Review Article

Stem Cell INVESTIGATION

Mechanisms determining the fate of hematopoietic stem cells

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Abstract: Successful *in vitro* expansion of hematopoietic stem cells (HSCs) will facilitate the application of HSC transplantation for the treatment of various diseases, including hematological malignancies. To achieve this expansion, the molecular mechanisms that control the fate of HSCs must be deciphered. Leukemia-initiating cells (LICs) or leukemia stem cells (LSCs) may originate from normal HSCs, which suggest that the dysregulation of the mechanisms that regulate the cell fate of HSCs may underlie leukemogenesis. Here we review the recent progress in the application of HSCs, the regulatory mechanisms of the fate of HSCs, and the origins of leukemia.

Keywords: Hematopoietic stem cells (HSCs); cell fate; leukemia

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Hematopoietic stem cells (HSCs) are able to give rise to an organism's entire blood system. They continuously differentiate into all of the blood cell lineages and possess the capacity for long-term self-renewal. In recent decades, remarkable progress has been achieved in the fight against hematological malignancies. Although novel chemotherapeutic regimens and targeted strategies have been developed, the most powerful weapon is HSC transplantation (HSCT). Since the first clinical trial of HSCT in the early 1960s, millions of patients with malignant or nonmalignant blood diseases have benefitted from HSCT, which is currently the most widely used therapeutic strategy involving stem cells worldwide (1,2). More than 50,000 patients are treated with allogenic or autologous HSCT each year. HSCs have been used to treat patients with leukemia and lymphoproliferative disorders. There are various types of leukemia including acute myeloid leukemia (AML), acute lymphoblastic leukemia, chronic myeloid leukemia (CML), chronic lymphoblastic leukemia, and myelodysplastic syndromes. Lymphoproliferative disorders include Hodgkin lymphomas, non-Hodgkin lymphomas (NHLs), and plasma cell disorders (3). In addition to hematological malignancies, HSCs have been used in the treatment of nonmalignant blood disorders (4), solid tumors (5), autoimmune diseases (6), and immune deficiencies such as human immunodeficiency virus disease (7).

Despite these advances, the application of HSCT is greatly hampered by the lack of sources of HSCs. One solution for this demand is to expand HSCs *in vitro*. Unfortunately, HSCs are easily differentiated and lose their long-term self-renewal activities *in vitro*, so the expanded HSCs are unable to reconstitute the recipient's hematopoietic system (8). Therefore, researchers in the fields of hematology and regenerative medicine have long sought the efficient expansion of functional HSCs. Thus, it is important to study the molecular mechanisms and regulatory networks that modulate the fate of HSCs to gain an understanding of hematopoiesis and to provide critical insight into the clinical applications of HSCs.

The expansion and maintenance of self-renewal in HSCs are regulated by several signaling pathways, such as the Notch (9), Wnt (10), bone morphogenetic protein (BMP) (11), mTOR (12), and Hedgehog (13) pathways, which are in turn regulated by both extrinsic and intrinsic mechanisms (*Figure 1*) (14). For example, the Wnt signaling required

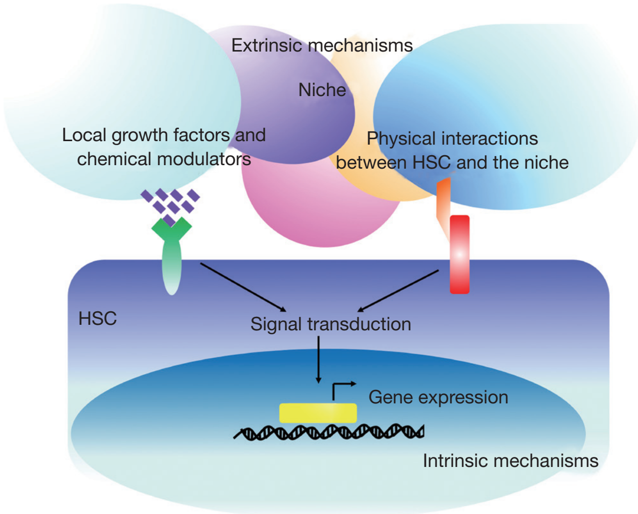

Figure 1 Extrinsic and intrinsic mechanisms that regulate cell fate of HSC. The expansion and maintenance of self-renewal in HSCs are regulated by both extrinsic and intrinsic mechanisms. Extrinsic mechanisms are dictated by niche, which provides physical interactions, growth factors and chemical modulators that trigger diverse signal transduction pathways. Intrinsic mechanisms are niche-dependent transcription factors that initiate expression of downstream target genes following extrinsic stimulations. TF, transcription factor.

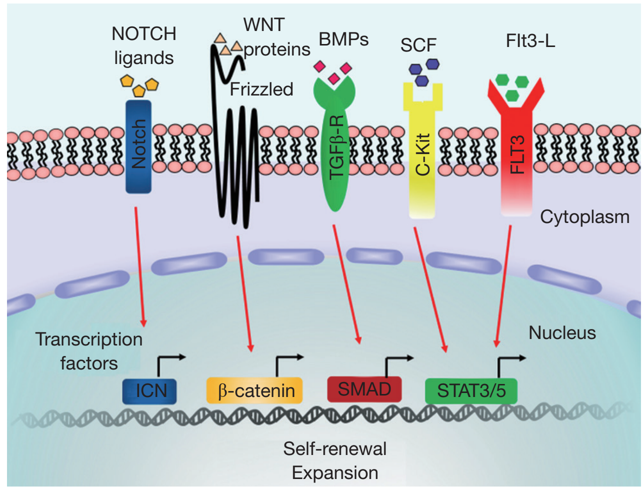

Figure 2 Signaling pathways involved in the self-renewal of HSC. Signaling pathways are initiated when growth regulators and chemical modulators bind to respective cell surface receptors. Signaling pathways lead to the translocation of the transcription factors from the cytoplasm to the nucleus. These transcription factors then bind to the appropriate DNA sequences to regulate the self-renewal of HSC. BMP, bone morphogenetic protein; SCF, stem cell factor; FLT-3, Fms-like tyrosine kinase 3; TGF-β, transforming growth factor-β.

