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Review

Stem/progenitor cells in the developing human liver: morphological and immunohistochemical features

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Abstract

Stem/progenitor cells in the adult liver are able to differentiate both into hepatocyte and cholangiocyte lineage. The identification and the role of human liver stem/progenitor cells has been a challenge topic in the recent scientific literature. The existence of stem/progenitor cells in the liver was first claimed in experimental animal models. CD34, c-kit, cytokeratin 7, cytokeratin 19, α-fetoprotein, OV6 and CD90 were the first markers shown. The major support for the existence of hepatic stem/progenitor cells has come from studies on liver carcinogenesis, human liver disease and cirrhosis. Where exactly the putative stem/progenitor cells reside in the normal liver is still controversial and their specific anatomical location is still unclear. Preliminary data from our lab indicate the portal tracts as the preferential site of the stem cell niches thanks to the expression of biliary-type cytokeratin 19, SOX9 and c-kit. Small undifferentiated cells were easily identified in H&E as well. Like in other organs, hepatic stem/progenitor cell niche was hypothesized and described as composed of numerous cell types that interact and cross-talk with hepatic stem/progenitor cells. Hepatic stem/progenitor cells represent a heterogeneous

population with a spectrum of morphological and immunohistochemical features ranging from bile duct cells to hepatocytes, including the multipotent hepatic stem/progenitor cells, the hepatoblasts, the committed progenitors and the diploid adult cells. Inside this complex and articulate spectrum, cells without hepatobiliary markers and hematopoietic stem/progenitor cells can be identified. The hepatic stem/progenitor cells exhibit specific population functions and can be identified by specific population immunohistochemical markers, including CD133, CXCR4, SOX9, SOX17, cytokeratins, Hedgehog proteins, MDR1 and many others. In conclusion, this study represents the basis for further studies, aimed at better characterizing these stem/progenitor cells and at identifying possible subtypes of hepatic stem progenitor cells.

Keywords

Stem cells, progenitor cells, human, liver, niche, morphogenesis.

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Introduction

Stem/progenitor cells are characterized by self-renewal ability, cellular plasticity and the aptitude to give rise to multiple differentiated cellular populations [1]. In the adult liver, the potential to regenerate after a severe liver injury or damage suggests the presence of hepatic stem/progenitor cells able to differentiate both into hepatocyte and cholangiocyte lineage [2, 3]. The identification of the role in response to parenchymal loss of human liver stem/progenitor cells has been a challenge and a contentious topic of the recent scientific literature. In addition to the most organized mature hepatocytes and cholangiocytes, liver stem/progenitor cells can also differentiate and give rise to extra-hepatic cell types such as intestinal, pancreatic [4, 5] and insulin-producing cells or could generate malignant transformation and liver cancer [6]. The regulatory factors Wnt, Hedgehog, and Notch have been reported to be related to hepatic stem/progenitor cell regulation [2, 3].

This work was aimed at giving an update on the morphological and immunohistochemical features of stem/progenitor cells in the developing human liver.

Historical and scientific background

The existence of stem/progenitor cells in the liver was first claimed in an experimental animal model, suggesting that prolonged and severe hepatic injury may be restored by pre-existing parenchymal cells derived from undifferentiated biliary cells [7]. In the first studies, liver stem/progenitor cells were not easily identified other than in the experimental animal models [8]. The treatment with acetylaminofluorene (AAF) was one of the most studied methods utilized to induce proliferation of hepatic stem/progenitor cells [4, 9]. Autoradiography from rats fed with AAF in a choline-devoid diet evidenced the presence of proliferating small periportal cells [9] with scant cytoplasm and ovoid nuclei, termed oval cells [10], that rapidly spread from periportal tracts across the liver [9]. Further experimental studies have then discovered a variety of toxins and carcinogens that, in combination or alone, were able to induce oval cells proliferation [4]. CD34, c-kit [4], cytokeratin 7, cytokeratin 19, α-fetoprotein, OV6 and CD90 [11, 12] were the markers shown to be expressed or up-regulated by rat oval cells. Moreover, in a rat model of extrahepatic cholestasis, proliferating oval cells were found to express cytokeratin 20, a cytokeratin never expressed in normal human liver [13]. Hepatic stem/progenitor cells from fetal, pediatric and adult human liver have been isolated and cultured using antibodies against c-kit and CD34 [4].

The major support for the existence of hepatic stem/progenitor cells has come from liver carcinogenesis studies [14]. Oval cell proliferation was observed in the intermediate stages of development of liver cancers in some transgenic mice expressing the SV40 antigen [15, 16]. Multiple evidences suggested that hepatocellular carcinoma and cholangiocarcinoma might derive from hepatic stem/progenitor cells after the loss of proliferation control [14, 17]. Several studies have identified hepatic stem/progenitor cells in hepatoblastoma [18] and hepatocellular carcinoma [19]. Numerous morphological studies have highlighted the presence of small hepatic stem/progenitor cells in

human liver disease [20] and cirrhosis [21]. Some investigators believe that, before hepatocellular carcinoma develops, hepatic stem/progenitor cells undergo developmental and differentiation arrest [22, 23]. Evidence for this hypothesis includes the fact that some carcinogenic protocols induce hepatic stem/progenitor cells proliferation without apparent proliferation of hepatocytes. Nowadays, many progresses in characterizing stem/progenitor cell population from distinct organs, such as bone marrow, nervous system as well as liver, have been made.

Where are hepatic stem/progenitor cells localized?

