Preliminary Study of Chronic Myeloid Leukemia Stem Cells

Thesis

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ABSTRACT

Chronic myeloid leukemia (CML) represents an important paradigm for understanding the molecular events leading to malignant transformation of primitive hematopoietic progenitors. LSCs are rare and divide less frequently, and thus, represent a reservoir for relapse and resistance to a molecularly targeted single agent the. LSCs are able to evade the majority of current cancer treatments that target rapidly dividing cells. The success of tyrosine kinase inhibitors at controlling the chronic phase disease is tempered somewhat by the persistence of the LSC pool in the majority of the patients. Combined therapies targeting aberrant properties of LSC may obviate therapeutic resistance and relapse in advanced phase and therapeutically recalcitrant CML.

CD7 antigen is expressed on immature myeloid progenitor of chronic phase-chronic myeloid leukaemia patients with inferior survival as a consequence of the more immature $CD34^{+}CD7^{+}$ stem cell carrying t(9;22).

Our work is to preliminary understand the origin of malignant stem cells in CML as well as to study the incidence of very primitive CML stem cells among different CML cases so as to anticipate the ontogeny as well as the prognosis. In-addition, this study aims to pave the way for further studies on CML initiating stem cells.

Key words: Chronic Myeloid Leukemia

CD34&CD7 +stem cells

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LIST OF ABBREVIATIONS

1. **ABL:** Abeslon leukemia virus
2. **ALL**: Acute lymphocytic leukemia
3. **AML**: Acute Myeloid Leukemia
4. **AP:** accelerated phase
5. **BC:** blast crisis
6. **BCR**: breakpoint cluster region
7. **BU/CY**: busulfan and cyclophosphamide
8. **CBL:** casitas B-lineage lymphoma
9. **CEL:** Chronic eosinophilic leukemia
10. **CFU-GMs:** colony forming unit-granulocyte-monocytes
11. **CFUs**: colony forming units
12. **CLL:** chronic lymphocytic leukemia
13. **CML:** Chronic myeloid leukemia
14. **CMML:** Chronic myelomonocytic leukemia
15. **CNL:** Chronic Neutrophilic Leukemia
16. **CRKL:** CRK-like protein
17. **DFS:** Disease-free survival
18. **EGBMT:** The European Group for Blood and Marrow Transplantation
19. **ELA2:** elastase
20. **FTBI-CY**: fractionated total body irradiation and cyclophosphamide
21. **GTP:** guanosine triphosphate
22. **GTPase**: guanosine triphosphatase
23. **GVHD:** graft-versus-host disease
24. **GVL**: graft-versus-leukemia
25. **HCT:** hematopoietic cell transplantation
26. **HCT**: Histocompatible transplantation
27. **HPRT:** hypoxanthine phosphoribosyltransferase

28. **IFN-α:** interferone alfa
29. **IM:** Imatinib
30. **IRIS:** International Randomized study of Interferon and STI571
31. **JMML:** Juvenile myelomonocytic leukemia
32. **KDa:** kilodaltons
33. **LAP**: leukocyte alkaline phosphatase
34. **LSCs**: Leukemic stem cells
35. **LTC-ICs:** long-term culture-initiating cells
36. **MAP:** mitogenactivated protein
37. **MCP**: monocyte chemotactic protein
38. **MIP:** Macrophage inflammatory protein
39. **NK**: Natural Killer cells
40. **NF:** Nuclear factor
41. **NOD:** nonobese diabetic
42. **PDGFR**: platelet derived growth factor receptor
43. **Ph chromosome**: Philadelphia chromosome
44. **PI3K:** phosphatidylinositol-3'-kinase
45. ***PR3*:** proteinase 3
46. **RAS**: Rat Sarcoma
47. **RT-PCR**: Real time polymerase chain reaction
48. **SCF:** Stem Cell Factor
49. **SCID:** severe combined immunodeficiency
50. **SIS:** simian sarcoma
51. **STAT:** signal transducers and activators of transcription
52. **TGF-ß**: transforming growth factor beta
53. **TKIs:** tyrosine kinase inhibitors
54. **TLS:** Tumor lysis syndrome

INTRODUCTION

Chronic myeloid leukemia is a hematopoietic malignancy which is resulted from an acquired genetic change in a haemopoietic stem cell. This altered stem cell proliferates and generates a population of differentiated cells that gradually displaces normal haemopoiesis and leads to a greatly expanded total myeloid mass.

Chronic myelogenous leukemia is classified as one of the myeloproliferative disorders. This group of diseases shares several distinct features:

1. They are clonal disorders of hematopoiesis that arise in a hematopoietic stem or early progenitor cell.
2. They are characterized by the dysregulated production of a particular lineage of mature myeloid cells with fairly normal differentiation.
3. They exhibit a variable tendency to progress to acute leukemia.

