Chronic Myeloid Leukemia: The Mutational Landscape

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Abstract

Chronic myeloid leukemia is 11% of all a malignant proliferative disorder in all leukemia. Basic causative agent of chronic myeloid leukemia is Philadelphia (ph) chromosome that is reported in 5%-10% of leukemia patients. It results due to translocation between two chromosomes, 5' sequences of BCR gene on chromosome 22 fuses to 3' sequences of proto-oncogene ABL on chromosome 9. This rearrangement of chromosome results fusion onco protein BCR-ABL culpable for pathogenesis of CML. This review focuses on the BCR-ABL activity harboring the oncogenic signaling and its treatment.

Key words: BCR-ABL, CML, Ph chromosome.

Introduction

Prevalence rate of CML is low around 0.002 % that is varying from 0.6 to 2.0 cases per 100,000 inhabitants [1]. It is commonly spread in men as compare to women and its risk is increases with age. Variability of incidences of CML is due to geographic and ethnic factors that contributing in its prevalence. It is estimated that its risk is increase with an approximate 20,000 in affecters every 10 years [2].

CML (Chronic myeloid leukemia) also known as differentiated myelo proliferative disorder caused by BCR-ABL tyrosine kinase (TK) onco protein [15] [8]. Primary molecular target to treat this disease is BCR-ABL signaling that endures the pathogenesis of CML. BCR-ABL a fusion transcript referred to as cytogenetic hallmark, that have tyrosine kinase activity responsible cellular and death decisions of leukemic patients. An aberrant gene present on chromosome "Philadelphia” that results the translocation between chromosome 9 and 22 chromosome acquire potential for promoting malignant transformation of hematopoietic stem cells.

BCR and ABL are the basic entities responsible for abnormally fused Philadelphia chromosome formation. ABL is non receptor TK onco protein that acts as shuttles between nucleus and cytoplasm where it binds to DNA and cytoskeleton. It plays important role in cell cycle regulation and cellular proliferation. BCR is a cytoplasmic protein [25] located on long arm of chromosome 22 responsible for
homotetramerization of BCR-ABL. The reciprocal translocation between corresponding genes gives a hybrid of 5′ c-BCR- 3′ ABL gene.

Hematopoietic Stem Cells

Approximately one fifth of all leukemia account for the chronic myeloid leukemia (CML) [3]. To identify the target cell in which the causative molecular lesion occurs is the integral sequel in cancer biology. Hematopoietic stem cells (HSCs) are capable for the continuous blood cell’s production and help sustain body’s immune function. This property is achieved by their exclusive self-renewal capacity or the ability to differentiate into various blood lineages. Several studies implicated Wnt-signaling pathway to be responsible for the regulation of such processes, but its exact role is an unsolved question for scientists [26].

By leukemic chromosomal translocation novel BCR-ABL fusion proteins are generated which deliver growth advantage to stem cells in which they reside in the bone marrow. By the activity of BCR-ABL fused gene, CML is characterized by the defective response or release of cytokines (regulators of hematopoiesis) and perturb the balance between intracellular signaling associations. BCR–ABL transcripts found as central mediators of myeloid proliferation. BCR-ABL mutation leads to an expanded progenitor population with self-renewal property resulting in leukemic stem cells (LSC) [22]. These findings commend that CML is not the sole reason for hematological disease but it originates from bone marrow-derived hemangioblastic precursor cell which gives rise to blood cells and endothelial cells [14]. VEGF (Vascular endothelial growth factor) plasma levels have been suggested to be higher in CML patients as compared to the normal controls. A number of VEGF1 bone marrow cells are reported to be significantly higher in CML patients as compared to the normal controls. Studies revealed that VEGF produced by the granulocyte macrophage colony-stimulating factor/interleukin 3 supported cell colonies of CML patients. VEGF receptor–1 messenger RNA also detected in all chronic phase CML samples [37]. Nuclear β-catenin a subunit of cadherin protein complex may also be the candidate responsible for self-renewal activity of Bcr-Abl chronic myeloid leukemia (CML) cells. It gets activated in granulocyte/macrophage progenitor population enhancing proliferative self-renewal activity and is responsible for progression to blast crisis allowing them to become leukemic stem cells [22].