In the adult human liver, stem/progenitor cells represent the activated progeny of a dormant cell compartment that are readily identified in persistent severe liver injury after chronic viral hepatitis [24], steatohepatitis [25] and other causes. The first suggestions focused the attention on a distinct population of periductal cells, located in the portal tracts [7, 26, 27]. However, where exactly these putative stem/progenitor cells reside in the normal liver is still controversial and their specific anatomical location is still unclear. Some authors hypothesized that adult hepatic stem/progenitor cells might originate from ductular cells of the terminal branches of the intrahepatic biliary system, connecting the canals of Hering with the bile canaliculi [7, 26, 27]. Otherwise, other studies recognized the periportal hepatocytes as the very one to possess stem/progenitor cells and metaplastic properties [5, 27]. Preliminary data from our lab on the human fetal liver indicate the portal tracts as the preferential site of the stem cell niches. At the periphery of developing portal tracts, ductal plate cells were easily identified and characterized by the expression of biliary-type cytokeratins (**Fig. 1**). Moreover, in the center of the developing portal tracts, small undifferentiated cells with oval nuclei

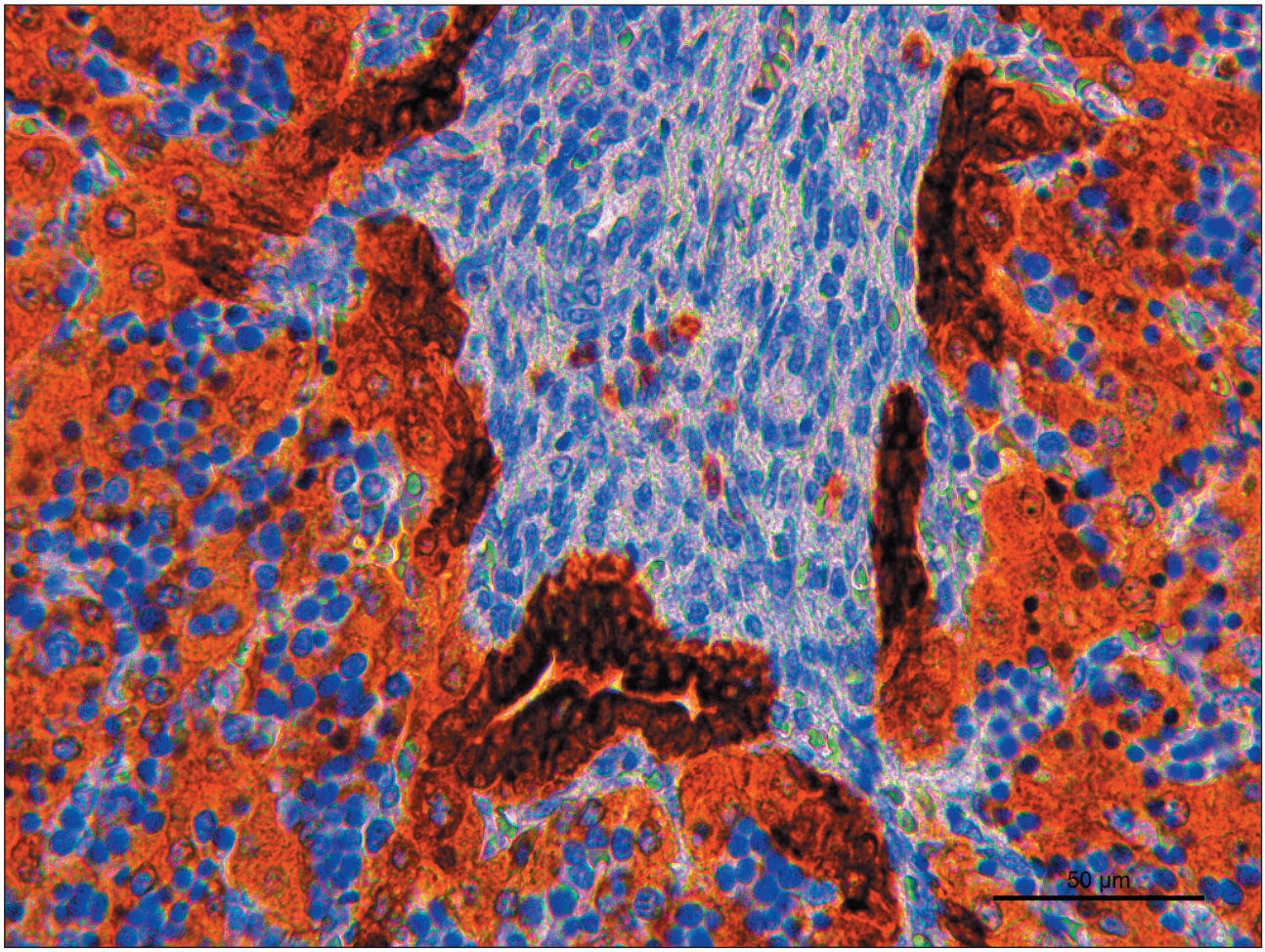

Figure 1. Cytokeratin 19 expressed in the hepatoblasts, in the intermediated hepatobiliary cells and the small undifferentiated cells of the mesenchyme of the portal tract; hematopoietic stem/progenitor cells are negative (40 HPF).

and scant cytoplasm, similar in shape to the rat oval cells, were easily identified in H&E-stained sections (**Fig. 2**). At immunohistochemistry, these hepatic stem/progenitor cells were characterized by the expression of SOX9 and c-kit (**Fig. 3** and **Fig. 4**).

