Limitations of CLL-1 (CLEC12A) as a putative marker of leukemic stem cells in acute myeloid leukemia

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BACKGROUND

Acute myeloid leukemia (AML) is the most common form of acute leukemia in adults¹. With help of chemotherapeutic drugs and stem cell transplantation, it is possible to achieve complete remission (CR). However, the majority will relapse within 2 years despite consolidation therapy including allogeneic stem cell transplantation.¹ Despite some improvement in survival for younger AML patients over the last few decades, long-term survival is still a big challenge.

The role of leukemia stem cells (LSCs) has been implied in leukemia initiation, progression, drugs resistance and relapse of the disease following remission^{2,3}. A number of cell surface markers to identify LSCs have been explored in the last few years⁴. Recently, human C-type lectin-like molecule-1 (CLL1 or CLEC12A), a myeloid lineage restricted cell surface marker has been shown to be overexpressed on blasts and LSCs making it an ideal target for development of new targeted therapies such as CAR-T-cells^{5,6}. However, there is still controversy about its role as LSC marker and its potential as target molecule.

AIMS

- To determine CLL1 expression on leukemic subpopulations defined by different CD34 and CD38 expression levels
- To determine whether CLL1^{hi} cells display high expression levels of genes associated with LSC activity
- To examine whether the expression levels of CLL1 at diagnosis may identify patients with adverse outcome.

METHODS

REFERENCES

1. Appelbaum FR, Gundacker H, et al: Age and acute myeloid leukemia. Blood 2006 107:3481- 3485.
2. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997; 3: 730–737.
3. Passegue, E. et al. (2003) Normal and leukemic hematopoiesis: are leukemias a stem cell disorder or a reacquisition of stem cell characteristics? Proc. Natl. Acad. Sci. U. S. A. 100, 11842–11849
4. Saito Y, Kitamura H, et al. Identification of therapeutic targets for quiescent, chemotherapy-resistant human leukemia stem cells. Sci Transl Med. 2010;2(17):17ra9.
5. Van Rhenen, A.; van Dongen, et al. The novel aml stem cell associated antigen CLL-1 aids in discrimination between normal and leukemic stem cells. Blood 2007, 110, 2659–2666.
6. Saygin C, Carraway HE. Emerging therapies for acute myeloid leukemia. J Hematol Oncol. 2017;10(1):93

Figure 1: (A) Surface expression levels of CLL1 in CD34⁺ vs CD34⁻ AMLs ($p < 0.01$). (B) Surface expression levels of CLL1 in different cellular compartments of CD34⁺ AML specimens ($p < 0.001$ for trend). (C) Probability of overall survival in AML patients according to the surface expression of CLL1. Patients with high CLL1 expression have a trend towards better survival. (D) Gene expression ratio of sorted CD34⁺CD38⁻dimCLL1^{hi} vs. CD34⁺CD38⁻dimCLL1^{lo} cells of LSC17 genes (an x-fold change > 1 indicates higher expression in CLL1^{hi} cells).

RESULTS

- CLL-1 expression was higher in CD34⁻ AML (n=42) as compared to CD34⁺ AMLs (n=108) (Fig 1a). Within the CD34-positive AMLs, CLL-1 was significantly up-regulated in the more mature CD34⁺38⁺ and CD34⁻ leukemic cell compartment (Fig 1b).
- Gene expression analysis revealed that most of LSC associated genes were differentially expressed between CD34⁺CD38⁻dimCLL1^{hi} and CD34⁺CD38⁻dimCLL1^{lo} cells. Most of LSC17 genes seem to be higher expressed in the CD34⁺CD38⁻dimCLL1^{lo} cells (Fig 1d).
- In 102 AML patients receiving intensive chemotherapy, patients with high CLL1 expression had a trend towards higher overall survival as compared to patients with low surface expression (Fig. 1c).

CONCLUSIONS

Among CD34-positive AML samples, CLL-1 expression was lowest in the LSC-containing CD34⁺38⁻ compartment and its surface levels inversely correlated with LSC-associated gene expression. Furthermore, CLL-1 expression did not impact on outcome in AML patients receiving intensive therapy. Thus, our data indicate that CLL-1 has limited potential as LSC marker in AML.