Philadelphia Chromosome

Ph chromosome is the causative agent in almost 90% of patients with CML. The translocation anomaly leads to an exchange of BCR and ABL genes on long arms of chromosome 22 and 9 respectively with high susceptibility for disease progression [7]. BCR-ABL gene association arises by a complex rearrangement. Break occurs at Abl gene upstream of exon a2 in almost tightly confined major break point cluster region of Bcr resulting in juxtaposition of 5′ of Bcr and 3′ position of Abl forming a hybrid gene that is further transcribed in chimeric BCR-ABL mRNA. Localization of parent cAbl-1 gene is at long arm (q) of chr 9 with molecular mass of 145 kd [10] expressed in several sub cellular sites including cytoplasm and nucleus where it interacts with a range of cellular proteins, including signaling, cell-cycle regulators, kinases, adaptors, phosphatases, cytoskeletal proteins and many transcription factors [18]. It has been implicated to function in various cellular processes, including regulation of cell survival and growth, DNA-damage responses and oxidative stress, cell migration and actin dynamics. It encodes a non-receptor tyrosine kinase with tight temporal activity [16].
Structurally abl gene carries 12 exons which span over 230 kilobases (kb) [39]. In CML break point occurs at 5’ end of exon2 (a2) of Abl (Fig 2).

In the end product tyrosine kinase activity is no longer tightly regulated and is responsible spatially for activating a multitude of survival signals that form an intricate chain of downstream reactions transducing the oncogenic signals to the nucleus. The antiapoptotic activation mechanisms depend upon the phosphotyrosine kinase activity of p210. It is believed to have an increased susceptibility to additional molecular changes in Ph-positive clone that underlie chronic disease progression.

**The Biology of BCR-ABL**

In the chimeric leukemic gene BCR-ABL, the kinase domain of ABL tyrosine kinase gets activated in the cytoplasm by BCR oligomerization portion via coiled-coil fused domain; constitutively induces activation of various distinct oncogenic events that drive diseases. BCR-ABL kinase activity is significant to emanate signaling pathway and cellular transformation that largely comprises of cytoplasmic signaling process [1]. Bcr-Abl downstream signaling regulates a balance between anti-apoptotic and pro-apoptotic proteins (Table: 1) during disease initiation phase [25].

**Ras Signaling**

Ras is foremost proto-oncoprotein that is aberrantly activated in BCR-ABL encode 21-kD G-protein inducing intracellular pathway that elicits leukemiaogenesis in CML[26]. Interaction of BCR-ABL and Ras include various adaptor and docking proteins which function as biological switches altering the activity of Ras by governing different signaling cascades. Increased Bcr-Abl levels activate Ras, Raf-1,phosphatidylinositol-3 kinase, and mitogen-activated protein kinase (MAPKK) that ultimately leads to the Osteopontin (OPN) expression[21].Adaptor proteins such as Grb2, Shc, Crkl and Cbl [40][14][30] support a connection or association between oncogene and Ras signaling. Grb2 binds to the tyrosine residue 177 by SH2 domain of BCR part of BCR-ABL. Ras in its activated GTP-bound state binds or activates effector molecules Raf, P13K, a typical protein kinase C(aPKC) [21] and MEK (Fig 2). Downstream events from Ras relay signals for MAPKs (mitogen-activated protein kinases) such as the Jun kinase pathway. Raf kinases undergo phosphorylation and activation of MAPK kinases pathway [31][36]. P 210 BCR-ABL phosphorylates STAT1 and STAT5, Akt and activates Bad. Strong genetic
evidences suggest that biochemical complexity with Ras plays crucial role in proliferation of CML.

JAK-STAT Signaling Pathway
The Janus kinase/signal transducer or activator of transcription (STAT) localized at chr 9 implicate a pivotal role in CML by inducing oncogenic activity [19]. Janus kinases, a family of 4 kinases have 2 highly homologous phosphate-transferring domains (JH1 and JH2) at carboxyl terminus. JNK is an inmost regulator of multiplicative cellular events, including programmed cell death (apoptosis). By the absence of NF-κB activation, prolonged JNK activation helps TNF-α induced apoptosis. JNK can underlie suppression of apoptosis held by negatively regulated phosphorylation of the proapoptotic Bcl-2 family protein Bad [34]. Thus, JNK has pro or antiapoptotic functions, depending on the cell type, nature of death stimulus, duration of its activation and the activity of other signaling pathways. C-Jun N-terminal protein kinase (JNK) is a subfamily of the mitogen activated protein kinase (MAPK) superfamily. Numerous convincing evidences show that JNK can function as a proapoptotic kinase [25]. Mutation in JH2 domain is capable for inducing downstream signaling pathways including JAK2-STAT5, ERK/MAPK, and Akt/PI3K. STAT which is a known transcriptional factor regulated by tyrosine phosphorylation undergoes cell proliferation, differentiation and apoptosis[19]. BCR-ABL tyrosine kinase activity is constitutively responsible for phosphorylation and activation of STAT-1/5. JAK2 provides docking site for STAT [6]. Phosphorylated STATs enters nucleus where the dimerized STATs bind specifically to the regulatory sequences constitutively to activation and repression oftranscription activity of targeted genes [30]. Further, activation of STAT-5 induces the anti-apoptotic gene expression as Bcl-xL (Fig 3).

