to hHpSCs in the ductal plate during fetal development. This could explain how extra and intrahepatic bile ducts are connected. Segmental, area and septal intrahepatic bile ducts share several common features with the extrahepatic bile ducts such as the presence of PBGs (7,8) and, interestingly, the presence of endoderm-like/progenitor-like cells that are EpCAM±/PDX1+/SOX17+/Lgr5+. These observations are in accordance with the possibility of isolating multipotent stem cells from fetal and adult livers of rodents and other mammals (9,10).

Bile ducts regeneration and hBTSCs homeostasis

The glands of the biliary epithelium represent niches of cells with classic phenotypic features of stem/progenitor cells of endodermal origin with respect to transcription factors, surface and cytoplasmic markers, capacity of proliferation, self-renewal and multipotential differentiative capacity (1-3). Recently, Spence *et al*., by a lineage tracing study, demonstrated that SOX17+/PDX1+ cells are the precursors of biliopancreatic cells (11). The recent study of the organization of cells within glands of the biliary epithelium by Carpino *et al*. has also shown that: (I) the progenitor-like cells and transit-amplifying cells are located at the bottom of the glands; (II) the cells with a phenotype intermediate between progenitor-like cells and mature cells are found in the middle of the glands; and (III) the fully differentiated cells are in continuum with the surface epithelium (2). Cells, secreting low levels of insulin, are found within PBGs, especially in the hepatopancreatic common ducts, whereas cells producing albumin are found in portions of the biliary epithelium near to liver. Endoderm-like/progenitor-like cells are also found in PBGs within intrahepatic large bile ducts, representing a candidate niche for the renewal of intrahepatic ducts. In PBGs, approximately 10% of the cells co-express PDX1, SOX17 and FOXa2 (forkhead box a2), a phenotype indicative of the early stem cell subpopulation (endoderm-like cells). Moreover, we have shown that glands of the biliary tree are also formed by populations of cells with a less primitive phenotype, being characterized by the loss of FOXa2 and PDX1 expression (biliary progenitor-like cells) or, alternatively, by the loss of FOXa2 and SOX17 (pancreatic progenitor-like cells). Both these cell populations maintained the expression of EpCAM, a more widely expressed marker. Several lines of evidence indicate that EpCAM expression is tightly regulated and only occurs when there is a temporary need for proliferation; it is immediately downregulated upon terminal differentiation (12,13). In adults, biliary tree stem cells could represent the remnant of a common stem/progenitor (PDX1+/SOX17+) for liver, bile duct system and pancreas, which exists at earlier stages of development (11), being considered to derive from ventral endoderm (14,15). Within the body of the PBGs, we identified the presence of a committed intermediate (transit-amplifying) compartment in which cells co-expressing both stem cell markers and markers of mature cells were present, i.e., secretin receptor (cholangiocytes), mucins (goblet cells), insulin (endocrine pancreas) and, rarely, albumin (hepatocytes). The relative proportion of cells expressing mature cell markers indicative of liver *vs*. bile duct *vs*. pancreas correlates with the location along the length of the biliary epithelium. Accordingly, PBGs are organized along the duct wall such that cells with an undifferentiated phenotype (EpCAM±/PDX1+/SOX17+/Lgr5+) are situated mostly in the gland base near the fibromuscular layer. The transit-amplifying progenitor population (EpCAM+/PDX1+/SOX17+/Lgr5-) is concentrated in the body of the glands (corresponding to the middle of the duct wall). Finally, mature cells (e.g., cholangiocytes, goblet cells, hepatocytes, pancreatic cells) are located at the neck of PBGs in close contact with the

surface epithelium: the type of mature cell depending on whether the portion of the biliary tree is near the liver (hepatocytes), in the middle of the biliary tree (cholangiocytes) or near the duodenum (pancreatic cells and goblet cells). The distribution of proliferating cells follows a similar gradient. Taken together, these data indicate that PBGs contain progenitor-like cells, which normally proliferate and are responsible for the renewal of the surface epithelium, generating mature cells such as cholangiocytes and goblet cells (in the middle of the biliary tree) or hepatocytes (near the liver) or islet cells (near the pancreas). The endoderm-like/progenitor-like cell compartment within PBGs resembles the organization of the stem cell niche within intestine. In the intestine, proliferation takes place at the base of intestinal crypts where Lgr5+ stem cells interspersed by Paneth cells are located (16,17). They give rise to progenitors produced from stem cells in the crypts (transit-amplifying population), migrate upwards and fully differentiate into mature cells. The phenotype of the mature cell (e.g., stomach, duodenum, small intestine or large intestine) depends on the position of the villus in the anterior to posterior axis along the intestine (18).

