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Advances in Acute Myeloid Leukemia Stem Cells

Xiaoxiao Yang, Xuewen Xu, Yanfang Liu, Aihua Gong,
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Abstract

As a common hematological malignant tumor, acute leukemia is believed to originate from a subpopulation of special cancer cells, named cancer stem cells. Cancer stem cells are recognized to be the main source of tumor origin, multidrug resistance, metastasis, and recurrence. Leukemic stem cells (LSCs) were first identified and confirmed to play an important role in the occurrence and development of leukemia. In this article, we summarize the following content: special markers and sorting methods for acute myeloid leukemia stem cells and the role of cancer stem cells in treatment resistance, metastasis and invasion, recurrence, and target treatment of acute leukemia.

Keywords: acute myeloid leukemia, cancer stem cells, leukemic stem cells, treatment resistance, metastasis

1. Introduction

Acute myeloid leukemia (AML) is a group of heterogeneous diseases characterized by the uncontrolled proliferation of myeloid precursor cells and the replacement of normal hematopoiesis in the bone marrow. According to the latest survey, AML is a common cancer in adults and the second most common leukemia in children, with relatively higher rates observed in countries with high Human Development Index in North America, Oceania, and Europe [1]. The annual incidence rate of AML in the world is 2.25/100,000, and the incidence increases with age. The number is below 1/100,000 for people under 30 years of age and 17/100,000 for those above 75 years of age. Therefore, AML is actually a middle-aged and elderly disease, accounting for 80–90% of adult acute leukemia, but only accounts for 15–20% of children leukemia. Men have a higher incidence of AML than women, especially in North America, Oceania, Europe, and Asia. Epidemiology shows that environmental, occupational, and genetic factors are closely related to the pathogenesis of AML. Genetic changes in tumor cloning lead to a cascade of reactivity at the molecular level that cause abnormal proliferation and differentiation of malignant cells and inhibit normal hematopoiesis.

Tumorigenesis has been long known to resemble organogenesis and is a heterogeneous process involving many phenotypically and functionally different cells. The cancer stem cell (CSC) concept was first reported more than a century ago and refers to a very small subset of relatively quiescent cells in the tumor that are endowed with the ability to self-renew and differentiate into non-stem daughter cells that make the bulk of tumor [2]. Leukemic stem cells (LSCs) were first

identified and confirmed to play an important role in the occurrence and development of leukemia. In 1994, Lapidot et al. reported that AML contains LSC. It is believed that only 0.1–1% cells have the ability to produce AML [3]. The researchers transplanted sublethal doses of CD34$^+$CD38$^+$ and CD34$^+$CD38$^-$ subpopulations isolated from the bone marrow of a patient with AML into non-obese diabetic mice with severe combined immunodeficiency disease (NOD/SCID mice). After 4–8 weeks, human AML cells isolated from the engrafted murine bone marrow expression both of CD34$^+$CD38$^-$. The recipient mouse, re-implanted with CD34$^+$CD38$^-$ cells, could survive and pass to the next generation. The researchers also found CD34$^+$CD38$^-$ cells can induce various subtypes of leukemia other than M3, thus indicating that this subpopulation of cells has stem cell-like strong self-renewal and reproductive ability. In 1997, Bonnet et al. confirmed the presence of LSC in NOD/SCID mice [4]. Inoculation of 10^6 LSCs resulted in the formation of human AML in animals; this finding suggested that the "source of all evils" is LSC (**Figure 1**) [5]. Since then, the existence of LSC has been recognized, which is a significant milestone. The presence of LSC has been confirmed not only in hematological malignancies but also in some solid tumors.

