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Cytokines regulating hematopoietic stem cell function

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Abstract

Purpose of review—Regulation of the multiple fates of hematopoietic stem cells – including quiescence, self-renewal, differentiation, apoptosis, and mobilization from the niche – requires the cooperative actions of several cytokines and other hormones that bind to receptors on these cells. In this review we discuss recent advances in the identification of novel hematopoietic stem cell supportive cytokines and the mechanisms by which they control hematopoietic stem cell fate decisions.

Recent findings—Several extrinsic factors that stimulate ex-vivo expansion of hematopoietic stem cells were recently identified by a number of experimental approaches, including forward genetic screening and transcriptional profiling of supportive stromal cells. Recent experiments in which multiple cytokine signaling pathways are activated or suppressed in hematopoietic stem cells reveal the complexity of signal transduction and cell-fate choice in hematopoietic stem cells *in vivo* and *in vitro*.

Summary—The study of genetically modified mice and improvements in the in-vitro hematopoietic stem cell culture system will be powerful tools to elucidate the functions of cytokines that regulate hematopoietic stem cell function. These will further reveal the complex nature of the mechanisms by which extrinsic factors regulate signal transduction and cell-fate decisions of hematopoietic stem cells.

Keywords

cytokines; ex-vivo expansion; growth factors; hematopoietic stem cells; signal transduction

Introduction

Cytokines are secreted proteins that regulate many aspects of hematopoiesis – immune responses and inflammation in particular. Many hematopoietic cytokines were identified and purified on the basis of their abilities to support in-vitro formation of hematopoietic colonies from progenitors; the functions of many of these cytokines and their receptors were confirmed and extended through studies of genetically modified mice. Subsequently, many cytokines were shown to bind directly to receptors on hematopoietic stem cells (HSCs) and regulate many HSC functions, including quiescence, self-renewal, differentiation, apoptosis, and mobility. HSCs reside in ‘stem cell niches’ in the bone marrow and in other tissues; stromal cells are thought to synthesize many HSC cytokines but, in general, we know little about which cells

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in a niche make specific cytokines. Many cell culture experiments have shown that HSCs respond to multiple cytokines and that the fate of an HSC – self renewal, apoptosis, mobilization from the niche, formation of differentiated progeny cells – depends on multiple cytokines, adhesion proteins, and other signals produced by stromal cells and likely other cells in the body. The study of HSC cytokines will facilitate the development of new strategies for HSC-based cell and gene therapies. Despite a great deal of progress in the past decade, identification and functional study of HSC cytokines are still in a primitive stage and many secreted or cell surface proteins that affect HSC function remain to be discovered.

Identification of hematopoietic stem cell cytokines

To isolate HSC cytokines, sensitive assays for HSC functions are critical. For many years, due to the rarity of HSCs, the lack of an efficient culture system for them, and the difficulty in measuring their activities, direct identification of cytokines that target HSCs was virtually impossible. Some HSC cytokines, including Wnts and bone morphogenic proteins (BMPs), were initially identified from studies of invertebrate development. Some, such as thrombopoietin (TPO), were isolated based on functions of cells other than HSCs.

Two spatially and likely functionally distinct bone marrow microenvironments, or HSC niches, have been proposed (reviewed in [1,2]). Recent work shows that the endosteal HSC niche contains osteoblasts as the main supportive cell type (reviewed in [2,3]). In the vascular niche, many HSCs in the bone marrow and spleen are associated with the sinusoidal endothelium [4].

