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Review Article

Hematopoietic Microenvironment in the Fetal Liver: Roles of Different Cell Populations

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Hematopoiesis is the main function of the liver during a considerable period of mammalian prenatal development. Hematopoietic cells of the fetal liver exist in a specific microenvironment that controls their proliferation and differentiation. This microenvironment is created by different cell populations, including epitheliocytes, macrophages, various stromal elements (hepatic stellate cells, fibroblasts, myofibroblasts, vascular smooth muscle and endothelial cells, mesenchymal stromal cells), and also cells undergoing epithelial-to-mesenchymal transition. This paper considers the involvement of these cell types in the regulation of fetal liver hematopoiesis.

1. Introduction

In mammals, the liver serves as the main hematopoietic organ during a considerable period of prenatal ontogeny. In murine liver, for example, hematopoietic cells first appear in 10-day embryos, with hematopoietic function of the organ reaching a peak on embryonic days 13–14 and ceasing during the first 2–4 postnatal days [1, 2]. Hematopoiesis requires specific microenvironment that produces chemical signals to attract hematopoietic cells and regulates their proliferation and differentiation via contact and humoral interactions. The hematopoietic microenvironment of the fetal liver is created by a complex of cell types, including epitheliocytes, resident macrophages, and several stromal cell populations of mesenchymal origin such as hepatic stellate cells, fibroblasts, myofibroblasts, vascular smooth muscle and endothelial cells, and mesenchymal stromal cells (MSCs). Let us consider the roles of different cell components of this microenvironment in the maintenance of hematopoietic activity in the developing liver.

2. Liver Epithelium

At early stages of liver development, its epithelium is represented by bipotent hepatoblasts, which subsequently differentiate into hepatocytes and cholangiocytes [1, 2]. Hepatoblasts can be identified by the simultaneous expression of both hepatic (cytokeratin 18, albumin) and biliary epithelial markers (cytokeratin 19) and also of E-cadherin [3]. The morphology and phenotype of liver epithelial cells change in the course of development, and these changes correlate with hematopoietic activity [4, 5]. Cells of the hepatocyte lineage appear to play an important role in the regulation of erythropoiesis: they closely interact with erythroblasts [5] and produce erythropoietic cytokines such as stem cell factor and erythropoietin [6, 7].

Localization of megakaryocyte lineage cells among hepatocytes described in human fetal liver [8] and thrombopoietin production by some lines of murine hepatocytes [9] both suggest a contribution of the hepatic epithelium to the control of megakaryocytopoiesis.

The involvement of the epithelium in maintaining the hematopoietic function of the liver is confirmed by the existence of hepatoblast and hepatocyte cell lines capable of supporting hematopoiesis in long-term culture via secretion of cytokines [9] or adhesive interactions with hematopoietic progenitor cells [10]. The biliary epithelium can also support both long-term proliferation of hematopoietic cells and production of committed erythroid or granulocyte/macrophage

progenitors by means of contact interactions via liver-regulating protein that is expressed on the surface of the epitheliocytes [11].

3. Macrophages

In the developing liver, macrophages first appear in sinusoids. At the stage of active liver hematopoiesis, they migrate to the parenchyma to form erythroblastic islands consisting of a central macrophage surrounded by erythroblasts and sparse lymphocytes [12, 13]. The interaction of erythroid cells with macrophages mediated by the erythroblast macrophage protein (Emp) is necessary for their enucleation [14]. Moreover, macrophages express vascular cell adhesion molecule VCAM-1, which also mediates their adhesive interactions with erythroblasts [15], and jagged-1, a ligand for the Notch signal system involved in regulation of hematopoiesis [13]. The central macrophages of erythroblastic islands degenerate as hematopoiesis in the liver ceases [12].