Although LCSs were identified and thought to be the main cause of leukemia origin, recurrence, and drug resistance, there is still controversy regarding the origin of this distinct population [6]. Several hypotheses have been proposed with regard to the origin of LCS: (1) from hematopoietic stem cells (HSCs) [7]; (2) from partially differentiated hematopoietic progenitor cells [8]; (3) from blood vascular stem cells and granulocyte macrophage precursors (GMPs) [9–11]; and (4) from relatively mature leukemia cells [12]. Although the number of LSCs is small, LSCs have the same potential for self-renewal, multidirectional differentiation, and unlimited proliferation, resistance to cell death, multidrug resistance, metastasis, and recurrence. Because they can escape inhibition by most chemotherapeutic
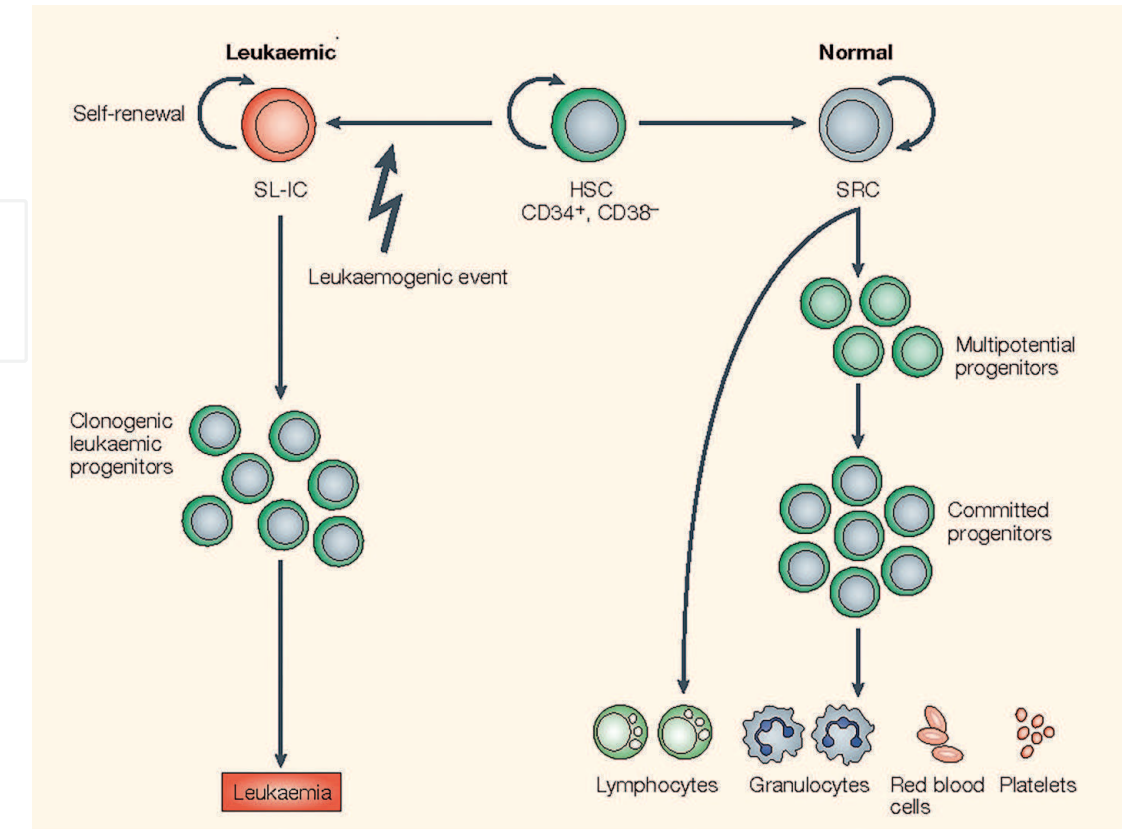

Figure 1.
Comparison of the normal and AML human hematopoietic systems.

drugs, LSCs in a relatively quiescent state can be latent for a long time. Once the conditions are appropriate, such as a certain stimulus into the cell cycle, they can escape the immune surveillance of the body, thus showing unlimited proliferation. Therefore, relevant research and analysis on the biological characteristics of LSCs may provide new ideas for therapeutic regimens. The discovery of LSCs has broadened the treatment of leukemia, and targeted therapy of related signaling pathways and niche may become a new research hotspot.

2. Expression of special surface markers in LSCs

Bonnet et al. revealed that the CD34$^+$CD38$^-$ subpopulation is similar to normal HSCs with surface markers and can be used to identify cells with unlimited proliferation and differentiation in AML [4]. Subsequent studies have shown that the surface markers of LSCs are extremely complex and vary from person to person. Previous experiments have demonstrated that in some cases, subpopulations of cells with different phenotypes have LSC activity [13–15]. CD34 and CD38 are no longer specific markers that define LSCs. Recent studies have identified various new markers such as CLL-1, CD96, TIM3, CD47, CD32, and CD25. The current study summarizes some of the specific markers expressed by LSCs (**Table 1**) [16] and has been utilized to successfully validate LSCs in recent clinical trials [17].

