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Regulators of the Proliferation of Hematopoietic Stem and Progenitor Cells During Hematopoietic Regeneration

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1. Introduction

Adult hematopoietic stem cells (HSCs) ensure the maintenance of the HSC pool via their self-renewal capacity, and replenish mature cells throughout life via their proliferation and differentiation into lineage-restricted cells [1, 2]. HSCs are the only stem cells that have been used in the clinic to treat diseases, such as leukemia, germ cell tumors, and congenital immunodeficiencies. Since HSCs were first proposed [3], advances in multicolor flow cytometry have allowed the purification of mouse HSCs close to homogeneity. Several groups have succeeded in long-term hematopoietic reconstitution by transplanting a single lineage of HSCs (e.g., CD34^{low/-} Kit⁺ Sca-1⁺ lineage marker-negative cells and CD150⁺ CD48⁻ Kit⁺ Sca-1⁺ lineage marker-negative cells), providing direct proof of the existence of HSCs [4-7]. More recently, two groups analyzed the cycling status of HSCs by monitoring their proliferation rate over several months in vivo. The results of these studies suggested the presence of dormant HSCs that divide only about five times throughout the mouse life span [8, 9].

In the bone marrow (BM), HSCs are located in a specialized microenvironment, called the niche. Under steady-state conditions, signals from niches maintain some HSCs in a dormant state. Acquisition of dormancy is critical for the preservation of the self-renewal ability of HSCs and for the prevention of premature stem cell exhaustion [10-12]. However, in response to external stresses, such as bleeding, myeloablative chemotherapy and total body irradiation, HSCs proliferate extensively to produce very large numbers of primitive progenitor cells, thereby enabling rapid hematological regeneration [13]. Once recovery from myelosuppression or other stresses has been achieved, the activated HSCs return to a quiescent state via a number of negative feedback mechanisms [14]. This ability is a hallmark of HSCs and is

fundamental for the maintenance of hematopoietic function throughout the life of the organism. Although this property has long been recognized and is very important for organism survival, the molecular basis underlying how HSCs react to a hematologic emergency remains enigmatic. However, some key players have been identified. In this chapter, we briefly review the recent advances in our knowledge of HSC cell-intrinsic and cell-extrinsic regulators that are critical for hematopoietic regeneration under stress hematopoiesis.

2. Cell-intrinsic factors

2.1. Hemoxygenase-1

Heme plays many important roles, such as in the promotion of proliferation and differentiation of hematopoietic progenitor cells (HPCs) [15], and in the stimulation of hematopoiesis [16, 17]. Heme oxygenase (HO) catalyzes the degradation of heme. HO-1, encoded by the *Hmox1* gene, is a stress-inducible isozyme of HO and is highly expressed in the BM and spleen [17]. Cao et al reported that the hematopoietic lineages of heterozygous HO-1-deficient mice (*HO-1^{+/-}*) display accelerated recovery from myelotoxic injury induced by 5-FU treatment. BM transplantation experiments also revealed that mice transplanted with *HO-1^{+/-}* BM cells reconstituted hematopoietic lineages more rapidly than those transplanted with *HO-1^{+/+}* BM cells. However, *HO-1^{+/-}* HSCs could not rescue lethally irradiated recipient mice or serially reconstitute irradiated mice [18]. These results suggest that HO-1 restricts the proliferation and differentiation of HSCs/HPCs under stress conditions, and that the dysregulation of HO-1 can lead to precocious HSC exhaustion.

2.2. PSF-1

PSF-1, a partner of *sld five-1*, forms a multiprotein complex, termed GINS. The GINS complex contains Psf2, Psf3, and Sld5 in addition to Psf-1 [19-21]. All genes encoding this complex are evolutionally conserved and are essential for cell growth [22]. Ueno et al isolated the mouse ortholog of *PSF1* from a BM Lin⁻c-Kit⁺Sca-1⁺ hematopoietic stem cell cDNA library and found that PSF1 was specifically expressed in immature cells including blastocysts and spermatogonia. They also generated *PSF1^{-/-}* mice lacking functional *PSF1*, which died in utero around the implantation stage [23]. In hematopoietic cells, PSF1 was highly expressed in CD34⁺ KSL progenitor cells but not in CD34⁻ KSL cells. In addition, proliferating CD34⁺ KSL cells sorted from BM 4 days after 5-FU exposure were PSF1 positive. Next, Ueno et al investigated the function of PSF1 in hematopoiesis using *PSF1^{+/-}* mice. The pool size of HSCs and progenitor cells was decreased in aged *PSF1^{+/-}* mice compared to wild-type mice. Whereas young *PSF1^{+/-}* mice showed normal hematopoiesis under steady-state conditions, 5-FU treatment was lethal in *PSF1^{+/-}* mice as a result of a delay in the induction of HSC proliferation after BM ablation [24]. These results suggest that PSF1 is essential for the acute proliferation of HSCs during the regeneration phase after BM suppression.