The hepatic stem/progenitor cell niche

The stem/progenitor cell niche hypothesis proposed that stem/progenitor cells reside within stationary compartments. The niche is not only the position where stem/progenitor cells are located, but it takes also account of the composition of the surrounding signals and intrinsic programs required to control proliferation and differentiation [28]. A stem/progenitor cell niche is the cellular and extracellular microenvironment, the interaction with other cell types, signaling and adhesion molecules which contributes to sustain self-renewal, supports and regulates stem/progenitor cell maintenance [29, 30]. Like in other organs, hepatic stem/progenitor cell niche is composed of numerous cell types, including portal myofibroblasts, hepatic stellate cells, endothelial cells, hepatocytes, cholangiocytes, Kupffer cells, Pit cells and immune cells. Any of these cell types can interact and cross-talk with hepatic stem/progenitor cells, thereby influencing proliferation and differentiation within the niche itself [11]. Numerous studies demonstrated the existence of the hepatic stem/progenitor cell niche in the canals of Hering, lined both by small cholangiocytes and hepatocytes. A maturation from stem/progenitor cells to mature ones occurs within the liver parenchyma, from periportal through the midacinar to the periterminal zone [31]. Stem/progenitor cell niches continuously generate from the portal field new small hepatocytes [32], that mature on their way to the central vein, supporting the concept of the "streaming liver" [28]. Parenchymal and mesenchymal cells and their maturation are strictly connected [33]. In order to understand the liver niche,

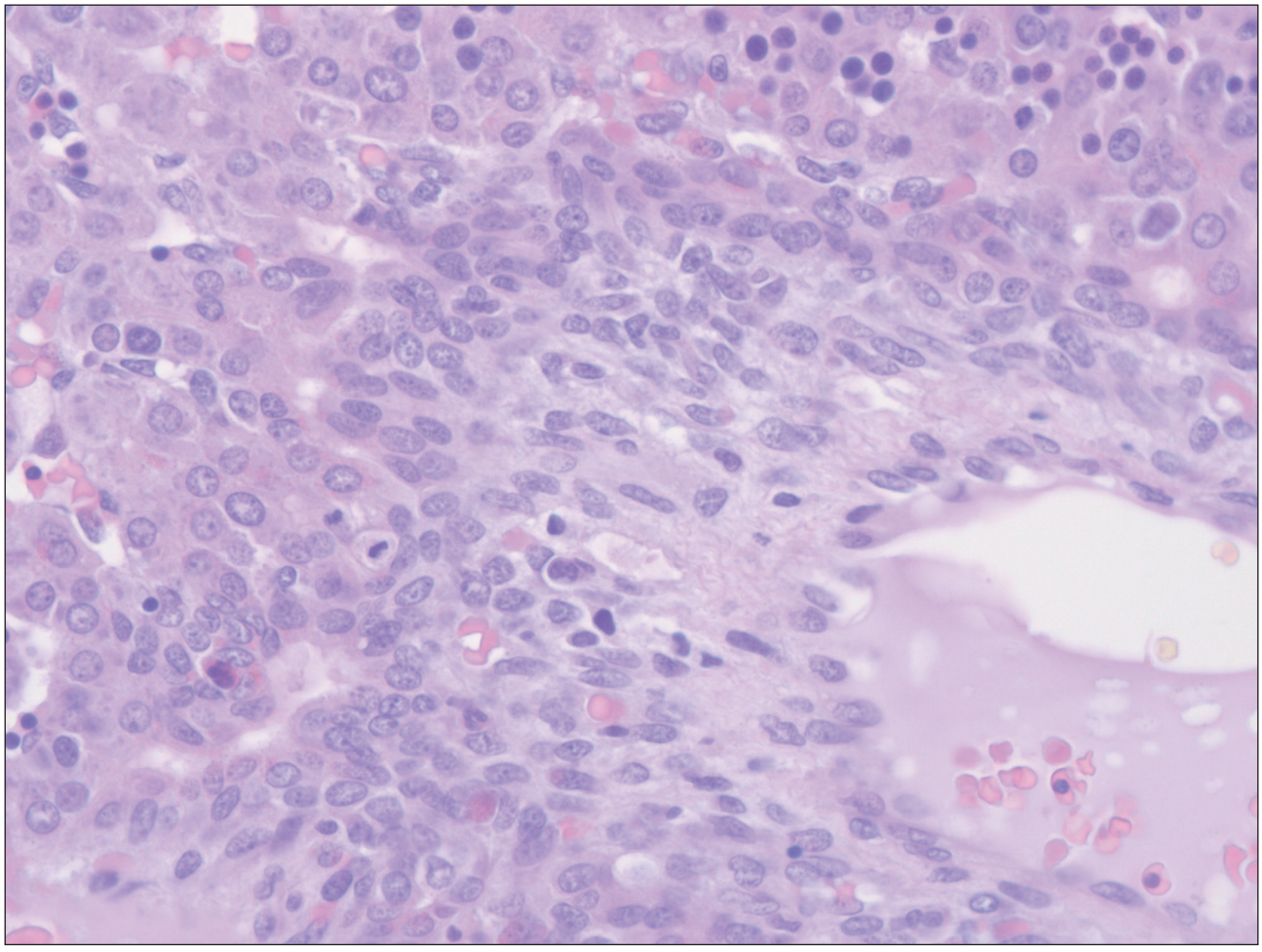

Figure 2. Small undifferentiated cells with oval nuclei and scant cytoplasm, similar in shape to the rat oval cells in H&E-stained sections (40 HPF).

