Changes in molecular biology of chronic myeloid leukemia in tyrosine kinase inhibitor era

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Abstract: Chronic myeloid leukemia (CML) is a clonal myeloproliferative disease characterized by a reciprocal translocation between long arms of chromosomes 9 and 22 t(9;22) that generates the BCR-ABL fusion gene. If left untreated, newly diagnosed chronic phase CML patients finally progress to accelerated and blastic phase. After the introduction of tyrosine kinase inhibitors (TKIs), treatment strategies of CML changed dramatically. However, the development of resistance to TKIs started to create problems over time. In this review, the current information about CML biology before and after imatinib mesylate treatment is summarized.

Keywords: Chronic myeloid leukemia, molecular biology, imatinib mesylate

Introduction

Chronic myeloid leukemia (CML) is a malignant myeloproliferative clonal disorder of haematopoietic stem cells resulting from a translocation between chromosomes 9 and 22 t(9;22) (q34;q11) or its variants t(V;9;22) [1]. This reciprocal translocation generates the shortened 22q known as the Philadelphia (Ph) chromosome and the new fusion oncogene is called as BCR-ABL (Breakpoint Cluster Region-Abelson Leukaemia). This oncogene encodes a chimeric 210 kD Bcr-Abl protein that incorporates an activated Abl tyrosine kinase domain, which is a well documented underlying reason for the malignant transformation in CML [1-3]. CML accounts for 15-20% of the newly diagnosed cases of adult leukemias. Most of the CML patients have been diagnosed in chronic phase (CP), but as a result of genomic instability, it progresses to ill-defined unstable accelerated phase (AP) and then to the terminal blastic crisis phase (BP) over time, becoming increasingly resistant to therapy [4].

Implementation of tyrosine kinase inhibitor therapy

Before 2000s, CML has been treated with hydroxyurea and interferon therapy that provide temporary disease control but do not alter progression to advanced disease with a median survival ranging 45-55 months from diagnosis [4]. Therefore, the most effective treatment strategy was allogeneic stem cell transplantation (ASCT). The recognition of the BCR-ABL oncogene and the corresponding protein led to the synthesis of small-molecule drugs, designed to interfere with BCR-ABL tyrosine kinase activation [5]. After the introduction of targeted treatment with tyrosine kinase inhibitors (TKIs), treatment strategies and outcomes were changed dramatically. Imatinib is the first used, generally well tolerated TKI that targets the tyrosine kinase activity of Bcr-Abl in CP-CM [6]. Imatinib was introduced into clinical practice in 1998. Imatinib is an ABL tyrosine kinase inhibitor of the 2-phenylamino pyrimidine class blocking the inactive conformation of BCR-ABL protein. This prevents the transfer of phosphate group from adenosine triphosphate (ATP) to substrates, and blocks the downstream signal transduction pathways. Thus, imatinib voids the inhibition of proliferation and induction of apoptosis [7]. Imatinib became the first choice for the treatment of CP-CML because of its high efficacy, low toxicity and ability to maintain durable hematological and cytogenetic responses. In the International Randomized Interferon
versus STI571 (IRIS) trial, the 8-year follow-up data revealed an estimated overall survival of 85% for imatinib suggesting a high and persistent efficacy of this TKI in CML-CP [8]. Despite high rates of hematologic and cytogenetic responses, primary refractory disease and drug resistance have been observed in 25% of patients with imatinib monotherapy, which is often caused by the domain mutations of BCR-ABL kinase, that prevent imatinib binding [4].

Current definitions of biology of chronic myeloid leukemia after imatinib

Imatinib has been shown to induce a complete haematologic response in CP-CML patients [9]. However, imatinib has been unable to completely eliminate BCR-ABL-expressing leukemic cells [10, 11]. It has been shown that imatinib prolongs survival of mice with BCR-ABL-induced CML, but does not cure the disease [12]. Shortly after the introduction of imatinib, the impressive success of the drug as a front-line therapy in CML has been tempered by problems such as disease persistence or relapse arising from different mechanisms, including duplications, mutations in the kinase domain of the BCR-ABL protein and mechanisms independent from BCR-ABL activity. Growing evidence has also suggested a pivotal role of persistent leukemic cancer stem cells, characterized by high self-renewal and pluripotency, in CML maintenance and/or relapse [13]. Stem cells may escape imatinib-mediated apoptosis due to the inability of imatinib to attack quiescent stem cells [10]. Although the heterogeneous development of imatinib resistance is challenging, the fact that BCR-ABL is active in many resistant patients suggests that the chimeric oncoprotein remains as a good therapeutic target. However, patients with clonal evolution are more likely to have BCR-ABL-independent mechanisms of resistance [14]. Imatinib-resistance mechanisms are shown in Table 1.

