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Multidrug resistance in chronic myeloid leukemia

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Abstract: Chronic myeloid leukemia (CML) is characterized by the accumulation of Philadelphia chromosome-positive (Ph+) myeloid cells. Ph+ cells occur via a reciprocal translocation between the long arms of chromosomes 9 and 22 resulting in constitutively active Bcr-abl fusion protein. Tyrosine kinase inhibitors (TKIs) are used against the kinase activity of Bcr-abl fusion protein for the effective treatment of CML. However, the development of drug resistance, directed by different genetic mechanisms, is the major problem of clinical applications of TKIs. These mechanisms include mutations in the TKI binding site of Bcr-abl, overexpression of Bcr-abl, overexpression of ATP binding cassette transporters, aberrant ceramide metabolism, inhibition of apoptosis, and changes in expression levels of microRNAs. Recently, many studies have focused on understanding the molecular mechanisms of drug resistance in cancer while targeting therapies providing reversal of resistance. Cancer stem cells also have roles in tumor initiation, maintenance, progression, metastasis, and drug resistance. Uncovering the mechanisms of drug resistance can provide more efficient treatment of cancer since these findings may provide novel targets for a complete cure. In this review, we discuss recent findings on the mechanisms of multidrug resistance and its reversal in CML.

Key words: Chronic myeloid leukemia, drug resistance, tyrosine kinase inhibitor, Bcr-abl

1. Introduction

Chronic myeloid leukemia (CML) is a hematological cancer characterized by the overproduction of mature or immature myeloid cells in the peripheral blood, spleen, and bone marrow. These cells are Philadelphia chromosome (Ph)-positive in more than 90% of CML patients. The Philadelphia chromosome results from a balanced reciprocal translocation between the Abelson gene (*Abl1*) on the long arm of chromosome 9 and the breakpoint cluster region gene (*Bcr*) on the long arm of chromosome 22, t(9;22)(q34;q11). This balanced translocation results in the *Bcr-abl1* fusion gene, a constitutively active chimeric tyrosine kinase (Al-Achkar et al., 2012; Calderón-Cabrera et al., 2013; Press et al., 2013). Different sizes of the Bcr-abl fusion protein are synthesized in different leukemias. A Bcr-abl protein of 210 kDa is observed in more than 90% of CML and 30%–35% of acute lymphocytic leukemia (ALL) patients. Bcr-abl proteins of 190 and 230 kDa are detected in ALL and chronic neutrophilic leukemia patients, respectively (Chan at al., 1987; Deininger et al., 2000; Kantarjian at al., 2006; Quintás-Cardama and Cortes, 2009). Bcr-abl tyrosine kinase activity causes malignant cell transformation. The Bcr-abl oncoprotein affects some downstream signaling pathways resulting in uncontrolled cell proliferation, decreased cell apoptosis, adhesion, and differentiation. All these changes form the phenotypic features of CML (Jagani et al., 2008). There are 3 phases in CML, known as the chronic, accelerated, and blast crisis phases. The transition from chronic to accelerated phase and to blast crisis phase results from secondary chromosomal aberrations such as trisomy 8, trisomy 19, an extra Ph chromosome, and isochromosome 17q (p53 gene on 17p is lost) (Al-Achkar et al., 2012; Jabbour and Lipton, 2013).

In the United States, the annual incidence of CML is estimated at 1.0 to 1.3 per 100,000 or approximately 4800 to 5200 new cases annually. The estimated prevalence of CML in the United States was approximately 25,000 to 30,000. Therapy with imatinib has changed the demographics of CML. The annual mortality was approximately 10% for the first 2 years and 20% to 25% in the following years (Huang et al., 2012).

Until radiotherapy was discovered in the 19th century, arsenic was used for the treatment of CML. In the 1960s, busulfan and hydroxyurea were used, while allogenic stem cell transplantation has been used since the 1980s. In the 1980s, patients not suitable for transplantation were treated with interferon alpha, resulting in a survival rate

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of approximately 35% (Frazer et al., 2007). Understanding the molecular mechanisms of CML resulted in the development of tyrosine kinase inhibitors (TKIs) (Hamad et al., 2013; Baccarani et al., 2014). Treatment with TKIs increased survival rates, decreased side effects, and improved life quality. As a result, the difficulties encountered with previous therapeutic approaches have been overcome (Nasr and Bazarbachi, 2012; Hamad et al., 2013). The 2-phenylaminopyrimidines were first reported as potent protein tyrosine kinase inhibitors with selectivity for the Abl and platelet-derived growth factor receptor (PDGF-R) tyrosine kinases (Buchdunger et al., 1995, 1996).