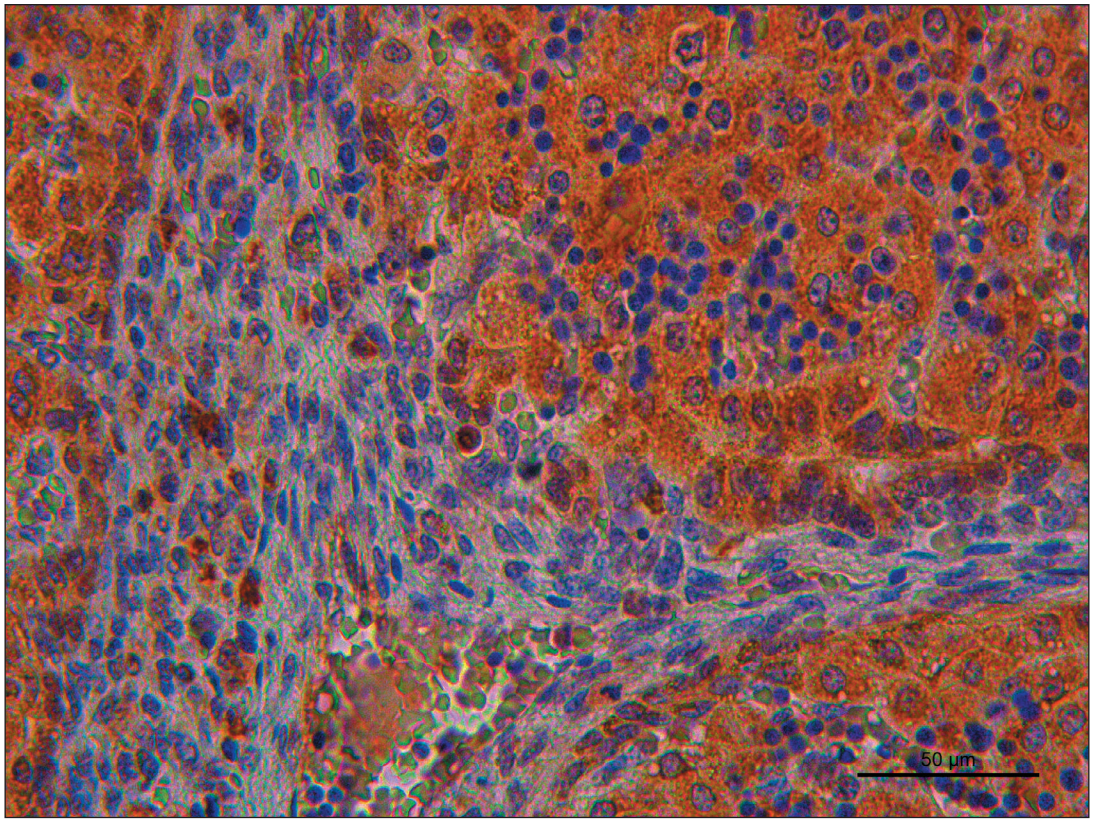

Figure 3. SOX9 highly expressed in the small undifferentiated cells of the mesenchyme of the portal tract and weakly expressed in the hepatoblasts, in the intermediated hepatobiliary cells; hematopoietic stem/progenitor cells are negative (40 HPF).

