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Mini Review

PPAR-gamma in overcoming kinase resistance in chronic myeloid leukemia

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Abstract: Peroxisome proliferator-activated receptor gamma (PPARγ) plays key roles in regulating cellular differentiation, proliferation and apoptosis pathways. As such, they are considered promising targets for anticancer drug development, especially for breast cancer, multiple myeloma and hematologic malignancies. Chronic myeloid leukemia (CML) is a myeloproliferative disorder arising from an oncogenic Bcr-Abl tyrosine kinase. Inhibitors of this oncogene by small molecules such as imatinib are effective only in 75% of the patient's population. One of the potential strategies to overcome this resistance is to devise combination therapy protocols with other therapeutic agents including PPAR ligands. Since PPAR ligands are potentially interesting in different hematologic malignancies, this article will review the potential of PPAR ligands for use in CML treatment.

Key words: Chemotherapy, CML, Drug resistance, imatinib, PPARγ.

Introduction

Chronic myeloid leukemia (CML) is a cancer of the white blood cells that starts in certain blood-forming cells of the bone marrow. CML is a myeloproliferative disorder arising from a translocation between chromosomes 9 and 22, which results in production of oncogenic Bcr-Abl tyrosine kinase (1). Such a discovery has led to the development of Bcr-Abl tyrosine kinase inhibitors such as imatinib, nilotinib and dasatinib. Imatinib was the first tyrosine kinase inhibitor of its kind to be used in patients with CML, but unfortunately it is only effective in 75% of the cases (2, 3). Therefore, alternative therapeutic agents or strategies are needed to overcome this problem. Considering the potential effects of peroxisome proliferator-activated receptor (PPAR) ligands in different hematologic malignancies, this article will review the potential of PPAR ligands for use in overcoming drug resistance in chronic myeloid leukemia CML treatment.

Drug resistance

As shown in Fig. 1, the efficacy of a given chemotherapy regimen is largely dictated by drug resistance, making it a frontline topic in cancer research (4). Drug resistance occurs during or shortly after chemotherapy and can cause rapid disease progression (5). In addition, drug resistance limits the treatment choices for patients, especially in in conditions where treatment options are few. Resistance to chemotherapeutics can be roughly divided into intrinsic (primary) and acquired (secondary) categories. In the so called intrinsic resistance, the factors causing resistance are present even prior to drug exposure in the tumor cells. On the other hand, sometimes tumor cells are initially sensitive to the drug and only during or after chemotherapy, they developed resistance due to some genetic mutations. A number of the main mechanisms that could be utilized by CML to resist cytotoxic drugs are summarized below.

Mechanisms of resistance: Bcr-Abl-dependent and independent mechanisms

There are five categories of resistance. Point mutations in the Bcr-Abl kinase domain (KD) can lead to resistance, particularly secondary resistance, and are responsible for treatment failure in many cases (6). Numerous mutations have been characterized throughout the Abl sequence, including the ATP phosphate-binding loop (P-loop), and substrate-binding site mutations (7).

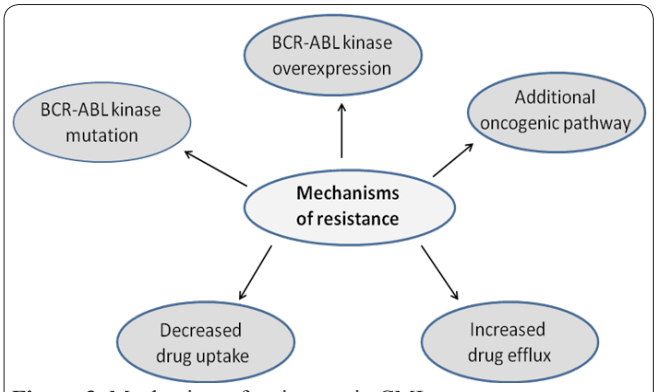

Figure 3. Mechanism of resistance in CML.