[Boham et al., 2002]

The risk of developing CML is slightly increased by exposure to high doses of irradiation, as occurred in survivors of the atomic bombs exploded in Japan in 1945, in patients irradiated for ankylosing spondylitis and women with uterine cervical carcinoma who required radiation therapy but, in general, almost all cases must be regarded as 'sporadic' and no predisposing factors are identifiable. In particular, there is no familial predisposition and no definite association with HLA genotypes has been recognized. No contributory infectious agent has been incriminated ***[Verfaillie et al., 1999].***

Chronic myelogenous leukemia (CML) is associated with the Philadelphia chromosome t(9;22)(q34;q11) Phladelphia chromosome resulted from a translocation between chromosome 9 and 22.The gene involved in this translocation was shown to result from fusion of BCR (breakpoint cluster region) gene on chromosome 22 to the ABL (Abeslon leukemia virus)gene on chromosome 9 ,With formation of the BCR-ABL fusion oncogene .

This oncogene codes for a constitutively active cytoplasmic tyrosine kinase, which now believed to be principle cause of the chronic phase of the CML.
This deregulated tyrosine kinase is implicated in the development of CML and has become a primary target for the treatment of this disorder ***[Groffen et al., 1984].***

Twenty to 50 percent of patients are asymptomatic, with the disease first being suspected from routine blood tests. Among symptomatic patients, systemic symptoms (fatigue, malaise, weight loss, excessive sweating), abdominal fullness and discomfort are frequent. Bleeding episodes due to platelet dysfunction are common. Tenderness over the lower sternum is sometimes seen. Acute gouty arthritis may also present at this time. Other frequent findings include splenomegaly and anemia ***[Savage et al., 1997].***

The clinical hallmark of CML is the uncontrolled production of granulocytes, predominantly neutrophils, but also eosinophils and basophils. The chronic phase is characterized by a large increase in the pool of committed myeloid progenitors, leading to peripheral blood leukocytosis and often thrombocytosis with a prominent left shift in the differential count and basophilia. White blood cell count is usually above 100,000/μL (52 and 72 %), and platelet count above 600,000 to 700,000μL (15 and 34%) ***[Spiers et al., 1977]***.

The leukocyte alkaline phosphatase (LAP) score is abnormally low. Bone marrow aspiration and biopsy in patients with CML in chronic phase shows granulocytic hyperplasia, a non diagnostic finding. Other non-specific bone marrow findings include an increase in reticulin fibrosis and vascularity ***[Lundberg et al., 2000].***

In the past, the diagnosis of CML was based largely upon clinical and laboratory criteria, including the presence of splenomegaly, neutrophilic leukocytosis with circulating immature cells of the granulocyte series, basophilia, and low to absent alkaline phosphatase activity in the circulating neutrophils.

However, current diagnostic criteria require detection of the Ph chromosome or its products, the BCR-ABL fusion mRNA or the Bcr-Abl protein. RT-PCR is a highly sensitive technique that employs specific primers to amplify a DNA fragment from BCR-ABL mRNA transcripts; it is becoming the diagnostic test of choice for Ph-positive leukemia ***[Goldman et al., 1999].***

The disease has a triphasic clinical course: a chronic phase, which is present at the time of diagnosis in approximately 85 percent of patients; an accelerated phase, in which neutrophil differentiation becomes progressively impaired and leukocyte counts are more difficult to control with myelosuppressive medications; and blast crisis, a condition resembling acute leukemia in which myeloid or lymphoid blasts fail to differentiate. Without treatment, CML progresses from an initial chronic phase followed by advanced phases of disease (accelerated phase and blast crisis) ***[Giles et al., 2004].***

Until the 1980s, CML was generally assumed to be incurable and was treated palliatively in the early days with radiotherapy, and more recently with alkylating agents, notably busulphan and hydroxyurea ***[Kennedy, 1992]***.

The introduction of imatinib into clinical practice in 1998 was an important therapeutic advance, as with this agent most patients achieve a complete cytogenetic response and may expect prolongation of survival compared with other methods of treatment ***[Michael, 2008]***.

Treatment options for patients with CML are varied and include: potential cure with allogeneic hematopoietic cell transplantation (HCT); disease control without cure using tyrosine kinase inhibitors (TKIs) or use of interferone. Factors influencing the choice of therapy include: the phase of CML; availability of a donor for HCT; patient age; the presence of medical co-morbidities affecting patient suitability for HCT; and, for patients in earlier phases of CML, the response to treatment with tyrosine kinase inhibitors (TKI)s ***[Kantarjian et al ., 2007].***