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The Proteasome as a Therapeutic Target in Chronic Myeloid Leukemia

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1. Introduction

Chronic Myeloid Leukemia (CML) appears as a consequence of the reciprocal translocation between chromosomes 9 and 22 which results in the expression of the Bcr-Abl oncoprotein in a hematopoietic stem cell. The tyrosine kinase activity of Bcr-Abl is the direct cause of the disease. Therefore, inhibitors of this kinase activity are being used in the treatment of CML. Imatinib was the first of such inhibitors and is the first line treatment in CML. However, the appearance of Bcr-Abl mutants that are resistant to these inhibitors has emerged as a problem in the treatment of CML. Consequently, the search for new drugs or therapeutic targets is underway.

The proteasome is a multiprotein complex where proteins are degraded in a much regulated manner. Protein degradation at the proteasome is dependent upon ubiquitylation, which, in turn, is mediated by ubiquitin-ligase complexes. By degrading specific proteins, the proteasome is involved in cellular processes as important as cell cycle regulation, proliferation, differentiation and survival. As such, its activity is also related to the transformation of tumor cells. In fact, it has been shown that tumor cells show an increase in proteasome activity. Specifically, Bcr-Abl tyrosine kinase activity results in an increase of proteasome activity and degradation of some negative regulators of cell cycle progression as $p27^{kip1}$. Therefore, proteasome inhibitors could be an alternative in CML treatment.

In this chapter, we will discuss the available data suggesting that proteasome inhibition could be used in the treatment of CML, including those cases in which Bcr-Abl inhibition is not a possibility.

2. Bcr-Abl and its control of the cell cycle and apoptosis

In the t(9;22)(q34;q11) translocation found in CML, the *bcr* (Breakpoint Cluster Region) and *abl* (Abelson Leukemia) genes get fused. As a result, the chimeric tyrosine kinase oncoprotein Bcr-Abl is expressed (Ben-Neriah et al., 1986). Unlike c-Abl that shuttles between the nucleus and the cytoplasm and whose kinase activity is finely tuned, Bcr-Abl is cytoplasmic and its kinase activity is increased and deregulated (Salesse & Verfaillie, 2002). Through phosphorylation of different substrates, Bcr-Abl activates different signal transduction pathways that participate in the control of cellular proliferation, differentiation, migration, adhesion and survival (Afar et al., 1994; Andreu et al., 2005; Bedi et al., 1994;

Gordon et al., 1987; Horita et al., 2000; Jiang et al., 2000; Puil et al., 1994; Salesse & Verfaillie, 2003; Sawyers, 1993).

Several different substrates and partners of Bcr-Abl have been identified so far. Current efforts are directed at linking these pathways to the specific pathologic defects that characterize CML. These defects include increased proliferation and/or decreased apoptosis of a hematopoietic stem cell or progenitor cell (which leads to a massive increase in myeloid cell numbers), premature release of immature myeloid cells into the circulation (probably due to a defect in adherence of myeloid progenitors to marrow stroma) and genetic instability which results in disease progression (Druker, 2008).

Pathways activated by Bcr-Abl include the Ras-Raf-MAPK, the PI3K/Akt and the JAK/STAT pathways. The Ras-Raf-MAPK pathway links Bcr-Abl to the increased proliferative rate. The PI3K/Akt pathway induces survival signals through different mechanisms such as the Ras-Raf pathway, the phosphorylation of Bad, the induction of Bcl-x_L expression and the inhibition of caspase activity. Besides, it controls the activity of several transcription factors involved in the expression of cell cycle regulatory proteins, such as FoxO, belonging to the Forkhead family of transcription factors, and NF-κB (Manning & Cantley, 2007; Medema et al., 2000). Akt also regulates cell growth through mTOR and the control of protein translation and synthesis (Manning & Cantley, 2007). The JAK/STAT pathway is also a target of Bcr-Abl kinase activity. Bcr-Abl induces the expression of the antiapoptotic protein Bcl-x_L through the activation of STAT5, which binds to the promoter of the *bcl-x_L* gene, thus increasing survival of Bcr-Abl expressing cells (Horita et al., 2000).