2.1 CLL-1

C-type lectin domain family 12 member A (CLL1, also known as CLEC12A)-positive cells show high tumorigenicity in immunodeficient mice, indicating that this cell subpopulation has the characteristics of LSCs. Moreover, the side population cells enriched in LSCs isolated by flow cytometry from patients with AML also express CLL-1 [18]. Jiang et al. have reported that CLL1-antibody-drug conjugate (CLL1-ADC) could become an attractive target therapy for AML [19]. The use of a

Marker	Expression on LSC	Reference
CD34	+/−	[4, 13]
CD38	+/−	[4, 12, 13]
CD90	−/+	[16]
CD123	++	[16, 22]
CD45RA	+	[15]
CD33	++	[90, 91]
CD13	++	[15]
CD44	++	[15]
CLL-1	+	[17, 18]
CD96	++	[16, 19]
TIM3	++	[15]
CD47	++	[23–26]
CD32	+	[27–30]
CD25	+	[27–31]

Table 1.
Summary of cell surface marker expression on AML LSCs.

DNA-binding payload in CLL1-ADC is critical because such a payload affords the ADC the ability to kill both proliferative and quiescent cells, thus making CLL1-ADC a very compelling candidate for the treatment of patients with AML.

2.2 CD96

CD96 is a member of the immunoglobulin superfamily, a transmembrane glycoprotein, and a T-cell surface-specific receptor 6. By using blood samples from 55 patients with AML, Du et al. found that CD96 (>10%)-enriched patients showed a poor response to chemotherapy [20]. Of note, CD96 was proved to be an efficient identical marker of LSCs in CD34$^+$CD38$^-$ groups.

2.3 CD44

CD44 is a surface glycoprotein and a receptor for hyaluronic acid, which is mainly involved in cell-cell interaction, adhesion, and migration [16]. CD44$^+$ cancer cells show higher sphere-forming ability and treatment resistance. CD44 is not only a special marker of LSC, but it is also a key regulator of LSC function that is essential for homing of LSCs to microenvironmental niches and for maintaining LSCs in a primitive state.

2.4 CD123 (IL3Rα)

Approximately 45% of AML cells that overexpress CD123 have higher proliferative activity and are more tolerant to apoptotic stimulation. Clinical studies have also demonstrated patients overexpressing CD123 usually have a poor prognosis. Williams et al. found that NK-92 preferentially inhibits leukemic stem cells compared with bulk leukemia cells [21]. NK-92 combined with the anti-CD123 antibody, 7G3, enhanced survival in a primary AML xenograft model when compared with control arms. Some other IL3R antibodies (DT388IL3, CSL362, and MGD006) can significantly prolong the survival rate of patients with AML [22, 23].

2.5 CD47

CD47 is a transmembrane glycoprotein that is widely expressed in human tissues. CD47 also functions as a marker of "self" on host cells within an organism. When expressed, CD47 binds to SIRPα on the surface of circulating immune cells to deliver an inhibitory "don't eat me" signal [24]. Higher expression of CD47 has been demonstrated in LSCs [25, 26]. Anti-CD47 antibody treatment has also been shown to act synergistically with cytarabine (Ara-C) chemotherapy in a model of AML. While Ara-C effectively eliminated TSP-1 cancer cells in the proliferative phase, anti-CD47 antibodies were putatively able to target quiescent LSCs that were not susceptible to Ara-C treatment but highly expressed CD47 [27].

2.6 CD25 and CD32

Saito et al. conducted microarray analysis and found that CD25 and CD35 were expressed on quiescent LSCs, but not on HSCs [28]. The activation of CD25, namely IL2Rα, can control cell proliferation, survival, and differentiation. CD32 is a member of the Fc-gamma receptor family and is mainly found on immune cells. Transplantation of CD34$^+$CD38$^-$CD25$^+$ cells and CD34$^+$CD38$^-$CD32$^+$ cells into NO/SCID mice can trigger leukemia and resistance to cytarabine. It has been reported that overexpression of CD25 in AML cells may be caused by the activation of STAT5

and MYC [29, 30]. Gönen et al. analyzed the correlation between the expression of CD25 (IL-2 receptor alpha) and prognosis in 657 patients with primary AML (≤60 years old); they concluded that CD25 can be used as a biomarker for poor prognosis of AML [31]. Cerny et al. also indicated that CD25 expression can be used as an indicator to predict early treatment failure in AML [32].

3. LSCs are the source of treatment resistance

The most fundamental reason for the relapse of AML is the existence of LSCs. It is necessary to investigate the key mechanisms of resistance of LSCs to the current treatment strategy for effective clearance of LSC.

