Cellular and Molecular Life Sciences

REVIEW

Survival regulation of leukemia stem cells

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Abstract Leukemia stem cells (LSCs) are a subpopulation cells at the apex of hierarchies in leukemia cells and responsible for disease continuous propagation. In this article, we discuss some cellular and molecular components, which are critical for LSC survival. These components include intrinsic signaling pathways and extrinsic microenvironments. The intrinsic signaling pathways to be discussed include Wnt/β-catenin signaling, Hox genes, Hh pathway, Alox5, and some miRNAs, which have been shown to play important roles in regulating LSC survival and proliferation. The extrinsic components to be discussed include selectins, CXCL12/CXCR4, and CD44, which involve in LSC homing, survival, and proliferation by affecting bone marrow microenvironment. Potential strategies for eradicating LSCs will also discuss.

Keywords CML · AML · LSC · Wnt · Hox · Hh · Alox5 · miRNA · Microenvironment

Abbreviations
ALL   Acute lymphoblastic leukemia
AML   Acute myeloid leukemia
B-ALL B cell acute lymphoblastic leukemia/lymphoma
BM   Bone marrow
CLL   Chronic lymphocytic leukemia
CML   Chronic myeloid leukemia
CSC   Cancer stem cell
GMP   Granulocytes-macrophage progenitors
GVHD   Graft versus host disease
HVGD   Host versus graft disease
HSC   Hematopoietic stem cell
LSC   Leukemia stem cell
T-ALL T cell acute lymphoblastic leukemia/lymphoma
Ph   Philadelphia chromosome
TKI   Tyrosine kinase inhibitor

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Introduction

It has become apparent that the initiation and propagation of some types of leukemia are driven by leukemia stem cells (LSCs), and LSCs share some biological features with normal hematopoietic stem cells (HSCs) [1–3]. LSCs self-renew and give rise to leukemia through certain degree of cellular differentiation. They are also believed to cause disease relapse and drug resistance [1–3]. Therefore, the development of effective therapeutic strategies for eradicating LSCs is essential for improving patient survival or even curing the diseases.

In general, leukemia includes a broader group of neoplasms, which usually initiates in bone marrow (BM) and results in high numbers of abnormal white blood cells in peripheral blood [4]. These abnormal cells also infiltrate various organs and tissues, and often have some defects in differentiation and compromised immune function. There are four major types of leukemia [4]: chronic myeloid leukemia (CML), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), and acute lymphoblastic

leukemia (ALL); there are a number of less common leukemias as well. CML is a myeloproliferative disorder with a high number of well differentiated neutrophils in peripheral blood and myeloid cells in BM. AML is a genetically heterogeneous clonal disorder characterized by rapid growth of abnormal myeloid linage cells accumulated in BM and blood. CLL comes from a group of white blood cells called lymphocytes, typically progresses more slowly than other types of leukemia, and most commonly affects older adults. Acute lymphoblastic leukemia (ALL), also called acute lymphocytic leukemia or acute lymphoid leukemia, is the most common malignancy in children. ALL includes precursor B cell acute lymphoblastic leukemia/lymphoma (B-ALL) and T cell acute lymphoblastic leukemia/lymphoma (T-ALL).

AML stem cell is the first one to prove the hypothesis of cancer stem cells (CSCs) [5] by using separated population of blasts from AML to transfer disease into immune-deficient mice [6]. Subsequently, LSCs also are testified in CML and ALL. Unlike CML and AML, the biological characteristics of ALL stem cells are not well studied to date. In this review, we will mainly focus on the biological characteristics of CML and AML stem cells, the roles of critical regulators and microenvironment for LSC self-renewal and survival. We will also describe current and prospective therapeutic strategies to target LSCs.

