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Mini Review

Multi-Targeted Approach to Treat Drug Resistant CML Using Natural Compounds: A Double Edged Sword

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Abstract

Chronic myelogenous leukemia (CML) is a hematopoietic stem cell disorder which arises due to translocation of ABL gene on chromosome 9 to BCR gene present on chromosome 22. This produces BCR-ABL oncogene which is the main cause of CML. Imatinib mesylate has been the choice for the treatment of initial chronic phase of the disease. However, through BCR-ABL gene over expression, kinase domain mutations, importantly T315I CML cells acquire resistance to imatinib and other second-generation tyrosine kinase inhibitors. In this mini-review, we will be analyzing the normal functions of c-Abl and the aberrant signaling pathways activated upon its loss of regulation. The causes of drug resistance and some of the possible routes to combat drug resistance is defined. The natural compounds and their significance in drug discovery and the efficacy of tyrosine kinase inhibitors by combinational treatment with natural compounds to overcome the adverse side effects and drug resistance also examined..

Keywords: Chronic myelogenous leukemia (CML); BCR-ABL; Imatinib; Drug resistance; Natural compounds; Tyrosine kinase inhibitors

Mini Review

Chronic myelogenous leukemia (CML) is characterized by excessive proliferation of myeloid lineage of cells. The abnormality is caused due to constitutive tyrosine kinase activity of fusion protein Bcr-Abl, resulting from reciprocal chromosomal translocations of ABL from chromosome 9 to BCR on chromosome 22 [1]. The normal cellular Abl (c-Abl) is a multi-domain protein that belongs to non-receptor tyrosine kinase family. It phosphorylates proteins at tyrosine residues. Phosphorylation activity of Abl kinase is under allosteric regulation by its SH2 domain [2,3]. Abl influences several protein-protein interactions and enzymatic activity and localization. C-Abl associates a variety of sub cellular proteins including signaling adaptors, kinases, phosphatases, cell cycle regulators, transcription factors and cytoskeletal proteins [2,4]. DNA damage response of c-Abl is mediated by Ataxia Telangiectasia Mutated (ATM) and Abl critically modulates the epigenetic and non-epigenetic regulators of DNA damage and apoptosis [5,6]. Normal c-Abl is auto-regulated both by inter and intra-molecular interactions of its N and C terminal domains [7]. The Bcr-Abl fusion protein, produced as a result of the translocation posses consistently elevated tyrosine kinase activity [8]. Normal cellular Abl p145 shuttles between nuclear and cytoplasm compartments. The fusion protein Bcr-Abl retains in the cytoplasm and activates aberrant cell signaling pathways that leads to the halt of apoptosis and induces proliferation [9]. Furthermore, Bcr-Abl protein directly or indirectly activates of Signal transducer and activator transcription 5 (STAT5)/B-cell lymphoma-extra-large (Bcl-xL), extracellular signal–regulated kinase 1/2 (Erk-1/2), phosphatidylinositide 3-kinase (PI3K)/Ak strain transforming (Akt), Src signaling molecules by Phosphorylation [9,10]. The auto-phosphorylated Bcr-Abl provides the docking site, tyr-177 of Bcr domain, for these signaling molecules [11]. Constitutively active Bcr-Abl further drives several survival pathways that provide proliferative advantage and drug resistance in CML [12]. Src kinases, such as Lyn, Hck, Fgr, gets activated downstream of Bcr-Abl signaling upon direct complex formation with the Bcr-Abl and also involved in the activation of other downstream signaling molecules thus Bcr-Abl requires Src kinases for its transforming activity [13]. Therefore, simultaneously targeting Bcr-Abl and Src kinases is proven an effective strategy by Dasatinib, a dual tyrosine kinase inhibitor [14]. Inhibition of Src family kinase (SFK) with pyrrolo pyrimidine inhibitor, A-419259, inhibits the cell growth and promote apoptosis in CML cell lines which indicates their transforming potential in CML progression [15]. The transition of chronic phase to accelerated blast phase in CML progression requires Src kinases Lyn, Hck, Fgr. Lyn plays a role in cytokine

signaling in a variety of cells and shown to have role in cell growth and apoptosis in hematopoietic cells [16,17]. Lyn over expression was observed in imatinib resistant CML cells it has inhibitory effect on apoptosis and can provide the resistance to the Imatinib and Nilotinib [18]. Both the Src kinases Lyn and Hck get activated upon complex formation with the Bcr-Abl by kinase independent mechanism. Src kinases phosphorylates tyr177 residue of Abl kinase thus substituting the role of auto-Phosphorylation activity of kinase defective Bcr-Abl [19]. Src kinases play different roles in different lineage of hematopoietic cells in Bcr-Abl dependant and independent mechanisms and provide survival advantage by the activation of different downstream targets [20].

