Mini-Review

Properties of Chronic Myeloid Leukemia Stem Cells: Strategy Based on the Kinetics during Treatment with ABL-Tyrosine Kinase Inhibitors

Yosuke Minami*
Division of Blood Transfusion, Kobe University Hospital, Kobe, Japan

Abstract

Chronic Myeloid Leukemia (CML) is effectively treated with Tyrosine Kinase Inhibitors (TKI) such as Imatinib Mesylate (IM) targeted against BCR-ABL, however, several mathematical models and ex vivo-examinations suggested that IM does not eradicate CML stem cells. We previously reported the investigation of residual CML diseases during TKI treatment using FACS-sorting and quantitative RT-PCR of BCR-ABL among each population; total mononuclear cells, hematopoietic stem cells, and myeloid progenitors. Our observations were supportive of the mathematical model that consists of the initial rapid $\alpha$-slope and the consequent $\beta$-slope correspondent to kinetics of residual cells. The observations also implied that the second-generation of ABL-tyrosine kinase inhibitors (2nd TKIs), dasatinib or nilotinib therapy can be more promising approach for efficient reduction of the CML stem cells. Moreover, we need to develop the evaluation method of the residual CML stem cells to establish rational TKI-cessation strategies in CML.

PROPERTIES OF CHRONIC MYELOID LEUKEMIA STEM CELLS

Chronic Myeloid Leukemia (CML) is a clonal myelo proliferative disorder that is characterized by the presence of a fusion oncogene, BCR-ABL, which encodes a protein with constitutive tyrosine kinase activity [1]. The mechanisms for TKI insensitivity of CML stem remains unclear; factors such as quiescence, high level of BCR-ABL expression, acquired mutations in the oncogene, and overexpression of membrane transporter proteins in these cells may play a role [2-4].

In the normal myelopoiesis is sustained through the life by the regulated proliferative and differentiation activity of a large pool of Hematopoietic Stem Cells (HSCs) (Figure 1A). Cells within hematopoietic hierarchy can be distinguished by their proliferative and differentiation activity which they display under conditions designed to optimally elicit these, either in vivo (where the most primitive cells are called long-term repopulating cells, LTC-ICs or in vitro (as long-term culture-initiating cells, LTC-ICs and CFCs) [5,6]. Surface markers, such as CD34 and CD38 are differentially expresses upon differentiation, progenitors being mostly CD34+CD38+ and HSCs exclusively CD34+CD38-. In patients with CML-Chronic Phase (CP), normal and leukemic cell population co-exist (Figure 1B) [1,4,8,9]. In the stem cell compartment, normal HSCs often outnumber the small numbers of their leukemic counterparts. However, current evidence suggests that the normal HSCs are outcompeted by the CML stem cells when these begin to proliferate and differentiate, which the CML stem cells also attempt more frequently due to their higher turnover and increased probability of differentiation. The autocrine secretion of IL-3 and Granulocyte colony stimulating factor (G-CSF) by primitive leukemic progenitors likely contributes to growth advantage of leukemic myeloid progenitors and mature cells in patients resulting in their dominance of peripheral blood and bone marrow of newly diagnosed CML patients with mature CML cells [6].

Residual CML stem cells during treatment with ABL-tyrosine kinase inhibitors

The use of Tyrosine Kinase Inhibitors (TKI) such as Imatinib Mesylate (IM) targeted against BCR-ABL has proven successful in CML and long-term survival has become a reality [10,11]. However, several mathematical models and ex-vivo examinations suggested that imatinib (IM) therapy does not eradicate CML stem cells [3,8,12-14]. We previously reported a method for investigation of CML-CP cases during TKI treatment using
Figure 1 CML-CP stem cells and leukemic myelopoiesis.
(A) Schematic representation showing myelopoiesis in normal adults. Surface markers, such as CD34 and CD38 are differentially expressed upon differentiation.
(B) Schematic representation showing how leukemic myelopoiesis is differently deregulated at different stages of hematopoiesis in patients with CML-CP. (Adapted from ref. 6)