We have also demonstrated that the biliary extracellular bio-matrix is characterized by a defined structural and molecular organization (19). This organization could support and regulate the lineages within the wall of bile ducts. Moreover, bile acids (20,21) and bile flow (22,23) are additional candidate regulators of the hBTSC niche homeostasis and activation. Signaling pathways involved in the homeostasis of intestinal stem cell niche and mature fate specification (24), such as WNT, Notch, BMP (bone morphogenetic protein), Hedgehog have been extensively studied in the biliary epithelium and found to play a pivotal role in many aspects of biliary physiopathology (development, congenital diseases such as Alagille syndrome and biliary atresia, and bile duct regeneration after injury). Unfortunately, in the biliary tree, these pathways have been scarcely investigated as mediators of hBTSCs self-renewal and maintenance in the niche or specification toward mature lineages (cholangiocytes or goblet cells).

Perspectives in biliary diseases and carcinogenesis

Hyperplasia and neuroendocrine differentiation of PBG cells occur in diseases of bile ducts such as primary sclerosing cholangitis (PSC) (8). PSC is a disease primarily affecting large intra and extrahepatic bile ducts (25). This disease involves the entire wall of the bile ducts as evidenced by the involvement of PBGs. From a clinical and pathological point of view, PSC is completely different with respect to primary biliary cirrhosis (PBC), a disease specifically triggering interlobular bile ducts (26). From a clinical point of view, PBC affects mostly women, whereas PSC affects mostly men (27). In PSC, different types of malignancies can develop, whereas this is not the case of PBC (28). Finally, ulcerative colitis is frequently associated with PSC but not PBC. From a pathological point of view, PBC electively targets interlobular bile duct in portal spaces, whereas PSC preferentially triggers large bile ducts (26,28). In biopsy specimens from PSC patients, hairy and enhancer of split 1 (Hes-1) was completely abolished while pancreatic and duodenal homeobox 1 (PDX-1), a transcription factor driving neuroendocrine cell differentiation, was over-expressed. PDX-1 activation was absent in diseases primarily affecting liver parenchyma including viral hepatitis, alcoholic liver disease, non-alcoholic steato-hepatitis and PBC (29). These data suggest that the transcription factors driving the activation and differentiation of the stem cell compartments after injury are different in PBGs of large ducts with respects to canals of Hering. The existence of lineage-dependant diseases could represent the background for hypothesizing also a lineage-dependent carcinogenesis. A large body of recent literature deals with the involvement of stem/progenitors in the oncogenesis of the liver (30-35). Human hepatic stem cells are actually considered to be the origin of some subtypes of hepatocellular carcinoma (HCC) (36) or intrahepatic cholangiocarcinomas (32,33,37). It has been shown that stem cell activators such as WNT/b-catenin, transforming growth factor (TGF)-b, Notch and Hedgehog signaling pathways also expedite liver tumorigenesis (34,35). Primary liver cancers arise in the context of hepatobiliary diseases of different etiologies (28,38,39). As for cells of the pancreatic duct glands (40), the cells of the PBGs are sites of increased vulnerability to diseases, especially in response to the death of the injured mature cells or to chronic stimuli, which represent the initial step of the oncogenic process. Similarly, cell proliferation and PBG hyperplasia are common features associated with pathologies affecting the large intrahepatic and the extrahepatic bile ducts, which are currently considered risk factors for development of cholangiocarcinoma (CCA) (28). Interestingly, it has been observed that diseases primary targeting large bile ducts, such as PSC and liver flukes, are associated with both intrahepatic (IH) and extrahepatic