3.1 LSCs are mostly in the G0 quiescent phase

Dean et al. showed that 96% of LSCs are in the G0 phase of the cell cycle [33]. Chemotherapeutic drugs acting on the cell cycle or on rapidly differentiating cells can inhibit only differentiated mature leukemia cells, while LSCs in the G0 phase cannot be completely inhibited because they do not divide. Once they are properly stimulated to re-enter the cell cycle, they will continue to proliferate and differentiate into daughter leukemia cells, thus causing recurrence. According to some studies, LSCs are much less sensitive to daunorubicin and cytarabine than differentiated leukemia cells.

3.2 LSCs highly express multidrug resistance genes and proteins

The expression of multidrug resistance genes on the surface of LSCs can induce the production of various membrane transporters that can pump a variety of chemotherapeutic drugs out of the cell, which results in lowering the concentration of the drug in the cancer cells. The ABC membrane transporter plays a pivotal role in this drug efflux process. The ABC transporter, namely the ATP-binding cassette transporter, has an ATP-dependent drug-release function [34]. The most representative multidrug resistance genes are ABCB1, ABCC1, and ABCG2, which encode P-glycoproteins (P-gp, P-170, and MDR1), multidrug resistance protein (MRP), and breast cancer resistance protein (BCRP), respectively. BCRP is preferentially expressed in $CD34^+CD38^-$ LSCs. The intracellular drug concentration after BCRP inhibition is increased, but it is much lower than that of cells expressing only BCRP. Therefore, it is indicated that the drug resistance of LSC is related to the interaction of multiple drug resistance proteins. Some other reports have revealed that LSC has higher MDR1, MRP, BCRP, and lung resistance related protein (LRP) expression relative to HSC, thus giving LSC a stronger drug resistance advantage. The high expression of multidrug resistance gene in LSCs is the main mechanism by which LSCs exhibit primary resistance to chemotherapeutic drugs [35, 36].

3.3 LSC display higher self-renewal ability

Hope et al. proved that LSCs have self-renewal ability, which is one of the most prominent features of CSCs [37]. The self-renewal ability of LSCs may be one of the key factors that promote the development and metastasis of leukemia, and the molecular regulation mechanism is very complicated. Bmi-1 is a member of the PcG (polycomb group) transcriptional repressor family and is an essential factor in maintaining HSC self-renewal. Raffel et al. showed that miR-126 overexpression renders AML cells more resistant to standard chemotherapy and that treatment of primary AML cells results in the enrichment of LSC-like cells with increased

miR-126 levels [38]. Moreover, leukemic cells with high miR-126 expression were selected in refractory patients after induction chemotherapy, thus correlating high miR-126 levels to LSCs and therapy resistance. miR-126 knockdown leads to the expansion of HSCs but impaired maintenance of LSCs, and its overexpression promotes LSC self-renewal, which is inhibited in HSCs [39, 40]. In addition, all genes and signaling pathways that contribute to HSC self-renewal may be involved in LSCs, such as Wnt, Notch, HOX, and Shh. Recent studies have revealed that the activation of the Shh signaling pathway in LSCs by upregulation of SMO is essential for LSC survival maintenance [41, 42].

3.4 The special microenvironment (niche)

The receptors CXCR4 on the LSC membrane and CXCL12 in the bone marrow microenvironment are required for LSC to maintain dormancy, self-renewal, differentiation, growth, and homing. However, targeted therapy for the niche will enhance the expression of the drug pump MDR1, which induced LSC insensitive to therapy and failed to achieve the goal of reversing its resistance [43].

3.5 Multiple signaling pathway abnormalities

Recent studies have demonstrated that abnormal activation of multiple signaling pathways is one of the key mechanisms of LSC multidrug resistance, such as Sonic Hedgehog, Bmi-1, Nocth, and WNT. Among these pathways, the abnormality of Hedgehog (Hh) pathway is closely related to CSC resistance, such as increased endogenous synthesis of ligand protein Hh, loss of PCTH activity, inhibition of smoothened (SMO) signaling protein, mutation of SUFU, and overexpression of the transcription factor GLI1, thus regulating the downstream target gene and participating in the maintenance of stem cell proliferation, which are related to multiple hallmarks of tumor cell resistance [44, 45]. Studies have revealed that Hh signaling is abnormally activated in LSCs, GLI1 can induce endogenous BCL-2 expression, and the Hh pathway also up-regulates BCL-2 by activating PI3K/AKT, thus leading to apoptotic disorder and drug resistance of LSCs.