The first developed TKI was imatinib mesylate (Glivec or Gleevec or STI571), which targets Bcr-abl protein. In CML cells, the kinase domain of Bcr-abl is phosphorylated at tyrosine residues and activated by ATP binding. Imatinib mesylate mimics ATP and inhibits its binding to the tyrosine kinase domain of Bcr-abl (Fausel, 2007). Different studies demonstrated that patients with accelerated or blast crisis phases can show resistance to imatinib (Gorre et al., 2001; Sawyers et al., 2002).

In order to solve this problem, second-generation TKIs were developed such as dasatinib (BMS-354825, Sprycel), nilotinib (AMN 107, Tasigna), and bosutinib (SKI-606). These agents showed better performance for the treatment of CML as compared to imatinib (Hamad et al., 2013). Nilotinib also binds to the ATP binding site of Bcr-abl and inhibits the signaling cascade essential for the proliferation of cells. The structure of nilotinib compared to imatinib is more compatible in terms of the ATP binding pocket site (Frazer et al., 2007). Unlike other TKIs, dasatinib binds to both the active and inactive conformations of the Abl kinase domain and targets some other kinases such as the Src family, c-Kit, PDGF-R, and ephrin-A receptor. Dasatinib is a prominent agent for imatinib-resistant CML patients (An et al., 2010). Furthermore, the newly developed second-generation drug bosutinib is used to treat solid tumors by blocking Src-family kinase and Bcr-abl activity. Unlike dasatinib, bosutinib does not target c-Kit and PDGF-R, but rather causes phosphorylation of cellular proteins and inhibits proliferation of CML cells (Weisberg et al., 2007; Cortes et al., 2012). Lastly, ponatinib is a third-generation drug specific for tyrosine kinase activity, which particularly binds to Bcr-abl. In contrast to other drugs, this treatment is efficacious against T315I-mutated CML patients (~20% of imatinib-resistant patients).

Aurora kinases, which have a significant role in mitosis, are overexpressed in cancer cells. Inhibition of these kinases causes the mitotic catastrophe of leukemia cells. Danusertib could be a substantial agent for new therapies by inhibiting all aurora and Bcr-abl tyrosine kinases (including T315I mutation) (Jabbour et al., 2013).

2. Drug resistance

Drug resistance is known as insensitivity of cancer cells and tissues to anticancer agents. When a cell shows a drug-resistance phenotype, it may also demonstrate resistance to chemically and structurally different anticancer agents. While clinical outcomes indicated the success of tyrosine kinase inhibitors, development of resistance in CML patients was reported as the major problem in treatment of CML. There are different Bcr-abl dependent and independent mechanisms contributing to multidrug resistance in cancer. These mechanisms include mutations in the TKI binding domain of Bcr-abl, overexpression of Bcr-abl, ATP binding cassette (ABC) transporters, aberrant ceramide metabolism, inhibition of apoptosis, and changes in expression levels of certain microRNAs.

2.1. Bcr-abl mutations

Point mutations in the Bcr-abl kinase domain decrease and/or inhibit the interaction of TKI and the oncogenic Bcr-abl protein depending on the location of the mutation (Figure 1). Alterations in critical contact points due to amino acid substitutions increase the failure of agent binding to the target site. In addition, drug treatment can induce mutations leading to the development of drug resistance and, thus, drug efficacy decreases during treatment of CML. Point mutations are found more frequently in advanced phase CML as compared to the chronic phase of the disease. Mutations in the genome can lead to dysfunction (An et al., 2010). It was shown that 4 regions are essential for high frequency binding of imatinib (P-loop, SH-3, SH-2, and A-loop). The P-loop is responsible for phosphate binding and mutations in this site were frequently observed in 43% of patients who were generally in the acute and blast crisis phases. The P-loop mutations Y253F and E255K increase the probability of transformation depending on Bcr-abl kinase activity. The most common mutation observed in imatinib-resistant CML patients (T315I) has isoleucine instead of threonine at the 315th amino acid in the Bcr-abl protein (Comert et al., 2013; Figure 1). In our in vitro studies, we determined that neither resistance to imatinib in K562 and Meg-01 cells nor resistance to nilotinib in K562 cells resulted from mutations in the TKI binding site of the Bcr-abl oncoprotein (Baran et al., 2007a, 2007b; Camgoz et al., 2013).