Biology of LSCs

During normal hematopoietic differentiation, stem and progenitor cells may accumulate some kinds of somatically genetic abnormalities, including specific gene mutations, chromosome number alterations, and chromosomal translocations. Genetic alterations are frequently detected in leukemia. These genetic lesions alter the mechanisms of self-renewal, proliferation, and differentiation eventually resulting in the development of leukemias. For example, in patients with CML, approximately 95 % of cases associate with the chromosome translocation of t(9;22)(q34;q11), also called philadelphia chromosome (Ph) [7]. Ph chromosome is also detected in 15–30 % of adult patients with ALL, ∼5 % children with ALL, and ∼5 % of patients with AML [8]. This genetic abnormality results in the formation of a fusion constitutive tyrosine kinase, BCR/ABL. *BCR/ABL1* alone is sufficient to cause CML in mice by expressing the fusion oncogene in a specific subpopulation of cells BM, and this cell population is required for *BCR/ABL1* to induce CML-like disease in mice. By using separated population of cells from CML patients or CML-like disease mice transduced by *BCR/ABL1* [9], it has been demonstrated that a subpopulation of leukemia cells called LSCs are responsible for CML continuous growth and propagation (Fig. 1). In CML patients, the Ph chromosome is believed to originate from a
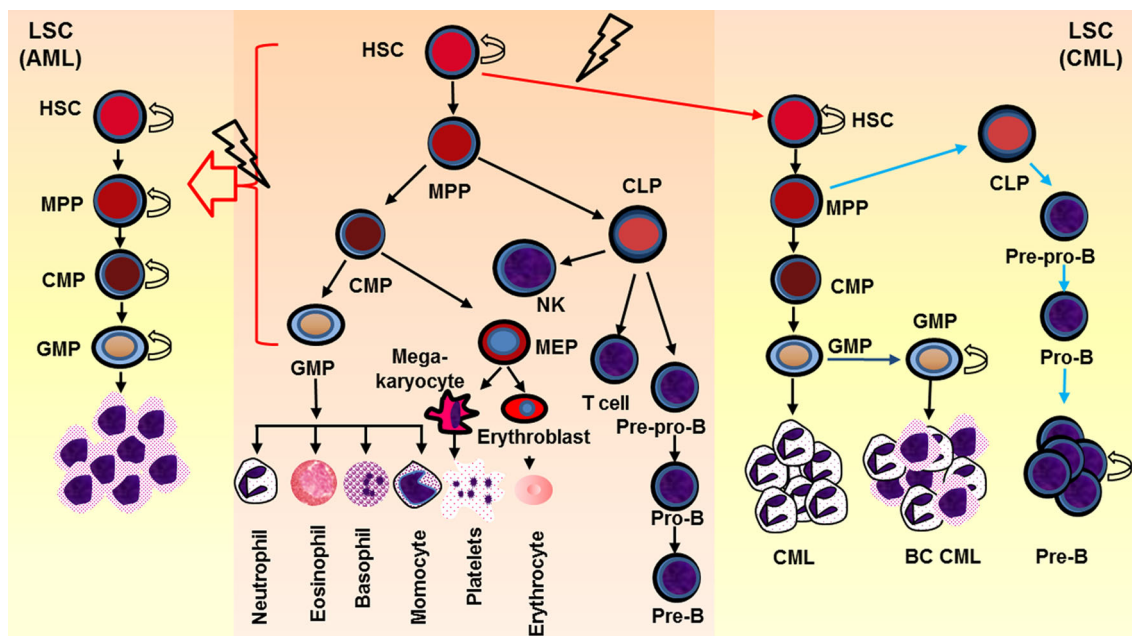
Fig. 1 Schematic illustration of the normal and leukemic hematopoietic hierarchies. Hematopoietic cells are organized in a hierarchy that is sustained by a small population of self-renewing hematopoietic stem cells (HSCs). HSCs differentiate to progenitors in a lineage-restricted manner. Progenitors retain limited self-renewal capacity which in turn produce functionally mature blood cells (*middle column*). If HSCs or progenitors accumulate transforming mutations resulting in acquisition of uncontrolled self-renewal capacity and giving rise to aberrant developmental hierarchy, finally leading high count abnormal white cells in blood and bone marrow, these cells are determined as LSCs. In AML, LSC can be initiated from HSC, MPP, and GMP (*left column*). In CML chronic phase, it is believed that LSC is generated at HSC level; in blastic phase, LSC possess some biologic characteristics of GMP (*right column*)

HSC for it can be clonally detected both in myeloid and lymphoid cells [10]. Both of LSCs and normal HSCs reside in a rare cell population among $CD34^+CD38^-$ cells [11, 12]. Importantly, new biomarkers that help to distinguish LSCs from normal HSCs have begun to be discovered; for example, dipeptidylpeptidase IV (CD26) has been found to be specifically expressed on the surface of LSCs in CML [13]. In a murine model of *BCR/ABL1*-induced CML-like disease, $Lin^-Sca-1^+c-Kit^+$ (LSK) cells have been demonstrated to function as LSCs [9]. During CML progression, LSCs do not reside in one cell population. For example, in the absence of treatment, CML has three clinical phases: chronic phase, accelerated phase, and blastic phase. In chronic phase, phenotypically LSCs exhibit some characteristics similar to those of normal HSC including morphology, biomarkers, and gene profiling [14]. In CML blastic phase (BC CML), granulocyte–macrophage progenitor (GMP) cells have been shown to acquire the properties of LSCs [15] (Fig. 1). In blastic phase, leukemia progenitor cells are thought to accumulate more genetic or epigenetic changes, which disrupt LSC differentiation and further alter critical signal pathways that regulate leukemia cell survival and growth.