Figure 2 Representative analysis of HSC/Progenitors in CML-CP bone marrow cells.
Using FACSaria, in CML-chronic phase (CP) bone marrow cells, we examined CD34+38− and CD34+38+ populations, and analyzed BCR-ABL transcripts among each sorted population; total mononuclear cells, HSC/Thy-1+, HSC/Thy-1−, common myeloid progenitors (CMP), granulocyte macrophage progenitors (GMP) and megakaryocyte erythroid progenitors (MEP).
FACS-sorting and quantitative RT-PCR of BCR-ABL among each population; total mononuclear cells, HSC, and myeloid progenitors (Figure 2)[9,15,16]. From each population, we collected at least 5,000 cells (most samples were over 20,000 cells), and the limited number of sorting cells was one critical reason for the methodological limitation regarding subtle quantitative evaluation. As internal control, we compared ABL, GAPDH, and BCR. For this kind of evaluation using limited number of cells, ABL was not the best from the aspect of expression, and BCR was more suitable. In the HSC population by this method, more than 30% cells are supposed to have stem cell potential, likely as LTC-ICs (Figure 1B). In optimal responders to IM therapy, BCR-ABL transcripts in the HSC populations tended to be more retentive than other populations. Treatment with the second-generation of ABL-Tyrosine Kinase Inhibitors (2nd TKIs), dasatinib or nilotinib induced more rapid reduction of BCR-ABL transcripts even in the HSC population, which implied that 2nd TKI therapy can be a more promising approach than IM treatment for early reduction of CML stem cells [16]. However, these observations also implied that there was a methodological limitation for subtle quantitative evaluation around Complete Molecular Remission (CMR) during 2nd-TKI treatments.

Mathematical models

The mathematical models based on the previous TKIs clinical studies, mathematicians made a standardized formula about the manner of the BCR-ABL decline, which consists of bi-exponential phases; α-slope with initial rapid decline and β-slope correspondent to kinetics of more residual cells. Our observations were similar with biphasic decreasing in the CD34+38- population. Combined with the results, we developed a hypothesis that the β-slope corresponds mainly to the partial (quiescent, imatinib-insensitive stem cells) CD34+38- population, not to the entire one. Our results showed treatment with 2nd-TKI induced a steeper α-slope in comparison with IM-treatment.

Strategy and issues based on the kinetics of CML stem cells

In the nonrandomized Stop Imatinib (STIM) study, IM treatment was discontinued in patients with CML who had achieved complete molecular remission (CMR) of more than 2-year duration [17]. Of the 69% of patients with complete follow-up, 61% relapsed from CMR states (nevertheless, all patients who relapsed responded safely to the reintroduction of IM). The remaining patients maintained CMR states, suggesting that TKI treatment may cure some proportion of patients with CML [18,19]. Ross et al. proposed the sensitive measurement of minimal residual disease using genomic PCR method with patient-specific primers [20]. Moreover, we need to develop the clinically-available biomarker and the evaluation method of the residual CML stem cells to establish rational TKI-cessation strategies in CML (Figure 3). At the same time, we should also continue to discuss about the classification and strategy based on the each clinical goal among CML patients.

ACKNOWLEDGEMENT

The preparation of this review was partially supported by Grants-in-Aid from the National Institute of Biomedical Innovation and from the Ministry of Education, Culture, Sports, Science and Technology on Scientific Research, Japan.

Conflict of interest disclosure

Y Minami received research grants from Bristol-Myers Squibb and Kyowa-Kirin. They did not in any way influence the content of the paper.

REFERENCES


20. Ross DM, Hughes TP, Melo JV. Do we have to kill the last CML cell? See comment in PubMed Commons below Leukemia. 2011; 25: 193-200.