(EH) cholangiocarcinoma (CCA) (28). On the contrary, parenchymal liver diseases such as cirrhosis of any etiology and chronic HCV- and HBV-related diseases, where only the stem cell niche in canals of Hering are activated (28), are exclusively associated with IH-CCA. A number of recent studies suggest that stem cell niches within the biliary epithelium could be involved in CCA development (41,42). In particular, endoderm-like stem cells within PBGs could represent the cells of origin of pure mucin-producing CCAs (43-45). This histological subtype of CCA is characterized by mucin-producing cells and typically arises at the hepatic hilum or at the level of large intrahepatic bile ducts (46). Our data demonstrated how PBGs contain mucin-producing cells and their location overlaps with the sites in which the mucinous CCA typically occurs. Moreover, CCAs express several markers in common with PBG cells such as EpCAM, OCT4 (POU class 5 homeobox 1), CD133 (prominin 1), Sall-1 (31). Finally, risk factors for hilar CCA, such as PSC and liver fluke infection, are characterized by the proliferation of PBGs (28,39,47). Based on new insights in pathological heterogeneity of CCA (43), one could hypothesize that carcinogenetic events involving the hHpSCs-derived lineage can give rise to combined HCC (hepatocarcinoma)-CCA and mixed-type CCA, whereas the involvement of the hBTSCs-derived lineage can give rise to pure mucin-producing CCA (44,45). The existence of two different stem cell compartments and the associated lineages may result in multiple cells of origin of CCA and could represent the basis of the clinico-pathological and epidemiological heterogeneity of CCA (45). Signaling pathways, such as WNT, Notch, Hedgehog have been extensively studied in the biliary epithelium and found to play a pivotal role in many aspects of biliary pathology and carcinogenesis. Unfortunately, the involvement of these pathways in carcinogenesis involving hBTSC dependent lineages has been so far neglected.

Perspectives in regenerative medicine of the liver

Patients affected by liver diseases need new cell sources to enable a better transition into clinic programs of cell therapy and regenerative medicine. Hepatocyte transplantation is the proof of concept for cell therapy of liver disease (48,49). Unfortunately, the limited supply of donor organs for the isolation of good quality hepatocytes and the low cell engraftment stimulated the search of alternative cell sources (48,49). Stem cells of different origins are currently under study as sources for cell therapy of liver diseases including Embrionic Stem (ES) cells, Induced Pluripotent Stem (iPS) cells, HpSCs, and Bone Marrow Stem Cells (BMSCs (48-50). Liver parenchymal repopulation with exogenous cells is the prerequisite for successful cell therapy (50). To this regard, hepatic stem/progenitor cells (hHpSCs) discovered both in fetal and adult life are endowed with high proliferative capability with respect to mature hepatocytes (51-53). hHpSCs reside in the ductal plate of fetal liver and in canals of Hering of adult liver, differentiate in parenchymal liver cells and contribute to hepatic tissue homeostasis (52). Immunogenicity of fetal hepatic stem/progenitor cells is limited due to the low or null expression of class I and II major histocompatibility complex (MHC) antigens (54,55). In addition, hHpSCs showed high tolerance to ischemia (52,54). One of the favorable properties of the stem cells derives from the metabolomic pattern. Indeed, high level of unsaturated molecules perpetuates embryonic stem (ES) cells pluripotency, whereas the increase of oxidized metabolites promoted cell differentiation (56). These regulatory mechanisms may modulate the hepatic stem cells proliferation, renewal and differentiation (57). In recent studies, isolated hepatic progenitor cells (hepatoblasts and hHpSCs) from fetal liver were used to treat lethal congenital liver diseases (58). Moreover second-trimester fetal liver cells, enriched of EpCAM+ cells were infused into the hepatic artery of 25 patients with end stage liver cirrhosis (58). A marked improvement of clinical and biochemical parameters has been observed and the mean Model for End-Stage Liver Disease (MELD) score significantly decreased during six month follow-up without any adverse immune reaction (58). Interestingly, cell tracing showed specific hepatic seeding (58). In general, clinical trials, using autologous bone marrow derived stem cells, provided interesting data in terms of safety and short-term benefits in the treatment of chronic liver diseases (59). However, the mechanisms of action of bone marrow-derived stem cells in the setting of liver disease treatment are unclear (60). We have recently demonstrated the presence of cells endowed with a constellation of markers suggestive of primitive endodermal phenotype in PBGs of extrahepatic bile ducts (1-3). These cells are easily isolated and cultivated and, show a stable phenotype during *in vitro* expansion (1). They differentiated between hepatocytic and biliary fates and pancreatic islets under defined mediums (1). Transplantation to the liver of SCID (Severe Combined Immunodeficiency) mice resulted in mature hepatic fate lineage restriction (1). hBTSCs are candidate