4. The role of LSCs in tumor metastasis and invasion

CSCs are thought to be the seed of tumor metastasis. CSCs that particularly express C-X-C chemokine receptor type 4 (CXCR4) preferentially disseminate [46]. The specific ligand for the CXCR4 chemokine receptor is termed matrix-derived factor-1 (SDF-1, also known as CXCL12). Both CXCR4 and SDF-1 are expressed in various tissues and cell types and regulate cell migration [47]. The SDF-1/CXCR4 axis is also involved in the migration of CSCs [48]. SDF-1 is a homeostatic chemokine secreted by stromal cells and is released into the interstitial space [49]. On the one hand, SDF-1 exerts effects through its unique physiologic cognate receptor CXCR4, which is known to mediate chemotaxis, hematopoiesis, angiogenesis, and tumor spread and metastasis. On the other hand, it also acts in a paracrine fashion on cells in the local microenvironment to stimulate directional migration of hematopoietic and nonhematopoietic normal and malignant cells [50–52]. Li et al. found that HERG K^+ channels were widely expressed in primary leukemic cells but not in normal lymphocytes [53, 54]. Blocking HERG K^+ channels by applying its specific inhibitor in hematopoietic cell lines and primary leukemic cells significantly reduced the migration of leukemic cells induced by SDF-1; this indicated a role for HERG K^+ channels in the progression of leukemia.

Currently, there is a lack of direct evidence linking LSCs to metastasis. There are some sporadic reports that LSCs may play a role in metastasis. In patients with AML, low levels of CXCR4 expression have been shown to be associated with better prognosis, longer recurrence-free period, and overall survival. It has also been suggested that CXCR4 is an independent prognostic predictor of disease recurrence and survival [55]. Another study has shown that overexpression of C-myc, Bmi-1, Oct4, and Nanog in precancerous and cancerous cells may initiate oncogenic epithelial-mesenchymal transition and tumorigenesis, which plays important roles in the genesis of CSCs, malignant tumor initiation and progression, cancer metastasis, and drug resistance [56]. Compared with the parental cells, chemotherapy-resistant MOLT4$^+$ cells expressed much higher levels of the stem cell surface marker CXCR4. It was found that the expression of CXCR4, related to tumor cell homing and migration, was significantly higher in MOLT4$^+$ cells than in MOLT4$^-$ cells. In addition, hMDSCs-MOLT4 cells seem to have a strong invasive potential in vivo, as demonstrated by strong interstitial and vascular tissues in tumor tissue sections.

It was confirmed that the niche was involved in metastasis. With respect to HSCs, two distinct niches have been defined: the osteoblastic niche and the vascular niche [57–59]. Tabe et al. hypothesized the presence of a "metastatic niche" that facilitates the survival, proliferation, and metastasis of LSCs [60]. Yang et al. demonstrated that vascular endothelial growth factor receptor 1 (VEGFR1) was involved in the initiation of a premetastatic niche and that cells expressing VEGFR1 home to tumor-specific premetastatic sites and form cellular clusters before the arrival of tumor cell clusters [61]. They can alter the local microenvironment and lead to the activation of integrins and chemokines. After treatment with anti-VEGFR1 antibodies, the supportive premetastatic cell clusters were abolished and metastasis was prevented, which indicated the importance of a metastatic niche.

5. The role of LSCs in tumor proliferation and anti-apoptosis

Various signaling pathways that stimulate proliferation or inhibit apoptosis are known to aberrant activate LSCs.

5.1 Hedgehog pathway

The Hh pathway is a highly conserved pathway that regulates the proliferation, migration, and differentiation of cells during development [62, 63]. Three distinct ligands, namely Sonic (Shh), Indian (Ihh), and Desert (Dhh) Hedgehog, exist in humans. Upon ligand binding to the receptor pat (Ptch), inhibition of smoothened (Smo) receptor is relieved. Smo then activates members of the Gli family of zinc-finger transcription factors, translocating them to the nucleus to regulate the transcription of Hh target genes including Gli1, Gli2, and Ptch, and regulators of cell proliferation and survival [64–66].

The Hh pathway promotes cell proliferation mainly by regulating cell cycle. Its regulation mechanism is as follows [67]: (1) Cyclin D1 and cyclin D2 act as downstream target genes for the transcription factor GLI1 and are involved in cell cycle G1 to S phase transformation; (2) PTCH regulates the activity of cyclin B, which is part of the mitosis promoting factor (MPF) compound. MPF is required for cell entry from the G2 phase to the M phase; and (3) SMO proteins block cellular dormancy by modulating P21, a cyclin-dependent inhibitory protein.

