

# The biomarkers of leukemia stem cells in acute myeloid leukemia

Yahui Ding<sup>1</sup>, Huier Gao<sup>2</sup>, Quan Zhang<sup>1</sup>

<sup>1</sup>State Key Laboratory of Medicinal Chemical Biology, College of Pharmacy and Tianjin Key Laboratory of Molecular Drug Research, Nankai University, Haihe Education Park, 38 Tongyan Road, Tianjin 300353, China; <sup>2</sup>State Key Laboratory of Experimental Hematology, Institute of Hematology and Hospital of Blood Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin 300020, China

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**Correspondence to:** Quan Zhang, State Key Laboratory of Medicinal Chemical Biology, College of Pharmacy and Tianjin Key Laboratory of Molecular Drug Research, Nankai University, Haihe Education Park, 38 Tongyan Road, Tianjin 300353, China. Email: zhangquan612@163.com.

**Abstract:** Acute myeloid leukemia (AML) is a heterogeneous disease characterized by morphology and chromosome aberrations with high mortality. Leukemia stem cells (LSCs) in AML played important roles in leukemia initiation, progression, and were considered to be the root of chemotherapeutic drug resistance and disease relapse. The identification and targeting LSCs depended on membrane markers like CD34, CD38, CD123, TIM3, CD25, CD32 and CD96. In addition, the transcription factors were also therapeutic targets in eradicating LSCs, such as histone deacetylases (HDACs), NF- $\kappa$ B, HIF-1 $\alpha$  and  $\beta$ -catenin. Besides membrane markers and transcription factors, intracellular reactive oxygen species (ROS), telomerase and microRNAs were identified to be new targets for ablating LSCs in AML.

**Keywords:** Leukemia stem cells (LSCs); biomarker; acute myeloid leukemia (AML); membrane marker; transcription factors

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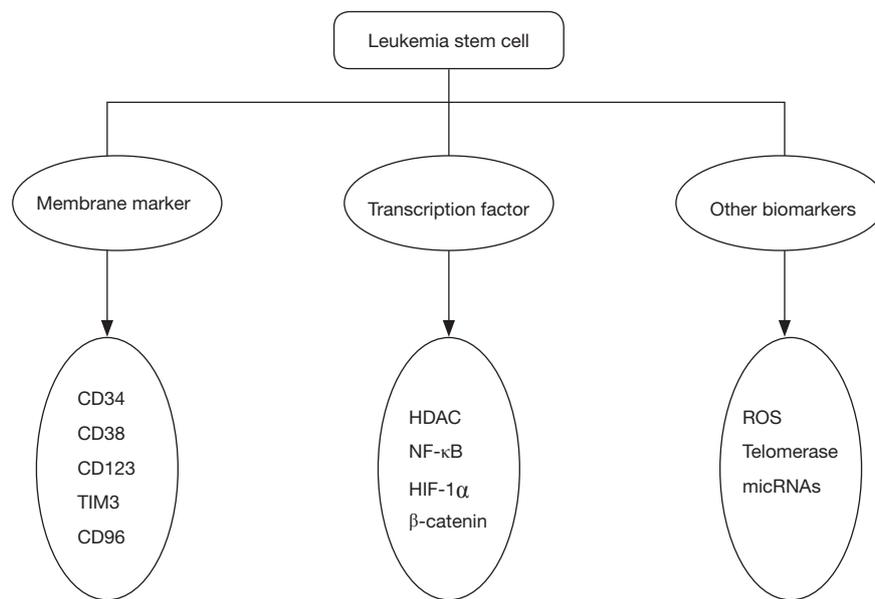
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Acute myeloid leukemia (AML), an aggressive malignant disease of hematopoietic system and the most common type of acute leukemia, remains high mortality. AML characterized with the accumulation of granulocytopenia in the bone marrow (1). The most commonly used therapy was chemotherapy and stem cell transplantation. However, the majority of the patients died of AML relapse (2,3). DNA alkylators, topoisomerase inhibitors, antibiotics, steroids were approved by FDA for treating AML patients recent years (4). However, AML had a very poor prognosis especially to elder patients (5). More and more evidence has suggested that the small population of leukemia initiating cells or leukemia stem cells (LSCs) is supposed to be the major reason of leukemia initiation, progression, chemotherapeutic drugs resistance and disease relapse (6,7). The mechanisms for LSCs leading to AML relapse were required to be identified (8-10). The targeting of LSCs was considered to be a potential strategy to improve the