Ras endures cellular mutations by impelling multiple proliferative pathways such as SAPK activation. Activated Ras also triggers SAPK (a stress activated protein kinase) pathway by an ability of recruitment and activation of GCKR that mediates cell transformation. Insight studies suggest that BCR-ABL viaRas pathway signals serine/threonine protein kinase GCKR (germinal center kinase related) that prompts SAPK activation.

Phosphotidylinositol 3-Kinase/Akt Signaling Pathway
Bcr-Ablonco protein expression recruits downstream signaling pathways of phosphatidylinositol-3 kinase (PI-3K)/AKT. The oncogenic Abl activity provokes PI-3K signaling required for the CML cell proliferation [29]. PI3K is a lipid kinase and a key player with decisive role in cell survival and proliferation. BCR-ABL gene activates PI3-K; phosphorylated PI3K (Fig 3) gets converted into PIP2 to PIP3 by phosphorylation which further activates Akt. Under normal conditions Akt regulates cell survival or proliferative mechanisms while its oncogenic activity is executive mediator of downstream activated PI3K [1]. Activated Akt underlies phosphorylation of many target proteins regulated by BCR-ABL downstream signaling

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<th>Enzymes</th>
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<tr>
<td>Bcl-2</td>
<td>Mutation caused uncontrolled cell proliferation</td>
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<tr>
<td>PI3K</td>
<td>Activation by BCR/ABL-mediated leukemogenesis</td>
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<td>PP2A</td>
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<td>FOXO</td>
<td>Supression cause inhibition of Apoptotic factor 1</td>
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<td>Myc</td>
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<td>STAT</td>
<td>Enhanced transcription of anti apoptotic cell cycle progression</td>
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<td>Bad</td>
<td>Survival function</td>
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Table: 1. Proteins regulated by BCR-ABL downstream signaling
molecules such as FOXO, Mdm2, caspase 9, mTor and Bad. Phosphorylated Bad retains in cytoplasm with halted effect and is unable to promote apoptotic effect [29]. PI3K and Akt mutants fulfill liberated cell proliferation demands.

Figure 3: A snapshot of deregulated signal transduction pathway: BCR-ABL exerts oncogenic signals by interacting multiple proteins that activate downstream signaling pathways by tyrosine kinase activity related to enhanced proteins expression, hematopoietic differentiation blockage, reduced apoptosis and emergence of drug resistance.

Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL)
TRAIL (tumor necrosis factor-related apoptosis-inducing) when receptors TRAIL-R1 and TRAIL-R2 interacts with its cell-surface induces death signals [28]. LIGAND IL-3 hematopoietic cytokines inhibits the transcription of pro-apoptotic TRAIL. By inhibiting the TRAIL function by siRNA, the cytokines deprived cells have been rescued partially (12). Notably, NFK-B speculated to be an apoptotic therapy for CML patients [38].

ERK5/MAPK Activation By Abl Kinase
MAPK (mitogenic activated protein kinases) are presumed to be the conserved enzymes present in all eukaryotes, activated by various stimuli and are responsible for activation of a large number of cellular processes [5]. MAPK mediates transcriptional regulation of various pro-survival factors that are esteemed to be the principal survival signals. Interaction of MAPK and tyrosine kinase c-Abl activates and regulates ERK5 wild type activity that enhances cell transformation [27].

Treatment Options
The significant efforts, combined understandings and knowledge regarding to molecular mechanism of signal transduction pathways yielded insights to cancer biology, drug trafficking across cell compartments, cyogenetic cellular processes and chemotherapeutic aspects mainly towards the development of molecular targeted therapies for CML. The integral goals for the CML treatment are associated with the complete cyogenetic and the hematological responses.

As the proteins tyrosine kinases are discerned to be the paramount regulators of BCR-ABL downstream signaling as they catalyzes the transformation of phosphate group of ATP to the tyrosine residue of protein substrate [23][24]. The overexpression and constitutive mutational kinase activity are engaged by unransfered proliferation and malfunctioned cell growth [13]. Studies on active involvement of PKTs in dysregulative cell proliferation in CML eventually lead to the revolutionized treatment of CML pointing those as premium therapeutic targets for cancer by ensuring small novel tyrosine kinase inhibitor molecules[13][32]. Formerly, there were limited CML treatment options.

