ORIGINAL RESEARCH PAPER
VOLUME-6 | ISSUE-7 | JULY-2017 | ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

GENETIC BASIS OF CHRONIC MYELOID LEUKEMIA

Anatomy

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KEYWORDS:

Chronic myeloid leukemia, ABR/BCL gene, FISH

Chronic myeloid leukemia is a malignancy characterized by proliferation of myeloid series of hematopoietic stem cell. It is characterized by an initial chronic phase followed by an accelerated phase leading to a late blast phase which happens 3-4yrs after the disease onset. It was initially believed that CML is a three step process but Jeral P.Radish proposed that it is a two-step process [1], with new gene expression changes occurring early in accelerated phase before the accumulation of increased numbers of leukemia blast cells. The chronic phase is characterized by accumulation of myeloid progenitors and mature cells in blood and extramedullary tissues. The blast phase shows a maturation arrest in myeloid or lymphoid lineage.[2] The only cure for this disease is allogenic stem cell transplantation if given in chronic phase but the therapy itself is a complex and potentially toxic modality. Treatment with Interferon α delays progression of the disease to blast crisis and in 10-20% of patients results in complete remission. Blast crisis is highly resistant to treatment, often leading to death secondary to complications like infection or thrombosis. The beginning of the use of Interferon as treatment for CML started in 1983 after a report by Tapez et al which proposed that leukocyte IFN induced cytoreduction in CML [3]. This proposal was approved by certain other studies [4,5,6]

There was a clear benefit in the survival of CML patient treated with IFN as compared to those who were given hydroxyurea or busulfan [7] Thus IFN became the gold standard of CML therapy in 90s.Then came the era of tyrosine kinase inhibitors the superiority of which was documented by IRIS trail in the treatment of newly diagnosed patient with CML in chronic phase [8]. In 2013, the Tyrosine kinase inhibitors Imatinib, Nilotinib and Dasatinib were approved as first line therapy in CML [9] while Ponatinib and bosutinib were approved as second /third line therapy in CML [10]. Although the efficacy of tyrosine kinase inhibitors has resulted in overall survival of >90%, they are not curative. Also there is no currently approved TKIs effective against T3151 BCR-ABL variant.

Chronic myeloid leukemia is a cancerous condition associated with an acquired genetic abnormality, the Philadelphia chromosome. The Ph has great historical interest because it was the first cytogenetic marker found to recurrently characterize a clinically delimited group of human.[11] Ph is present in >90% cases and is result of rearrangement between BCR and ABL gene. The BCR-ABL fusion gene, is seen in upto 95% of CML patient and translates into an oncoprotein, $p210^{BCR/ABL}$ [12].

The genetic events that cause progression of chronic phase to blast crisis are largely unknown. A variety of genetic abnormalities has been demonstrated which includes the acquisition of additional chromosomal abnormalities, for instance duplication of the Ph isochromosome 17(p) resulting in duplication of the Ph isochromosome 17(p) resulting in disruption of TP53 and less frequently, the deletion of p15/p16 tumor suppressor genes (especially in lymphoid blast crisis) and the RUNX1-EV11 fusion

Molecular mechanisms of BCR-ABL

Approximately 90% of patients with CML have an acquired genetic abnormality, the Philadelphia chromosome which is a result of a rearrangement between the BCR and Abelson genes. Before discussing the abnormality, let's discuss about the normal phenomenon. The product of normal Abelson gene is p145 protein which is a tyrosine kinase and this protein is essential for cell growth[13] ,induction of apoptosis [14] ,and involved in DNA repair [15] .Although $p145^{ABL}$ is largely found in cell nucleus, there is suggestive evidence that it plays a role in cell signaling from integrins as well as other cell surface receptors such as B cell receptors and CD19[16] In mice studies it was observed that $Abelson^{-/-}$ mice died soon after birth due to severe runting and impaired lymphoid development. However $ABL^{+/-}$ mice lived normally. Likewise BCR-/- mice developed septic shock when given lipopolysaccharide due to significantly increased and dysregulated neutrophilic oxidative burst. BCR +/- mice are normal. Thus this study suggests loss of one allele in ABL or BCR gee does not play a role in pathogenesis of CML. CML results due to reciprocal translocation between long arms of chromosome 9 and 22 t(9;22 q34:q11.2). In this translocation, the c-ABL protooncogene is transposed from its normal position i.e. chromosome 9 to a 5.8kb major breakpoint cluster region M-BCR on chromosome 22 forming a BCR-ABL fusion gene. This new gene encodes $p210^{BCR/ABL}$, an oncoprotein which has increased tyrosine kinase activity [16,17], and increased binding to actin cytoskeleton [18] compared with the p145 Abelson protein. Transplantation of BCR-ABL transduced hematopoietic stem cells or transgenic expression of $p210^{BCR/ABL}$ induces leukemia, lymphomas and CML like syndromes.

p210BCR/ABL kinase phosphorylates JAK 2 and STAT1/STAT5 [19] proteins that transfer signals from non TK cytokine receptors to the nucleus. RAS and STAT activation contributes to growth factor independent of cell lines containing BCR-ABL gene. Moreso p210 BCR/ABL activates the PI(3)K/Akt pathway [20], increases expression of BCL-2 [21] and phosphorylates STAT5 [22] leading to increased resistance.