Duplications

The development of imatinib-resistance was firstly described in 2000 through BCR/ABL oncogene amplification [15]. Mahon, Weisberg and le Coutre demonstrated an amplification in the Abl sequence in vitro by generating imatinib-resistant cell lines using BCR-ABL-transformed murine hematopoietic cells and BCR-ABL-positive human cell lines [16-18]. The same groups identified elevated Abl kinase activity due to a several-fold increase in the amount of BCR-ABL protein, but the value of these data was limited since these results were obtained in vitro.

Mutations

Mutations in the tyrosine kinase inhibitor binding site of BCR/ABL is another important mechanism of drug resistance. The kinase domain mutation frequency is 23% in naive CML patients [19]. 40-60% of CML patients under prolonged imatinib exposure that have clinical resistance, harbour BCR-ABL kinase domain mutations [20]. Point mutations were observed in approximately 35-70% of patients displaying imatinib resistance, either spontaneously or through the evolutionary pressure of imatinib [21]. If mutations are detected prior to or early after administration of imatinib treatment, they generally predict the clinical imatinib-resistance and progression. Soverini et al reported the frequency of mutations according to disease phase at the time of diagnosis and they found that 52% of patients with AP, 75% of BP and 27% of CP CML patients had mutations. Thus, mutational frequencies appear to increase in imatinib resistance and progress from CP to BP
Mutations were observed in the critical contact points of imatinib to BCR-ABL or the most relevant biological consequence is the incapability of imatinib to inhibit the kinase activity of BCR-ABL due to prevention of BCR-ABL from adopting the inactive conformation. These mutations may also lead to disturbed function of BCR-ABL that would lead to death of the cell and would not be detectable, resulting in restoration of the BCR-ABL function and clonal selection of mutated cells resulting in reduced kinase activity. This is sufficient to allow cellular survival with imatinib-resistance and mutations of the activation loop which may result in an activated conformation of ABL insensitive to imatinib [14, 22, 23].

Hochaus and Shindler et al firstly demonstrated four regions that clustered to acquired mutations which lead to substitutions of amino acids that are important for specific binding of imatinib. These mutations were P-loop, a highly conserved region responsible for phosphate binding; T315, a non-conserved residue that is in part responsible for the selective inhibition of ABL by imatinib; and M351 and E355, mutations of the activation loop, resulting in an activated conformation of ABL insensitive to imatinib [7, 14]. Soverini et al demonstrated that 85% of all imatinib-resistant mutations are associated with amino acid substitutions at seven residues (P-loop: M244V, G250E, Y253F/H and E255K/V; contact site: T315I; and catalytic domain: M351T and F359V) [22]. In recent papers, >90 different amino acid substitutions were identified in imatinib-resistant patients as shown in Figure 1 [24].

The most frequently observed mutation identified in imatinib-resistant CML patients is, T315I mutation [25]. T315I mutation is a single C→T nucleotide substitution at position 944 of the Abl gene, resulting in a threonine to isoleucine substitution at amino acid 315 (Th315—Ile315; T315I) in the Bcr/Abl protein [26]. It was shown that the T315I mutation is associated with poor prognosis by increasing oncogenicity and promoting progression, or related to the pleiotropic resistance to TKI, in several studies [27-29].

Figure 1. Map of all the amino acid substitutions in the Bcr-Abl kinase domain identified in clinical samples from patients reported to be resistant to imatinib in published papers [24].
In the GIMEMA study, P-loop mutations were found in 43% of patients. The frequency of P-loop mutations clearly increases in AP and BC as well as with disease duration. Therefore, patients with CML in these phases tend to develop imatinib-resistance mutations. P-loop mutations in Y253F and E255K exhibited an increased transformation potency that correlates with intrinsic BCR-ABL kinase activity [30]. P-loop mutations were reported as detectable 2.8 months before the development of resistance in patients having imatinib treatment as compared to 6.3 months for T315I mutations and 10.8 months for M351T mutations [31]. P-Loop mutations have also been suggested to cause worse outcome in terms of time to progression and inferior overall survival [20, 22]. Thus, earlier detection of the P-loop mutations may provide clinical benefit for patients by earlier reconsideration of the therapeutic interventions [32]. Mutational frequencies appear to increase the progress from CP to BP in several studies [15, 22]. Hochhaus et al did not find any difference in the setting of hematologic resistance between patients with and without a mutation regarding the time to progression [14].

Capdeville et al reported that 40% of late CP CML patients fail to reach a major cytogenetic response (MCgR) on imatinib therapy, and these patients experienced the disease progression to BC more quickly than those who obtain cytogenetic remission [33]. This poor prognosis was also confirmed in terms of survival [22]. In vitro studies suggested that different mutations confer different degrees of resistance to imatinib [34]. Although some mutations like T315I confer a true resistant phenotype, thereby suggesting withdrawal of imatinib in favor of alternative treatment options, others (ie, M351T) might be overcome by dose escalation.