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Point mutations can cause conformational changes in the imatinib binding site of Bcr-Abl, thus blocking imatinib binding and reducing its efficacy.

More than 100 different point mutations have been determined in Bcr-Abl, the most important of which are T315I, Y253H and F255K (8, 9). The T315I mutation for example, results in a threonine to isoleucine substitution at amino acid 135. Subsequently, an oxygen molecule which is required for hydrogen bonding between imatinib and the Abl kinase is eliminated (10). This mutation results in resistance to imatinib, nilotinib and dasatinib and has been associated with the long-term outcome of chemotherapy (11). On the other hand, the clinical relevance of other mutations discussed above, is still under debate and research.

Amplification of the Abl kinase oncogene has been observed in several studies (12, 13). However, in the majority of patients, it was not shown to be primarily responsible for treatment failure. Reactivation of Bcr-Abl signal transduction is another mechanism of resistance and has been associated with both Bcr-Abl point mutations and gene amplification (14).

Bcr-Abl-independent mechanisms of resistance to imatinib include i) increased expression of P-glycoprotein pumps which enhances drug efflux, , ii) decreased expression of the drug uptake transporter, human organic cation transporter 1 (hOCT1), iii) increased levels of serum protein α1-acid glycoprotein, which binds to imatinib and inhibits its binding to to Abl, iv) low concentration of drug in serum, and v) activation of alternative signaling pathways through Ras/Raf/MEK kinase, STAT, Erk2, or SFK phosphorylation of Bcr-Abl. Moreover, the overexpression of prostaglandin-endoperoxide synthase 1/cyclooxygenase 1, which encodes an enzyme that metabolizes imatinib, can be also involved in primary resistance (15).

Peroxisome proliferator-activated receptor gamma

Four most important domains of A/B, C, D and E/F have been determined in PPARγ structure. The N-terminal A/B domain docks a ligand-independent activation function 1 (AF-1), the E/F C-terminal domain contains ligand-dependent activation function 2 (AF-2). The C domain consists a DNA binding domain (DBD) (16). The D domain is between DBD and the ligand-binding domain (LBD). Crystallographic studies have shown that DBD contains two zinc-fingers with α-helical DBD (17). In turn, the C-terminal LBD is composed of 13 α-helices and a 4-stranded β-sheet (18). The relatively big size of LBD in PPARs allows them to interact with a wide variety of ligands. Upon ligand binding, LBD undergoes conformational changes and is heterodimerized with another nuclear hormone receptor, retinoid X receptor (RXR), and recruits co-activators such as steroid receptor co-activator 1 (SRC-1) and PPAR binding protein (PBP) with histone acetylase activity, eventually resulting in induction of transcription (16). PPAR-RXR heterodimer recognizes and binds to the peroxisome proliferator response elements (PPRE) in the promoter region of target genes containing the consensus sequence 'AGGTCA' and initiates the transcription. PPARγ is essential players in lipid metabolism and mostly recognize free fatty acids and their derivatives (19). PPARγ can interact with a gamut of endogenous and synthetic ligands (Table 1).

Table 1. Ligands for PPARγ.

Endogenous	Linoleic acid, Arachidonic acid, 13-HODE, 15d-PGJ2 9-HODE, 15-HETE Oxidized LDLs
Synthetic	JTT501, TZDs (Ciglitazone, Rosiglitazone, Pioglitazone, Troglitazone,balaglitazone), LG-100754, GW 1929