Unlike LSCs in CML, AML stem cells are diversified and heterogeneous. AML LSCs originate from different steps of hematopoietic progenitors [6] (Fig. 1), and were initially proposed to establish cancer stem cell (CSC) concept [16]. In that study, a subpopulation of $CD34^+CD38^-$ human AML cells were shown to serially transplant leukemia in a mouse xenograft model. In contrast, more committed progenitors ($CD34^+CD38^+$) lack such potential [6, 17]. Recent studies showed that a subpopulation within $CD34^+CD38^{--}$ $CD90^+$ cells in human cord blood contains a non-HSC multipotent progenitor (MPP), which is postulated to the origination of human AML [18] (Fig. 1). This result was further confirmed in an AML1/ETO-transduced AML murine model, in which AML1/ETO was expressed in a clonal HSC population, but the MPP population was responsible for AML development [19]. The diversities of LSC in AML may be related to the somatically genetic abnormalities occurring in myeloid progenitors. Some studies performed in murine models generated by retroviral transformation provide direct evidence that some oncogenes favor transforming particular cell populations to turn them into LSCs. For example, rearrangements of the mixed-lineage leukemia (MLL) gene contribute to approximately 10 % of adult AML, which are also detected in more than 70 % of infant leukemia and many cases of secondary acute leukemias [20]. Studies from one of these rearrangements, MLL/AF9, show that MLL/AF9 can turn GMP cells to LSCs, whereas, the combination of HoxA9 and Meis1 (myeloid ecotropic viral integration site 1 homolog, a Hox cofactor) transforms LSK cells to cause AML [21].

Critical signaling mechanisms for LSC survival

Survival mechanisms of LSC survival are not well understood. Available studies indicate that LSCs share some signaling pathways with their normal stem cell counterparts for survival and proliferation. However, LSCs also require some pathways that are unique and specific to LSCs in self-renewal and proliferation [1–3]. Here we selectively describe some signaling pathways that play a critical role in survival regulation of LSCs.

Wnt signaling

The Wnt glycoproteins are highly expressed in BM and play an important role in the self-renewal and long-term maintenance of normal HSCs [22]. To date, several members of Wnt family have been identified, including Wnt1, 3a, 5a, and 1b. Wnt signaling is mediated through their intracellular components. Canonical Wnt signaling is initiated by the binding of a Wnt protein with its receptor, frizzled (Fz) family, and a co-receptor of the low-density-lipoprotein-receptor-related-protein family (LRP5 or LRP6) [23]. In signal transduction, β-catenin acts as a major molecule in this pathway [24, 25]. Upon activation, β-catenin proteins accumulate and locate to nucleus, then displace co-repressors in the groucho-related gene family (GRG) bound to T cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors, resulting in the induction of expression of target genes such as *c-Myc*, *c-Jun* and *cyclinD1* [26] (Fig. 2). Increasing evidence supports that Wnt signaling plays an important role in the development of AML and CML. For example, Wnt/β-catenin pathway is highly up-regulated in both AML [27–29] and CML [30, 31]. In mouse models of AML induced by co-expression of Hoxa9 and Meis1a oncogenes or by fusion oncoprotein MLL/AF9, it has been showed that Wnt/β-catenin signaling pathway is required for self-renewal of LSCs that are derived from either HSC or GMP [21]. During leukemogenesis, β-catenin is essential for AML initiation from HSC or GMP [21]. Similarly in CML, it has been demonstrated that β-catenin and Wnt pathway associated genes are highly expressed in LSCs compared to normal HSCs [32]. Studies performed in a *BCR/ABL1*-transduced CML murine model show that donor cells with depletion of β-catenin primarily impairs the ability of *BCR/ABL1* to cause CML in adoptive hosts and LSCs cannot be serially transplanted to cause CML, indicating that LSC self-renewal and long term maintenance rely on β-catenin [30, 31]. Furthermore, the up-regulation of β-catenin is not converted by BCR/ABL tyrosine kinase inhibitor (TKI), which highlights its importance as a therapeutic target in combination with TKI. All these previous studies support that β-catenin plays a critical role in LSC persistence, and
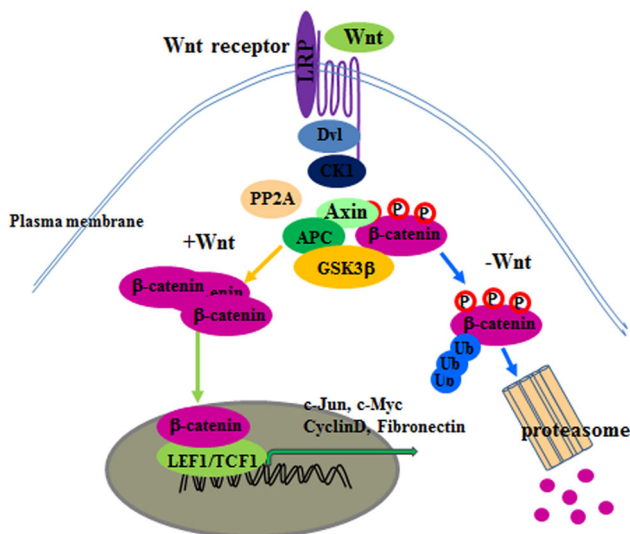
Fig. 2 Wnt family members bind to their receptors, Frizzled protein alone or with the low-density lipoprotein-receptor-related proteins (LRPs). Frizzled receptors recruit intracellular signaling molecules, the Dishevelled (Dvl) family including Dvl-1, Dvl-2, and Dvl-3, for further intracellular signaling transduction. Dvls function as molecular adaptors on Frizzled receptors due to their protein-protein interaction domains and/or heterotrimeric G-proteins. Beta-catenin is associated in a cytoplasmic complex together with the adenomatous polyposis coli (APC) protein, the cytoplasmicserine/threonine kinase GSK-3β, and axin. Axin is dephosphorylated by the protein phosphatase 2A (PP2A). A complex of axin with one of the three isoenzymes of casein kinase I (CKIα, δ, or ε) phosphorylates β-catenin on serine 45. This step is independent of (GSK-3beta) and initiates the phosphorylation-degradation cascade of β-catenin. In the following step, beta-catenin is phosphorylated at serine 33/37 by GSK-3β. The phosphorylated β-catenin is, subsequently, recognized by the E3 ubiquitin ligase and thereby, targeted for ubiquitination and subsequent degradation by the proteasome. In the presence of Wnt signaling, the phosphorylating activity of GSK-3β is inhibited leading to the stabilization of β-catenin. The APC-GSK-3beta-axin activity is dissociated and unphosphorylated β-catenin accumulates in the cytoplasm, translocates into the nucleus, where it interacts with TCF and LEF transcription factors binding on the promoter of Wnt target genes and mediates their transcription