About a decade ago stromal cell lines were established from in-vivo fetal or adult hematopoietic organs, including aorta-gonado-mesonephros (AGM), fetal liver, yolk sac, and bone marrow. Moore *et al*. [5] established numerous fetal liver stromal cell lines and used them to isolate secreted proteins that support HSCs. Recently, this group isolated HSC supportive stromal cell lines from the AGM region [6]. The Williams lab isolated yolk sac stromal cell lines that support HSC and progenitor activities [7]. We identified primary mouse fetal liver $CD3^{+}Ter119^{-}$ cells as a novel HSC supportive population, and by transcriptional profiling we identified insulin-like growth factor 2 (IGF-2) and a group of angiopoietin-like proteins (Angptls) as HSC growth factors [8–10]. We also showed that IGF binding protein 2 (IGFBP2), a protein secreted by HEK 293T cells, supports HSC expansion (Huynh HD *et al.*, unpublished data), and we anticipate that cancer cells will be a rich source of other HSC-stimulating proteins.

Discussion of individual factors

Below we discuss very recent results for several cytokines that affect HSC fates.

Stem cell factor

Stem cell factor (SCF), also known as steel factor, is a cytokine expressed by a number of cell types. SCF functions by binding to c-Kit, a tyrosine kinase receptor expressed on all HSCs. Expression of a defective c-Kit leads to a decrease in repopulating HSCs [11]. Although SCF may not be essential for the generation of HSCs [11], numerous studies [12] have shown that it prevents HSC apoptosis. Almost all cytokine combinations used to date for culturing HSCs include SCF. SCF potentiates the greater ability of fetal liver HSCs than adult HSCs to undergo symmetric self-renewal in culture [13•]; this activity likely needs the cooperation of other factors. The membrane-bound form of SCF is also an adhesive molecule for HSCs to the bone marrow environment [14], and an increased number of osteoclasts was associated with HSC mobilization. Receptor activator of nuclear factor (NF)-κB (RANK) ligand and cathepsin K mediate the cleavage of membrane-bound SCF; this decreases the abundance of SCF and, therefore, increases HSC mobilization [15]. The involvement of SCF in survival, mobility, and

possibly self-renewal of HSCs in culture and in the HSC niche likely reflects the complex relationship of different cell fates of HSCs.

Thrombopoietin

TPO is the primary cytokine that regulates megakaryocyte and platelet development. TPO and its receptor Mpl also exert profound effects on primitive hematopoietic cells. All HSCs express Mpl; TPO−/− or Mpl−/− mice have a decreased number of repopulating HSCs [16]. In-vitro culture studies [17] also indicate a role of TPO in promoting the survival of repopulating HSCs. Through study of AGM and fetal liver Mpl−/− HSCs, Petit-Cocault *et al.* [18•] showed that TPO contributes to both generation and expansion of HSCs during definitive hematopoiesis. An intracellular adaptor, Lnk, induces a negative signaling pathway downstream of TPO in HSCs [19,20]. A recent study [21] on mice that express Mpl lacking the C-terminal 60 amino acids revealed a pivotal role of an unknown signal emanating from the membrane proximal region of the Mpl receptor or from JAK2 that is critical for maintenance of HSC activity.

Notch ligands

In addition to their major roles in lymphopoiesis, the Notch ligands Delta and Jagged are involved in the generation, antidifferentiation, and expansion of HSCs (reviewed in [22]). Importantly, Calvi *et al.* [23] and Duncan *et al.* [24] demonstrated that the Notch signaling pathway plays a role in the osteoblast bone marrow HSCs niche. Notch ligands have positive effects on ex-vivo expansion of HSCs: activated Notch is able to immortalize primitive mouse hematopoietic progenitors and Notch ligands support HSC expansion in culture (reviewed in [22]). Recently, by culturing human cord blood cells in serum-free medium supplemented with SCF, TPO, Flt3L, IL-3, IL-6/sIL-6R, and Delta 1, Suzuki *et al.* [25] reported an approximate six-fold increase in SCID-repopulating cell (SRC) number. It is noteworthy that there exists a dose effect for Notch ligands in HSC culture. Whereas a low amount of Delta 1 supports human cord blood SRC expansion, high amounts of the cytokine induce apoptosis (reviewed in [22]). This emphasizes the complicated relationship among the different fates of HSCs. As conditional knockouts of Notch1 and Jagged1 have normal in-vivo HSC activities [26], there likely is functional redundancy of different Notch isoforms and their ligands.

