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Editorial

On the origin of stellate cells: mesodermal, endodermal or neuro-ectodermal?

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In humans, the embryonic liver is formed from endodermal cells of the ventral foregut, 24–26 days post-conception [1,2]. Endodermal cells grow out and form a bud, the hepatic diverticulum, that gives rise to the parenchyma (hepatocytes) and bile duct system (biliary epithelial cells). The hepatic diverticulum grows into the mesenchymal framework of the septum transversum. Within the septum transversum, spaces appear that will be surrounded by flat, evenly aligned, mesodermal cells, which resemble endothelial cells in the capillaries or sinusoids. The endodermal sprouts from the diverticulum (the hepatoblasts) will extend between the endothelium-lined spaces, thus establishing the basic composition of liver tissue: cords of epithelial cells, separated by sinusoidal vessels. These developments are well underway by the 32nd day of human embryonic development. By using SE-1 monoclonal antibody to label sinusoidal endothelial cells and Factor VIII antibodies to mark endothelial cells of the larger veins, Morita and colleagues have provided experimental support that sinusoidal endothelial cells do not derive from ingrowing vitelline veins [3].

Active macrophages (Kupffer cells) and stellate cells become identifiable during the second half of the third month (75–90th day) of human development [4]. These early Kupffer cells may derive from primitive macrophages which are first present in the yolk sac and colonize the liver [5]. In adult life, the early macrophages coexist with macrophages from extrahepatic sources [6]. Stellate cells also appear in the second half of the third month of human embryonic development. They are located in the space of Disse and contain 1–2 lipid droplets. In the 5-month human fetus, stellate cells contain small lipid droplets [7]. At birth, stellate cells have not reached their final shape and size [8]. In the 1-day neonatal rat, stellate cells are rather rounded with slim and scanty processes. Two to three weeks after birth, the cell processes extend with the formation of perforated membranous appendages. Five weeks after birth, rat stellate cells have reached their definitive dendritic configuration with elongated perisinusoidal and thicker intersinusoidal extensions.

Hepatic stellate cells represent 5–8% of all human liver cells and 13% of the volume of sinusoidal cells. They have long cytoplasmic processes which run parallel to the sinusoidal endothelial wall. Second order branches sprout out from the processes, embrace the sinusoid and penetrate between hepatocytes reaching neighbouring sinusoids. Some stellate cells are in close contact with nerve endings. In normal liver, stellate cells are mainly involved in storage of vitamin A. In addition, they synthesize extracellular matrix molecules, matrix degrading metalloproteinases, cytokines and growth factors. Following acute or chronic liver injury, stellate cells get activated. They undergo a process of transdifferentiation leading to a myofibroblastic phenotype. Activated stellate cells are characterized by the loss of vitamin A-rich lipid droplets, increased proliferation, enhanced contractile properties, release of pro-infammatory, pro-fibrogenic and pro-mitogenic cytokines, migration to sites of injury, increased secretion of extracellular matrix, and altered matrix proteinase activity providing the fundamental requirements for tissue repair. In acute or self-limited liver damage, these changes are transient whereas in the case of persistent injury, they lead to chronic inflammation and enhanced deposition of extracellular matrix leading to fibrosis and cirrhosis. In addition, stellate cells are involved in the pathogenesis of portal hypertension [9], and in progression of primary or metastatic liver cancer [10].

The embryonic origin of stellate cells is as yet unresolved with some studies favouring a mesenchymal [11], some others an endodermal [12,13] and yet others a neuro-ectodermal origin [14–16].

The view that stellate cells are of mesenchymal origin is largely based on electron microscopic studies of the embryonic human and rodent liver [11], and on the fact that stellate cells express vimentin [17,18]. Transmission electron

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microscopy shows that when the liver primordium grows into the mesenchymal tissue of the septum transversum, 'mesenchymal' cells with myofibroblastic morphology get trapped between the ingrowing hepatoblasts and sinusoidal endothelium. The 'trapped' cells develop subsequently lipid droplets and are therefore interpreted as precursors of mature stellate cells. The mesenchymal nature of the 'trapped' cells is based on morphological similarities between the latter and the cells present in the septum transversum. Other studies have shown that rodent and human stellate cells express vimentin [17,18]. This observation supports but does not prove the mesenchymal origin of stellate cells. The literature contains numerous examples of epithelial–mesenchymal transition characterized by loss of expression of keratins in favour of expression of vimentin, or vice-versa [19].

Two groups have reported that around day 12–13 of rat embryonic development, some non-hematopoietic liver cells are stellate-shaped and co-express cytokeratins 8 and 18 (hepatoblast markers) and desmin (stellate cell marker). This co-expression ceases to exist as from day 18 [20,21]. This data suggests that hepatocytes and stellate cells may derive from a common endodermal precursor. In early embryonic mouse liver, however, such common precursor cells have not been confirmed [22].

Quiescent stellate cells contain a host of neural marker proteins: GFAP, nestin; the nerve growth factors NGF, BDGF, NT-3 and NT4/5; the nerve growth factor receptors p75, Trk-B, Trk-C; the cell adhesion protein N-CAM; the prion protein PrP^{C} and synaptophysin (for review, see [23]). Moreover, they display a dendritic morphology similar to astrocytes rather than to the spindle-shape of quiescent skin fibroblasts. These observations have led to the speculation that stellate cells could be of neuro-ectodermal origin [24,25]. Embryonic neural tissues contain multipotent cells that can form different sublineages under the influence of growth factors. These multipotent cells will become glial cells when exposed to neuregulin isoform I, or neurons under the influence of BMP-2, or myofibroblasts/smooth muscle cells when incubated with TGF-β [26,27].