2.2. Overexpression of Bcr-abl

Overexpression of the Bcr-abl oncogene is another mechanism of imatinib resistance. Bcr-abl transformed murine hematopoietic cells and Bcr-abl positive human cells were used to show amplification in the *Abl* gene (An et al., 2010; Comert et al., 2013). In our studies, we determined significant overexpression of Bcr-abl mRNA and protein in imatinib-resistant K562 and Meg-01 cells (Baran et al., 2007a, 2007b). On the other hand, a more

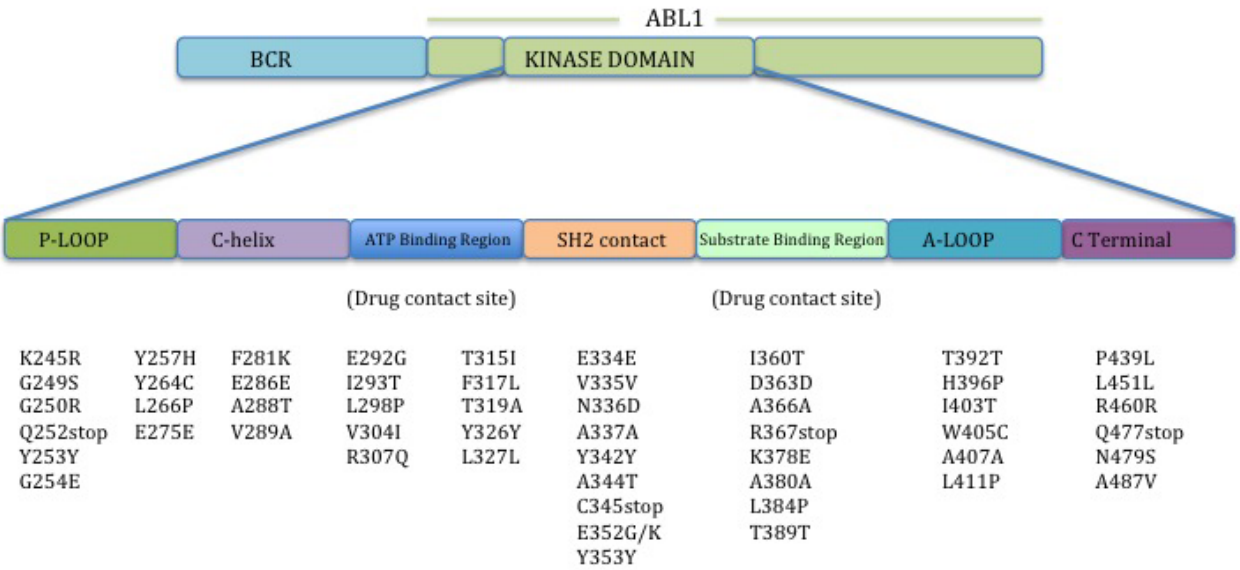

Figure 1. Distribution of the mutations with respect to the main regions of the Bcr-abl kinase domain.

recent study revealed that there was also an important increase in mRNA levels of Bcr-abl in nilotinib-resistant K562 cells (Camgoz et al., 2013). More interestingly, our group showed that there were also significant increases in protein stability of Bcr-abl in imatinib resistant cells (Salas et al., 2011).

2.3. ABC transporters

ABC transporters in the cell membrane are another important mechanism of Bcr-abl independent drug resistance (Eechoute et al., 2011). ABC transporters, encoded by 49 genes, are a highly conserved transmembrane protein family and import/export the substrate by hydrolyzing ATP. Amino acids, sugars, inorganic compounds, and hydrophobic substances are imported/exported into or out of the cells by these transporters. In addition, ABC transporters provide drug efflux across organelles and the cell membrane (Vasiliou et al., 2009). ABC transporters have 7 subfamilies, including ABC-A, ABC-B, ABC-C, ABC-D, ABC-E, ABC-F, and ABC-G. The ABC-B subfamily consists of 11 genes. The first and best characterized ABC transporter is ABCB1 [known as the multidrug resistance (MDR1) transporter] and it has a role in the multidrug resistance mechanism (Juliano and Ling, 1976). It was reported that expression levels of MDR1 are increased in imatinib-resistant K562 cells (Peng et al., 2012). Single nucleotide polymorphism (SNP) analysis in the *MDR1* gene could be effective to predict imatinib efficacy in the treatment of CML patients. A recent study demonstrated that genetic variations in the *MDR1* gene affect the drug transportation process. The relationship between MDR1 polymorphism and leukemia risk was determined according to alleles T and G at the SNP. The heterozygous genotype (GT) is related to drug resistance of imatinib. However, it was determined that recessive TT genotyped patients have developed a mechanism against resistance to imatinib (Elghannam et al., 2014). Changes in the expression levels of the *MDR1* gene resulting in increased P-glycoprotein (P-gp), the product of the *MDR1* gene, are linked to resistance in chemotherapy (Widmer et al., 2003). It was demonstrated that treatment of doxorubicin-resistant K562 cells with 1 μM imatinib in combination with the p-glycoprotein inhibitor verapamil significantly suppressed cell growth (Mahon et al., 2003). In addition, the ABCA subfamily genes (*ABCA2*, *ABCA3*, *ABCA6*) also have a role in the drug resistance mechanism (Dean et al., 2001; Vasiliou et al., 2009). It was found that expression of the *ABCA3* transporter gene and drug resistance are correlated. After the expression level of the *ABCA3* gene was decreased with specific small interfering RNA (siRNA), imatinib activity was increased in K562 and LAMA 84 CML cells (Chapuy et al., 2009).