Wnts

Wnt signaling is involved in HSC generation and expansion (reviewed in [27•]). The Wnt pathway supports mouse HSC expansion and has most recently been shown to be important for self-renewal of stem cells of B-cell antigen receptor (BCR)-ABL-induced chronic myelogenous leukemia [28]. Nemeth *et al.* [29•] showed that the noncanonical Wnt5a supports HSC repopulation by inhibiting canonical Wnt signaling in HSCs and maintains HSCs in a quiescent state. Wnt also promotes generation of hematopoietic cells from human embryonic stem cells [30•]. In gene-altered mice, prolonged activation of Wnt/β-catenin signaling results in an increase of phenotypical HSCs but failure of hematopoietic functions, including blocks in HSC differentiation and loss of repopulating activity [31,32]. Deletion of both β-catenins and γ-catenins in mice does not change HSC repopulation activity [33,34]. Nevertheless, Wnt signaling is still maintained in these double-deficient HSCs [34]. Most recently, Zhao *et al.* [28] showed that β-catenin-deficient HSCs have defects in long-term growth and maintenance.

Bone morphogenic proteins and transforming growth factor-β

Transforming growth factor (TGF)-β potently inhibits HSC activity *in vitro* (reviewed in [3, 35••]). However, a TGF-β signaling deficiency *in vivo* does not affect proliferation of HSCs. BMPs, members of the TGF-β superfamily, play important roles in HSC specification during development. A negative role of BMP signaling in maintenance of mouse HSCs was shown by its control of the size of the HSC endosteal niche [3]. BMP4 supports HSC expansion in

culture and partially mediates the effects of Sonic hedgehog on cultured human HSCs [36]. Recently, the Karlsson lab characterized the expression of TGF-β superfamily ligands, receptors, and Smads in mouse HSCs; primary HSCs and the Lhx2-HPC cell line express most of the proteins required to transmit signals from several TGF-β family ligands [37]. In addition, Pimanda *et al.* [38•] demonstrated the integration of BMP4/Smad pathway and Scl and Runx1 activity in HSC development. Importantly, Trowbridge *et al.* [39] showed that a glycogen synthase kinase 3 (GSK-3) inhibitor that can modulate Wnt, Hedgehog, and Notch pathways enhances HSC repopulation.

Fibroblast growth factors

All long-term repopulating bone marrow HSCs express a fibroblast growth factor (FGF) receptor [40]; both FGF-1 and FGF-2 support HSC expansion when unfractionated mouse bone marrow cells are cultured in serum-free medium [40,41]. Crcareva *et al.* [42] confirmed that FGF-1 stimulates ex-vivo expansion of HSCs and showed that the expanded cells were efficiently transduced by retrovirus vectors. Conditional derivatives of FGF receptor-1 have also been used to support short-term HSC expansion and long-term HSC survival in culture [43]. However, the role of the FGF pathway in regulating adult HSCs or embryonic hematopoietic development is controversial as Schiedlmeier *et al.* [44•] showed that the treatment of purified mouse HSCs that ectopically express HoxB4 with the fibroblast growth factor receptor (FGFR) inhibitor SU5402 enhanced HSC repopulating activity. Similar results were obtained using primitive hematopoietic colonies derived from embryonic stem cells [44•]. These inconsistent results were obtained from different starting cell populations and under different culture conditions, suggesting that the crosstalk of FGF signaling with other pathways is complex.

Angiopoietin-1

The angiopoietin (Ang) family of growth factors is composed of four members that bind to the Tie-2 tyrosine kinase receptor; Ang growth factors are important modulators of angiogenesis. Members of the angiopoietin family of proteins contain an N-terminal coiled–coil domain that mediates homo-oligomerization and a C-terminal fibrinogen-like domain that binds Tie-2. The Tie-2/Ang1 signaling pathway plays a critical role in the maintenance of HSCs in a quiescent state in the bone marrow niche [45]. This pathway also supports SRC activity of human bone marrow $CD34^{-}$ cells in culture [46].