The Hh signaling pathway regulates apoptosis mainly through the following mechanisms: (1) Regulate the activity of the BCL-2 family. The BCL-2 family is divided into anti-apoptotic proteins (such as BCL-2, BCL-XL, and MCL-1) and

pro-apoptotic proteins (such as BAX, BAD, and BAK). The ratio between the two types of proteins will directly affect the stability of the mitochondrial membrane and is the most important regulator of the mitochondrial apoptosis pathway. Overexpression of BCL-2, an increase in the ratio of BCL-2 to BAD, leading to defects in mitochondrial apoptosis, is one of the important mechanisms for LSC multidrug resistance and poor prognosis of AML [68]. BCL-2 is the target gene downstream of the Hh pathway, and Hh pathway blockers can induce apoptosis by downregulating BCL-2 [69]. Kobune et al. found that cyclopamine induces apoptosis of drug-resistant CD34$^+$ AML cells by downregulating BCL-2 and makes them sensitive to Ara-C [70]. MCL1 has also emerged as a mechanism of resistance to apoptosis and to BCL-2/BCL-XL inhibitors, and therefore, it is considered as a potential therapeutic target [71]. (2) Regulation of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) mediated apoptosis. TRAIL-R3 is a blank receptor that lacks a functional death region and is highly expressed in CD34$^+$CD38$^-$ LSCs; the downregulation of TRAIL-R3 increases apoptosis [72]. (3) Regulation of FAS protein express in the death receptor pathway. Studies have found that the Hh pathway inhibitor GDC-0449 promotes tumor stem cell apoptosis by upregulating FAS protein [73].

5.2 NF-κB pathway

NF-κB is a significant transcriptional activator located upstream of the IRF-1 gene. It is aberrantly activated by LSCs. NF-κB not only inhibits apoptosis but also regulating the expression of cytokine genes. Furthermore, apoptosis can be inhibited by inducing and upregulating antiapoptotic genes. Therefore, NF-κB plays an essential role in maintaining LSC growth and survival. Inhibition of this signaling pathway not only promotes LSC apoptosis but also enhances the sensitivity of LSCs to chemotherapeutic drugs [74, 75]. At present, the targeted drugs for NF-κB are mainly proteasome inhibitors MG-132 and Bortezomib (VELCADE, PS-341), which can better target LSCs without any significant effect on normal HSC. It was reported that PTL can specifically induce apoptosis of LSCs by inhibiting NF-κB activity. At present, the PTL analog DMAP has been developed, and its experimental effect is remarkable [76, 77].

5.3 PI3K/Akt pathway

The PI3K/Akt pathway is an intracellular pathway that plays a critical role in apoptosis and cancer, whose components are often altered in cancer, leading to dysregulated apoptosis and chemoresistance [78]. Chen et al. demonstrated that the PI3K inhibitor LY294002 can directly target LSCs without adverse reactions to normal HSCs, and they found that PI3K and NF-κB may coexist in the same signaling pathway [79]. Further, it has been reported that the mammalian target of rapamycin (mTOR) is a substrate for PI3K that regulates the survival of LCSs after etoposide treatment. Mise et al. showed that the inhibitory effect of rapamycin on mTOR significantly reduced the survival rate of AML cells, and rapamycin enhanced the effect of etoposide on these cells [80]. It is found that PTEN that negatively regulates the PI3K pathway and is essential for maintaining normal hematopoiesis [81]. However, PTEN deletion has no significant effect on HSC differentiation survival, while PTEN deletion in LSCs can lead to the production and proliferation of LSCs. In addition, rapamycin, an inhibitor of the PI3K pathway downstream regulator of mTOR was found to inhibit LSCs and protect against normal HSC failure.

6. Treatment avenue for LSC

6.1 Niche of LSCs

Niche is involved in stem cell self-renewal, survival, chemotherapy tolerance, and metastasis of leukemia cells [82]. In the mice model, it was found that the homing of HSCs to the bone marrow is regulated by chemokine CXCL12 expressed in mesenchymal stem cells, and its receptor is CXCR4 [83]. Inhibition of CXCL12-CXCR4 interaction helps to reduce chemotaxis, thus affecting the movement, adhesion, and metastasis of LSCs. In vitro studies have shown that the anti-leukemia active peptide CXCR4 inhibitor LY2510924, as a single agent or in combination chemotherapy, can rapidly and permanently destroy the CXCL12-CXCR4 axis, thereby inhibiting the proliferation of AML cells and leading to apoptosis [84]. Fully human IgG4 monoclonal antibody BMS-936564 against CXCR4 showed high safety and antitumor activity in relapsed and refractory patients with AML [85]. However, because of the similar biological properties of LSCs and HSCs, the non-selection of related inhibitors has become another major clinical problem.