Another population of macrophages in the fetal liver consists of Kupffer cells, which line the sinusoids. Some recent findings suggest that they derive from the yolk sac and are not a progeny of definitive hematopoietic stem cells [16]. Their functions in the maintenance of hematopoiesis consist in phagocytosis of the nuclei extruded from late-stage erythroblasts [17, 18] and secretion of erythropoietin [19]. The presence of dividing and maturing erythroblasts in the vacuoles of Kupffer cells, which has been observed in the fetal liver [18], may also indicate the role of these cells in the regulation of erythropoiesis.

4. Hepatic Stellate Cells

The hepatic stellate cells, or Ito cells, are located in the perisinusoidal space of Disse. Quiescent stellate cells contain retinoid lipid droplets. When activated, they lose these droplets and acquire morphological and phenotypic feature characteristics of myofibroblasts, including the expression of smooth muscle actin [20]. Activation of stellate cells occurs upon liver damage [21, 22]. They also become activated in monolayer cultures but remain quiescent when cultured on collagen gel [21].

The stellate cells of the fetal liver express desmin [23, 24], β3-integrin, nestin [24], CRBP-1 [25], N-CAM [26], and reelin [27]. Some researchers consider that these cells are derived from mesenchymal cells of septum transversum, which form the submesothelial layer under the liver capsule [26, 28, 29], but their epithelial origin cannot be excluded [30]. The number of hepatic stellate cells increases in the course of development [25]. In the fetal period, they are associated with hematopoietic cells [23] and apparently regulate hematopoiesis by secreting chemoattractants (such as stromal cell-derived factor-1) as well as by means of contact interactions mediated by VCAM-1 [24]. Hepatic stellate cells are also known to secrete erythropoietin [6] and stem cell factor [31]. Therefore, these cells can be regarded as an important component of the hematopoietic microenvironment.

5. Fibroblasts and Myofibroblasts

In either adult or fetal liver, fibroblasts (myofibroblasts) are located in the region of portal triads [20, 25], around central veins, and in the Glisson capsule [22] or, in the fetal liver, in the submesothelial layer of mesenchymal cells [29]. Portal myofibroblasts express smooth muscle actin and desmin and are morphologically similar to activated stellate cells [20, 32]. However, they are not related by origin to hepatic stellate cells [19, 32]; unlike the latter, they express CD90 [32, 33], gremlin [33], fibulin-2, and interleukin-6 [34] but do not express reelin [35].

Perivascular fibroblasts of the fetal liver are cells of mesodermal origin [29]. All stromal cells of portal triads at early developmental stages express smooth muscle actin, but they are subsequently substituted by fibroblasts characteristically expressing vimentin (but not smooth muscle actin) [25]. Thus, myofibroblasts disappear during development and are absent in the normal adult liver [22].

Morphological and immunohistochemical analysis of fetal liver reveals myelopoiesis mainly around the blood vessels, that is, in places where the fibroblasts and myofibroblasts are located [8, 36]. These findings may reflect their important role in the regulation of myeloid differentiation. However, the role of myofibroblasts and fibroblasts in the regulation of hematopoiesis has not been studied sufficiently. Some data suggest their involvement in organizing the hematopoietic microenvironment by producing extracellular matrix components, including fibronectin and collagen [37]. Adhesion to fibronectin appears to stimulate proliferation of both hematopoietic stem/progenitor and erythroid cells [38, 39], which is confirmed by correlation between the content of this protein in the periportal region and the activity of hematopoiesis in human fetal liver [40]. In murine fetal liver, myeloid cells are associated with perivascular and subcapsular collagen, that may suggest a significance of its production by stromal cells for supporting myelopoiesis [5].

6. Myoid Cells

Differentiated smooth muscle cells in the human fetal liver have been found only in the tunica media of hepatic artery branches [25]. However, the fetal mouse liver has served as the source of numerous stromal cell lines expressing markers of different stages of smooth muscle cell differentiation. Many of these lines, especially those at early or middle differentiation stages, can maintain hematopoiesis in long-term culture and probably correspond to pericytes located around venous capillaries [41, 42]. Immature cells of this lineage are likely to produce hematopoietic cytokines, as it has been shown for myoid cells of bone marrow stroma [43], whereas more mature (contractile) cells may control migration of hematopoietic cells by modifying the permeability of intercellular spaces between endotheliocytes [42].