for regenerative medicine of liver and endocrine pancreas. The observation that hBTSCs can engraft and differentiate in mice livers is encouraging for future studies, where BTSCs could be transplanted in much higher numbers via vascular or intrasplenic routes. There is an increasing range of potential applications of stem cells in liver diseases, with many clinical studies already undertaken (61). In general, the cell types so far proposed for regenerative medicine of the liver have encountered a number of obstacles including: (I) limited sourcing for mature liver cells (48,49); (II) side effects such as emboli, ectopic distribution of cells, and transient effects for adult hepatocytes (48,49); and (III) high oncogenic potential for ES cells and induced Pluripotent Stem (iPS) cells (48,49,59,62). The large availability of adult biliary tree tissues and their biological properties guarantee an ideal source of easily isolable and cultivable stem cells and progenitors candidate for regenerative medicine of the liver. Finally, hBTSCs can be easily produced under GMP conditions. The fetal human biliary tree contains progenitor/stem cells of endodermal origin within the surface epithelium and bud of PBGs, which likely represent the fetal precursors of hBTSC populations persisting in adult biliary tree throughout life (63). The hBTSCs from foetal tissue could, theoretically, be considered as a source for cell therapies of liver and pancreas (63). As far as clinical programs are concerned, hHpSCs from foetal livers have been successfully used in clinical trials without requirement of immune-suppression (58). The isolation of both hepatoblasts and hBTSCs from the foetal liver should furnish obvious advantages in terms of cell yield and therapeutic targets (64).

Potential role of hBTSCs in the regenerative medicine of pancreas

Islet transplantation is viewed as an ideal treatment for type I diabetes, but it is constrained by the limited yield of quality donor pancreata that can be used to isolate islets (65). Recently, a new source of islet precursors has been identified in the biliary epithelium of donors of all ages (1-3). It is noteworthy that lineage restriction of ES cells, iPSCs or mesenchymal stem cells (MSCs) to an islet fate requires transfection with key transcription factors (e.g., PDX1) and sequential treatments with a panel of growth factors and matrix components for 4 to 6 weeks (66,67). By contrast, the biliary stem cells are already at the stage 4 of the 5 stages of the differentiation process previously described (68) and are poised to rapidly generate islets in an appropriate microenvironment and without gene manipulation. Given the assumption that pancreatic stem cells are lacking in postnatal tissues, a number of cell sources have been studied including: ES cells (66,69,70), iPS cells (64,65), MSCs (from bone marrow or cord blood) (71), adipose tissue or amniotic fluid-derived stem cells (72,73), transdifferentiation of acinar cells to islet cells by genetic manipulations (74,75). They will not lead to clinical programs until a number of challenges are overcome. In contrast to these different proposals, the biliary tree, in both fetal and adult life is replete with large numbers of multipotent stem cells (1-3,63,64). The ready availability of biliary tree tissues from fetal and adult liver and the extensive expansion potential of the biliary tree stem cells in culture under wholly defined conditions makes biliary tree stem cells a viable option for clinical programs in the treatment of diabetes and other pancreatic diseases.

hBTSCs and pancreas ongoing organogenesis

Peribiliary glands (PBGs) in bile duct walls, and pancreatic duct glands (PDGs) associated with pancreatic ducts, in humans of all ages, contain a continuous, ramifying network of cells (76). We show that proximal (PBGs) to distal (PDGs) maturational lineages start near the duodenum with cells expressing markers of pluripotency [NANOG (Nanog homeobox), OCT4, SOX2], proliferation (Ki67), self-replication (SALL4) and early hepato-pancreatic commitment [SOX9, SOX17, PDX1, Lgr5 (leucine-rich repeat-containing G protein coupled receptor 5)] and transit to PDG cells with no expression of pluripotency or self-replication markers, maintenance of pancreatic genes (PDX1), and expression of markers of pancreatic endocrine maturation [NGN3 (neurogenin 3), MUC6 (mucin 6, oligomeric mucus/gel-forming), insulin] (76). The biliary tree-derived stem cells and their connections to pancreatic-committed progenitors constitute a biological framework for life-long pancreatic organogenesis (76). In a recent paper by Banga *et al*. (77), SOX9+ cells in the liver have been reprogrammed to pancreatic cells by the overexpression of three transcriptional factors [PDX1, NGN3 and MafA (v-maf musculoaponeurotic fibrosarcoma oncogene homolog A)]. The Authors (77) observed the appearance within the intrahepatic biliary tree of ectopic duct-like structures where cells express a variety of endocrine pancreatic markers associated with the improvement of glycemic profile. In addition, chromatin configuration of mature liver cells still allows access to pancreatic transcription factors and, so their overexpression can be