In addition to participating in the hematopoietic function, the bone marrow niche is also an important place for the presence of immune cells. There is a group of activated leukemia-specific immune cells in leukemia bone marrow, and regulatory T cell (Treg) is one of the important members [86]. Fujisaki et al. found that hematopoietic stem/progenitor cells and Treg can coexist on the endosteum of murine bone marrow, and HSPC disappears shortly after Treg cell depletion [87]. This experiment successfully demonstrated the involvement of Treg cells in the formation of bone marrow niche. Treg is a dynamic cell population that regulates the immune response. Stem cells evade immune surveillance by recruiting Treg cells and using their regulatory functions [88]. Therefore, it is speculated that these cells will likely become new targets for eliminating LSCs (**Figure** 2) [89].

6.2 LSCs-related signaling pathways

Leukemia is characterized by selective overgrowth of LSCs and interferes with the differentiation of HSCs. Chemotherapy kills rapidly dividing cancer cells, but does not eliminate reservoirs of LSCs that cause relapse. LSCs have a variety of regulatory abnormal signaling pathways, including WNT/β-catenin, JAK/STAT, PI3K/AKT, RAS, NF-κB, and Notch. WNT is involved in the maintenance of properties of LSCs. Riether et al. discovered that tyrosine kinase inhibitors induced CD70 expression on LSCs during targeted drug therapy, while CD70 inhibited WNT/β-catenin signaling pathway [90]. STAT is an important transcription factor regulating cell growth, proliferation, and inhibition of apoptosis. Activation of the JAK/STAT signaling pathway is associated with sustained activation of the proto-oncogene AHI-1 in CD34 cells, regulating CML-LSC autonomous growth in vitro and inducing leukemia [91].

In recent years, studies on micro-RNA and transcription factors in leukemia patients have become increasingly mature. For example, the transcription factor MYC can inhibit the expression of the shared target gene FLT3 by miR-15a-5p, and FLT3 plays a crucial role in activating the STAT5A pathway and promoting tumor cell proliferation [92, 93], but its specific mechanism of influence on the development of tumor remains to be further investigated. Targeted drugs in mounting numbers for LSCs signaling pathways are being developed, but most of them are still in the stage of animal experiments, and more research is needed to determine the safety and efficacy in humans.
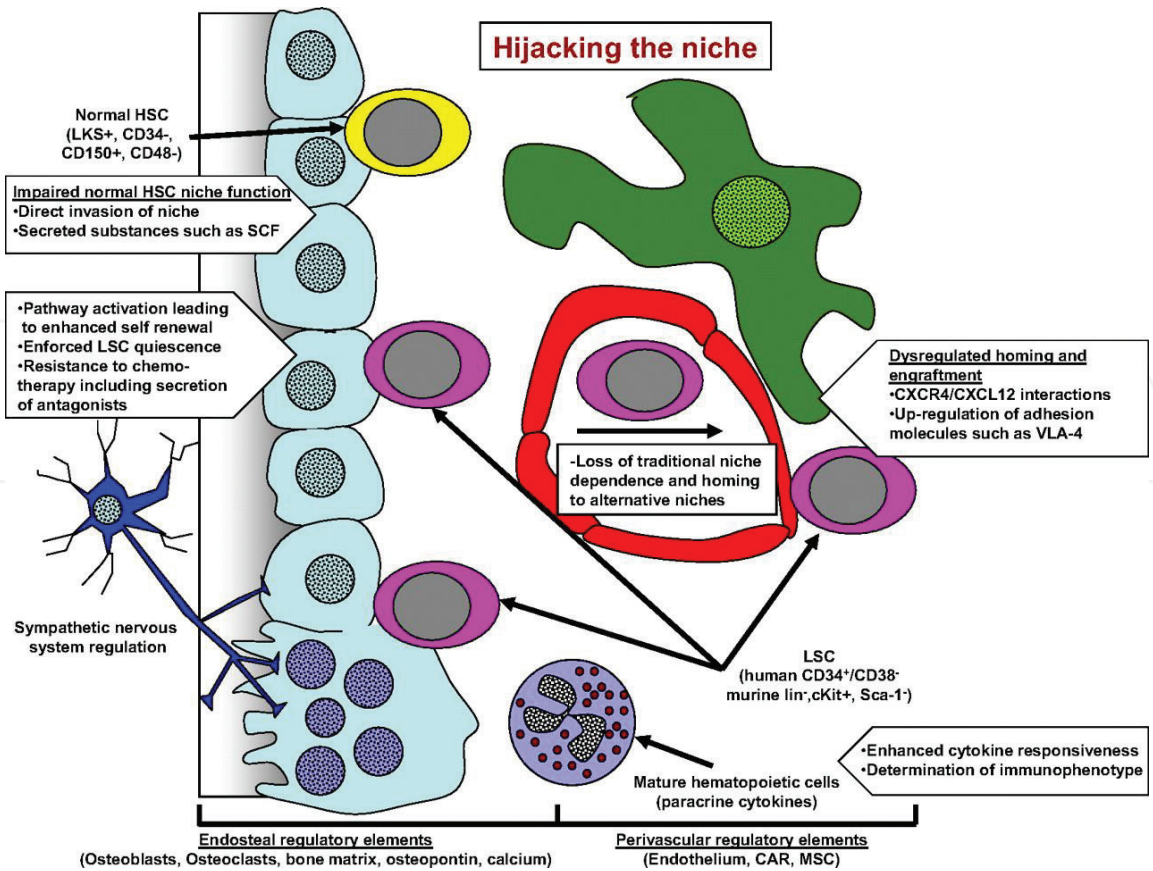
Figure 2.
The niche of leukemic stem cell. The niche provides support for self-renewal, quiescence, homing, engraftment, and proliferative potential for HSCs. LSCs may impair the function of the normal HSC niche. In addition, LSCs can infiltrate these niches and may hijack these normal homeostatic processes.