2.4. Organic cation transporters (hOCT1)

Human organic cation transporter (hOCT1) controls the uptake of substances through the cell membrane. Imatinib is one of the substrates of hOCT1 and is affected by expression levels of this transmembrane protein. Decreasing hOCT1 levels cause a low intracellular concentration of imatinib in the cytoplasm, and therefore the therapeutic activity of the drug is weakened in the cell (Wang et al., 2008). As a second-line treatment agent, nilotinib is administered to imatinib-resistant patients with CML. Molecular analyses revealed that transport of nilotinib is not related to hOCT1 or MDR1 (Davies

et al., 2009). The high efficacy of nilotinib in MDR1-overexpressed patients directs the treatment line in the case of resistance to imatinib (Agrawal et al., 2013). Another second-generation TKI, dasatinib, is also effective in terms of cytogenetic and hematological responses in imatinib-resistant patients. A recent study showed that inhibition of pump activities does not change the inner concentration of dasatinib despite the fact that it is a substrate of MDR1 and ABCG2 transporters (Hiwase et al., 2013).

2.5. Aberrant ceramide metabolism

Sphingolipids are bioactive metabolites that have essential roles in cellular functions such as cell cycle regulation, proliferation, metabolism, and drug resistance. Sphingolipid metabolism contains ceramide, sphingosine, glycosylceramide (GC), ceramide-1-phosphate (C1P), sphingomyelin (SM), and sphingosine-1-phosphate (S1P) (Hannun and Obeid, 2008).

The backbone of sphingosine metabolism is ceramide. Metabolism and generation of ceramide determines the fate of a cell. Conversion of SM to ceramide by sphingomyelinase is generally regulated by stress conditions (Gilbert et al., 2006; Figure 2).

Stimulation of ceramide production is mediated by Fas/CD95 triggered cell death (Lin et al., 2000). Moreover, sphingosine is synthesized from ceramide by ceramidase enzymes while the reverse reaction occurs via ceramide synthase. Ceramide is the central molecule of sphingolipid metabolism mediating programmed cell death (Figure 3). DNA fragmentation analysis as an indicator of apoptosis indicates the potency of this sphingolipid derivative on leukemia cells. This analysis allows determination of ceramide-related double-stranded DNA degradation by separation of apoptotic DNA fragments using gel electrophoresis (Jarvis et al., 1996). Moreover, application of external ceramides in combination with imatinib (Baran et al., 2007a), nilotinib (Camgoz et al., 2011), or dasatinib (Gencer et al., 2011) resulted in synergistic apoptotic effects of sensitive and drug-resistant CML cells.

In sphingolipid-mediated signaling, whereas ceramide directly recruits and activates protein kinase-C (PKC), sphingosine has the potential to inhibit PKC, so low levels of sphingosine might be responsible for noninhibition of PKC (Shirahama et al., 1997). S1P, the product of the sphingosine kinase (SK) enzyme, is another derivative that is responsible for differentiation, proliferation, and antiapoptotic regulation (Figure 2). S1P has the reverse activity of ceramide by preventing cell death triggered by extrinsic factors. The ceramide/S1P rheostat is a tightly regulated process with regard to its antagonist effect. Activation of the oncogenic enzyme SK and increased