for the self-renewal of HSCs is activated by extracellular proteins (e.g., WNT3A), which then up-regulate some of the genes implicated in self-renewal (e.g., HoxB4 and Notch1) and arrest the HSCs in an undifferentiated stage (10). The stemness of HSCs is niche dependent; it is essential for adult HSCs located in the bone marrow niche (osteoblastic niche and bone marrow vascular niche) (15,16), which include osteoblasts, osteoclasts, perivascular stromal cells, endothelial cells, macrophages, sympathetic neurons, and nonmyelinating Schwann cells (17). It provides physical interaction and secretes many growth factors and chemical modulators, such as NOTCH ligands (JAGGED-1/2) (18,19), WNT proteins (WNT3A) (10,20), BMPs (11), angiopoietin-like factors (21), thrombopoietin (22), stem cell factor (23,24), retinoic acid (25), CXCL12 (26), and E-selectin (27), that activate the regulatory pathways and maintain the self-renewal of HSCs or promote their proliferation. Recently, we found that angiopoietin-like 7 derived from a stromal cell line is capable of promoting the expansion of human HSCs and increasing their repopulation activities via Wnt signaling. This finding provided new insight into the regulation of the fate of HSCs and a new method for *in vitro* culture of HSCs (28).

In intrinsic mechanisms, under the cascade of these signaling pathways, transcription factors play the primary role in determining the gene expression profiles of stem cells. The current view is that the fate of HSCs is regulated by competition between transcription factor complexes (29). It is well established that transcription factors such as ICN (18,19), β-catenin (10,20), Myc (30), SMAD (11), STAT3/5 (31), CEBPα (32), HOXB4 (33), GATA2 (34), PU.1 (35), JUNB (36), and GFI1 (37) are necessary for the self-renewal process of HSCs (*Figure 2*) and that *ex vivo* over-expression of these code genes may result in expansion of the HSCs by restricting cell differentiation, resetting the cell cycle, and mediating cell division. Self-renewal is activated by diverse signals and regulated by many transcription factors, but these transcription factors are not the sole mediators; for example, Myc, NOTCH, and leukemic fusion proteins together stimulate self-renewal (38,39). Therefore, signaling through multiple pathways is likely to trigger a set of cellular events associated with self-renewal; the transcription factors then make a proper response to these signals and endow a moderate self-renewal process with HSCs. Therefore, self-renewal and expansion occur autonomously in HSCs and are also affected by the niche; the HSCs must remain in a tightly controlled and precisely balanced stage.

Many studies have suggested that leukemia is a stem cell-based disease (40,41). Although the existence and relevance of leukemia-initiating cells (LICs) or leukemia stem cells (LSCs) in acute lymphoblastic leukemia have remained elusive (42,43), LICs have been fairly well described in AML and CML by several research groups (41,44-46). LICs are a subset of cells that have the capacity to self-renew, to give rise to more differentiated progeny, and to maintain the leukemia for long periods. Although LICs and HSCs differ in their production of differentiated cells, they have striking similarities. For example, like HSCs, LICs account for only a small subset of leukemic cells that are capable of extensive proliferation *in vitro* and *in vivo*. For most subtypes of AML, the cells capable of transplantation have a ($CD34^+$, $CD38^-$) phenotype, similar to that of HSCs (41,47). In addition, LICs are niche dependent, and xenograft transplantation assays have proven the role of niches in resistance to chemotherapy and in the cell cycle regulation of LICs (48,49). Furthermore, both normal stem cells and LICs depend on SDF-1-mediated CXCR4 signaling for homing and mobilization (50). In addition, many molecular mechanisms that enable self-renewal, such as the Notch (51), Wnt (52,53), angiopoietin (54), and FGF (55) signaling pathways, are common to both normal stem cells and LICs. Increasing evidence suggests that certain subtypes of human leukemia may arise from mutations that accumulate in normal HSCs. For example, in CML, BCR–ABL fusion resulting from t(9;22) was found in HSCs (40). In addition, it has been reported that human CML can be induced in mice by introducing the BCR–ABL fusion protein into normal HSCs (56). Translocation of chromosomes 8 and 21 in HSCs results in RUNX1–ETO fusion and leads to AML (57). Furthermore, preleukemia clones with somatic mutations have been found in the HSCs of patients with AML (58). It has also been reported that the genetic alterations specific for T-cell lymphoma (59), follicular lymphoma (60) and hairy cell leukemia (61) could be traced to the HSC stage. The regulatory network tightly controls and maintains normal HSC function. Disturbances to these systems can lead to dysregulation of the HSCs, impairing their differentiation (62), increasing cell survival (63), and ultimately resulting in the abnormal proliferation of leukemic cells. Therefore, it is reasonable to assume that LICs may be derived from normal HSCs, and further studies should focus on the molecular mechanisms that transform HSCs into LICs.

Some of the biological features of HSCs have now been recognized, but the molecular mechanisms that underlie these properties are still not clearly understood. Investigation of the regulatory mechanisms of HSCs may help us to understand not only the origin of LICs but also to determine a means of expanding functional HSCs *in vitro*, which would have many beneficial clinical uses.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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