• REVIEW •

Hepatic stem cells: existence and origin

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Abstract

Stem cells are not only units of biological organization, responsible for the development and the regeneration of tissue and organ systems, but also are units in evolution by natural selection. It is accepted that there is stem cell potential in the liver. Like most organs in a healthy adult, the liver maintains a perfect balance between cell gain and loss. It has three levels of cells that can respond to loss of hepatocytes: (1) Mature hepatocytes, which proliferate after normal liver tissue renewal, less severe liver damage, etc; they are numerous, unipotent, "committed" and respond rapidly to liver injury. (2) Oval cells, which are activated to proliferate when the liver damage is extensive and chronic, or if proliferation of hepatocytes is inhibited; they lie within or immediately adjacent to the canal of Hering (CoH); they are less numerous, bipotent and respond by longer, but still limited proliferation. (3) Exogenous liver stem cells, which may derive from circulating hematopoietic stem cells (HSCs) or bone marrow stem cells; they respond to allyl alcohol injury or hepatocarcinogenesis; they are multipotent, rare, but have a very long proliferation potential. They make a more significant contribution to regeneration, and even completely restore normal function in a murine model of hereditary tyrosinaemia. How these three stem cell populations integrate to achieve a homeostatic balance remains enigmatic. This review focuses on the location, activation, markers of the three candidates of liver stem cell, and the most importantly, therapeutic potential of hepatic stem cells.

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INTRODUCTION

The considerable excitement surrounding the stem cell field is based on the unique biological properties of these cells and their capacity to self-renew and regenerate tissue and organ systems[1-3]. Although the existence of a liver stem cell has been debated for many years, it is now generally accepted that the liver contains cells with stem-like properties and that these cells can be activated to proliferate and differentiate into mature hepatic epithelial cells under certain pathophysiologic circumstances[4-8]. Cellular therapy with liver stem cells and their progeny is a promising new approach which will contribute to gene therapy of liver diseases[9-11].

HEPATOCYTES AS HEPATIC STEM CELLS

It has been estimated that the liver is replaced by normal tissue renewal approximately once a year. The participation of putative liver stem cells has never been demonstrated in this process[12,13]. Replacement of lost liver tissues is accomplished by proliferation of mature hepatocytes (and supporting sinusoidal cells)[14]. In the classic partial hepatectomy (PH) experiments, the loss of two-thirds of the rat liver is replaced within 2 weeks by proliferation of hepatocytes[15-17]. Although periportal hepatocytes appear to proliferate early after PH, all hepatocytes, including those immediately adjacent to the central vein, may undergo mitosis and proliferate promptly, continuously replenish the lost cells. Overturf et al[18] performed serial transplantation of a limited number of unfractionated adult parenchymal hepatocytes in fumarylacetoacetate hydrolase (FAH) deficiency mice. The results document that such cells can divide at least 69 times without loss of functions. So it can be concluded that hepatocytes are highly proliferative and have growth potential similar to that of hematopoietic stem cells.

A cell population that has an extensive self-maintaining capacity is the definition of stem cells. In this context, the adult liver, having the extensive capacity of maintaining parenchymal cell number throughout the life span of the organism, can be considered as a single lineage stem cell system in which the hepatocyte is the stem cell. Potten and Loeffler[19] proposed stem cells as actual stem cells, potential stem cells, and committed stem cells. So hepatocytes appear to be "committed stem cells" that are normally quiescent, but can be activated to produce progeny whose only differentiation option is hepatocytic.

HEPATIC STEM CELLS IN CANAL OF HERING

In the development of liver, the early fetal hepatocytes or hepatoblasts are progenitors for both adult hepatocytes and bile epithelial cells, which suggests that hepatoblasts are at least bipotential precursors[20]. The question then arises whether either or both of the cell lineages derived from the hepatoblast retain the "bipotential capacity" of the precursor cells. There is at present no substantial evidence indicating that adult hepatocytes are more than a unipotential committed stem cell system, while adequate data have been accumulated to show that there really exist so-called "oval cells" in adult liver. Oval cells have lineage options similar to those displayed by hepatoblasts in early stages of liver development[21]. As such, oval cells can be regarded as "bipotential precursors" for the two hepatic parenchymal cell lineages. They are able to differentiate into mature hepatocytes or cholangiocytes in response to various types of stress or injury.