Unexpectedly, the fetal liver in different species has proved to contain precursor cells of skeletal muscles showing spontaneous fusion into myotubes in vitro [44–46]. These cells may enter the liver and other organs of the embryo when they migrate from the dermomyotome to populate

areas where skeletal muscles are to be formed. However, in view of the data that skeletal myoblasts secrete a wide range of regulatory molecules, including stromal cell-derived factor-1 and hematopoietic cytokines such as macrophage colony-stimulating factor [47], their specific role in the maintenance of liver hematopoiesis cannot be excluded.

7. Vascular Endothelium

The endothelium of blood vessels in different parts of fetal liver acini differs in structure: it forms a continuous layer in portal vessels but is fenestrated in central veins [48]. Regarding the hematopoietic function of the liver, of special interest is the endothelium of sinusoids, which mature blood cells must penetrate to enter the circulation. The sinusoids at early stages of development are lined with a continuous endothelium, but its structure subsequently changes so that it becomes highly permeable to blood cells and regulatory molecules. The basal membrane disappears, composition of the extracellular matrix changes [49], and diaphragmed or open fenestrae, intercellular fissures, and temporary migration pores are formed [17, 48]. The porosity of the sinusoidal endothelium decreases by the end of prenatal ontogeny, when its structure and phenotype approach those in the adult liver [48, 49].

Due to the expression of cell adhesion molecules such as E-selectin and VCAM-1 [50, 51] and chemoattractants such as stromal cell-derived factor-1 [52], the sinusoidal endothelium in the fetal liver can control the homing of hematopoietic cells, their retention in the niche, and release into the circulation. In the fetal liver, hematopoietic stem cells interact with the sinusoidal endothelium via activated protein C. This interaction facilitates self-renewal of the stem cells and prevents their apoptosis [53]. Moreover, endothelial cell lines or medium conditioned by them maintain in vitro differentiation of erythroid and granulocyte-macrophage lineage cells [54, 55], which is evidence for the ability of endotheliocytes to regulate hematopoiesis via contact interactions with hematopoietic cells and secretion of cytokines. Fetal liver endothelial cells can also promote B-lymphopoiesis from primitive hematopoietic cells [51].

8. Mesenchymal Stromal Cells (MSCs)

Multipotent MSCs are plastic-adhesive cells with a specific antigenic phenotype (for human cells, $CD105^+$ $CD73^+$ $CD90^+$ $CD45^-$ $CD34^-$ $CD14^-$ $CD11b^-$ $CD79a^-$ $CD19^-$ $HLA\text{-}DR^-$) and potential for osteogenic, adipogenic, and chondrogenic differentiation [56]. They were first identified by Friedenstein et al. [57] in the mouse bone marrow, spleen, and thymus as fibroblast colony-forming units. To date, MSCs have been revealed in many organs (including the fetal liver), where they apparently reside in vascular walls [58]. MSCs of the fetal liver have certain distinctive features, compared to such cells from other organs. Thus, they show higher proliferative activity than MSCs from the adult bone marrow [59, 60], but their osteogenic and adipogenic potential is lower [61]. With respect to osteogenic capacity, they are also inferior to MSCs from other fetal organs [62, 63].

In the course of embryonic development, MSC supposedly migrate to the liver from the aortic-gonad-mesonephros region [64], although their de novo formation from the septum transversum mesenchyme is also possible. The amount of these cells in the liver changes during development in correlation with hematopoietic activity [65–67], which is evidence for their important role in organization of the hematopoietic microenvironment. The cessation of hematopoiesis in the liver is accompanied by a decrease in not only the number of MSCs but also in their proliferative activity [59] and differentiation potential [67].