Wnt/β-catenin pathway offers an attractive therapeutic strategy for targeted therapies of CML and AML [21, 30].

Hox family genes

Gene expression analysis and functional studies have shown that homeobox family genes (Hox) play important roles in the regulation of early stages of hematopoiesis, including self-renewal of HSCs, and also impact leukemic transformation [33–37]. Multiple Hox genes have been identified as partners of chromosomal translocations in leukemias [38]. Gene profiling studies demonstrate that Hox genes and their cofactors are globally deregulated in cells from leukemia patients [38–40], indicating that Hox genes and their cofactors play important roles in a wide spectrum of human leukemia. The clustered Hox family genes are an evolutionarily highly conserved group of genes that encode DNA-binding transcription factors [41]. Hox genes exhibit a high degree of homology to the clustered homeotic genes (HOM-C) of *Drosophila melanogaster* [38]. The highly conserved homeobox sequence motif is comprised with a 60 amino-acid helix–turn–helix DNA-binding domain, which is the common element defining this family genes [38]. Hox genes are particularly regulated by their upstream regulators, caudal-type homeobox genes (Cdx) genes, Cdx1, Cdx2, and Cdx4. Cdx genes are involved in Wnt and bone morphogenic protein (BMP) signaling [42]. The crosstalk between Hox genes and Wnt signaling suggest the roles of Hox genes in survival regulation of LSCs. Substantial evidence shows that aberrant expression of Hox genes contributes to the pathogenesis of myeloid malignancies, especially self-renewal of LSCs [39, 43]. For example, in CML, HoxA9 is required for LSC self-renewal and its expression correlates with a poor prognosis [40]. In most AML patients, deregulation of Hox genes were detected particularly due to the aberrant expression of Cdx2 [44]. Studies using murine models also demonstrate the roles of Hox gens in the pathogenesis of AML. For example, fusions of the HoxA9 or HoxD13 with NUP98 can sufficiently recapitulate AML in mouse [45, 46]. Overexpression of individual Hox family members, including HoxB3 [47], HoxB8 [48], or HoxA10 [49], by retroviral transduction or retroviral insertion mutagenesis also generates AML in murine models. Except for Hox family genes directly involving in leukemogenesis, they are also deregulated in other oncogene-transduced leukemias. The expression of Hox genes is significantly up-regulated in MLL-rearrangements involved leukemias [50, 51].

Without any doubt, elucidation of the roles of Hox genes in leukemogenesis helps to understand the mechanisms for LSC self-renew and differentiate, and ultimately for developing new strategies for targeting LSCs [52].

Hedgehog (Hh) pathway

The Hedgehog (Hh) pathway plays important roles in numerous developmental processes, including determination of cell fate, patterning, proliferation, survival, and differentiation [53]. Mammals have three Hedgehog homologues, Sonic Hh (SHh), Desert Hh (DHh), and Indian Hh (IHh) [53]. Recent studies point out that Hh signaling regulates adult stem cells in the maintenance and regeneration of adult tissues [54]. Hh aberrant activation is associated with the development of several types of solid tumors [55–58], and regulates the proliferation of CSCs and increases tumor invasiveness [54].