While p145 ABL is primarily localized in nucleus , $p210^{BCR/ABL}$ is localized almost exclusively in cytosol due to a loss of a nuclear localizing signal and has increased binding to actin as compared with p145ABL [23]. This results in phosphorylation of a number of neighbouring cytoskeletal proteins including FAK [24] and paxillin [25] all of which contribute to abnormal adhesion receptor function and may explain the premature circulation of progenitors and precursors in the blood [26].

Events occurring during disease progression

At cellular level

Like any other malignancy , this disease is associated with decreased apoptosis. In CML, multiple mechanisms contribute towards resistance against apoptosis. The major mechanism being phosphorylation and activation of the PI(3)-K/Akt pathway [27,28], PI (3) kinase activation also via protein kinase B results in the phosphorylation and inactivation of the pro-apoptic protein BAD a member of the BCL-2 family.[29]

Another mechanism is activation of NF-κB (seen in transgenic models of CML) which provides protection against Apoptosis.[30]

Lastly the RAS pathway may be involved in BCR-ABL mediated inhibition of apoptosis [31] and elevated cytokine production [32] which may have inhibitory effect in apoptosis.

Protection against apoptosis is relatively less in chronic phase CML but there is some evidence suggesting that BCR-ABL mediates resistance to apoptosis in a dose dependent fashion . The disease progression is usually associated with increased expression of the BCR-ABL mRNA. But decreased apoptosis despite being a characteristic feature of CML cannot be the sole factor for disease

progression and transformation.

Also it has been observed that the cell differentiation gets blocked in blast phase but not in chronic phase [33]. Thus this can have a therapeutic implication and differentiating agents can be tested in treatment of the disease in blast crisis.

Both MHC restricted and MHC unrestricted mechanism play a vital role in natural control of the Ph clone in chronic and blast phase of CML[34]

At genetic level

Molecular and cytogenetic variations occur in most of the patients during transformation to blast crisis. Approximately 70-80% of patients with classical Ph positive CML exhibit additional non random chromosomal aberrations [35] involving chromosomes 8.17.19 and 22 with duplication of Ph chromosome or Trisomy 8 being the frequent.

At the molecular level, the most frequent abnormality is a deletion of p53. The deletion of this tumor suppressor gene located on short arm of chromosome 17 is the main culprit leading to myeloid blast crisis [36].

About 50% of patients with lymphoid blast crisis have a homozygous deletion of the p16INK4a gene located on chromosome 9. Other less frequent acquired genetic abnormalities are deletions of the retinoblastoma gene, overexpression of c-MYC or N-RAS and generation of an oncogene AML-EVI-1.

Cytogenetic Monitoring of CML

Cytogenetic analysis of bone marrow or peripheral blood cells leads to identification of the Ph translocation and other chromosomal aberrations that can be correlated with leukemic process. As the leukemic cells are naturally dividing, the addition of mitogens such as phytohemagglutinin is unnecessary and in CML may induce non leukemic populations of lymphocytes. It is at the stage of metaphase of cell cycle that the translocation t(9;22) (q34;q11.2) is best visualized. After treating with trypsin staining with giemsa , identification of t (9;22) and other abnormalities is achieved by karyotype analysis , when chromosomes are arranged according to International Nomenclature system[38]

FISH monitoring of CML

FISH is a modification of conventional cytogenetic procedures and enables microscopic assessment of the number, size and location of specific DNA sequences in single cells [39]. The target cell preparation are initially treated with a protease such as pepsin to clear away all unwanted proteins that may obstruct probe access to chromosomal DNA target sites, further nuclear DNA is denatured and hybridized with fluorescently labelled and denatured DNA probes. The signal patterns are now visualized by fluorescent microscopy.

Both the techniques i.e. cytogenetic(karyotype) and FISH are required for assessment of BCR-ABL1 status in CML vary according to disease stage and treatment. The karyotype assessment of bone marrow will establish presence of the t(9;22)(q34;q11.2), complex Ph translocation variants and any supplementary karyotype changes that may suggest advancing disease. On the other hand, FISH of same cytogenetic preparation using appropriate BCR and ABL1 probes will confirm BCR-ABL1 fusion gene status and assist to identify unusual metaphase and interphase signal configurations with complex BCR-ABL1 variants or associated genomic deletions. The greatest sensitivity of FISH has been seen with application of dual colour, double fusion probes that identify BCR-ABL1 and ABL1-BCR fusion gene signals[40]. Modern probes typically include a green labelled BCR probe (G) and a red labelled ABL1 probe (R). Fusion of parts of the BCR and ABL1 gene is represented as yellow fusion (F) signal.

Conclusion

A cytogenetic analysis should be performed at the time of diagnosis, on the initiation of treatment to decide the line of treatment and also to monitor the course of disease during treatment. The analysis helps in deciding the line of treatment so that early remission of the disease can take place. The future of the CML lies in its rapid diagnosis and discovering a cure which causes less morbidity and mortality and thereby increasing life expectancy of patient.

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