In the fetal liver, MSCs are a probable source of stromal cells similar in their characteristics to smooth muscle cells [42]; it is also not excluded that they can differentiate into myofibroblasts [68] and endothelial cells [69]. Apparently, the role of these cells is not limited to their differentiation into more specialized components of the hematopoietic stroma. It has been shown for bone marrow MSCs that they can produce stromal cell-derived factor-1 [70], interact with hematopoietic cells via surface molecules (VCAM-1, cadherins, integrins and, etc.), and regulate their proliferation and differentiation by secreting wide range of cytokines [71, 72]. Such regulatory functions are also likely for MSCs of the fetal liver, although experimental evidence for their ability to maintain hematopoiesis is as yet scarce [66, 73].

9. Epithelial-to-Mesenchymal Transition (EMT)

In addition to cell populations with distinct epithelial or mesenchymal phenotypic traits, the developing liver contains cells undergoing epithelial-to-mesenchymal transition (EMT), which coexpress mesenchymal markers (e.g., vimentin, N-cadherin, Stro-1, osteopontin, collagen type I, smooth muscle actin), and markers of the liver epithelium (alpha-fetoprotein, cytokeratins 7, 8, and 18, albumin, E-cadherin) [74–76]. The apparent sources of these cells are liver mesenchyme and parenchyma; their origin from hematopoietic stem cells or pluripotent progenitor cells is less probable [74]. In particular, coexpression of epithelial and mesenchymal markers has been described in periportal liver cells [77], stellate cells [30], and hepatic stem cells [76].

Numerous EMT cells are found in the liver at the stage of active hematopoiesis, but they gradually disappear during late prenatal development and are scarce or absent in adult liver [74, 76]. They are regarded as an important component of the hematopoietic microenvironment. Thus, cells with the EMT phenotype maintain the undifferentiated state of hematopoietic stem cells in vitro [75], and the hematopoietic supportive ability of EMT clonal cell line AFT024 is lost after its hepatocytic differentiation induced by oncostatin M [74]. A noteworthy fact is that a major regulatory role is attributed to fibronectin production by cells of portal triads, which have certain traits of EMT [77].

10. Stromal Regulation of Hepatogenesis

Hematopoiesis in the fetal liver is coincident with histogenesis of its epithelial tissue. Available data indicate that

the stromal cell populations described above take part in the regulation of hepatogenesis as well as of hematopoiesis. In particular, there is evidence for secretion of hepatocyte growth factor by hepatic stellate cells [24], periportal connective tissue cells, and endothelial cells of the fetal liver [78] and for stimulation of the survival and proliferation of hepatic stem cells by stellate cells [79] or endotheliocytes [80] in cocultures. Paracrine signals from stellate cells stimulate hepatocytic differentiation in vitro [81], and co-culturing of hepatocytes with a certain fibroblast population induces them to form hepatic cordlike structures [82]. There is also evidence that the interaction of portal myofibroblasts and epithelium is important for the development of intrahepatic bile ducts [83]. Moreover, hematopoietic cells also affect hepatogenesis through the production of oncostatin M that stimulates the functional maturation of hepatocytes [84].

By conclusion, the hematopoietic microenvironment in the fetal liver is created by a complex of different epithelial and mesenchymal cell types. They produce cytokines, chemoattractants, extracellular matrix components, and so forth. and directly interact with hematopoietic cells, thereby providing for the functioning of the liver as a hematopoietic organ during a considerable period of prenatal development. To date, the niche for hematopoietic stem cells in the fetal liver has been characterized in sufficient detail, but further studies are needed to gain a deeper insight into the roles of different cell populations in the regulation of hematopoietic and hepatocyte differentiation.

Abbreviations

MSCs: Mesenchymal stromal cells
EMT: Epithelial-to-mesenchymal transition.

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