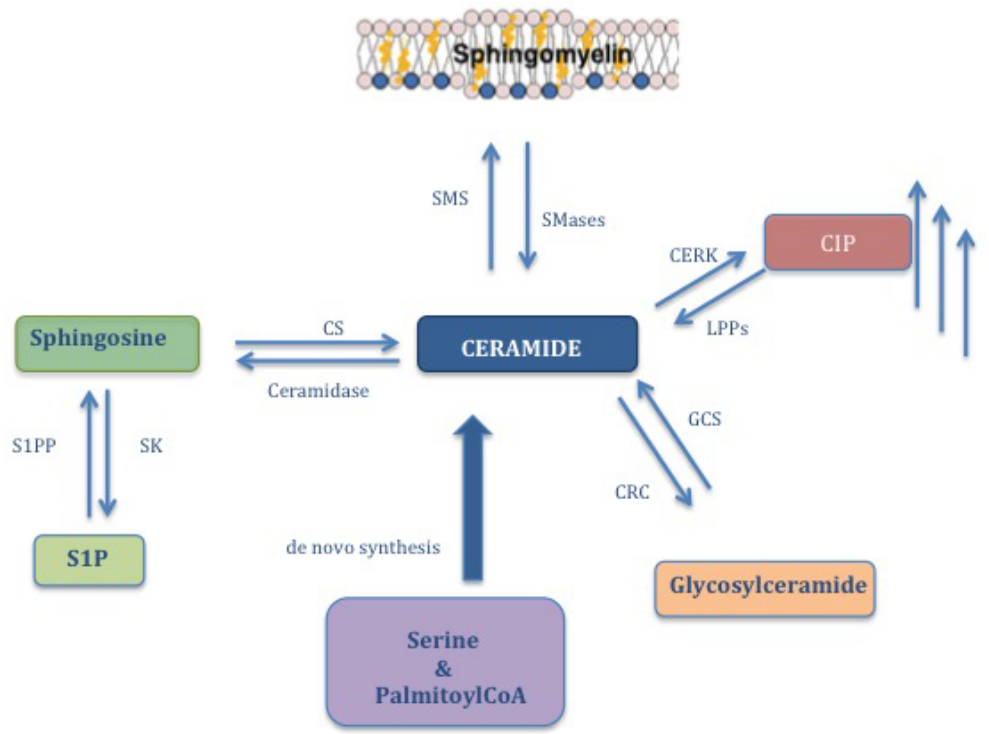

Figure 2. De novo synthases and metabolism of ceramide in sphingomyelin pathway. SMS: Sphingomyelin synthase, S1P: sphingosine-1-phosphate, S1PP: sphingosine-1-phosphate phosphatase, SK: sphingosine kinase, GCS: glucosylceramide synthase, CRC: cerebrosidase, CS: ceramide synthase, CERK: ceramide kinase, LPPs: lipid phosphate phosphatases, C1P: ceramide 1 phosphate.

concentrations of S1P reduce proapoptotic ceramide levels in the cell (Figure 3). On the other hand, an increasing level of ceramide diminishes cell survival by inducing proapoptotic molecules (Bonhoure et al., 2008). It was shown that inhibition of SK1 by siRNA or application of a SK1 inhibitor enhanced cell death and increased ceramide levels in imatinib resistant CML cells (Baran et al., 2007a, Salas et al., 2011). S1P accumulation prevents the degradation of Bcr-abl1 protein and inhibits programmed cell death; therefore, the resistance mechanism is triggered against imatinib (Ekiz and Baran, 2010).

Transfer of a glycose molecule to ceramide by glycosylceramide synthase (GCS) generates GC, an important metabolite of bioactive sphingolipids (Figure 2). High GCS activity is a significant factor in cancer progression and, more importantly, in drug resistance. This resistance becomes more advanced with the conversion of proapoptotic ceramide to antiapoptotic GC (Huang et al., 2011). It was demonstrated that T315 mutant CML cells became more sensitive after treatment with the GCS inhibitor. Inactivated glycogen synthase kinase-3 (GSK-3) in Bcr-abl signaling is reactivated by the GCS inhibitor and initiates apoptotic pathways. Therefore, the therapeutic potential of GCS inhibitor could be a novel strategy for drug-resistant patients (Liu et al., 2010). We also demonstrated that mRNA and protein levels of GCS are increased in imatinib- and nilotinib-resistant K562 cells (Baran et al., 2011). On the other hand, inhibition of GCS by application of GCS inhibitor resulted in increased sensitivity of drug-resistant cells to imatinib (Baran et al., 2011), nilotinib (Camgoz et al., 2011), and dasatinib (Gencer et al., 2011) in sensitive and drug-resistant cells.