Insulin-like growth factor 2

Recently, we identified a novel cell population that supports HSC expansion: $CD3^{+}Ter119-$ cells isolated from embryonic day 15 (E15) mouse fetal livers [8]. DNA array experiments showed that, among other proteins, IGF-2 is specifically expressed in these cells but not in several other cell types that do not support HSC expansion in culture. We showed that all fetal liver and bone marrow HSCs express receptors for IGF-2. IGF-2 stimulates ex-vivo expansion of both fetal liver and bone marrow HSCs [8]. The inclusion of IGF-2 with SCF, TPO, and FGF-1 supports an eight-fold increase of highly enriched HSCs in culture [8]. Whether IGF-2 acts on self-renewal, apoptosis, differentiation, or homing of HSCs is unclear. Interestingly, IGF-2 was found to bind and stimulate self-renewal of human embryonic stem cells [47•].

Angiopoietin-like proteins

Angptls are a family of seven secreted glycoproteins that share sequence homology with the angiopoietins [48]. Similar to the angiopoietins, each Angptl contains an N-terminal coiled-coil domain and a C-terminal fibrinogen-like domain. However, unlike angiopoietins, Angptls do not bind to Tie-2 or Tie-1 and their receptors are unknown. This suggests that Angptls may have functions different from the angiopoietins. Limited studies [48] on Angptls have shown

that several members play a role in regulating angiogenesis, metabolism, and tumorgenesis. Angptl7 was suggested to be a target of the Wnt/β-catenin signaling pathway [49]. However, most of the physiological activities of the Angptls remain unknown. Recently we identified Angptl2 and Angptl3 as growth factors that stimulate ex-vivo expansion of bone marrow HSCs. We further showed that several other analogues, including Angptl5, Angptl7, and Mfap4, also support ex-vivo expansion of HSCs. These studies have enabled us to optimize our serum-free culture system such that we now obtain up to 30-fold expansion of long-term-reconstituting mouse HSCs in culture [10]. Angptl5 also stimulates ex-vivo expansion of human cord blood SRCs [50•]. The mechanism by which Angptls regulate HSCs expansion is under investigation.

Other factors

The IL-10 receptor is expressed on mouse Lin -Sca-1^+c-Kit^+ (LSK) and side population (SP) cells and IL-10 stimulated a three to four-fold increase in the frequency of repopulating HSCs in culture [51]. mKirre is a membrane protein identified in the stem cell supportive OP9 stromal cells. The cleaved extracellular domain acted as a factor that supports HSC expansion *in vitro* [52]. A matricellular protein 'nephroblastoma overexpressed' (also called NOV or CCN3) was identified from $CD34^+$ human cord blood cells; recombinant Nov protein enhances SRC activity and its knockdown abrogates SRC function. This suggests that Nov is a regulator of human hematopoietic stem or progenitor cells [53••].

Conclusion

One can make two general observations from studies on known HSC cytokines. One is that there are differences in the roles of some cytokines in embryos and in adults. This is not surprising considering the differences between fetal and adult HSCs and the dissimilarities in their microenvironments. Another is that most, if not all, cytokines affecting HSCs exhibit pleiotropy and redundancy. Pleiotropic actions appear to be the result of their different activities in conjunction with other cytokines. Their redundancy may be explained by convergence of different intracellular signaling pathways to 'master regulators' for different stem cell fates, self-renewal for example. The study of genetically modified mice and improvements in the in-vitro HSC culture system will be powerful tools to elucidate the functions of cytokines that regulate HSC function. Through studying how the different cell fates of HSCs are regulated by extrinsic factors, we will gain exciting insights into the molecular mechanisms by which the environment controls the fates of HSCs, as well as the pathophysiological origins of many disorders. The knowledge we obtain from these studies promises to result in better development of hematopoietic cell and gene therapies.

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