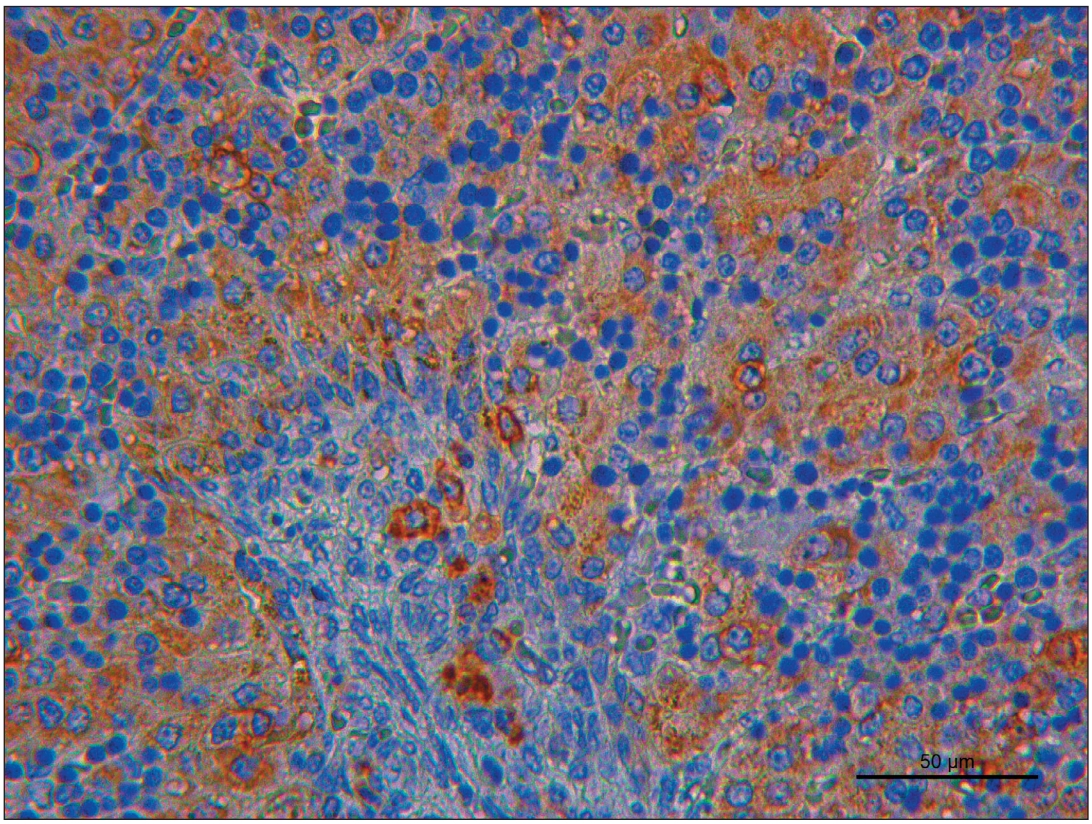

Figure 4. c-kit highly expressed in the small undifferentiated cells of the mesenchyme of the portal tract and weakly expressed in the hepatoblasts, in the intermediated hepatobiliary cells; hematopoietic stem/progenitor cells are negative (40 HPF).

the model of the intestinal stem cell niche could be used: at the bottom of the gland are placed the stem/progenitor cells, in the middle the intermediate cells and within the surface epithelium the differentiated cells [34]. As in the stem/progenitor cell niches of other organs, including bone marrow, intestine, and brain, the signaling pathways Wnt, Notch, and Hedgehog work in concert in order to regulate the maintenance of stem/progenitor cell quiescence, control the proliferation and manage cell fate. In particular, Notch and Wnt signals regulate the switch between cells division or differentiation [30] (**Fig. 5**).

How many are the liver stem progenitors?

Hepatic stem/progenitor cells constitute an average 2% of the parenchyma of fetal livers [34] and represent a heterogeneous population of duct-forming and non ductular cells that show varying expression patterns [35], with a spectrum of morphological and immunohistochemical features ranging from bile duct cells [36] to hepatocytes [37]. In addition, inside this complex and articulate spectrum, cells without hepatobiliary markers [32] and hematopoietic stem/progenitor cells can be identified [32, 38].

Hepatic stem/progenitor cells include the following subtypes:

1. multipotent hepatic stem/progenitor cells;
2. hepatoblasts;
3. committed progenitors, differentiated into:
   a. committed hepatocytic progenitors;
   b. small cholangiocytes;
4. diploid adult cells, consisting of:
   a. diploid adult;
   b. large cholangiocytes;
5. null cells;
6. hematopoietic stem/progenitor cells.

Shape and morphology

1. *Multipotent hepatic stem/progenitor cells* range in size from 7 to 10 μm in diameter and have a high nucleus-to-cytoplasm ratio [39, 40].
2. *Hepatoblasts* are diploid bipotent large cells, ranging from 10 to 12 μm, showing higher amounts of cytoplasm than hepatic stem/progenitor cells [31].
3. Among the *committed progenitors*, the *committed hepatocytic progenitors* are diploid, unipotent, immature cells from 12 to 15 μm [41], while the *small cholangiocytes* range in size from 6 to 8 μm in diameter and are diploid cuboidal cells, the nucleus-to-cytoplasm ratio is high and the endoplasmic reticulum small [31, 42].
4. *Diploid adult hepatocytes* are similar but smaller compared with normal adult hepatocytes [43]. *Large cholangiocytes* display a columnar shape, a smaller nucleus and a larger cytoplasm; the Golgi apparatus is more abundant [31, 44].
5. *Null cells* do not exhibit neither hepatocellular nor biliary cells features [32].
6. Stem and progenitor cells committed to hematopoietic, endothelial, epithelial, and mesenchymal lineages are clearly identifiable in livers [34]. *Hematopoietic stem/progenitor cells*