2.6. Inhibition of apoptosis

Progression of CML through blast crisis is related to drug resistance that emerges by the inhibition of apoptosis. This resistance mechanism is accompanied by different genes or proteins that have a role in apoptotic signaling pathways. On the other hand, several polypeptides are selectively degraded by proteases. The precursor proteases, caspases, direct the apoptotic process in the cell. The Bcl-2 antiapoptotic protein family contributes to the intrinsic pathway while the inhibitor of apoptosis (IAP) protein family has a role in regulation of downstream apoptotic processes. Survival mechanisms of CML cells require the coordination of proteins to modulate apoptosis (Rumjanek et al., 2013). The *Tp53* tumor suppressor gene encodes the p53 protein and has several functions such as cell cycle regulation, DNA repair, programmed cell death, and genomic stability, making p53 one of the essential molecules in the cell (Naccarati et al., 2012). It was shown that CML progression is related to p53 mutation. CML patients whose exon 8 region of the *Tp53* gene is mutated have higher accelerated phase and blast crisis values. In addition, the molecular response is decreased during treatment with imatinib, thus increasing the influence of the mutation on CML (Mir et al., 2013). Stabilization of p53 also triggers apoptosis in CML.

Mitochondria-dependent cell death is mediated by the Bcl-2 protein family. The intrinsic apoptotic pathway is triggered by antiapoptotic Bcl-2 and proapoptotic Bax, Bim, and Bid-like proteins, which regulate cytochrome-c release. Overexpression of Bcl-2 encourages aggressive tumor progression. In an in vitro study it was indicated that K562 cells treated with imatinib have higher levels of

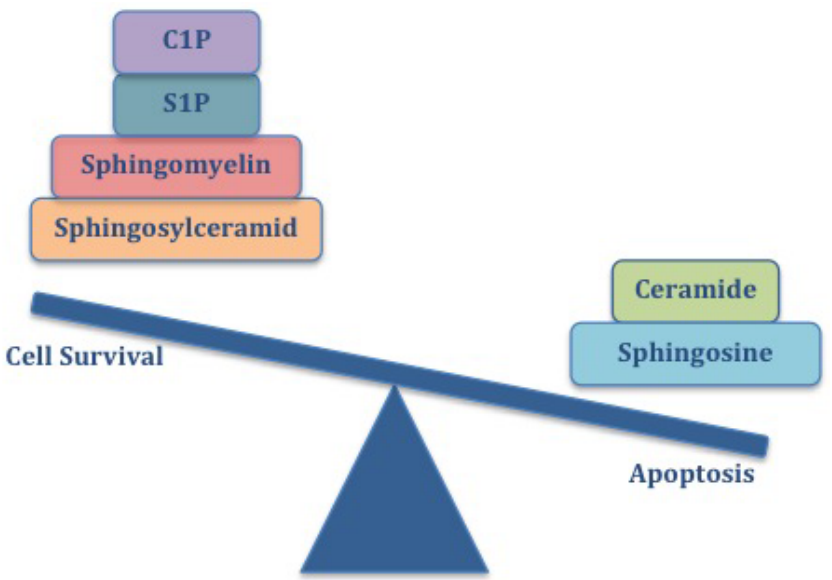

Figure 3. The balance between sphingolipids determines the cell fate mechanism (cell survival or cell death).

Bim. In addition, agents designed against Bcl-xl and Bcl-2 antiapoptotic proteins are a novel therapeutic option for the treatment of leukemias (Cirinnà et al., 2000; Kuribara et al., 2004). Extrinsic pathway-dependent apoptosis is mediated by death receptors. For instance, Fas receptors include a highly protected DISC domain inducing apoptosis through activation of caspase cascade. CD95L-stimulated tumor development supports the idea that apoptosis and tumor growth might use different pathways (McGahon et al., 1995; Traer et al., 2012; Rumjanek et al., 2013). IAP is associated with the inhibition of programmed cell death. IAP and XIAP (another inhibitor protein) are highly expressed in CML cells and have a strong association with Pgp/ABCB1 (Conte et al., 2005; Silva et al., 2013). Signal transducer and activator of transcription (STAT) proteins are cytoplasmic transcription factors that coordinate the cell proliferation activated by Janus kinase. In leukemia cells, STAT proteins are activated and enhance the survival and growth of cells. Therefore, the STAT signaling pathway is highly potent in therapeutic applications. In our study, we demonstrated that inhibition of STAT5A through the use of siRNA increased the apoptotic effects of imatinib in both sensitive and drug-resistant CML cells (Baran et al., 2010).