2.3. Necdin

Necdin belongs to the melanoma antigen family of molecules, whose physiological roles have not been well characterized [25]. Necdin was originally identified as a gene product induced in neurally differentiated embryonal carcinoma cells [26]. Interestingly, recent genetic analyses demonstrate that aberrant genomic imprinting of *NDN* on the human 15q11-q13 chromosomal region is, at least in part, responsible for the pathogenesis of Prader-Willi syndrome [27-29], a disease associated with a mildly increased risk of myeloid leukemia [30]. Necdin interacts with multiple cell cycle-related proteins, such as SV-40 large T antigen, adenovirus E1A, E2F1, and p53 [31-34]. We reported that necdin is one of 32 genes that show higher expression in HSCs than in differentiated hematopoietic cells [35]. Other groups also found that necdin is highly expressed in HSCs [36, 37]. Necdin-deficient mice show accelerated recovery of hematopoietic systems after myelosuppressive stress, such as after 5-FU treatment and BM transplantation, whereas no overt abnormality is seen under conditions of steady-state hematopoiesis. As necdin is a potential negative cell cycle regulator, the enhanced hematologic recovery in necdin-null mice was suggested to result from an increase in the number of proliferating HSCs and progenitor cells. As expected, after 5-FU treatment, necdin-deficient mice had an increased number of HSCs, but this increase was transient and was observed only during the recovery phase [35]. These data suggest that the repression of necdin function in HSCs could form the basis of a novel strategy for the acceleration of hematopoietic recovery, thereby providing therapeutic benefits after clinical myelosuppressive treatments (e.g., cytoablative chemotherapy or HSC transplantation). Necdin is a p53 target gene, and *in vitro* overexpression and knockdown experiments demonstrated that necdin plays a role in the maintenance of HSC quiescence and self-renewal [37]. However, another group reported that necdin overexpression does not result in enhanced HSC quiescence [38].

2.4. Slug

Slug is a member of the highly conserved Slug/Snail family of zinc-finger transcriptional repressors found in diverse species ranging from *C. elegans* to humans. SLUG is a target gene for the E2A-HLF chimeric oncoprotein in pro-B cell acute leukemia [39]. Slug is highly expressed in immature hematopoietic cells, and a study using *Slug*^{-/-} mice revealed that slug is essential for the radioprotection of HPCs [40]. Slug is induced by p53 and protects immature hematopoietic cells from apoptosis triggered by DNA damage. Slug exerts this function by repressing Puma, a proapoptotic target of p53 [41]. Sun et al. investigated the effects of Slug under steady-state and stress hematopoiesis [42]. The numbers of HSCs (LSK, Flk2-LSK, SLAM, and EPCR⁺) and progenitor cells (multipotent progenitors: CD150⁻CD48⁻CD244⁺; lineage-restricted progenitors: CD150⁻CD48⁺CD244⁺) were comparable regardless of the Slug genotype under steady-state conditions. Consistent with previous reports [40, 43], they found that hematopoiesis in the BM was normal in *Slug*^{-/-} mice, suggesting that Slug is not required for steady-state hematopoiesis. On the other hand, an *in vivo* competitive repopulation assay revealed that Slug-deficient BM cells had a higher long-term reconstitution capacity. However, HSC homing and differentiation were not affected by the deficiency of Slug. These results suggest that Slug deficiency increases HSC self-renewal. Next, to assess whether Slug dosage

affects HSC self-renewal capability, they performed a serial transplantation assay. *Slug*^{-/-} BM cells showed an enhanced repopulation capacity during serial transplantation. Furthermore, the repopulating and proliferation potential of *Slug*^{-/-} HSCs treated with 5-FU were also examined in vivo and in vitro. Slug deficiency increased the reconstituting potential of 5-FU-activated HSCs in vivo and accelerated HSC expansion in vitro. Taken together, these results suggest that Slug negatively regulates HSC self-renewal under stress hematopoiesis.