In normal hematopoiesis, Hh proteins are produced in BM niche and bind to their receptors, patched (Ptch) 1 and 2. In the absence of Hh proteins, Ptch inhibits the activity of smoothened (Smo), a membrane protein associated with G protein-coupled receptors. Current study suggests that Ptch functions as a pump to remove oxysterols from Smo, resulting in inhibiting Smo activity [59]. Upon Hh binding, the pump function of Ptch is switched off. The accumulation of oxysterols allows Smo to be activated or stay on cell membrane for a longer period of time. Smo released from Ptch inhibition results in activation of Gli family transcription factors. Activated Gli members translocate into nucleus and activate the transcription of Hh target genes (Fig. 2). It has been shown that Hh signaling preserves and increases the short-term repopulating capacity of human HSCs, and deletion of the Hh gene impairs their renewal [60].

In neoplasms, Hh has been implicated in the growth of B cell lymphoma [61] and multiple myeloma [62]. Abnormal Hh signaling has also been associated with CML and AML [63]. In a BCR/ABL1-transduced CML murine model, the Hh pathway genes (such as Gli1 and Ptch) are up-regulated in LSCs compared to normal HSCs. Similar to the findings in the murine disease model, these genes have also been shown to be up-regulated in CD34$^+$ cells from CML patients compared to CD34$^+$ cells from healthy donors [64]. In addition, Hh signaling plays a role in regulating LSCs of CML, because loss of Smo caused the depletion of LSCs [60]. In contrast to CML, Hh signaling is not required for the development of AML induced by MLL/AF9 in a murine model [65]. However, targeting Smo with a small molecule attenuates AML by modulating cell cycle progression and self-renewal of LSCs, and also improves AML therapy through sensitizing dormant LSCs to chemotherapy and overcoming drug resistance derived from BM microenvironment [66].

Alox5 pathway

The arachidonate 5-lipoxygenase (Alox5) pathway represents a unique signaling mechanism that is required specifically for survival regulation of LSCs of CML with a minimal role in normal HSCs. The Alox5 gene encodes an important catalytic enzyme known as 5-lipoxygenase (5-LO) that is responsible for the biosynthesis of leukotriene A4 (LTA4) from arachidonic acid [67, 68]. 5-LO is activated by 5-lipoxygenase-activating protein (FLAP), a nuclear membrane-bound protein that binds to arachidonic acid, for leukotriene synthesis. Alox5 is primarily expressed in polymorphonuclear leukocytes, peripheral blood monocytes, macrophages, and mast cells [67, 69]. Phosphorylation of Alox5 at Ser271 and Ser663 by MAPKAP2 and ERK family kinase increases Alox5 enzymatic activity

in vivo [70, 71]. Alox5 plays a role in numerous physiological and pathological processes, including oxidative stress response, inflammation, and cancer [72]. Alox5 overexpression is detected in some cancer cells and is associated with poor diagnosis [67, 73]. Microarray studies of human CML cells showed that Alox5 was highly expressed in CD34$^+$ CML cells [74], suggesting that Alox5 plays a role in regulating LSCs in CML patients. The essential role of Alox5 in CML LSCs was originally discovered from a study using a murine model of BCR/ABL1-induced CML [75]. In that study, the mRNA level of Alox5 was shown to be up-regulated in LSCs compared to normal HSCs, and the up-regulation was not restored by imatinib, a BCR/ABL kinase inhibitor. Furthermore, BCR/ABL1 failed to induce CML in the absence of Alox5, and inhibition of Alox5 function by an Alox5 inhibitor or in combination with imatinib significantly prolonged the survival of CML mice [75]. On the other hand, Alox5 has also been shown to be highly up-regulated in AML by RUNX1-ETO9a in cooperating with KLF6, although it is still unknown whether Alox5 plays a role in AML-initiating cells [76].

The role of microRNAs

Recent studies have suggested that microRNAs (miRNAs) play roles in leukemogenesis. For example, the dysregulation of miRNAs was found in human AML and CML [77–79]. Han et al. recently reported the role of miR-29a in HSC and LSC of AML [80]. They found that miR-29a was expressed at high levels in all tested AML samples. Furthermore, it has the capability to convert non-self-renewing myeloid progenitors into self-renewing populations. Overexpression of miR-29a in hematopoietic progenitors leads to biased myelopoiesis and a myeloproliferative disorder (MPD), eventually that progresses to AML [80], suggesting that miR-29a can transform AML by converting myeloid progenitors into self-renewal LSCs. The level of miRNA-150 is significantly lower in both CML and AML patient cells than in healthy BM cells [78, 81]. miRNA-150 exerts its effects partially by targeting the transcription factor MYB. MYB plays a role in maintaining self-renewal of hematopoietic progenitor cells, and its expression is decreased in fully mature myeloid cells. During the pathogenesis of AML, lower level of miRNA-150 leads to high level of MYB protein, resulting in acquisition of self-renewal capacity of LSC counterpart progenitors [78, 81]. Several studies have confirmed that a large number of miRNAs exhibit altered expression in AML [77]. To date, only a small fraction has been confirmed as functional mediators of AML or CML development or maintenance. Well understanding the mechanisms of microRNAs in

hematopoiesis and leukemogenesis will help in establishing the strategies for therapies of hematopoietic system diseases.