PPARγ in overcoming CML drug resistance

The improvement in the knowledge of molecular mechanisms of cancer cells has led to the introduction of many therapeutic agents with different modes of action including general cytotoxic agents such as nitrogen mustards, natural products such as Vinca alkaloids and anthracyclins and finally use of antibodies and immunotoxins (20, 21). One important issue in chemotherapy is serious toxicity to normal cells, because cytotoxic compounds lack selective effect on tumor cells. Another vital issue is the development of resistance to chemotherapy. This can result from two possible causes: inherent resistance of tumor cells due to genetic characteristics and acquired resistance following by treatment with drugs. Typically 1 of 10^6-10^7 tumor cells have inherent resistance to drugs, while most of them develop resistance to repeated treatment to anticancer agents and then become resistant to similar or different kinds of chemotherapy which is known as multidrug resistance (MDR) (22). Multidrug resistance causes a reduction in anticancer drugs concentration within cells by limiting uptake, increasing efflux or affecting membrane lipids (23). One of the most important mechanisms of drug resistance against antineoplastic agents is by the activity of membrane proteins that efflux cytotoxic drugs and limits their accumulation below cytotoxic threshold. These transporters belong to the ATP-binding cassette (ABC) superfamily (24, 25). Among them, some transporters are thought to contribute the most in drug resistance such as P-glycoprotein (P-gp), multidrug-resistance associated protein (MRP), breast cancer resistance protein (BCRP) and lung resistance protein (LRP) (26). In disseminated cancers such as hematological malignancies, chemotherapy is the preferred therapeutic method other than surgery and radiotherapy. So overcoming multidrug resistance to chemotherapy in hematologic malignancies is very important. CML is a myeloproliferative disease making up about 15% of all leukemic cases (27). This disease is easily diagnosed because patients have an abnormal chromosomal translocation (9;22) known as Philadelphia chromosome which encodes an active tyrosine kinase Bcr-Abl. Imatinib mesylate, the specific Bcr-Abl tyrosine kinase inhibitor, is the standard treatment for CML. Although, the majority of patients respond well to imatinib a subset of them relapse or grow resistance to this therapy through different mechanisms (28). Therefore, later studies were devoted to the identification of novel therapeutic agents to overcome the observed resistance.

One of the strategies to overcome the resistance was to identify novel tyrosine kinase inhibitors capable of suppressing mutant Bcr-Abl. Combining imatinib with other therapeutic agents such as PPARγ ligands would also be another option.

PPARγ is highly expressed in adipose tissue and is essentially involved in adipocyte differentiation, insulin sensitization and lipid metabolism (29). PPARγ is also expressed in many types of cancer cells including hematologic cells of bone marrow myeloid, erythroid and monocyte progenitors and has an important role in inhibiting cell growth (30). Sakamato and colleagues showed that resistance to chemotherapy in human myeloid leukemia is correlated with an increased level of glyoxalase I (GLO-1), which detoxifies metabolites formed due to increased oxidant production caused by anthracyclines such as doxorubicin, one of the drugs used in treatment of myeloid leukemia. They showed that inhibition of GLO-1 enzyme activity circumvents resistance to Dox in resistant human myeloid leukemia (31). Later to examine the effects of PPARγ ligands on GLO-1 expression, Davies and coworkers utilized troglitazone (TRG), a member of thiazolidinedione family of antidiabetic drugs, in a number of cell types such as Dox resistance human myeloid leukemia cells. They found that TRG downregulated GLO-1 gene expression and reasoned that TRG might be a possible adjunct therapy to overcome Dox resistance in human myeloid leukemia cells (32).

PPARγ agonists are also capable of affecting multidrug resistance proteins. As mentioned above, drug efflux transporters belonging to the family of ABC transporters are the most common mechanism drug resistances that actively pump the chemotherapeutic agents out of the cells leading to the reduced intracellular accumulation of drugs. PPARγ activation by TRG in Dox-resistant human myeloid leukemia cells downregulated the expression of MDR-1 gene product, P-gp, and restored the sensitivity to Dox treatment. Use of a PPARγ inhibitor, GW9662, in these cells reduced the expression of P-gp, suggesting that the observed effects of TRG were PPARγ-independent and TRG may downregulate P-gp expression by indirectly antagonizing PPARγ (33).