long-term survival of AML patients (11). Therefore, the biological features for identification of LSCs were important for the drug discovery, targeting therapy and contributed to a better understanding of the molecular mechanism of disease (12,13). Meanwhile, the identification of LSCs in AML is especially significant in disease diagnosis, prognosis, monitoring and drug screening of AML. The identification and targeting of LSCs were depending on the membrane markers, the transcription factor and other specific mechanisms to selectively eliminate LSCs while sparing normal hematopoietic stem cells (HSCs) (*Figure 1*).

## Membrane markers of LSCs in AML

Bonnet and Dick in 1997 reported that the population of cells characterized by the phenotype of CD34<sup>+</sup>CD38<sup>-</sup> was able to reconstitute human AML in NOD/SCID mice, which was the first report of LSCs (14,15). This



**Figure 1** Different biomarkers of leukemia stem cell.

work identified that the subpopulation with the surface antigens CD34<sup>+</sup>CD38<sup>-</sup> could be regarded as the specific feature of LSCs in AML. Hematopoietic tissues of AML patients included both LSCs and residual normal HSCs. However, normal HSC shared the same surface markers of CD34<sup>+</sup>CD38<sup>-</sup>. Therefore, the identification of LSCs from normal HSCs was important for scientific research and clinical investigation. As reported, the antigen expression level of CD123, interleukin-3 receptor alpha chain, was negatively related to the outcome of chemotherapy and prognosis in AML patients (16). Meanwhile, CD123 has been reported to prominently express on CD34<sup>+</sup>CD38<sup>-</sup> cells in leukemia while not normal CD34<sup>+</sup>CD38<sup>-</sup> hematopoietic cells (17,18). Therefore, CD123 is an important marker for the identification and targeting of LSCs (19). TIM3 (T-cell Ig mucin3), a negative regulator of Th1-T-cell immunity (20,21), was found to be an important marker used for LSCs and HSCs discrimination (22). Jan reported that TIM3 was highly expressed on the surface of multiple specimens of LSCs, not on normal bone marrow HSCs (23). TIM3 has been identified as a unique AML stem cell surface marker. Saito's study demonstrated that CD32 and CD25 were highly expressed on the surface of primary human LSCs (24). Meanwhile, elimination of CD32 and CD25 expression on normal human HSCs did not damage the function of normal hematopoietic development. Furthermore, the expression

of CD32 and CD25 on human LSCs were sustained after chemotherapy, which suggested that targeting these two surface markers may be effective therapeutic strategies for treatment of AML (24-26). CD96, a trans-membrane glycoprotein, has been reported to express merely on T and NK cells. AML LSCs could be distinguished from normal HSC by the expression of CD96 (27,28). These findings suggested that CD96 was a LSC-specific marker in human AML and excellent candidate target for targeting LSCs (29,30). Targeting IL3R (CD123) with diphtheria toxin (DT)-IL3 fusion proteins was in phase II clinical trial (31). Targeting specific surface markers of LSCs is considered to be a great potential strategy for selectively eliminating LSCs.