Microenvironment and LSCs

The extrinsic components mediated by bone marrow microenvironment play critical roles in leukemogenesis, LSC survival, and drug resistance [2]. Bone marrow homing is an important biological process for HSC function, and is governed by a cascade of molecular interactions. First, migrating cells are captured from the fluid stream onto BM tissue endothelium, during which the most effective mediators are the selectins, including E-selectin (CD62E, expressed on endothelium), P-selectin (CD62P, expressed on platelets and on endothelium) and L-selectin (CD62L, expressed on leukocytes and hematopoietic stem cells). Several other molecules including CD44 and some integrins [e.g. LPAM-1 ($\alpha_4\beta_7$)] are also involved by mediating the tethering and rolling adhesive interactions [82]. After intimately contacting with the endothelium, cells are exposed to chemical signals (principally chemokines, but also cytokines and other inflammatory agents) present in the local milieu, resulting in the activation-dependent up-regulation of integrin adhesiveness that leads to firm adhesion, followed by endothelial transmigration. The major mediators of firm adherence are the integrins, very-late activation protein 4 (VLA-4), and lymphocyte function-associated antigen 1 (LFA-1). Finally, HSCs home and locate to BM niches.

Besides regulating HSC functions, BM niche may similarly regulate LSCs. In fact, studies from different research groups have demonstrated that both the osteoblastic and vascular niches are critical for LSC survival, proliferation, and differentiation [2, 83–86]. By comparing the homing of the human leukemia cells with normal cord blood CD34$^+$ cells, the transplanted leukemia and cord blood cells initially localized on the surface of osteoblasts in the epiphysial region and then expanded to the inner vascular and diaphysial regions [87]. Furthermore, after transplantation, leukemia cells initially migrated toward the CXCL12-positive vascular niches in the BM, which is similar to the homing mechanism for normal HSCs [88, 89]. CXCR4 level is significantly elevated in leukemic cells from patients with AML [90], and CXCR4 expression is associated with poor outcome [91, 92]. The high level CXCR4 enhances LSC homing to BM microenvironment by interacting with its ligand, CXCL12 (expressed on the surface of BMSCs). Blocking CXCR4 with antibody can dramatically decrease the engraftment of primary AML cells into NOD/SCID mice, but did not significantly affect engraftment of normal human progenitor cells [93]. Except

for providing a leukemic niche for LSC survival and leukemic hematopoiesis, BM MSCs also mediate LSC resistance to targeted therapy. For example, it has been shown that BM MSCs render CML LSCs apoptosis triggered by TKI treatment through N-Cadherin and Wnt/β-catenin signaling [94]. In addition, inhibition of CXCR4 with its antagonist, such as Plerixafor, can enhance the efficacy of other drugs anti-leukemic effects [95, 96].

CD44 has been shown to play a critical role in conducting LSCs homing to BM niches and maintaining a primitive state [97]. As shown in a CML murine model, CD44 deficiency reduced homing of donor cells to recipient BM, resulting in decreasing LSC engraftment and impairing the induction of CML by *BCR/ABL1*. This defect was rescued by intra-femoral injection of or co-expression of human CD44 into the CD44 null donor cells [98]. These studies indicate that CD44 is required for LSC homing, suggesting that BM microenvironment plays a critical role in regulating self-renewal and survival of LSCs. Integrins are also required for LSC homing to the BM niches. AML cells migrate to BM microenvironment through interaction between VLA-4 on leukemic cells and fibronectin on MSCs [99], and integrin ligation triggers activation of β-integrins that promote LSC survival, which is associated with an increased phosphorylation of Akt in a PI3K-dependent manner [100].

Genetic changes in BM microenvironment play a role in leukemic transformation in some situations. For example, when phosphatase and tensin homolog (Pten) is deficient in both donor hematopoietic cells and recipient microenvironment, myeloproliferation and progression to leukemia/lymphoma occur. In contrast, when Pten-deficient hematopoietic cells are transplanted into a wild-type BM microenvironment, recipient mice remained leukemia-free [101]. In addition, dysfunction of retinoblastoma (Rb) protein or retinoic acid receptor gamma (RARγ) in the BM microenvironment contributed to development of pre-leukemic myeloproliferative disease [102, 103]. Mechanistically, BM microenvironment with Rb or RARγ deficiency promoted the expansion of HSCs and progenitors, which may be resulted from loss of inhibitory signals provided normally by the osteoblastic niche [102, 103]. Furthermore, osteoblast-specific deletion of Dicer1, a gene required for RNA and microRNA processing, caused cytopenia, multilineage dysplasia, and increased apoptosis of primitive hematopoietic progenitors in mice [104], phenotypically resembling myelodysplastic syndromes (MDS). Importantly, in some animals, the dyspoietic changes lead to the development of clonal myeloid sarcomas and AML, suggesting that perturbations of niche signaling selects for the acquisition of secondary genetic changes in the neighboring HSCs [104].