High throughput screening (HTS) and combinatorial chemistry in the 90's, leads the anti-cancer drug discovery towards targeted therapies [21]. Gleevec and Herceptin are the successful out comes from the targeted therapies [22,23]. In the case of CML, selective inhibition of Abl kinase is an attractive strategy in the CML treatment. Targeted inhibition of deregulated Abl kinases enzymatic activity using ATP binding site inhibitors, interfere with Abl substrate Phosphorylation and thereby inhibit tumor cell proliferation, progression and survival. Highly selective imatinib is ineffective upon mutation which brings the conformational flexibility to the Bcr-Abl. T315I point mutation is one that brings conformational change at the ATP binding pocket of Bcr-Abl there by resistance to the imatinib. The acquired resistance for one targeted drug could be compensated with an alternative drug or by simultaneously targeting another essential protein involved in the pathogenesis process [24]. In silico screening of drug like molecules for the Bcr-Abl wild type and T315I mutant results in identification of some of the novel leads to combat drug resistance [25].

Currently, there is a need to identify novel tyrosine kinase inhibitors that target the different region of Bcr-Abl and the other downstream molecules of Bcr-Abl activated pathways. In addition, new ways of TKI utilization must be investigated in order to overcome adverse side effects involved with treatment and to improve patient survival.

Traditional Chinese Medicine (TCM) is used as alternative or complementary medicine and has been used for years to treat different cancers. TCM components stabilize tumor lesions, improve symptoms, enhances the quality of life, prolong survival time [21]. Natural compounds of TCM are highly diverse and chemically complex and the rich source for the identification of novel molecules for different cellular targets. Natural inhibitors identified from ZINC database by In Silico virtual screening against Bcr-Abl were shown to be promising upon in vitro testing [26]. Natural compounds possess privileged structures and are chemically complex and diverse that interacts with several cellular targets simultaneously to bring the optimal outcome. Using natural compounds as drugs has been proven as safe and exhibits less side effects [27].

Current tyrosine kinase inhibitors imatinib, Nilotinib, Dasatinib, Bosutinib and Ponatinib are potent in Bcr-Abl targeted treatment of CML but the side-effects and drug-resistant mutations in BCR-ABL limit their capability. Natural compounds that inhibit Bcr-Abl or other downstream targets involved in progression of CML need to be identified as an alternative or as a complementary to these synthetic drugs. Some reports showed combination of natural compounds with synthetic drugs minimizes the dosage toxicity of the drug and optimizes the response compared to single agent alone [28]. The drug combination of current synthetic tyrosine kinase inhibitor ponatinib and natural compounds produced in the roots of the plant Coleus forskohlii showed effectiveness towards highly drug resistant mutant T315I along with other CML cell lines [29]. A triterpenoid Stelletin B isolated from marine sponge Jaspisstellifera were shown to be effective as a single agent or in combination with imatinib on CML cell lines. It mediates apoptosis in CML cells through the inhibition of STAT-5, PI3K and also it modulates the drug efflux mechanism of multidrug resistance proteins MDR1 and MRP1 in CML cells [30]. Similarly α-bisabolol, a small plant-derived oily sesquiterpene alcohol also revealed striking synergism with imatinib and nilotinib on Bcr-Abl positive cell lines. It illustrated the pro-apoptotic effect by Bcr-Abl independent cell death mechanism caused by the loss of plasma and mitochondrial membrane integrity [31]. Homoharringtonine is another natural alkaloid produced from various Cephalotaxus species. In CML its effectiveness is independent of Bcr-Abl mutations. It showed to have potency over the T315I mutant Bcr-Abl [32].

Cancer cells exhibit abnormal ROS level with high oxidative stress. Some natural compounds further promote the oxidative stress by the generation of ROS and induce oxidative stress-mediated cell death. Pharmacological ROS modulation perspective could be utilized for selective killing of cancer cells [33]. Natural compound β-phenyl ethyl isothiocyanate (PEITC) sensitizes CML cells and induce cell death by the generation of ROS. It demonstrated to have effectiveness in killing Gleevec resistant cells harboring T315I mutation [34]. The fungal metabolite chaetocin inhibits thioredoxin reductase-1 thereby induces oxidative stress and showed potency in the eradication of leukemic stem cells (LSCs) which are resistant to the drug imatinib. The enhanced efficacy of chaetocin observed in the presence of bone marrow stromal cytokines and growth factors (BMSFs) [35]. Hydroxychavicol (HCH) a component present in the alcoholic extracts of Piper betel leaves induces the apoptosis in CML cells including drug-resistant T315I cells. It mediates apoptosis by ROS dependent JNK activation [36]. The tyrosine Phosphorylation activity of Bcr-Abl increased upon treatment with ROS generating H2O2 [37] but interestingly the natural compounds that generate ROS inhibit the growth of Bcr-Abl positive cells by Bcr-Abl independent mechanism. It indicates natural compounds potential to modulate ROS mediated signaling towards the desired therapeutic effect. The mechanism by which they mediate the therapeutic effect requires thorough investigation.

Conclusion

The clear understanding of disease pathology and involvement of different downstream singling molecules could facilitate the design of new treatments for CML. Natural compounds could interact with multiple targets simultaneously and bring the

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potential outcome. In addition, natural compounds can be used as an adjunct with TKI inhibitors, providing one of the best approaches to treat drug-resistant CML.

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