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RESEARCH HIGHLIGHT

To go or not to go: the "Itchy" effect on the destiny of hematopoietic stem cells

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Protein ubiquitylation is an evolutionarily conserved post-translational covalent modification that forms an isopeptide bond between the activated ubiquitin and the target protein [1]. It is consisted of three-step enzymatic processes involving E1 ubiquitin-activating enzymes, ubiquitin-conjugating enzymes (E2s), and E3 ubiqutin ligases (E3s). E3s contribute to the specificity of this system by selectively targeting a particular target(s) and to the regulation of many cellular physiological processes such as cell proliferation, apoptosis and differentiation. Aberrant expression or dysfunction of E3s results in autoimmune diseases , tumorigenesis , and neurodegenerative disorders. However, it is not until recently that some E3s are recognized to function in the homeostasis of hematopoietic stem cells (HSCs) [2-5].

The production of blood cells throughout life depends on the regenerative ability of a rare population of HSCs with Lineage$^-$Sca-1$^+$c-Kit$^+$ (LSK) phenotype. HSCs are functionally defined by self-renewal capacity and pluripotential differentiation. LSK population is heterogeneous and can be classified into long-term reconstituting HSCs (LT-HSCs), short-term HSCs (ST-HSCs) and multipotent progenitor cells (MPPs), based on their differential self-renewal capacities [6]. At steady state, most HSCs are kept in a quiescent state and only a few percentages of them are entering cell cycle within bone marrow. Such a cell cycle regulation on HSCs is essential to supply mature blood lineages in response to the stress such as exposure to chemotoxic agents or infection without exhaustion of HSCs. It is thought that HSC homeostasis is regulated by cytokines such as thrombopoietin (TPO), bone morphogenetic protein 4 (BMP4), or transforming growth factor-β (TGF-β) in bone marrow niche. In addition, the direct interaction between HSCs and osteoblasts also affects the self-renewal of HSCs [7].

In particular, Notch signaling is well characterized as one of the major pathways [7]. Notch receptor family is consisted of four members (Notch1-4), which are heterodimeric receptors in mammals. The extracellular domain of receptors contains EGF-like repeats that bind their ligands, such as Jagged1, 2, Delta-like1, 3 or 4. The intracellular domain of Notch (ICN) has RAM domain followed by ankyrin repeats that bind transcription factor CSL (CBF-1 in human, RBP-J in mice, Suppressor of hairless in *Drosophila* and Lag-1 in *Caenorhabditis elegans*), nuclear localization signals and PEST motif. Notch signaling is initiated by the interaction between ligand and receptor, followed by two proteolytic cleavages mediated by ADAM metalloproteases and γ-secretase. These processes lead to the release and translocation of ICN to the nucleus where it binds CSL to form transcription complexes [8].

It has been previously observed that Jagged1 expressed on osteoblasts promotes HSC proliferation through the activation of Notch1 expressed on HSCs in bone marrow niche [9]. The activation of Notch1 signaling by enforced ICN expression enhanced HSC proliferation *in vitro* and *in vivo* [10]. Consistent with these findings, inhibition of Notch1 signaling by γ-secretase inhibitor or enforced expression of dominant negative mutant form of CBF-1 suppressed self-renewal of HSCs [9,11]. Although activated Notch1 signaling may apparently contributes to HSC self-renewal, it has been largely unknown how Notch1 signaling is regulated in HSC homeostasis.

A recent study by Rathinam *et al.* in *Nature Immunology* sheds the light on the molecular mechanism regulating Notch1 signal transduction on HSC homeostasis by the E3 ligase Itch-mediated ubiquitination [5]. Of all LSK populations, the primitive LT-HSCs, which are defined as CD150$^+$CD48$^-$, contained much more Itch at mRNA levels. When they investigated HSC pool derived from *Itch*$^{+/+}$ or *Itch*$^{-/-}$ (or Itchy) mice, LSK population was significantly increased in *Itch*$^{-/-}$ mice. This was due to

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the hyper-proliferation of Itch-deficient HSCs. Notably, the frequencies of each LSK subsets such as LT-HSCs, ST-HSCs and MPPs were quite similar between *Itch*$^{+/+}$ and *Itch*$^{-/-}$ mice. These data suggest that Itch negatively regulates HSC pool size, but does not affect progenitor differentiation. To address whether these proliferative phenotypes are intrinsic to *Itch*$^{-/-}$ HSCs, the authors transplanted LSK cells from *Itch*$^{+/+}$ or *Itch*$^{-/-}$ mice into lethally irradiated congenic recipients. The frequencies of HSCs derived from *Itch*$^{-/-}$ mice were higher than those from *Itch*$^{+/+}$ mice. Moreover, these phenomena were observed under competitive conditions. Taken together, these results suggest that Itch negatively regulates the reconstitution capacity of HSCs, independent of hematopoietic microenvironment.

The next question is what is the mechanism of regulating HSC homeostasis by Itch? It was documented that Notch is a target substrate for Itch a decade ago [12] and the two genetically interact with each other in the development of autoimmune disease in a Notch ICN transgenic mouse model [13]. However, the physiological relevance remained unclear. Given the similarities between Itch deficient and Notch1 overexpressed HSCs [5,10], they hypothesized that augmented proliferation in Itch-deficient HSCs was due to the activated Notch1 signaling. They utilized mice with transgenic Notch reporter crossed with *Itch*$^{-/-}$ mice and clearly demonstrated that Notch1 signaling was strengthened in Itch deficient HSCs. The protein levels of Notch1 were increased and the mRNA expression of downstream target genes such as *Hes1*, *Myc* and *Dtx* were upregulated in *Itch*$^{-/-}$ HSCs. Finally they showed that knockdown of Notch1 by RNAi attenuated the proliferative phenotypes of *Itch*$^{-/-}$ HSCs both *in vitro* and *in vivo* [5].

To keep the HSCs function as the source of all blood cells, the cell cycle is tightly regulated in these cells. Especially it is considered that the transition from quiescent G0 to G1 phase is important for HSCs to maintain these functions. A number of studies argued that aberrant HSC proliferation might cause the impairment of self-renewal capacity, subsequently leading to the exhaustion of HSCs, as revealed in the experimental models of gene-targeted mice combined with bone marrow transplantation [14]. Notably, although HSCs derived from *Itch*$^{-/-}$ mice were hyper-proliferative, because of accelerated cell cycle entry, these HSCs showed repopulation capacity through two rounds of sequential transplantation without HSCs exhaustion [5]. More detailed analysis of Notch1 signal transduction through the ubiquitin system by Itch on HSC homeostasis might lead to better understanding of the underlying molecular mechanisms by which the cell fates of HSCs are decided when they enter cell cycle.

Many studies have demonstrated that critical regulators of HSC homeostasis are involved in leukemogenesis [14]. In fact, it was observed that activated Notch1 signaling induces T-cell acute lymphoblastic leukemia (T-ALL) in bone marrow transplanted mouse models [15]. Itch-deficient mice did not show the development of leukemia, and the frequencies of myeloid, erythroid and lymphoid lineages were similar in *Itch*$^{+/+}$ and *Itch*$^{-/-}$ mice [5]. With regard to these paradoxical evidences, activated Notch1 signaling in Itch-deficient HSCs may not be sufficient enough to cause leukemia. In addition to these findings, *Itch*$^{-/-}$ mice showed augmented hematopoietic recovery compared with *Itch*$^{+/+}$ mice after myeloablation with 5-fluorouracil administration. Taken together, the study by Rathinam *et al.* implies that Itch could be a potentially therapeutic target for HSC-based disorders.

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