Together, these findings underscore the importance of interactions between LSCs and BM microenvironment in

functional regulation of LSCs, and emphasize the presence of additional genetic mutations within the BM microenvironment.

Targeting LSCs

The findings outlined above suggest the following strategies for eradicating LSCs: (1) inhibition of signaling in LSC survival pathways; (2) inhibition of LSC self-renewal; (3) targeting of extrinsic components in microenvironment.

In the case of *BCR/ABL1*-induced CML, BCR/ABL kinase itself serves as a rational target for treating CML patients. The first BCR/ABL tyrosine kinase inhibitor (TKI) is imatinib mesylate that specifically inhibits BCR/ABL kinase activity by binding close to the ATP binding site in the BCR/ABL1 kinase domain. Later on, several other notable TKIs, such as dasatinib, nilotinib, bosutinib, and ponatinib, have been developed to treat CML with imatinib resistance. However, it has been generally recognized that a TKI alone will not completely eradiate LSCs to cure CML. We have shown that LSCs persisted in CML mice after the treatment with imatinib or dasatinib [9], suggesting that combined therapy with a TKI and another LSC-targeting agent is a legitimate strategy for treating CML. Several molecules are potential anti-LSC targets, including Alox5, Wnt/β-Catenin pathway genes, Hh, Hox family genes, etc. Specifically, selective Alox5 inhibitors have been shown to reduce proliferation and induce apoptosis of CML cells both in vitro and in vivo [75, 105]. It is to emphasize that Alox5 pathway is specifically required for the survival of CML LSC, and an Alox5 inhibitor, an anti-inflammatory drug called Zileuton (Zyflo) is currently being tested in a clinical phase I trial in combination with TKI for testing an inhibitory effect on LSCs in CML. In addition, the Wnt/β-catenin pathway is required for self-renewal of LSCs [30, 31, 58], and inhibition of Wnt signaling reduced survival of LSCs in AML and tumor cell viability in lymphoma and myeloma cell lines in vitro [106]. Furthermore, inhibition of Wnt signaling resulted in a decreased tumor growth and prolonged overall survival [106]. It should be mentioned that the inhibition of the Wnt/β-catenin signaling could be "risky" because the signaling also plays critical role in tissue homeostasis [107].

As described above, Hox family genes play critical roles in the survival regulation of LSCs, serving as potential targets for leukemia therapy. On the other hand, some studies have demonstrated that several genes (such as Notch, Wnt, and rearranged MLLs), which play important roles in leukemogenesis and LSC survival regulation, function upstream of Hox genes. Targeting these genes or pathways will decrease Hox gene expression and affect

LSC survival [108]. In addition, MLL-rearrangements enhance the expression of Hox genes through DOT1, suggesting a strategy for suppressing Hox gene expression in LSCs by targeting ODT1.

Hh pathway is required for the maintenance of normal HSCs and LSCs in CML. Although, inhibition of Hh signaling can be achieved by direct binding of cyclopamine or vismodegib to Smo. Itraconazole (Sporanox) targets Smo through a mechanism distinct from cyclopamine and vismodegib [109]. Itraconazole inhibits mutant forms of Smo, which are resistant to vismodegib and other cyclopamine-competitive antagonists [110]. Inhibition of Ptch and Gli3 (5E1) with antibodies also leads to inhibition of the pathway [111, 112]. In addition, arsenic trioxide (Trisenox) inhibits Hh signaling by interfering with Gli transcription and protein function [113, 114] (Fig. 3).

Although LSCs and normal HSCs share some common signal pathways, LSCs appear to also utilize some unique pathways for regulation of their survival and proliferation. For example, the NF-κB pathway is continuously activated in LSCs and plays a critical role in survival regulation of LSCs, as inactivation of NF-κB with chemical compounds eradiated LSCs [115]. In addition, constitutive activation of Akt is also commonly observed in AML, and inhibition of Akt activity by using PI3K inhibitors, such as wortmanin or LY294002, impaired LSC survival [116, 117]. Furthermore, some anti-apoptotic proteins, such as Bcl-xl and Bcl-2, are highly expressed in leukemic progenitor cells, and inhibition of the Bcl-2 family members with BH3-mimetic ABT preferentially inhibited survival of LSCs in AML mice [118].