In this issue of the Journal, Baba and colleagues [28] as well as Suskind and Muench [29], report new data that shed a different light on the origin of stellate cells. Baba and colleagues use bone marrow of green fluorescent protein (GFP) transgenic mice which is administered to age-matched C57BL/J mice. Subsequently, hepatic stellate cells are isolated from the livers of the recipient mice. GFP-expressing cells with intracytoplasmic lipid droplets comprise 33.4% of the total number of isolated cells. The GFP expressing cells also contain stellate cell lineage markers such as desmin, GFAP and α-SMA. The authors conclude that bone marrow is a source of stellate cell precursor cells.

In the light of the controversy that has arisen about the recruitment of hepatocytes from bone marrow precursor cells, the data generated by Baba et al. should be interpreteted with caution. Initial reports claimed recruitment of new hepatocytes from extrahepatic precursor cells such as bone marrow stem cells [30–32]. However, initial enthusiasm has been dampened by several studies that showed cell fusion of extrahepatic precursor cells with pre-existing hepatocytes [33–36]. The resulting cells were aneuploid containing chromosomes from both recipient and donor, and had acquired hepatocytic differentiation markers as a result of merger rather than by differentiation of precursor cells into bona fide diploid hepatocytes.

In the light of the hepatocyte experience, the conclusions by Baba et al., although not unreasonable in themselves, should be followed up by experiments to exclude (i) that pre-existing desmin-positive or GFAP-positive liver cells from the recipient mice fuse with GFP expressing bone marrow cells from the donor mice, and (ii) that desmin-negative or GFAP-negative cells undergo unexpected transdifferentiation as a result of the experimental procedures, thereby expressing an abnormal set of intermediate filament proteins.

Suskind and Muench have examined by flow cytometry the question whether midgestational embryonic human liver contains a common precursor cell that gives rise to both resident liver cells and hematopoietic cells. Contrary to their initial hypothesis, the authors identified a population of $CD34^{+}$ cytokeratin (CK)$7/8^{+}$ precursor cells that were different in several aspects from $CD34^{+}CK7/8^{-}$ haematopoietic precursor cells. $CD34^{+}CK7/8^{+}$ cells were positive for CD13, CD59, NGFR, desmin and α-SMA. In flow cytometry, their forward and side scatter were higher than those of $CD34^{+}CK7/8^{-}$ cells indicating that the $CD34^{+}CK7/8^{+}$ cells were larger and had a more elaborate cytoplasm. In culture, $CD34^{+}CK7/8^{+}$ cells projected short cytoplasmic extensions. The authors conclude that $CD34^{+}CK7/8^{+}$ cells are embryonic stellate cell precursors. One issue that should be addressed before this conclusion can be accepted is why 94% of $CD34^{+}CK7/8^{+}$ cells is desmin-positive. Several previous immunohistochemical studies have shown that human stellate cells are desmin-negative [17,18]. The authors also conclude that $CD34^{+}CK7/8^{+}$ cells form a lineage which is distinct from the haematopoietic lineage. Moreover, these data reinforce the view, but do not definitively prove, that stellate cells derive from endodermal precursor cells. Alternative interpretations of the presented data may be that a precursor cell common to $CD34^{+}CK7/8^{+}$ and $CD34^{+}CK7/8^{-}$ cells exists in early embryonic development, but that this common precursor has disappeared by midgestation, or that $CD34^{+}CK7/8^{-}$ cells evolve into $CD34^{+}CK7/8^{+}$ cells by means of transdifferentiation whereby cells become larger and express a different set of intermediate filament proteins. Furthermore, future studies should address the question in which micro-anatomical position $CD34^{+}CK7/8^{+}$ cells are located, and how these cells relate to 'oval cells'. The latter cells have been reported to be positive for both CD34 [37,38] and for CK7/8 [39]. Therefore, the question arises whether hepatocytes, biliary

epithelial cells and stellate cells derive from a common precursor cell?

The papers by Baba et al. and by Suskind and Muench, provide the Stellate Cell Community with new food for thought. Since 30 years, stellate cells are in the spotlights. Stellate cells play a prominent role in the pathogenesis of clinically important conditions such as hepatic fibrosis and cirrhosis, portal hypertension and liver cancer. Understanding the basic biology of stellate cells will contribute to our understanding of the pathogenesis of the above conditions. Identification of the embryonic origin of stellate cells will improve our understanding of their relationship to other fibrogenic liver cells such as portal fibroblasts, periductular fibroblasts and 'second layer cells'. It will also improve our insights into the relationship between hepatic and extrahepatic (pancreas, kidney, digestive canal, lung, spleen, adrenal, ductus deferens, uterus, vocal cords) stellate cells. Do all these stellate cells derive from the same precursor cells? Knowledge of the embryonic origin of stellate cells will also raise the possibility of using cell-lineage specific promoters to drive transgene expression selectively in stellate cells in vivo with a view of applying gene therapy. It will also open the prospect of reconstituting stellate cells from precursor cells in view of repopulating the liver with quiescent stellate cells in the event these cells have perished through apoptosis. Both Baba and colleagues, as well as Suskind and Muench, have brought these prospects closer to reality.

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