2.7. microRNAs

MicroRNAs (miRNA) are small noncoding RNAs that play important roles in the transcriptional and posttranscriptional regulation of gene expression. miRNAs match the target mRNAs and inhibit their translation. miRNAs affect many physiological and pathological processes such as apoptosis, cell proliferation, cell division, tumorigenesis, and development. Abnormal expression of miRNAs was observed in hematological malignancies including chronic myeloid leukemia, chronic lymphocytic leukemia, multiple myelomas, and B-cell lymphomas. Some miRNAs act as tumor suppressors and others may be oncogenic. miRNA levels in the cell are very important for developing new treatments (Undi et al., 2013). Different types of miRNAs are also involved in drug-induced apoptosis and drug resistance in CML (Table). For instance, miR-17-19 is downregulated in imatinib-treated CML cells. miR-21 causes the inhibition of cell migration, cell proliferation, and division, and it also induces apoptosis. Methylated miR-203 in acute myeloid leukemia (AML), CML, ALL, and chronic lymphoblastic leukemia leads to inhibition of Bcr-abl expression. miR-451 is important for erythroid homeostasis (Table). ABL1 and Bcr-abl1 are inhibited by miR-29b, and cell growth and colony formation are also inhibited (Venturini et al., 2007; Hu et al., 2010; Chim et al., 2011; Çelik et al., 2013).

3. Chronic myeloid leukemia stem cells

There are 2 basic models concerning the origins of cancer. The clonal origin suggests that tumors can be initiated by any cells in a population. The other model indicates that only certain cells in the population [defined as cancer stem cells (CSC)] can initiate tumor occurrence. Evidence for CSCs was first presented in leukemias and myelomas. It was reported that a part of purified leukemic stem cells separated from hematopoietic stem cells can give rise to new tumorigenic tissue (Park et al., 1971). The first characterization of leukemic stem cells was reported by Bonnet and Dick in AML. When CD34+/CD38– cells were isolated from AML patients and injected into NOD/SCID mice, initiation of AML and leukemic blasting in mice was observed (Bonnet and Dick, 1997).

Major problems encountered during the treatment process are tumor relapses and drug resistance, which are thought to originate from CSCs. CSCs are mainly responsible for tumor initiation, maintenance, angiogenesis, metastasis, drug resistance, and recurrence

Table. The roles of miRNAs in chronic myeloid leukemia.

ncRNA(s)	ncRNA class	Target	Clinical relevance	Citation
miR-7, -23a, -26a, -29a, -29c ,-30b, -30c, -100, -126, -134, -141, -183, -196b, -199a, -224, -362, -422b, -520a, -191	miRNA	N/A	Predictive response to therapy	San José-Enériz et al. 2009
miR-31 downregulation	miRNA	E2F2	Predictive response to therapy	Rokah et al., 2012
miR-564 downregulation	miRNA	E2F3, Akt2	Predictive response to therapy	Rokah et al., 2012
miR-155 downregulation	miRNA	E2F2, cyclin D1, K-ras, PIK3R1, SOS1	Predictive response to therapy	Rokah et al., 2012

ncRNA: noncoding RNA, N/A: not available.

of disease. It was also documented that CD34+ leukemia stem cells are insensitive to imatinib and dasatinib, and therefore these applications would be ineffective unless directly targeting leukemic stem cells to induce apoptosis (Graham et al., 2002; Hu et al., 2006).

Furthermore, the existence of CSCs is reported in other solid tumors. Breast cancer is the first solid tumor in which CSCs with the CD44+/CD24– surface marker was identified (Al-Hajj et al., 2003). Many CSCs have been identified and characterized for brain tumors, lung cancer, colon cancer, pancreas cancer, and prostate cancer so far (Singh et al., 2003; Kim et al., 2005; Ricci-Vitiani et al., 2007; Li et al., 2009; Goldstein et al., 2010).

Signaling pathways such as BMI-1, Notch, and Hedgehog have important roles in stemness and also regulate the activities of CSCs. After developing mice deficient in β-catenin in the hematopoietic cells, HSC and CSCs were isolated. Results showed a lack of the capacity for self-renewal, indicating the requirement of Wnt signaling in CSC maintenance (Zhao et al., 2007). The Hedgehog signaling pathway is as important as the Wnt signaling pathway in terms of stem cell regulation and embryonic formation. Suppression of Smoothened (Smo) decreased the triggering of CML stem cells in human (Zhao et al., 2009). In addition, it was shown that there is crosstalk among Sonic Hedgehog, Hox, and Notch signaling to induce the potential of CSCs (Sengupta et al., 2007).