Alternative approaches

Without any doubt, specific targeting of LSCs while sparing normal HSCs is an attractive approach to eradicating LSCs, although there is still a long way to go in this direction. Alternatively, both LSCs and normal HSCs in patients can be ablated and replaced by transplanted donor HSCs. In addition, induced pluripotent stem cells (iPSCs) provide a way of producing functional HSCs using somatic cells from a patient to overcome some shortcomings in HSC transplantation, including limited allogeneic donors, graft versus host disease (GVHD), and host versus graft disease (HVGD). iPSCs have been shown to have the capabilities of self-renewal, large-scale expansion, and differentiation into all three germ layers [119], and it is hopeful that functional HSCs can be generated from iPSCs for human use (Fig. 4). In fact, HSCs derived from iPSCs can be transplanted into patients with genetic hematologic disorders [120]. However, the challenges remain, partially because it is difficult to reproduce the microenvironment

Fig. 3 In the absence of Hh, Ptch inhibits the activation of Smo by removing oxysterol from Smo, resulting in Smo inactivity and degradation. In the presence of high level of Hh ligand binding with Ptch, the pump function of Ptch is turned off allowing oxysterols to accumulate around Smo, leading to Smo accumulation at the plasma membrane. This accumulation of sterols allows SMO to become active or stay on the membrane for a longer period of time. Activated Smo stimulates Hh pathway, ultimately leading to the transcription of Gli target genes, Gli1, Ptch1, Hhip, Bcl2, CyclinD, and Snail
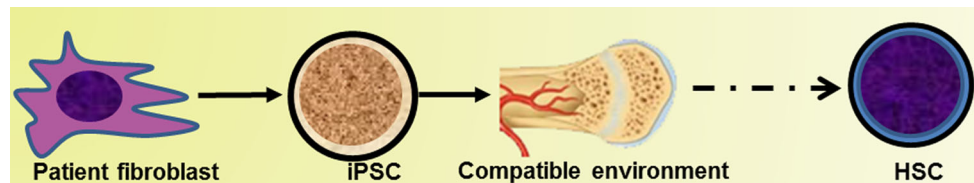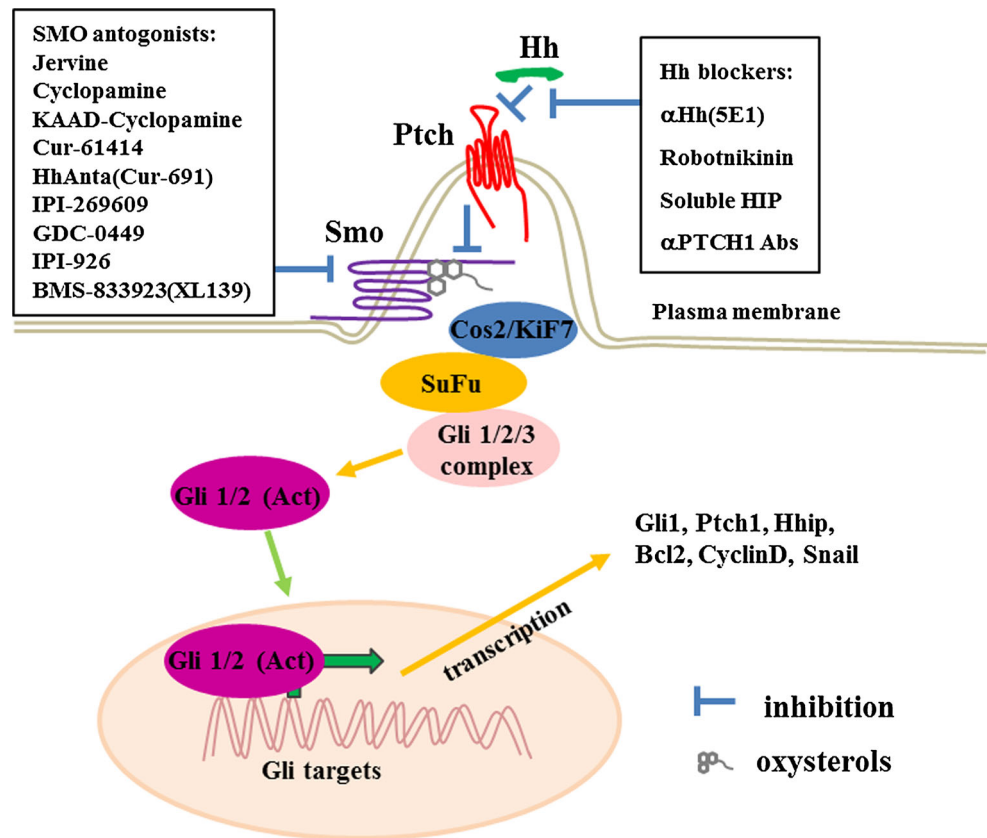
Fig. 4 Schematic illustration of the process to generate functional hematopoietic stem cells (HSCs). Fibroblasts are obtained from patient and induced to iPS with genetic or epigenetic modifications. iPS are guided to fully functional HSCs

necessary for the development of various hematopoietic cell lineages during embryogenesis [120]. Also, recapitulation of an adult HSC niche in vitro has also been complicated by the fact that not all cell types in BM niches are known [120]. Nevertheless, we are closer to understanding the biology of LSCs, and have begun to develop effective strategies for targeting them.

Compliance with ethical standards

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