**Mutation-independent resistance to imatinib**

Imatinib-resistance arises very rarely in patients that are treated with imatinib in early CP-CML. This implies that BCR-ABL independent factors such as the cellular context of BCR-ABL expression and stage of the disease decisively control the evolution of imatinib-resistance [20].

The Bcr-abl protein is composed of many domains. The Abl sequences encode a tyrosine kinase domain as well as Src-homology (SH3 and SH2) domains, a DNA binding domain, nuclear localisation signals and a nuclear export signal. The BCR/ABL fusion protein acts as an oncoprotein by activating several signaling pathways that lead to transformation. Myc, Ras, c-Raf, MAP/ERK, SAPK/JNK, STAT, nuclear factor kappa-B (NF-κB), phosphoinositole 3-kinase (PI-3K) and c-Jun are included as signal cascade molecules regulated by the Bcr/Abl activity [35]. It has also been shown in vitro that mutations outside the kinase domain in the neighbouring linker, SH2, SH3, and Cap domains can confer imatinib-resistance. In the context of ABL, these domains have an autoinhibitory effect on the the kinase activity and mutations in this region can activate the enzyme. BCR-ABL independent survival in the presence of IM reflects the inherent/innate or acquired features of BCR-ABL+ leukemic populations. Whereas acquired, the BCR-ABL independent resistance refers to signalling pathways that are activated in response to imatinib exposure [22]. Some additional mutations in substrate binding site, C-helix, A-loop, and C-terminal lobe have been characterized throughout the Abl sequence [22, 30, 36, 37].

Burchet et al also demonstrated a heterogeneous Akt-signaling-cascade activation during the manifest of imatinib-resistance independently from BCR/ABL-kinase mutations [38]. Activation of the PI-3K/Akt pathway is crucial for survival and proliferation of leukemic cells in CML [39]. Imatinib treatment activated the PI-3K/Akt/mammalian target of rapamycin (mTOR) pathway in BCR/ABL positive LAMA cells in a CP CML patient in vivo. In fact, the PI-3K/Akt activation critically mediated survival during the early phase of imatinib-resistance development before the manifestation of BCR/ABL dependent strong imatinib resistance such as through a kinase mutation [38]. Activation of the PI-3K/mTOR signaling increased the formation of reactive oxygen species and thereby contributing to BCR/ABL transformation. Thus, selective mTOR pathway activation may translate into a differential effectiveness of mTOR inhibitors in overcoming the imatinib-resistance [38].

Expression of the MDR-1 gene that encodes the multidrug resistance protein, P-glycoprotein...
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(Pgp), was implicated in the mechanism of drug resistance in most chemotherapies. Mahon et al demonstrated that reduced imatinib intake is also mediated by the overexpression of the Pgp efflux pump in cells derived from a BP-CML patients [16].

Zhao et al demonstrated that when BCR-ABL transformed cells were selected for imatinib-resistance in vitro, the cells that grew out displayed higher BCR-ABL expression comparable to an increase observed in accelerated forms of the disease. BCR-ABL transformation is associated with cell-autonomous proliferation. This enhanced expression of BCR-ABL was associated with an increased rate of glycolysis but a decreased rate of proliferation. The higher levels of BCR/ABL expression in the selected cells correlated with a non-hypoxic induction of HIF-1α that was required for cells to tolerate enhanced BCR/ABL signaling. HIF-1α induction resulted in an enhanced rate of glycolysis but reduced glucose flux through both the TCA cycle and the oxidative arm of the pentose phosphate pathway (PPP). The reduction in oxidative PPP mediated ribose synthesis was compensated by the HIF-1α-dependent activation of the non-oxidative PPP enzyme, transketolase, in imatinib-resistant CML cells. The increased glycolysis and decreased cell proliferation in resistant cells were both found to depend on the non-hypoxic activation of HIF-1α [40].

Other target pathways

Kinase domain mutations constitute 30-50% of the reason for imatinib-resistance. There are different molecular determinants that contribute to the sensitivity and resistance of tumour cells to imatinib-induced apoptosis. It has recently been suggested that p53 may be an important mediator of the imatinib induced apoptotic response, and, a deficiency in p53-signaling pathway antagonizes this response and mediates imatinib-resistance [41]. Increased expression of bcl-2 due to loss of ICsBP also mediates imatinib-resistance [20]. Sun et al showed that BCR/ABL independent activation of the JAK-2/STAT-5 signal transduction cascade causes imatinib-resistance due to antiapoptotic effect of this cascade [42]. JAK-2 also plays a role in cytokine induced BCR/ABL independent imatinib-resistance [43]. The organic cation transporter-1 (OCT-1) is the major active influx pump responsible for the transport of imatinib into target BCR/ABL positive cells. Imatinib is a substrate for OCT-1, and thus alterations in the expression and function of OCT-1 have a role in imatinib-resistance in progenitors [44, 45].