2.5. Erg

The E-twenty-six (ETS)-related gene (ERG) belongs to the ETS family of transcription factors [44]. ERG rearrangement has been reported in acute myeloid leukemia (AML) [45] and Ewing sarcoma [46]. ERG overexpression has also been observed in prostate cancer [47]. A recent study revealed that overexpression of ERG is an adverse prognostic factor in AML with a normal karyotype [48]. More recently, the role of Erg in hematopoietic development and normal hematopoiesis was investigated. *Mld2*, an allele of the murine *Erg* gene with a missense mutation in the ETS domain-encoding region, disrupts Erg transactivation of gene expression. Mice homozygous for the *Erg*^{Mld2} allele die at midgestation because of a failure in definitive hematopoiesis [49, 50]. Ng et al studied hematopoiesis in mice heterozygous for the *Erg*^{Mld2} mutation [51]. While *Erg*^{+/^{Mld2}} mice showed normal steady-state hematopoiesis, *Erg*^{+/^{Mld2}} BM cells exhibited defective HSC self-renewal in BM transplantation or during recovery from exposure to sublethal γ -irradiation. The TPO/c-Mpl pathway is critical for the self-renewal and proliferation of HSCs [52–54]. Next, the phenotype of *Erg*^{+/^{Mld2}}*Mpl*^{-/-} mice was examined because the *Mld2* mutation was originally uncovered during a sensitized ENU mutagenesis screen of *Mpl*-deficient mice [49]. The double mutant mice died of BM failure following an exacerbation of a defect in HSC proliferation. Thus, Erg is required for HSC self-renewal during stress hematopoiesis. ERG is also expressed in endothelial cells [55, 56]. A recent study showed that ERG plays a role in endothelial tube formation and angiogenesis [57]. More recently, Yuan et al identified Rhoj, a Rho GTPase family member, as a novel downstream target of ERG [58]. Interestingly, Rhoj is also highly expressed in HSCs [35].

3. Cell-extrinsic factors

The interaction between HSCs and their microenvironment (niche) is essential for HSC maintenance, self-renewal, and survival. However, recent studies have revealed that the cell-extrinsic factors provided by the BM niche are also important for HSC responses during hematopoietic regeneration. Some key players have been identified.

3.1. Connexin-43

The exchange of ions, metabolites, and other small molecules (up to ~1,200 Da) occurs via gap junctions. Gap junctions are configured by a large family of proteins known as connexins (Cx). In the connexin family, connexin-43 (Cx43) is highly expressed in BM stromal cells [59], endothelial cells [7], osteoblasts [60], and mesenchymal stem cells [61]. HSCs also express Cx43

[36]. Cx43-deficiency in the BM (*Mx1-Cre/Cx43^{flox/flox}*) led to impaired hematopoietic recovery after 5-FU treatment [62]. To clarify the mechanism of impaired hematopoietic regeneration after myeloablation by 5-FU, Taniguchi-Ishikawa et al generated hematopoietic-specific Cx43 (H-Cx43)-deficient mice (*Vav1-Cre/Cx43^{flox/flox}*) and analyzed their hematopoietic phenotype [63]. The lack of Cx43 in hematopoiesis did not impair long-term competitive repopulation capacity but impaired hematopoietic recovery after 5-FU administration. 5-FU-treated H-Cx43-deficient HSCs failed to enter the cell cycle and showed decreased cell survival. More detailed analyses revealed that enhanced quiescence in H-Cx43-deficient HSCs treated with 5-FU is associated with up-regulation of the expression of quiescence markers, p16^{INK4a} [64] and p38 [65], and with an increased level of intracellular reactive oxygen species (ROS).

The same group also investigated the role of Cx43 in the BM osteoblastic niche. For this, Gonzalez-Nieto et al used conditionally osteoblast lineage-specific Cx43-deficient mice: *Col1- α 1-Cre; Cx43^{flox/flox}* mice (OB/P Cx43-deficient mice) [66]. The OB/P Cx43-deficient mice showed normal hematopoiesis under steady-state conditions. However, engraftment and migration of normal HSCs was impaired by the loss of Cx43 in the osteoblast lineage. Interestingly, in nonmyeloablated mice, OB/P Cx43 deficiency did not cause a homing defect but increased the endosteal lodgment of HSCs, which was associated with the expansion of Cxcl-12-expressing mesenchymal/osteolineage cells in the BM niche [67]. Another group reported that Cx43 and Cx45 gap junctions mediate the secretion of CXCL12 from BM cells, resulting in HSCs adhesion to stromal cells [68].