Table 1 Areas of interest arising from the discovery of human biliary tree stem/progenitor cells

Development of liver, biliary tree and pancreas	Biliary tree and pancreas regeneration and homeostasis	Biliary pathology and cancerogenesis	Regenerative medicine of liver and pancreas
The theory of the branching tissues and the hilum-towards-periphery organogenesis suggests that hBTSCs and hHpSCs in the ductal plates during fetal development are functionally connected	PBGs resemble the organization of the stem cell niche within the intestine. Stem cells located at the bottom of PBGs could be involved in the regeneration and homeostasis of biliary and pancreatic ducts	Factors driving the activation of stem cells are different in PBGs with respect to canals of Hering. The existence of two different stem cells and associated lineages may result in multiple cells of origin of CCA	hBTSCs can be induced to differentiate in hepatocytes, cholangiocytes and pancreatic islets. hBTSCs from adult and fetal tissues are candidates for regenerative medicine of liver and endocrine pancreas

The recent discovery of human biliary tree stem/progenitor cells (hBTSCs) is significant in the study of hepatology, gastroenterology, pathology, and stem cells as it provides new insights in the areas of embryology of the liver, biliary tree, and pancreas; biliary tree and pancreas regeneration and homeostasis; biliary physiology and pathology; biliary cancerogenesis; and, finally, regenerative medicine of the liver and pancreas. BTSCs, biliary tree stem/progenitor cells; CCA, cholangiocarcinoma; HpSCs, hepatic stem/progenitor cells; PBGs, peribiliary glands

effective at phenotypic reprogramming (77). Interestingly, in accordance with the local microenvironment, insulin-positive cells were rarely found in intrahepatic PBGs while they are numerous in the hepato-pancreatic ampulla (2). *In vitro*, the differentiation of BTSCs towards endocrine pancreatic cells is characterized by the loss of pluripotency markers and the sequential acquisition of endocrine committed progenitor markers (NGN3 and MafA), recapitulating the embryologic development of endocrine pancreas from definite endoderm (1-3). *In situ*, a proximal-to-distal axis lineages were present, characterized by a shift from PBG stem cells with pluripotency gene expression to committed progenitors with NGN3/MafA expression in the pancreatic duct glands (76). In the paper by Banga *et al*. (77), insulin-positive cells were mostly located in large intrahepatic bile ducts and associated PBGs and, probably, derive from SOX9+ cells showing the phenotype of BTSCs (SOX17+/PDX1+). This suggests that candidate cells responsible for the permanent reprogramming event are the BTSCs of intrahepatic bile ducts, which are easily driven *in vivo* to differentiate toward pancreatic lineage, recapitulating the embryological development and the possible pancreatic organogenesis throughout life.

Conclusions

The discovery of human biliary tree stem/progenitor cells is opening a new scenario on many aspects of liver and pancreas pathophysiology (*Table 1*). These cells could represent, in the next future, an ideal cell source for the regenerative medicine of liver and pancreas. They are currently the objects of extensive investigations as cells of origin of bilio-pancreatic cancers. Finally, their involvement in the homeostasis and renewal of biliary and pancreatic ducts as well in the pathogenesis of chronic liver diseases, including PBC and PSC is under evaluation.