Defects in apoptosis signaling are another mechanism that leukemic cells develop resistance against anticancer drugs. Thiazolidinediones have been also shown to have beneficial effects in CML cells by affecting cell proliferation and apoptosis. PPARγ agonist rosiglitazone (RGZ) inhibited cell proliferation and induced apoptosis in K562 cells by caspase-3 activation, downregulation of the anti-apoptotic protein Bcl-2, upregulation of the pro-apoptotic protein Bax and decreasing telomerase activity in a dose-dependent manner (34). In a similar study which was carried out by Zhao and colleagues the growth inhibitory effects of carotenoids in combination with RGZ was examined on K562 CML cells. They showed that RGZ and carotenoids combination synergistically upregulated p21, downregulated cyclin D1 and finally inhibited cell proliferation in k562 cells greatly. Inhibition of PPARγ by GW9662 attenuated all these effects, indicating that PPARγ signaling pathway has an important role in the anti-proliferative effects of RGZ and carotenoids combination (35).

TZD18 and compound 48 are both novel dual PPARα and γ ligands that can affect proliferation and apoptosis in human myeloid leukemia cells. TZD18 downregulated cyclin D2, cyclin E and SDK-2 and upregulated CDKI $p27^{kip1}$ as well as activating caspase 8 and 9 leading to apoptosis induction and G_0/G_1 cell cycle arrest in many of human CML cell lines including K562, KU812 and KCL22. Combination of TZD18 with imatinib enhanced the anti-proliferative effects of imatinib synergistically. Inhibition of PPARγ by GW9662 and PPARα by MK882 could not reverse these effects, therefore, it is estimated that these effects are mediated through a mechanism independent of PPARγ and PPARα activation (36). Combination of compound 48 with imatinib could also inhibit the proliferation and induced apoptosis in both imatinib-sensitive and imatinib-resistant K562 and KU812 CML cells when compared to single treatment with each agent alone. Treating these cells with compound 48 could inhibit the PI3K/AKT and JAK/STATs signaling pathways, the two major downstream of Bcr-Abl, suggesting a possible mechanism for the anti-proliferative effects of compound 48 (37, 38).

In addition to impaired apoptosis and increased efflux of chemotherapeutics, reduced uptake is another mechanism contributing to resistant phenotype in CML cells. It has been shown that imatinib uptake in to leukemic cells is mainly dependent on human organic transporter 1 (hOCT1) and low expression of this transporter makes CML cells resistant to imatinib therapy (39). To examine novel tyrosine kinase inhibitors uptake by hOCT1, dasatinib a second generation tyrosine kinase inhibitor was used in hOCT1 transfected high expressing KCL22 cells. Although, dasatinib uptake was higher in hOCT1 overexpressed cells in comparison to control cells, inhibition of hOCT1 did not decrease imatinib uptake, suggesting mechanisms for dasatinib uptake other than hOCT1 transporter (40). PPAR ligands were also tested on hOCT1 and subsequent imatinib uptake in CML cells. Another major problem in imatinib therapy is the maintenance of quiescent CML stem cells and thus fewer than 10% of patients achieve complete molecular response (CMR). Combination of imatinib with a PPARγ agonist, pioglitazone, have recently described as a novel strategy for CML stem cells depletion. STAT5 is phosphorylated and activated by Bcr-Abl which is critical for the persistence of normal hematopoietic stem cells and CML stem cells. Pioglitazone reduced the expression of STAT5 and its downstream targets, HIF2A and CITED2 which are the key regulators of quiescence and stemness of CML cells, resulting in leukemic stem cell pool eradication. In the clinic, pioglitazone was added to imatinib therapy in three CML patients who had never achieved CMR. All three patients achieved sustained CMR up to 4.7 years even after withdrawal of pioglitazone. These results indicated that combination of thiazolidinediones with imatinib can be a possible strategy for quiescent CML stem cells eradication (41).

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