Morphologically, oval cells are small in size (approximately 10 mm), with a large nucleus-to-cytoplasm ratio, with an oval-shaped nucleus (hence their name). Oval cells are heterogeneous, and may display features of both bile duct cells and hepatocytes. Oval cells are activated to proliferate after hepatocyte loss in the mature liver if liver damage is extensive and chronic, or if proliferation of hepatocytes is inhibited, such as by viral infection. Then their progeny extended across the liver lobule and differentiated into either hepatocytes or bile duct cells, ultimately rebuilt the liver[22,23]. In rodents, the concept of the bipotential cell, the so-called oval cell, is now

widely accepted, the existence of a human equivalent remains controversial. In Heather' s experiments[24], immunolocalization of OV-6 and two biliary markers, cytokeratin 19(CK19) and human epithelial antigen 125(HEA-125) was compared in normal adult human livers and in primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) liver sections. It is proposed that the small OV-6-positive oval cells are analogous to those seen in rat models and may represent human liver progenitor cells that may differentiate into OV-6-positive ductal cells or lobular hepatocytes. The most commonly recognized tissue reaction in support of oval cells in human is the appearance of "ductular reactions"[25]. Ductular reaction is the proliferative response to many types of liver injury in human, characterized by an increase of bile duct-like structures. In a number of morphological studies, the presence of cells with a distinctive "small" or oval cell-like appearance have been reported in diseased human liver tissue[26,27]. This includes severe hepatic necrosis, alcoholic cirrhosis, focal nodular hyperplasia, hepatoblastoma, and biliary diseases such as primary biliary cirrhosis or biliary atresia. These oval cell-like cells are proliferative and can differentiate into hepatocytes and cholangiocytes.

The anatomic location of oval cell has also been a subject of controversy. Results from a detailed time course study of activation of hepatic stem cells in the AAF/PH model, utilizing a combination of immunohistochemistry with OV-6 and desmin antibodies and autoradiography after [3H]thymidine administration shortly after the PH, indicate that the earliest population of proliferating OV-6 positive cells is located in the small bile ductules. In addition, these early population of OV-6 positive cells express albumin and α-fetoprotein(AFP)[28]. Therefore, it seems likely that the major source of oval cells, at least in the AAF/PH model, is derived from the lining cells of the biliary ductules and that these cells constitute the dormant/facultative hepatic stem cell compartment. Theise *et al*' s experimental work[29] suggests that oval cells lie within or immediately adjacent to the canal of Hering(CoH), which is the anatomic juncture of the hepatocyte canalicular system, and the terminal branches of the biliary tree.

Oval cells express similar markers to hepatocytes or bile duct cells, like AFP, certain keratin markers (e.g., cytokeratin19 [CK19]), and γ-glutamyl transpeptidase. Monoclonal antibodies such as OV-6, OC2, and BD1 also aid in their characterization. High levels of certain mRNAs like AFP and stem cell factor (SCF) can also be expressed by oval cells. OV-6 identifies a cytokeratin of molecular weight 56 000, with epitopes shared on cytokeratins (CKs) 14 and 19. It is present in rat liver on bile ducts, oval cells, and nodular hepatocytes as well as transitional hepatocytes[30]. In human liver, OV-6 identifies cells in the ductal plate, oval cells and bile ducts and ductules in fetal tissue, and oval cells found in focal nodular hyperplasia[31,32]. AFP is an abundant serum glycoprotein in developing mammals. During embryonic development, AFP is first detected in the yolk sac and later in the fetal liver. The full-length AFP RNA and protein are highly expressed in the primitive hepatoblasts and postnatal hepatocytes[33]. Full length AFP has been shown to be expressed in oval cells and small basophilic hepatocytes during the early stages of carcinogenesis[34]. Hence, AFP expression can be used as an indicator for an early hepatic lineage, and has also served as an important marker for the activation of the hepatic stem cell compartment. SCF, also called c-kit ligand, encodes a transmembrane tyrosine kinase protein and belongs to the subfamily of platelet derived growth factor. The SCF/c-kit system is believed to play an important role in stem cell biology during hematopoiesis, gametogenesis, and melanogenesis[35,36]. Studies have shown that both SCF and c-kit are expressed in the bile duct cells and the expression of both the genes is increased in

oval cells in the 2-acetylaminofluorene and partial hepatectomy model[37].

HEPATIC STEM CELLS FROM BONE MARROW

In the experiment by Yavorkovsky *et al*[38], periportal necrosis of allyl alcohol toxicity resulted in a "null cell" proliferation that was negative not only for hepatocyte markers, but also for cholangiocyte markers. This phenomenon suggests that oval cells are not the only source of hepatic stem cells. This hypothesis might be correct as suggested by the research in transdifferatiation of marrow stem cells[39]. The ability of marrow stem cells to give rise to cells of different organs is being increasingly identified, for example, bone marrow turning into skeletal muscle[40,41], renal[42] ,or into brain[43].