3.2. TIMP-3

Metalloproteinases (MMPs) modulate the extracellular matrix (ECM) environment [69-71]. Several studies have indicated that MMP-9 and MT1-MMP are important for the cleavage and inactivation of the KIT ligand (Stem cell factor: SCF) and CXCL12 during G-CSF-induced mobilization and hematopoietic recovery after cytotoxic stress in the BM [72-75]. Tissue inhibitors of the metalloproteinase (TIMP) family consist of four members (TIMP-1 to -4), all of which are endogenous regulators of metalloproteinases (MMPs) [76, 77]. Although TIMPs were initially identified as inhibitors of MMPs, recent findings suggest that they might have more diverse functions [78, 79]. Previous work suggested that TIMP-1 deficiency or enforced expression of TIMP-1 or TIMP-2 does not alter steady-state hematopoiesis and stress hematopoiesis, such as those induced by G-CSF stimulation and myelotoxic insult, respectively [80]. However, Rossi et al recently found that increased expression of p53 in *TIMP-1^{-/-}* HSCs resulted in dysregulation of the transition from G1 to S phase of the cell cycle, indicating that TIMP-1 has a role in controlling the cell cycle dynamics of LT-HSCs [81].

Among TIMP family members, TIMP-3 has unique properties. TIMP-3 binds firmly to ECM, a disintegrin [82-84], and inhibits metalloproteinase domain-containing proteins, such as ADAMs and ADAMTSs (ADAM proteins with Thrombospondin Motifs) [85]. Nakajima et al found decreased expression of TIMP-3 in immune suppressor factor (ISF)/short form of ISF (ShIF)-transfected cell lines, and partial reduction of HSC-supporting activity following the restoration of TIMP-3 expression in stromal cells expressing ISF [86]. These authors also investigated the role of TIMP-3 in HSC regulation. TIMP-3 expression in BM was increased

after 5-FU injection, and addition or overexpression of TIMP-3 resulted in enhanced proliferation of HSCs in vitro. BM regeneration after myelotoxic stress was impaired in TIMP-3-deficient mice, but was accelerated by enforced expression of TIMP-3 in vivo [87]. Another group also studied the role of TIMP-3 in hematopoiesis. They found that TIMP-3 was highly expressed in the endosteal region of the BM, the HSC niche, whereas its expression was low in HSCs and progenitor cells. They also examined the effect of human TIMP-3 (huTIMP-3) overexpression in HSCs in vivo. TIMP-3 overexpression resulted in increased myelopoiesis and decreased lymphopoiesis. Consistent with the study of Nakajima et al [87], HSC proliferation was increased by huTIMP-3 overexpression in vitro and in vivo [88]. These results suggest that TIMP-3 is important for the cellular response to myelosuppression.

3.3. Tenascin-C

Tenascin-C (TN-C) is a large extracellular matrix (ECM) glycoprotein that is expressed mainly in the developing embryo [89]. In the adult BM, expression of TN-C is restricted to the endosteal region [90, 91]. Although TN-C-deficient mice exhibit grossly normal development, the colony-forming capacity of *TN-C*^{-/-} BM cells is lower than that of wild-type BM cells [92]. This suggests that TN-C makes a significant contribution under stress hematopoiesis because the mononuclear cell count and BM architecture of TN-C-deficient mice are essentially normal. Nakamura-Ishizu et al studied the function of TN-C during hematopoiesis in vivo using TN-C knockout mice. First, they examined the expression pattern of various ECM proteins in the BM under different conditions (steady-state, immediately after myeloablation, and during the hematopoietic recovery phase). TN-C was predominantly expressed in stromal cells and endothelial cells, which are components of the BM niche, and was markedly up-regulated in the BM during hematopoietic regeneration. *TN-C*^{-/-} mice showed defects in hematopoietic recovery after BM ablation caused by 5-FU treatment and sublethal irradiation. The transplantation of wild-type BM cells into *TN-C*^{-/-} recipient mice demonstrated that a supporting ability of hematopoiesis in BM microenvironment lacking TN-C is inadequate for the proliferation of transplanted wild-type BM cells for the regeneration of hematopoiesis [93]. These findings suggest that TN-C is a critical component of the BM microenvironment during hematopoietic regeneration.

4. Conclusions

In this chapter, we have briefly summarized regulators that have recently been shown to be involved in the control of HSCs and progenitors during the hematopoietic regeneration phase. Recent studies have revealed that, when myelopoiesis is compromised following infection, HSC proliferation involves not only the factors described in this review, but also inflammatory signaling molecules such as interferons [94-96], tumor necrosis factor- α [97, 98], and Toll-like receptors [99-101]. Because HSC proliferation potential is critical for organism survival during stress conditions, further understanding of the mechanism of stem cell activation will be needed before stem cells can be used in regenerative medicine.

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