Since potential drugs target cancer cells instead of CSCs, drug resistance remains the major problem during treatment. In order to prevent the production of new cancer cells by cancer stem cells and to overcome reversal of resistance, recent studies have focused on targeting CSCs. It was agreed that imatinib and other TKIs could not be effective on cancer stem cells due to disease relapse in the long-term (Corbin et al., 2011; Perl and Carroll, 2011). Distinguishing cancer stem cells from normal stem cells is another crucial point for the success of treatment. It is possible to eliminate normal stem cells by targeting the B-lymphoid kinase gene (*Blk*), which acts as a tumor suppressor in leukemic stem cells. However, this gene does not show any activity in normal hematopoietic stem cells. Decreased levels of *Blk* resulted in high potency of leukemic stem cells, while high levels of *Blk* caused inhibition of CSCs. Suppression of *Blk* by targeting its upstream regulator Pax5 or downstream effector p27 could be a possible target for elimination of CSCs (Zhang et al., 2012). Jak2/STAT5 is another potential target for CSCs and is related to drug resistance and CSC activity in leukemia cells (Jørgensen and Holyoake, 2007; Samanta et al., 2011). Compared to normal stem cells, SIRT1 (NAD+ dependent deacetylase), an inactivator of p53, is overexpressed in leukemic stem cells. It was reported that SIRT1 knockdown combined with imatinib triggered p53 activation and apoptosis synergistically in CML stem cells (Li et al., 2012). It was also shown that imatinib treatment increased the survival rate in SIRT1 gene knockout mice. Therefore, SIRT1 could be a novel target for reversal of drug resistance in CML (Yuan et al., 2012).

4. Reversal of resistance

Drug resistance is the major problem of the clinical process, causing disease reoccurrence and tumor relapse. In recent years, there have been increasing studies to overcome the problem of drug resistance. Researchers have focused on the reversal of resistance and many techniques have been developed. There are various methods such as signaling pathway targeting, direct protein targeting, nanotechnology, or knockdown/knockout techniques.

TKIs and their effects on MDR were shown as potential agents for reversal of drug resistance. The combination of imatinib and 5-bromotetrandrine has a significant reversal effect on the K562/A02 cell line by decreasing the *MDR1* gene and downregulating P-gp expression while increasing apoptosis (Chen et al., 2010). It was also indicated that nilotinib reverses resistance by blocking ABCB1 and ABCG2 transporters (Tiwari et al., 2009). On the other hand, salinomycin was found to be an effective agent to overcome ABC transporter-mediated drug resistance and apoptosis resistance in leukemic stem cells (Fuchs et al., 2010; Riccioni et al., 2010). In vivo studies have also demonstrated that imatinib combined with vincristine significantly suppresses tumor initiation in multidrug-resistant CML cells in a human-nude mouse xenograft model (Gao et al., 2006). In another study, imatinib was a highly effective agent for P-glycoprotein-mediated resistance, whereas, in imatinib-resistant cell lines, cepharanthine was reported as able to overcome the resistance of K562/MDR cells (Mukai et al., 2003).

The Hedgehog signaling pathway prominent during cell proliferation was affected by suppression of the *B4GALT1*, gene which resulted in overcoming multidrug resistance in human K562 adriamycin-resistant cells (Zhou et al., 2012). The phosphatidylinositol-3-kinase/protein kinase B (PI3-K/Akt) signaling pathway is one of the important signaling pathways for cell survival. In human leukemia cells, LY294002, an inhibitor of PI3-K, reverses P-glycoprotein-mediated resistance (Zhang et al., 2009). Human K562 leukemic cells are resistant to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) mediated apoptosis. It was shown that it is possible to reverse resistance by knocking down the DNA-PKCs/Akt pathway activated by TRAIL-induced apoptosis (Kim et al., 2009).

Nanotechnology has become an important tool for cancer treatment and reversal of resistance. Many studies

in this area have used nanoparticles. For example, as a system for targeted drug delivery, magnetic nanoparticles were used with wogonin and Fe_3O_4 for the reversal of MDR by downregulating MDR1 in K562 cells (Cheng et al., 2012). It was also indicated that the combination of daunorubicin and 5-bromotetrandrine or imatinib and 5-bromotetrandrine loaded onto iron oxide nanoparticles could overcome MDR (Chen et al., 2010; Cheng et al., 2011). Furthermore, magnetic nanoparticles with daunorubicin increased apoptosis and reversed MDR in K562-n/VCR cell vaccinated nude mice in in vivo studies (Chen et al., 2009). Targeting CSC-specific miRNAs with curcumin or epigallocatechin-3-gallate was reported as a potential technique for reversal of resistance (Wang et al., 2010).

5. Conclusion and future perspectives

Leukemia is a heavily investigated type of cancer for the development of new therapy strategies to cure the disease or increase patient quality of life. Although patients may respond to chemotherapy in the short term, after treatment, relapse can be observed. Rather than the development of new agents, it is better to focus on drug resistance and its mechanisms. A better understanding of the mechanisms of drug resistance could open new research areas and take us one step forward in cancer treatment.

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