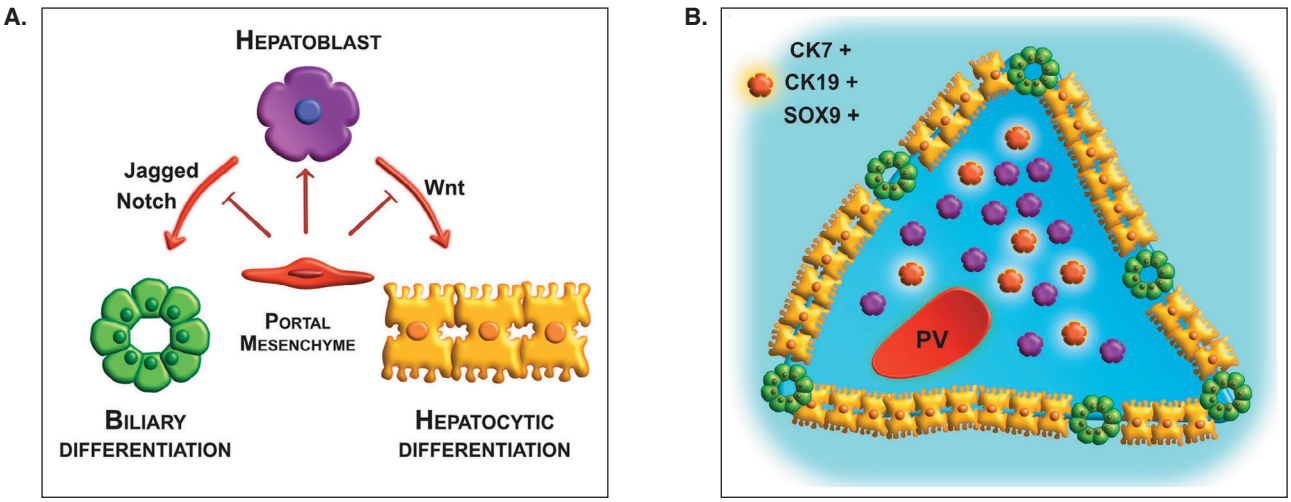

Figure 5. A. Schematic representation of the hepatic stem/progenitor cell niche and the interaction and cross-talk influencing proliferation and differentiation within the niche. **B.** Schematic representation of the expression of cytokeratins and SOX9 in stem/progenitor cells located in the mesenchymal portal tract.

show erythroid, myeloid and megakaryocytic lineage.

Identification

1. The *multipotent hepatic stem/progenitor cells* express epithelial cell adhesion molecule (EpCAM), neural cell adhesion molecule (NCAM), CD133, CXCR4, SOX9, SOX17, FOXA2, cytokeratins 8/18, cytokeratin 19, Hedgehog proteins (Sonic and Indian), intranuclear telomerase protein, claudin 3, MDR1, weak positivity of albumin and MHC antigens. Cytokeratin 19 is observed in punctuate form [45]. Albumin is synthesized but not packaged as in later lineage stages [31]. They do not express α-fetoprotein, intercellular adhesion molecule (ICAM-1), P450s, markers for hematopoietic, endothelial or mesenchymal cells [42, 45, 46]. It remains unclear whether c-kit (CD117), expressed in the liver's stem/progenitor cell niches [27, 47, 48], is on hepatic stem/progenitor cells or associated angioblasts, wrongly selected by flow cytometry [42, 45].
2. The *hepatoblasts* have a phenotypic profile that overlaps with hepatic stem/progenitor cells [31, 45, 46] by expressing CD133, CXCR4, SOX17, cytokeratins 8/18, cytokeratin 19, Hedgehog proteins (Sonic and Indian), MDR1 and not expressing P450s (P450-3A) or markers for hemopoietic, endothelial or mesenchymal cells [31, 49]. Differences with hepatic stem/progenitor cells include: reduction in levels of EpCAM; cytokeratin 14 and cytokeratin 19 in filamentous form [27, 47]; albumin in elevated levels [49] with discrete cytoplasmic packaging [7]; switch from NCAM to ICAM-1; expression of early P450s (P450-A7), cytokeratin 7, α-fetoprotein [49], gamma-glutamyl transpeptidase, and the placental form of glutathione-S-transferase (GST-P) [39-41].
3. *Committed progenitors* lose most stem/progenitor cell gene expression (NCAM, Hedgehog proteins), and express either hepatocytic or biliary markers [41].
   a. *Committed hepatocytic progenitors* express albumin, enzymes associated with glycogen synthesis (glucose-6-phosphate) and lack biliary markers (cytokeratin 19) and α-fetoprotein [31].
   b. *Small cholangiocytes* express anti-apoptotic proteins (annexin V and bcl2), endothelin receptors type A (EDNRA) and type B (EDNRB), endogenous opioid peptides, insulin, histamine (H1), acetylcholine (M3), α-1-adrenergic agonists, aquaporin 4, cystic fibrosis transmembrane conductance regulator (CFTR). Small cholangiocytes are negative for the Cl/HCO3-exchanger, secretin and somatostatin receptors [31, 50].
4. *Diploid adult cells*
   a. *Diploid adult hepatocytes*
   b. *Large cholangiocytes* express cystic fibrosis transmembrane conductance regulator (CFTR), Cl/HC03-exchanger, aquaporin 4, aquaporin 8, secretin and somatostatin receptors, receptors for hormones and neuropeptides, Na-dependent bile acid transporter ABAT (apical bile acid transporter), MDR (multidrug transporter) and MRP (multidrug resistance associated proteins) [31].
5. *Null cells* are characterized by the absence of hepatobiliary markers [32].
6. *Hematopoietic stem/progenitor cells* are recognizable through their specific phenotype profiles, expressing CD34 and CD45 [51]. Liver becomes the predominant site of hematopoiesis by 11.5 days in the mouse and 15 gestational weeks in humans and stays so until the end of gestation. The reason the liver is the major hematopoietic site during fetal life is not clear [52]. Endothelial stem/progenitor cells are vascular endothelial growth factor-2 (KDR) and CD146 positive, CD45 is negative. Mesenchymal stem/progenitor cells show CD73, CD105, CD90 (Thy-1) positivity and CD45 negativity [51]. Within the human liver, c-kit (CD117)-positive pluripotent hematopoietic stem cells (HSCs), residing in the perisinusoidal Disse spaces, give rise to all lineages of leukocytes and red blood cells [34, 53]. These mesenchymal-derived liver mononuclear cells can reconstitute the thymus, liver leukocytes, splenocytes, lymph nodes and bone marrow cells in mice [54].