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References

1. Cardinale V, Wang Y, Carpino G, et al. Multipotent stem/progenitor cells in human biliary tree give rise to hepatocytes, cholangiocytes, and pancreatic islets. Hepatology 2011;54:2159-72.
2. Carpino G, Cardinale V, Onori P, et al. Biliary tree stem/progenitor cells in glands of extrahepatic and intraheptic bile ducts: an anatomical in situ study yielding evidence of maturational lineages. J Anat 2012;220:186-99.
3. Cardinale V, Wang Y, Carpino G, et al. The biliary tree--a reservoir of multipotent stem cells. Nat Rev Gastroenterol Hepatol 2012;9:231-40.
4. Carpentier R, Suñer RE, van Hul N, et al. Embryonic ductal plate cells give rise to cholangiocytes, periportal hepatocytes, and adult liver progenitor cells. Gastroenterology 2011;141:1432-8,1438.e1-4.
5. Zhang L, Theise N, Chua M, et al. The stem cell niche of human livers: symmetry between development and regeneration. Hepatology 2008;48:1598-607.
6. Antoniou A, Raynaud P, Cordi S, et al. Intrahepatic bile ducts develop according to a new mode of tubulogenesis regulated by the transcription factor SOX9. Gastroenterology 2009;136:2325-33.
7. Terada T, Morita T, Hoso M, et al. Pancreatic enzymes in the epithelium of intrahepatic large bile ducts and in hepatic bile in patients with extrahepatic bile duct obstruction. J Clin Pathol 1994;47:924-7.
8. Nakanuma Y, Hoso M, Sanzen T, et al. Microstructure and development of the normal and pathologic biliary tract in humans, including blood supply. Microsc Res Tech 1997;38:552-70.
9. Suzuki A, Zheng YW, Kaneko S, et al. Clonal identification and characterization of self-renewing pluripotent stem cells in the developing liver. J Cell Biol 2002;156:173-84.
10. Yang L, Li S, Hatch H, et al. In vitro trans-differentiation of adult hepatic stem cells into pancreatic endocrine hormone-producing cells. Proc Natl Acad Sci U S A 2002;99:8078-83.
11. Spence JR, Lange AW, Lin SC, et al. Sox17 regulates organ lineage segregation of ventral foregut progenitor cells. Dev Cell 2009;17:62-74.
12. Trzpis M, McLaughlin PM, de Leij LM, et al. Epithelial cell adhesion molecule: more than a carcinoma marker and adhesion molecule. Am J Pathol 2007;171:386-95.
13. Maetzel D, Denzel S, Mack B, et al. Nuclear signalling by tumour-associated antigen EpCAM. Nat Cell Biol 2009;11:162-71.
14. Wandzioch E, Zaret KS. Dynamic signaling network for the specification of embryonic pancreas and liver progenitors. Science 2009;324:1707-10.
15. Zong Y, Stanger BZ. Molecular mechanisms of bile duct development. Int J Biochem Cell Biol 2011;43:257-64.
16. Pech MF, Artandi SE. TRAPping telomerase within the intestinal stem cell niche. EMBO J 2011;30:986-7.
17. Schepers AG, Vries R, van den Born M, et al. Lgr5 intestinal stem cells have high telomerase activity and randomly segregate their chromosomes. EMBO J 2011;30:1104-9.
18. Sancho E, Batlle E, Clevers H. Live and let die in the intestinal epithelium. Curr Opin Cell Biol 2003;15:763-70.
19. Wang Y, Cui CB, Yamauchi M, et al. Lineage restriction of human hepatic stem cells to mature fates is made efficient by tissue-specific biomatrix scaffolds. Hepatology 2011;53:293-305.
20. Sakata R, Aoki T, Ueno T, et al. Morphological observation on extrahepatic bile duct of golden hamsters fed a lithogenic diet: histochemical, ultrastructural and cell kinetic studies. Digestion 1994;55:253-9.
21. Zhang Y, Hong JY, Rockwell CE, et al. Effect of bile duct ligation on bile acid composition in mouse serum and liver. Liver Int 2012;32:58-69.
22. Kurumado K, Nagai T, Abe H. Mucus production of choledochal epithelial cells due to bile stagnation in the rat. Dig Dis Sci 1996;41:263-71.
23. Sutton ME, op den Dries S, Koster MH, et al. Regeneration of human extrahepatic biliary epithelium: the peribiliary glands as progenitor cell compartment. Liver Int 2012;32:554-9.
24. Medema JP, Vermeulen L. Microenvironmental regulation of stem cells in intestinal homeostasis and cancer. Nature 2011;474:318-26.
25. Terasaki S, Nakanuma Y, Unoura M, et al. Involvement of peribiliary glands in primary sclerosing cholangitis: a histopathologic study. Intern Med 1997;36:766-70.
26. Burak KW, Angulo P, Lindor KD. Is there a role for liver biopsy in primary sclerosing cholangitis? Am J Gastroenterol 2003;98:1155-8.
27. Alvaro D, Mancino MG, Onori P, et al. Estrogens and the pathophysiology of the biliary tree. World J Gastroenterol 2006;12:3537-45.
28. Cardinale V, Semeraro R, Torrice A, et al. Intra-hepatic and extra-hepatic cholangiocarcinoma: New insight into epidemiology and risk factors. World J Gastrointest Oncol 2010;2:407-16.

