New insight of leukemic stem cell

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Human acute myeloid leukemia (AML) derives from rare leukemic stem cells (LSCs). Relapse of disease can be ascribed to LSCs to some degree. Currently, a number of surface markers of AML LSCs have been identified, such as CD123, CD44, CLL-1. Some monoclonal antibodies aimed at cell surfaces have demonstrated efficacy in xenotransplantation models. In our recent work, we found that N-cadherin and Tie2 positive CD34+CD38-CD123+ populations could develop acute myeloid leukemia more effectively in NOD/SCID mice than their negative counterparts at the same doses. Meanwhile, the blast cells from the bone marrow of leukemic mice are transplantable. It is speculated that FLT3-ITD mutation could make the LSCs more capable of expanding in the environment. These data suggested that N-cadherin and Tie2 were very important in development of leukemia as LSC markers.


Most of acute myeloid leukemia (AML) patients can achieve complete remission (CR) after initial induction chemotherapy, but 5-years overall survival (OS) still remains low at 20-30%, and a majority of patients will relapse inevitably. The reason for that can be ascribed to the existence of leukemic stem cells (LSCs) to some degree. The concept of LSCs in human AML was firstly proposed by Lapidot et al. [1], and initial phenotype of LSC was identified, namely CD34+CD38-.

Recently, growing investigations were focused on LSCs, particularly in the exploration of surface marker. It is anticipated that identification of specific phenotype may contribute to the development of LSCs specific immunotherapy. As we known, hematopoietic stem cells (HSCs) and LSCs share some same cell surface markers, for example, they both express CD34, but not CD71, CD38 and HLA-DR. However, LSC has some unique surface markers, currently, a number of surface markers of AML LSC have been identified, such as CD123, CD44, CLL-1, CD96, CD47, CD32, CD25 and TIM-3 [2-8]. Meanwhile, monoclonal antibodies targeting some markers have demonstrated efficacy in xenotransplantation models, such as CD44, CD123 and CD47 [3, 7, 9]. All these advancements have shown that the research of LSC surface marker is a promising filed, that can be translated into clinical application in the future.

As we known, the interaction between HSCs and bone marrow microenvironment is indispensable for development of HSCs, and therefore it is supposed that adhesion molecules involed in the niche may play an important role in the development of leukemia. Our laboratory has been studying the two adhesion molecules these years, namely Tie2 and N-cadherin.

Tie2 is originally identified as the second member of an orphan RTK subfamily and participates in the process of vascular generation and reconstructing. N-cadherin locates between osteoblasts cells and the long-term HSCs (LT-HSCs), contributes to maintaining the normal function...
of HSCs. In our previous studies, we found that N-cadherin and Tie2 positive CD34⁺CD38⁺CD123⁺ populations are relatively less sensitive to chemotherapeutic drugs than their negative CD34⁺CD38⁻CD123⁻ counterparts, and they could be enriched by chemotherapy. Based on these observations, we supposed that N-cadherin and Tie2 molecules may facilitate the quiescence of the LSC. To test the above prediction, in our recent study, through NOD/SCID transplantation assay, we found that N-cadherin and Tie2 positive CD34⁺CD38⁺CD123⁺ populations can initiate leukemia development more effectively in NOD/SCID mice than their negative counterparts at the same dose. Meanwhile, the blast cells from the bone marrow of leukemic mice were transplantable. Therefore, we hypothesized that N-cadherin and Tie2 could mediated adhesion and homing of LSCs to bone marrow microenvironment when AML LSCs were transplanted into NOD/SCID mice, facilitated the LSCs self-renewal and expansion. N-cadherin or Tie2 positive CD34⁺CD38⁺CD123⁺ LSCs had advantage to survive in the new microenvironment over their negative counterparts.

In the exploration process of LSC surface markers, we have four issues to discuss with the global colleagues. First, as we known, the population of LSCs was very low in the majority of AML patients; therefore the LSCs population may be too low to reach the lowest threshold that could induce leukemia. In fact, we had previously performed the same transplantation experiments with the sorted cell number of N-Cadherin⁺ or Tie2⁺ CD34⁺CD38⁺CD123⁺ LSCs derived from AML samples <10⁴, but unfortunately, all the transplanted mice failed to develop leukemia after half a year. Therefore, to acquire enough LSC, we set up two criteria to screen appropriate AML samples, namely the white blood cell (WBC) count of patients at diagnosis was above 50×10⁹/L and CD34⁺CD38⁺ proportion above 10%, all the samples had to meet both criteria at the same time. The final data showed that the leukemia development was observed in those mice that were transplanted with LSCs as high as 10⁴ derived from AML patients. So, it is very important to get enough LSC population before transplantation for the expansion of LSC.

Second, it is reported that LSCs are most restricted to CD34 negative compartment when NPM1 mutation was positive [12]. But our data clearly demonstrated that the N-cadherin⁺ LSC and Tie2⁺ LSC population derived from CD34 positive compartment of the patient with positive NPM1 mutation could develop leukemia successfully. The result was further in accord with that the LSC was a heterogeneous population, it could also exist in CD34 positive compartment when NPM1 mutation was present. But this conclusion still needs more observations to support it.

Third, it is conceivable that LSC still needs more cytogenetic alteration to speed up its expansion. In our study, it was surprisingly found that the LSC from the patient with FLT3-ITD mutation could induce leukemia more successfully than the patient without FLT3-ITD mutation, and the FLT3-ITD mutation could subsequently be detected in the bone marrow and spleen of the leukemic mice. It is speculated that FLT3-ITD mutation could make the LSC more capable of expanding in the microenvironment. As we known, the patients with normal karyotype and FLT3-ITD mutation have poor prognosis with only a 30-40% chance of cure even after hematopoietic cell transplantation. Constitutive activation of FLT3 by ITD mutations is one of the most common molecular alterations known in AML [13-15]. Based on our data, FLT3-ITD mutation may occur in the early phase of leukemia pathogenesis, even blast cell with FLT3-ITD mutation are eliminated by chemotherapy, the aberrant mutation that pre-existed in LSC could still play an indispensable role in the relapse of the leukemia, and our data provided new insights into the pathogenesis of LSC.

Fourth, through surface marker comparison, the phenotype of blast cells from the leukemic mice of primary transplantation and secondary transplantation were almost the same with the phenotype of clinical sample. It strongly implied that the LSC can follow the pre-fixed model to initiate leukemia development, regardless of concrete microenvironment.

Though our study confirmed that N-cadherin and Tie2 were very important in development of leukemia as LSC markers, we have to confess that these two molecules are not sufficient to be LSC markers currently, because CD34⁺CD38⁺CD123⁺ population were already considered to be LSC, we only add new molecules on basis of that. So we will further explore the role of CD34⁺CD38⁺N-cadherin⁺ or CD34⁺CD38⁺Tie2⁺ cell populations in the development of leukemia. The exploration of surface maker of AML LSC is a promising field, but it is not sufficient to understand the origin and outcome of LSC if we only focus on surface marker. As we supposed above, the LSC with specific phenotypes may have the advantage for surviving, but it also needs other “hits” to become leukemic blast cells, such as cytogenetic mutations. In addition to surface markers, many other aspects of LSCs are also explored by investigators, such as signal pathway, ALDH and drug resistance, etc. We have confidence to believe that these investigations will be helpful to overcome acute leukemia in the future.

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Conflict of interest

Jianxiang Wang acts as consultant of Novartis and Bristol Myers Squibb.

References