Stromal cells are demonstrated to expressed in the hematopoietic florid phase both mesenchymal markers (vimentin, osteopontin, collagen I, α smooth muscle actin, thrombospondin-1, EDa fibronectin, calponin, Stro-1 antigens, myocyte-enhancer factor 2C) and epithelial (α-fetoprotein, cytokeratins 8 and 18, albumin, E-cadherin, hepatocyte nuclear factor 3α) markers. The expression of both mesenchymal and epithelial markers is suggestive for epithelial-to-

mesenchymal transition (EMT) that is frequently described in organogenesis and in liver development as well as during the hematopoiesis but not at the end of gestation and in the adult nonhematopoietic liver. EMT cells are rare considered a new unusual stromal cell type since they may be generated from circulating HSCs or both hepatic endodermal and mesenchymal stem cells. Oncostatin induces hepatocytic maturation and the loss of hematopoietic ability in EMT cells [52].

Function

1. *Multipotent hepatic stem/progenitor cells* are located within the liver's stem/progenitor cell compartment, along the portal tracts in a starlike shape, in the ductal plates and canals of Hering. This site represents the anatomic and functional link between the biliary tree and the intralobular hepatocytes biliary canaliculi. Hepatic stem/progenitor cells constitute about 0.5-2% of the parenchyma of livers of all age donors [39, 40]. Multipotent hepatic stem/progenitor cells are tolerant of ischemia, they can remain viable in cadaveric livers for about 6 days after death [39, 40]. Hepatic progenitors can potentially be stimulated by various components of the inflammatory response, such as lymphotoxin-β, IFNγ, TNF-α and histamine. Furthermore, it has been proposed that the proliferative ability can decrease with the resistance to transforming growth factor (TGF)-β, which allows progenitors to divide under conditions that would normally inhibit hepatocyte proliferation [55].
2. *Hepatoblasts* are undifferentiated hepatic cells revealed by developmental biology in the developing liver, some of them are able to differentiate into hepatocyte and cholangiocyte lineages [56]. Hepatoblasts reside throughout parenchyma of fetal and neonatal livers or as single cells and small cell aggregates bound to the ends of canals of Hering in adult livers [47]. The hepatoblasts can give rise both to hepatocytic and cholangiocytic cells [49], and are connected with other precursors, such as endothelia, hepatic stellate cells, and the hepatic transit amplifying cells [42]. Jagged/Notch signaling is crucial for biliary differentiation of the hepatoblasts [57]. During liver development, Notch signaling is required for biliary specification of embryonic hepatoblasts. Wnt signaling drives the hepatoblasts to hepatocyte differentiation [57]. In the developing human fetal liver, the differentiation of hepatoblasts into biliary epithelium is regulated by signals from the portal mesenchyme [58]; beta-catenin regulates multiple critical events during the hepatic morphogenesis process, including hepatoblast maturation, expansion, and survival [59, 60]; hepatoblasts not in contact with portal mesenchyme mature into differentiated hepatocytes [4].
3. *Committed progenitors* give rise to only one adult cell type and are especially found in fetal and neonatal tissues or chronic liver diseases (viral, alcoholic and non-alcoholic fatty liver diseases, autoimmune hepatitis, cholangiopathies), while they are not in normal adult liver [41].
   a. *Committed hepatocytic progenitors* are also called intermediate hepatocytes, they are associated with endothelial cell precursors and are located *in vivo* in the liver plates between the hepatoblasts and the diploid adult hepatocytes [31]. Committed hepatocytic progenitors replicate during normal liver growth and newly formed cells do not migrate [61]. Other investigators favor the alternative theory that all hepatocyte can replicate regardless of their position. This theory has been based primarily on the fact that a partial hepatectomy induce one [62, 63] or more rounds of replication of most hepatocytes within 48 h, implying that all have proliferative potential. An alternative theory states that the hepatocyte can develop into hepatocellular carcinoma if a sufficient number of mutations accumulate. This theory is supported by the fact that the administration of some carcinogenic agents results in the appearance of foci of replicating hepatocytes with altered gene expression before the development of cancer [64].
   b. *Small cholangiocytes* are localized together with hepatic stem/progenitor cells, in the stem/progenitor cell niche, within the canals of Hering, intralobular hepatocytes biliary canaliculi and bile ductules below 15 μm. The current hypothesis that characterizes the small cholangiocytes as committed biliary progenitors is supported by the links between bile ductules and canals of Hering, since bile ductules may cross the limiting plate through the periportal intralobular segment in addition to the intraportal site [31]. Small cholangiocytes are associated with hepatic stellate cell precursors [31, 42].