Correlation between bone marrow and liver has been discovered. (1) Hematopoiesis and hepatic development share common stages[44]. During fetal development, hematopoietic stem cells move out of the yolk sac and into the developing liver. The liver remains hematopoietic during the entire fetal period and for approximately the first week after birth in the neonates. Simultaneously with the appearance of hematopoiesis, hematopoietic stem cells (HSCs) can be detected in the fetal liver. In the latter part of gestation and after birth, the hematopoietic function of the liver is considerably reduced, if not totally absent. Although the liver loses its hematopoitic functions, hematopoiesis often returns in adult life in disease states. (2) Some studies show that hepatic oval cells and hematopoietic stem cells share CD34, Thy-1, and c-kit mRNA and protein[45-49]. Oval cells also express mRNA for the flt-3 receptor, previously reported to be restricted to hematopoietic stem cells[50]. (3) In Theise ND *et al*' s research work[51], a cohort of lethally irradiated female mice received whole bone marrow transplants from age-matched male donors. Fluorescence *in situ* hybridization (FISH) for the Y-chromosome was performed on liver tissues. Y-chromosome positive hepatocytes were identified in all animals sacrificed 2 months or longer post-transplantation. Simultaneous FISH for the Y-chromosome and albumin messenger RNA confirmed male-derived cells were mature hepatocytes. The experiments show that bone marrow cells transplanted from male donors to syngeneic recipients are able to localize in the two largest lobes of the liver, differentiating into mature hepatocytes carrying the Y-chromosome. In human experiment, biopsy and autopsy liver specimens from recipients of therapeutic bone marrow or liver transplants were analyzed for marrow-derived hepatocytes and cholangiocytes[52,53]. In view of these bone marrow-liver inter-relationships, it is expected that a closer relationship might exist in the two lineages[54]. Hepatocytes and cholangiocytes could derive from bone marrow stem cells. In both of human subjects and in the experimental animals, engraftment by bone marrow cells as hepatocytes could be seen in the absence of severe injury, suggesting that such movement might occur as a low level, baseline, physiologic phenomenon.

The mechanism of entry and differentiation of the transplanted bone marrow cells within the liver cell plates might also occur in different ways. First, circulating. Marrow-derived cells might enter the liver plates directly from the sinusoidal circulation. These cells are most often scattered throughout the parenchyma and intercalate randomly into pre-existent liver cords directly as hepatocytes. On the other hand, given previous demonstrations that oval cells derive from CoH, and that not only hepatocytes, but oval cells derive from marrow cells[55-57], we can speculate that marrow cells might enter into liver through the circulation, then translocate across basement membranes into CoH and/or the terminal branches of the biliary tree, and differentiate into cytokeratin 19-positive small cholangiocyte-like cells. Proliferation and differentiation into

hepatocytes would follow in proportion to the type and extent of hepatocyte injury. Thus, hepatic regeneration through marrow cells may have multiple and perhaps overlapping pathways to accomplish cell replacement and organ repair.

MSCs are not only highly self-regenerative, but also can transdifferentiate into liver endogenous stem cells and liver parenchymal cells. So MSCs can repair damaged liver. Compared with liver transplantation and hepatocyte transplantation, there are much more marrow donors than liver and liver cells donors. If we utilize a patient's own MSCs as a new therapeutic option to treat liver diseases, immunological rejection will be avoided completely. Now MSCs has been used in animal experiments and clinical trails[68,59]. Orlic *et al*[60,61] sorted lineage-negative (Lin-) bone marrow cells from transgenic mice expressing enhanced green fluorescent protein by fluorescence-activated cell sorting on the basis of c-kit expression. Then they injected these Lin- c-kit^{pos} cells into the contracting wall bordering the infarct shortly after coronary ligation. The results show that newly formed myocardium occupied 68 % of the infarcted portion of the ventricle 9 days after transplanting the bone marrow cells. The developing tissues comprised proliferating myocytes and vascular structures. Their studies indicate that it is possible to rebuild heart-attack-damaged hearts with adult stem cells from bone marrow. Also, in order to determine whether bone marrow contains cells that can correct liver diseases, Lagasse *et al*[62] used bone marrow transplantation in the fumarylacetoacetate hydrolase (FAH)-deficient mouse, an animal model of fatal hereditary tyrosinemia type I. The results show that bone marrow transplantation rescued the mouse and restored the biochemical functions of its liver.

PROSPECT

The liver is an organ known to have tremendous regenerative capacity. It is confirmed that liver stem cells exist in humans and that they can be of endogenous (hepatocytes or oval cells) or exogenous (most likely bone marrow) origin. The marked degree of hepatic engraftment from extrahepatic cells in cases of severe liver injury indicates that there may be therapeutic utility for bone marrow transplantation to correct defects in hepatocyte metabolic or synthetic function. The researches mentioned above represent significant advances in liver stem cell biology. However, questions regarding some respects of liver stem cells remain open and need to be resolved: how to activate liver stem cells[63,64]? How to isolate and characterize a plenty of liver stem cells with high purification[65,66]? How to set up a tracking system to make clear the ultimate fate of liver stem cells *in vivo*[67,68]? How to develop a stable *ex vivo* culture system of liver stem cells and adjust their differentiation[69,70]? Excellent *in vitro* and *in vivo* researches will pave the way for a much broader understanding of the biological properties and clinical use of liver stem cells.

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