Bioactive sphingolipids are important second messengers regulating important cellular functions such as growth, proliferation, cell cycle, apoptosis, drug resistance, senescence and quiescence [46]. It was shown by our group that there were significant increases in expression levels of glucosyl ceramide synthase (GCS) and sphingosine kinase-1 (SK-1), converting the apoptotic ceramide to antiapoptotic glucosyl ceramide and sphingosine-1-phosphate (S1P), respectively, in imatinib resistant cells as compared to sensitive CML cells [47]. Interestingly, we have also shown that sphingosine kinase-1 regulates the expression levels of BCR/ABL and its protein stability through S1P receptor-2 (S1PR-2) [48]. More importantly, we have shown that inhibition of GCS and SK-1 by molecular or biochemical approaches restored imatinib sensitivity [47-49]. Interestingly, down-regulation of SK-1 and S1PR2 by siRNAs inhibited the expression levels of BCR/ABL and induced apoptosis both in wild type and mutant form of BCR/ABL positive CML cells [48]. On the other hand, imatinib by itself increased the intracellular concentrations of apoptotic ceramide through the induction of the ceramide synthase genes [47].

CML stem cell: is it real?

CML was the first cancer shown to be initiated at the hematopoietic stem cell (HSCs) level by BCR/ABL, and thus it is a hallmark for understanding the molecular evolution of cancer. Stem cells have some functional properties such as the capacity to become dormant, acquire multi-lineage differentiation potential, survival and self-renewal [50]. Granulocyte-macrophage progenitors have been identified as potential leukaemic stem cells for human CML myeloid blastic crisis [51, 52]. The function of the BCR/ABL expressing HSCs as the stem cells in mice was tested by Hu and coworkers. When C57BL/6 (B6) bone marrow cells transduced with BCR/ABL retrovirus were sorted into two separate populations (Sca-1+ or Sca-1−), only the BCR/ABL-transduced Sca-1+ cells transferred lethal CML to secondary B6 recipi-
ent mice, suggesting that early bone marrow progenitors contain CML stem cells [53]. Discontinuation of imatinib even in the rare patients with complete molecular response causes recurrence of the disease [54]. This suggests the existence of stem cells in CML. Malignant bone marrow progenitors play an integral role in disease progression and TKI resistance in CML by reprogramming of the progenitors [55]. Abnormal activation of the signal transduction pathways and epigenetic events that regulate survival, differentiation and self-renewal can occur with this reprogramming (Figure 2).

Strategies for overcoming CML stem cell: where are we now?

Discovery of the TKIs targeting the BCR/ABL1 kinase, revolutionized CML therapy. However, TKIs are still unable to eradicate the disease due to the presence of a drug-insensitive stem cell population that sustains continued growth of the malignant cells [56]. Although the kinase domain mutations are infrequently detected in newly diagnosed CP CML patients, these mutations were found in a substantial number of patients when the CD34⁺ stem cells were analyzed [57]. Corbin et al reported that human CML stem cells do not depend on BCR/ABL activity for survival and are thus less responsive to imatinib therapy and act as a reservoir for the emergence of imatinib-resistant subclones. Imatinib inhibited the BCR/ABL activity to the same degree in all stem (CD34⁺CD38⁻CD133⁺) and progenitor (CD34⁺CD38⁻) cells, and in quiescent and cycling progenitors from newly diagnosed CML patients [58]. This is an important step towards understanding how to approach the persistent disease with the ultimate goal of leukemic stem cell eradication as a means to achieve a cure. When imatinib therapy is discontinued in patients with undetectable BCR/ABL levels by RT-PCR, they usually experience recurrence of active leukemia. Leukemic cells persist in most patients even when the disease burden is reduced below the detectable limits. Therefore, the current recommendation is lifelong continuance of the therapy [53].

The detection of pre-existing mutations in primitive stem/progenitor (CD34⁺) cells may have therapeutic and prognostic implications and is likely to be helpful in optimizing the management of CML patients.

Conclusion and future perspectives

TKIs implantation is a breakthrough in the treatment of CML but the resistance is still a significant clinical problem. TKI resistance mechanisms are therefore are well investigated. Resistance resulting from CML stem cell is
becoming more important over time. Therefore, the determination of the molecular mechanisms of TKI resistance can provide an important avenue for the more effective treatment of CML patients through biochemical or molecular targeting of these mechanisms that provide survival advantage for the leukemic cells.

Disclosure of conflict of interest

None of the authors have any interests which might influence the compilation of the current literature in this subject. We apologize to the authors whose valuable studies were not included here due to space limitations and the concentrated scope of the review.

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