The foremost drug used for treatment of CML is imatinib instead of conventional chemotherapy. Lately a second-generation of drugs namely TKI, has shown much better responses than imatinib and is being considered for frontline treatment. It was seen that patients having T315I mutation displayed a poorer prognosis; this type of outcome could be tackled by allogeneic stem cell transplantation. New approaches being used for targeting leukemic cells comprise of TKIs the low molecular weight compounds as well as antibodies and RNA interference technologies like antisence mRNAs, siRNAs & miRNAs, these specifically target the particular gene responsible for causing leukemia by destroying its mRNA before it could be translated into mutated proteins which lead towards tumourigenesis. But still the treatment required to treat chronic CML is yet to

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be discovered who has a better prognosis, for this reason modern technologies are being utilized.

**From Imatinib To RNAi**

Imatinib, the standard therapy for CML has also been reported to develop resistance in patients over time [2]. This resistance to drug is commended by the mutation in BCR-ABL oncogene. Some previous work revealed a possible mechanism of the LYN kinase activation for this developed mutation-resistance [35]. Lyn kinase is a key enzyme that regulates the cell activation. BCR-ABL interacts to Lyn kinase, promotes cell survival and shows resistance to the apoptotic stimuli in blastic stage [20]. Consequently, over-expression of Lyn kinase is thought to be associated with Imatinib resistance in CML patients.

**Conclusion**

By compiling all the information it is summarized that various biochemical mechanisms that execute transforming effects of BCR-ABL turn on different signaling pathways initiated by the interaction between first exon of BCR and C-ABL or other proteins with SH3/SH2 domains. Most prominent of these includes the upregulation of RAS and RAS-independent MYC-dependent pathway. This oncogenic expression of BCR-ABL involves in the inhibition of apoptosis leading to prolonged CML progenitor’s cell survival and acquisition of the secondary genetic changes. Despite the availability of tyrosine kinase inhibitors such as imatinib and highly potent dasatinib and nilotinib as accepted standard approaches to the treatment of CML diagnosed patients, the management of CML becomes more complex than earlier. Success of any drug treatment for CML patients ultimately depend upon how minimal residual disease can be and whether needs to be eliminated or not. Currently SCT is a feasible procedure for CML patients in early or late chronic phase under 40 years of age [11]. Most recent strategy opted by the researchers is to treat CML by using RNA interference (RNAi) by gene silencing mediated by small interference RNA (siRNA) but is limited due to the cytotoxicity of existing delivery methods. The expression vector of siRNA could be used in vitro for targeting specific RNA to reduce levels of specific gene product in the target cells. Thus RNAi is thought to be a potential application for the treatment as it is directed against BCR-ABL with effectively induced apoptosis [17].

**Future Prospects**

Chronic myeloid leukemia has set a paradigm in the field of cancer research and therapy. Imatinib affirmed as first-line treatment for CML does not able to eradicate all leukemia cells. Furthermore, patients attain resistance to the drug. To conquer such problems, various strategies have been developed that include combinations with other agents, dose escalation and novel Bcr-Abl inhibitors. Persisting to such scientific discoveries leads to advanced therapies [10]. The success behind drug treatment of CML ultimately depends upon the elimination or to be minimal residual disease. For CML patients, choice of primary treatment in chronic phase becomes exceptionally difficult. If suggested for transplantation only a minority of patients are eligible which still carry risk of death. At present, no long-term outcome of new promising therapies as tyrosine kinase inhibitor and transplants are known. As well as there is no sterling way to predict the response of a particular therapy to a patient. Development of therapies having potential to target and eliminate CML stem cells may only be facilitated by understanding complete biological properties, molecular and signaling pathways that enable these cells to self-renewal combined with insight into interaction of these cells to their bone marrow microenvironment [7]. Shift from cytotoxic drugs to the targeted therapeutic agents require an effort to understand disease at molecular level to guide therapy choice for the patients. . New generation of eloquent and unique molecules which are responsible to exploit clinically relevant oncogenes, are all engendered by the advances in cancer therapy [3]. Current technological tactics have aided to identify predictive genes or protein signatures, concerning their clinical significance (14). The study done by [26] revealed the biology of BCR-ABL stem cells and their of biologically relevant biomarkers, which might facilitate to devise better treatment outcome in CML patients. Limited molecular markers are available at present that could verify...
the position of a patient in the spectrum of CML diagnosis [4]. Another potential approach is to target the specific genes which are over expressed in CML stem cells. Recently, RNA interference (RNAi) has been promising tool developed for sequence-specific gene silencing of the disease-associated genes. The phenomenon of growth inhibition and cancer cells progression is a challenging task with impactful outcomes. Hence RNA interference (RNAi) is a flourishing technology with hopeful therapeutic results for the down regulation of related genes.

Overall, the treatment options for CML patients are increasing, and that is likely to continue in future.

References


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