6.3 Cell cycle of LSCs

In patients with drug resistance, most of their LSCs are in the quiescent phase (G0 phase) and therefore cannot be effectively eliminated by chemotherapy. Hence, some people consider that LSCs in stationary phase can be eliminated by two-step method: (1) Stimulate LSCs from the G0 phase into the cell cycle proliferation and then use specific tumor-targeted therapeutic drugs to eliminate LSCs and (2) Let LSCs stay in the G0 phase. It is worth noting that although the cells in the G0 phase are dormant, they have the ability to proliferate; thus, this can only delay survival and avoid recurrence. Experiments in vitro have shown that cyclin-dependent kinase (CDK6) can be involved in the regulation of cell cycle, and inhibition of CDK6 may cause leukemia stem cells to dormant and inhibit cell proliferation [94].

6.4 Immunophenotype of LSCs

Several immunophenotypes of LSCs have been identified, such as CD34, CD38, CD123, CD117, CD71, CD44, HLA-DR, TIM3, CLL-1, CD96, CD47, CD32 and CD25. Although these surface molecules are not expressed in all LSCs, their high expression may lead to a significant deterioration of the disease prognosis. It is also because of the difference in markers and functions between LSCs and HSCs that targeted therapy for leukemia stem cells is possible. CD33 is the first AML targeted therapeutic antigen approved by the US FDA, which is highly expressed in AML but not in normal HSCs. The monoclonal anti-tumor drug Gemtuzumab ozogamicin, consisting of the CD33 antibody, hP67.6, and the cytotoxic drug, is

a good candidate for selective killing of CD33⁺ LSCs [95]. In addition, in recent years, tumor-specific chimeric antigen receptor T-cell immunotherapy (CART) against CD33⁺ cells has become increasingly popular [96]. Busfield et al. detected that the anti-CD123 monoclonal antibody CSL362 has a good tumor cell killing effect in the AML mouse model [22].

Although the current monoclonal antibodies against the LSCs phenotype have achieved initial clinical success, it is undeniable that LSCs are diverse among different patients, and even in the same individual, the phenotypic differences are quite different. This brings new challenges to clinical treatment. Moreover, studies have shown that in patients with newly diagnosed AML, the distribution of LSCs is uniform and the number is small, but once the patient relapses after chemotherapy, the number of LSCs can be significantly increased, and some new phenotypes appear [97]. The phenotypic changes in LSCs at different stages of the same patient's disease also lead to difficulties in clinical application of this targeted LSC immunophenotypic treatment strategy. Therefore, the current targeted therapy based on this strategy is still in the exploration stage, and the development of related drugs is significantly limited due to the plasticity of the immunophenotype of LSCs.

7. Summary

LSCs play an important role in the origin, recurrence, and drug resistance of leukemia. Although the current research on LSCs has made some progress, the biological characteristics of LSCs and its mechanism in the pathogenesis of leukemia remain unclear, and the treatment strategy for targeted clearance of LSCs is still in its infancy. Therefore, clarifying its biological characteristics and developing drugs for targeted therapy of LSCs is an important direction for leukemia research in future.

Acknowledgements

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