4. *Diploid adult cells* are considered the sole parenchymal cells able to significantly proliferate under all known conditions both *in vitro* and *in vivo*. The genetic reprogramming through chromatin demethylation is the exception, occuring in tyrosinemia and in massive injury (> 80%) of mature parenchymal cells involving a transgene due to the mechanism for restoring cytokinensis [31, 65, 66].
   a. *Diploid adult hepatocytes* are partnered with endothelia, in culture they can undergo more than 6 division in 3 weeks, but have limited sub-cultivation capacity [43].
   Hedgehog signaling is essential for survival of this progenitor cells, since the stem/progenitor cells express and activate the specific receptor Patched (PTC) [67]. It was shown that Wnt and Notch signaling are also active in the adult human liver to drive proliferation and differentiation of the progenitor cells into the hepatocyte or cholangiocyte lineage [2].
   b. *Large cholangiocytes* are partnered with stellate cells and line interlobular ducts located in the portal triads. The connections of multipotent hepatic stem/progenitor cells in canals of Hering to the septal and segmental bile ducts have not yet been investigated. The markers expressed by septal, segmental and larger ducts are similarly found in the stem/progenitor cell niches of the biliary tree in peribiliary glands [68]. The acute large cholangiocytes are damaged, due for example to carbon tetrachloride (CCl4) or GABA, whereas the small cholangiocytes begin to proliferate, then change their phenotypical and functional features and acquire the ones of large cholangiocytes [69, 70]. This suggests that the population of small cholangiocytes within the ductules and the canals of Hering can be considered as the precursors of the cells lining the larger ducts. The microarray through the integration of the different gene expression profile in the small and large normal cholangiocytes demonstrates that the large cholangiocytes express genes related to function and differentiation, whereas the small cholangiocytes highly express the genes associated with cell proliferation [44]. This is consistent with many studies showing that bile duct injury due to CCl4 and GABA administration or the bile duct growth after hepatic resection leads to small cholangiocyte proliferation activated in order to restore the bile ducts. For that reason the small cholangiocytes are considered less mature then large cholangiocytes. While large cholangiocytes are more differentiated and contribute chiefly to bile secretion and absorption, small cholangiocytes demonstrate proliferative activities and high resistance to apoptosis. Therefore, cholangiocytes proceed in the opposite direction than hepatocyte, since cholangiocytes proceed from canals of Hering/ductules toward larger ducts, while hepatocytes from periportal areas toward the central vein [31].
5. *Null cells* were also identified, closed to portal tracts in addition to the other cell types, and characterized by the absence of hepatobiliary markers [32]. Null cells might represent stem/progenitor cells from extrahepatic sites and may originate from circulating bone marrow cells [32, 38].
6. *Hematopoietic stem/progenitor cells* expressing thymus cell antigen-1 were already reported to contribute to liver regeneration [71]. Conflicting results emerged from this and other studies demonstrating that in the liver a population of hematopoietic stem/progenitor cells originating in the bone marrow, under certain physiologic conditions, may give rise to cells potentially able to further differentiate into hepatocytes and/or ductal cells [71, 72]. Other data demonstrated that in the injured liver stem/progenitor cells do not arise through transdifferentiation from bone marrow cells, but from endogenous liver progenitors [73].

Discussion

The human fetal liver represents a major source of stem/progenitor cells. Preliminary data from our lab on the human fetal liver identified the composition and the location of the hepatic stem cell niche, which has been the subject of several previous studies in recent years [28-30]. We identified the vast majority of stem cells within the immature portal tracts, located in the mesenchyme, in close proximity to the ductal plate progenitor cells. These hepatic stem/progenitor cells appeared as undifferentiated cells, with oval large nuclei, and scant cytoplasm, isolated or arranged in small groups. On a morphological ground, these cells appeared similar to oval cells described in the rat liver after treatment with AAF. The major

differences with that experimental model regards the location of stem cells. Whereas in the rat liver oval cells spread from the portal tract towards the liver acini, in our study stem cells with oval nuclei were exclusively observed inside the developing portal tracts.

Other than the morphological features, we also found immunoreactivity of the stem/progenitor oval-like hepatic cells for SOX9 and c-kit, two typical markers of stem/progenitor cells; this underlines their stemness, and indicates these cells as the putative stem/progenitor cells of the human liver. Further studies are needed in order to better specify the immunoreactivity of these hepatic stem/progenitor cells for other typical markers of stem/progenitor cells, in order to verify the existence of subtypes of liver progenitors, characterized by the expression of different stem cell markers. Preliminary studies in our lab confirm previous data on the reactivity of liver stem cells for cytokeratin 9 [45].

Regarding the liver stem/progenitor cell niche, our data support the hypothesis that the portal tracts represent the preferential site of the niche in the developing liver. In previous studies on liver development, the ductal plate was considered as the principal site of liver progenitors, and a distinct population of periductal cells located in the portal tracts has been indicated by multiple authors as the putative stem/progenitor cells of the liver [7, 26, 27]. Moreover, the periportal hepatocytes were indicated as the liver cells able to possess stem/progenitor cell properties [5, 27]. In our experience, cells with morphological and immunohistochemical features of stem/progenitor cells have been identified, at the best of our knowledge for the first time, inside the portal tracts, suggesting the existence of oval-like cells even in the fetal human liver.

Our data may lead to a new interpretation of the composition of the stem/progenitor cell niche in the developing human liver. On the basis of our findings, multiple cell types appear to contribute to the hepatic stem cell niche, including oval-like cells, portal stromal cells, the endothelium of the developing portal vein, hematopoietic cells located in portal tracts, ductal plate cells, and periportal hepatoblasts. This picture underlines the complexity of the liver stem cell niche, given the presence in the same structure of so many cell types with so different differentiation stages. The relationships among all these cell types and stem cells are, at least in part, unknown, and represent a new challenge for hepatologists.

In conclusion, our study first evidenced the presence of a large number of undifferentiated stem/progenitor cells in the portal tracts of the developing human liver, and characterized these cells as immunoreactive for SOX9, c-kit and cytokeratin 19, allowing perinatal pathologists to evidence and mark them. In our opinion, this study represents the basis for further studies, aimed at better characterizing these stem/progenitor cells and at identifying possible subtypes of hepatic stem progenitor cells. Another challenge is represented by a better characterization of the borders and the exact composition of the liver cell niche, mainly focused on the relationships existing among the so different cell types cooperating with stem cells for assuring liver regeneration. With the hope that these studies might represent a contribution to the development of regenerative medicine in adult patients affected by chronic liver disease and liver insufficiency.

Declaration of interest

The Authors declare that there is no conflict of interest.

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