29. Marzioni M, Saccomanno S, Agostinelli L, et al. PDX-1/Hes-1 interactions determine cholangiocyte proliferative response to injury in rodents: possible implications for sclerosing cholangitis. J Hepatol 2012. [Epub ahead of print].
30. Reya T, Morrison SJ, Clarke MF, et al. Stem cells, cancer, and cancer stem cells. Nature 2001;414:105-11.
31. Komuta M, Spee B, Vander Borght S, et al. Clinicopathological study on cholangiolocellular carcinoma suggesting hepatic progenitor cell origin. Hepatology 2008;47:1544-56.
32. Ji J, Yamashita T, Budhu A, et al. Identification of microRNA-181 by genome-wide screening as a critical player in EpCAM-positive hepatic cancer stem cells. Hepatology 2009;50:472-80.
33. Yamashita T, Ji J, Budhu A, et al. EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features. Gastroenterology 2009;136:1012-24.
34. Marquardt JU, Raggi C, Andersen JB, et al. Human hepatic cancer stem cells are characterized by common stemness traits and diverse oncogenic pathways. Hepatology 2011;54:1031-42.
35. Woo HG, Park ES, Thorgeirsson SS, et al. Exploring genomic profiles of hepatocellular carcinoma. Mol Carcinog 2011;50:235-43.
36. Roskams T. Liver stem cells and their implication in hepatocellular and cholangiocarcinoma. Oncogene 2006;25:3818-22.
37. Vander Borght S, Komuta M, Libbrecht L, et al. Expression of multidrug resistance-associated protein 1 in hepatocellular carcinoma is associated with a more aggressive tumour phenotype and may reflect a progenitor cell origin. Liver Int 2008;28:1370-80.
38. Welzel TM, Graubard BI, El-Serag HB, et al. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma in the United States: a population-based case-control study. Clin Gastroenterol Hepatol 2007;5:1221-8.
39. Tyson GL, El-Serag HB. Risk factors for cholangiocarcinoma. Hepatology 2011;54:173-84.
40. Strobel O, Rosow DE, Rakhlin EY, et al. Pancreatic duct glands are distinct ductal compartments that react to chronic injury and mediate Shh-induced metaplasia. Gastroenterology 2010;138:1166-77.
41. Cardinale V, Wang Y, Carpino G, et al. Mucin-producing cholangiocarcinoma might derive from biliary tree stem/progenitor cells located in peribiliary glands. Hepatology 2012;55:2041-2.
42. Carpino G, Cardinale V, Reid L, et al. Cells of origin and cancer stem cells in cholangiocarcinoma. Transl Gastrointest Cancer 2012;1:33-43
43. Komuta M, Govaere O, Vandecaveye V, et al. Histological diversity in cholangiocellular carcinoma reflects the different cholangiocyte phenotypes. Hepatology 2012;55:1876-88.
44. Cardinale V, Carpino G, Reid L, et al. Multiple cells of origin in cholangiocarcinoma underlie biological, epidemiological and clinical heterogeneity. World J Gastrointest Oncol 2012;4:94-102.
45. Cardinale V, Carpino G, Reid LM, et al. Cholangiocarcinoma: a cancer in search of the right classification. Hepatology 2012;56:1585-6; author reply 1586.
46. Blechacz B, Komuta M, Roskams T, et al. Clinical diagnosis and staging of cholangiocarcinoma. Nat Rev Gastroenterol Hepatol 2011;8:512-22.
47. Nakanuma Y, Sato Y, Harada K, et al. Pathological classification of intrahepatic cholangiocarcinoma based on a new concept. World J Hepatol 2010 27;2:419-27.
48. Puppi J, Strom SC, Hughes RD, et al. Improving the techniques for human hepatocyte transplantation: report from a consensus meeting in London. Cell Transplant 2012;21:1-10.
49. Dhawan A, Puppi J, Hughes RD, et al. Human hepatocyte transplantation: current experience and future challenges. Nat Rev Gastroenterol Hepatol 2010;7:288-98.
50. Muraca M. Evolving concepts in cell therapy of liver disease and current clinical perspectives. Dig Liver Dis 2011;43:180-7.
51. Schmelzer E, Zhang L, Bruce A, et al. Human hepatic stem cells from fetal and postnatal donors. J Exp Med 2007;204:1973-87.
52. Turner R, Lozoya O, Wang Y, et al. Human hepatic stem cell and maturational liver lineage biology. Hepatology 2011;53:1035-45.
53. Gridelli B, Vizzini G, Pietrosi G, et al. Efficient human fetal liver cell isolation protocol based on vascular perfusion for liver cell-based therapy and case report on cell transplantation. Liver Transpl 2012;18:226-37.
54. Kubota H, Reid LM. Clonogenic hepatoblasts, common precursors for hepatocytic and biliary lineages, are lacking classical major histocompatibility complex class I antigen. Proc Natl Acad Sci U S A 2000;97:12132-7.
55. Rao MS, Khan AA, Parveen N, et al. Characterization of hepatic progenitors from human fetal liver during second trimester. World J Gastroenterol 2008;14:5730-7.
56. Yanes O, Clark J, Wong DM, et al. Metabolic oxidation