### The transcription factors of LSCs in AML

Recently, several transcription factors were identified to affect the activity and function of LSCs in AML. As reported, LSCs were greatly associated with refractory AML, multi-drug resistance and relapse. The alternative p53-inactivating is the main mechanism for survival and continued evolution of LSCs during and after chemotherapy (32). Furthermore, the activity of p53 could be regulated by histone deacetylases (HDACs) (33). Therefore, the HDACs protein modulators could be exploited to control the activity of p53 and enhanced the

response to chemotherapy and targeting of LSCs. Hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) is an important regulator for low oxygen level in AML. Meanwhile, HIF-1 $\alpha$  played important roles in the self-renew of HSCs and LSCs in AML (34). Bonnet and his colleagues suggested that HIF-1 $\alpha$  or HIF-2 $\alpha$  was necessary for the survival of LSCs and may be potential therapeutic targets for eradicating LSCs in AML (35).

NF- $\kappa$ B is an important transcription factor in cell survival, proliferation and differentiation. The expression of NF- $\kappa$ B in HSCs is low, while it is significantly over expressed in LSCs (36). Therefore, NF- $\kappa$ B could distinguish HSCs and LSCs, and may serve as a potential therapeutic target for the selective elimination of LSCs sparing HSCs (37,38). Dimethylaminoparthenolide (DMAPT), a NF- $\kappa$ B inhibitor, could selectively eradicate LSCs, which prompted it to be in clinical trials for the treatment of AML, acute lymphoblastic leukemia (ALL), and chronic lymphocytic leukemia (CLL) in the United Kingdom (38,39). DMAMCL (ACT001) was able to eliminate LSCs by inhibiting the activity of NF- $\kappa$ B. ACT001 was in clinical trial in Australia (36,40).

$\beta$ -catenin is a key molecule of Wnt/ $\beta$ -catenin signaling pathway which is crucial for LSC self-renewal, tumor occurrence, development, recurrence, and drug resistance (41,42). The Wnt/ $\beta$ -catenin was active in human LSCs and in HSCs while  $\beta$ -catenin was unnecessary for the self-renew of adult HSCs (43-45). Meanwhile, pharmacological inhibition of  $\beta$ -catenin impaired LSC function and significantly reduced the growth of human MLL leukemic cells (46,47). Therefore, all these transcription factors might be considered to be potential targets for selectively ablating LSCs.

### Other biomarkers and genes

Membrane markers and transcription factors are the main characteristics of LSCs in AML. There are some other types of biomarkers which were reported to be related with the maintenance and survival of LSCs. Intracellular reactive oxygen species (ROS), reactive metabolites containing oxygen, played important roles in stem cell sustaining and function. Meanwhile, ROS had effect on leukemia initiation and progression in a certain degree (48). The level of intracellular ROS in LSCs was relatively lower than that of in HSCs. With this result, agents, inducing overproduction of ROS, can selectively eradicate AML stem cells via modulating ROS production. ROS could be

an optimal therapeutic target of LSCs in AML (49). LSCs depended on telomerase to sustain the self-renew and extensive proliferation. Targeting the telomerase might be a perfect therapeutic strategy to eliminate LSCs in AML (50). Moreover, microRNAs like miR-34a, miR-126, miR-21, miR-196b and miR-17-92 played key roles in the regulation of LSCs, which would be developed as novel therapeutic agents against LSCs (51-54).

### Conclusions

LSCs in AML were considered to be the root of chemotherapeutic drug resistance and disease relapse. LSCs are the subpopulation cells featured with membrane markers like CD34, CD38, CD123, TIM3, CD25, CD32 and CD96. In addition, the transcription factors were also therapeutic targets in eradicating LSCs, such as HDAC, NF- $\kappa$ B, HIF-1 $\alpha$  and  $\beta$ -catenin. Besides membrane markers and transcription factors, intracellular ROS, telomerase and microRNAs were identified to be new targets for ablating LSCs in AML. Identification of the specific features of LSCs will greatly prompt the discovery of potential agents that can selectively eradicate LSCs in AML, which would greatly improve the response to drug resistant and refractory/relapsed AML.

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### Footnote

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

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