regulates embryonic stem cell differentiation. Nat Chem Biol 2010;6:411-7.
57. Cardinale V, Carpino G, Cantafora A, et al. Metabolic oxidation controls the hepatic stem cells (HpSCs) fate and the hepatic lineage organization in physiologic and pathologic conditions. Hepatology 2012;56:2006-7.
58. Khan AA, Shaik MV, Parveen N, et al. Human fetal liver-derived stem cell transplantation as supportive modality in the management of end-stage decompensated liver cirrhosis. Cell Transplant 2010;19:409-18.
59. Houlihan DD, Newsome PN. Critical review of clinical trials of bone marrow stem cells in liver disease. Gastroenterology 2008;135:438-50.
60. Xu YQ, Liu ZC. Therapeutic potential of adult bone marrow stem cells in liver disease and delivery approaches. Stem Cell Rev 2008;4:101-12.
61. Forbes SJ, Newsome PN. New horizons for stem cell therapy in liver disease. J Hepatol 2012;56:496-9.
62. Kiskinis E, Eggan K. Progress toward the clinical application of patient-specific pluripotent stem cells. J Clin Invest 2010;120:51-9.
63. Semeraro R, Carpino G, Cardinale V, et al. Multipotent stem/progenitor cells in the human foetal biliary tree. J Hepatol 2012;57:987-94.
64. Semeraro R, Cardinale V, Carpino G, et al. The fetal liver as cell source for the regenerative medicine of liver and pancreas. Ann Transl Med 2012;1:2.
65. Mineo D, Pileggi A, Alejandro R, et al. Point: steady progress and current challenges in clinical islet transplantation. Diabetes Care 2009;32:1563-9.
66. D'Amour KA, Agulnick AD, Eliazer S, et al. Efficient differentiation of human embryonic stem cells to definitive endoderm. Nat Biotechnol 2005;23:1534-41.
67. Kroon E, Martinson LA, Kadoya K, et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol 2008;26:443-52.
68. Murry CE, Keller G. Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. Cell 2008;132:661-80.
69. D'Amour KA, Bang AG, Eliazer S, et al. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. Nat Biotechnol 2006;24:1392-401.
70. Ku HT, Zhang N, Kubo A, et al. Committing embryonic stem cells to early endocrine pancreas in vitro. Stem Cells 2004;22:1205-17.
71. Prabakar KR, Domínguez-Bendala J, Molano RD, et al. Generation of glucose-responsive, insulin-producing cells from human umbilical cord blood-derived mesenchymal stem cells. Cell Transplant 2012;21:1321-39.
72. De Coppi P, Bartsch G Jr, Siddiqui MM, et al. Isolation of amniotic stem cell lines with potential for therapy. Nat Biotechnol 2007;25:100-6.
73. Furth ME, Atala A. Stem cell sources to treat diabetes. J Cell Biochem 2009;106:507-11.
74. Zhou Q, Brown J, Kanarek A, et al. In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature 2008;455:627-32.
75. Zhou Q, Melton DA. Extreme makeover: converting one cell into another. Cell Stem Cell 2008;3:382-8.
76. Banga A, Akinci E, Greder LV, et al. In vivo reprogramming of Sox9+ cells in the liver to insulin-secreting ducts. Proc Natl Acad Sci U S A 2012;109:15336-41.
77. Wang Y, Lanzoni G, Carpino G, et al. Biliary tree stem cells are precursors to pancreatic committed progenitors: evidence for possible pancreatic organogenesis throughout life. Stem Cells 2012. [Epub ahead of print].

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