Bcr-Abl regulates the expression and activity of the cell cycle inhibitor $p27^{kip1}$ through the PI3K/Akt pathway. The inhibition of Bcr-Abl and the inhibition of the PI3K/Akt pathway both result in an increase of $p27^{kip1}$ levels. Bcr-Abl also induces the expression of cyclins D and E and the activity of the cyclin E associated kinase Cdk2, resulting in the phosphorylation and inactivation of the tumor suppressor Retinoblastoma protein (Rb). Consequently, Bcr-Abl expressing cells show a deregulation of one of the main mechanisms controlling cell cycle progression along G1 and into S phase (Andreu et al., 2005).

Therefore, by activating several different signal transduction pathways, Bcr-Abl induces cell proliferation and suppresses apoptosis in CML cells.

3. The proteasome as a therapeutic target

Protein synthesis and degradation are key mechanisms by which protein levels are regulated inside cells. In fact, specific protein degradation has emerged as a very active field of research. Two different machineries are involved in this process: the autophagosome (which will not be considered in this chapter) and the proteasome.

3.1 Protein degradation at the proteasome

The 26S proteasome is a multiprotein complex that can be found in the cytoplasm and in the nucleus of eukaryotic cells. It is composed of a catalytic 20S core and two 19S regulatory subunits. Proteasome assembly and protein degradation are both ATP dependent (Almond & Cohen, 2002; Baumeister et al., 1997; Baumeister et al., 1998). The catalytic core has three activities: chymotryptic, tryptic, and post-glutamyl peptide hydrolytic-like activities.The regulatory subunits are responsible for substrate recognition and unfolding. The proteasome is involved in a wide variety of cellular processes including apoptosis, cell cycle and

division, differentiation and development, all of them related directly or indirectly to tumoral transformation (DeSalle & Pagano, 2001).

For a protein to be degraded at the proteasome, it must be labelled with ubiquitin. Ubiquitin is a small polypeptide of 76 aminoacids which is highly conserved in all eukaryotes. Protein ubiquitylation not only can lead to degradation but is also a mechanism involved in intracellular transport. Ubiquitin can be added to proteins as single molecules to one (monoubiquitylation) or several (multiubiquitylation) Lysine residues or as polyubiquitin chains (polyubiquitylation) using one of several Lysine residues of the ubiquitin molecule to form the chain.

Ubiquitylation requires the sequential activity of three enzymes. The C-terminal Glycine of ubiquitin is activated in an ATP dependent process by an E1 ubiquitin-activating enzyme. This reaction results in the formation of a thioester covalent linkage between ubiquitin and a Cysteine residue in E1. Activated ubiquitin is then transferred to the ubiquitin-conjugating enzyme E2 by a trans-esterification reaction. Finally, a ubiquitin-ligase E3 enzyme transfers the ubiquitin molecule to the specific target protein to be degraded, thus acting as the substrate recognizing enzyme. This conserved ubiquitylation cascade allows for different combinations of E1-E2-E3 molecules leading to the recognition, labeling and degradation of multiple target proteins (Hershko & Ciechanover, 1998).

3.2 The proteasome and the cell cycle control

Along the cell cycle, two different E3 complexes are involved in the degradation of regulatory proteins. The SCF complex contains four different subunits: Skp1, Cul1, Roc1/Rbx1 and one of several different F-box proteins. This E3 complex is necessary, for instance, for the degradation of G1 cyclins, leading to the G1/S transition. G2 cyclins, on the other hand, are ubiquitylated by the E3 APC/C (Anaphase Promoting Complex/Cyclosome). The APC/C complex is activated by phosphorylation and its activity is required for mitosis (Murray, 2004).

The F-box protein in the SCF complexes interacts with specific substrates targeting them for degradation. Interaction with Skp1 requires the conserved F-box region. The substrate that is to be degraded needs to be previously phosphorylated. SCF complexes containing the F-box protein β-Trcp ($SCF^{\beta\text{-}Trcp}$) are involved in the degradation of proteins of the NF-κB pathway and of β-catenin. Another important F-box protein is Skp2 (S-phase kinase-interacting protein 2), identified as a protein interacting with cyclin A/Cdk2 complexes (Zhang et al., 1995). Many key regulators of the cell cycle, including the cell cycle inhibitor $p27^{kip1}$, are ubiquitylated by SCF^{Skp2} complexes (Carrano et al., 1999; Sutterluty et al., 1999; Tsvetkov et al., 1999).

The cell cycle inhibitor $p27^{kip1}$ binds to Cdk2-containing complexes, inhibiting its kinase activity and therefore blocking cell cycle progression along G1 and into the S phase. It can also bind to cyclin D-containing complexes, where $p27^{kip1}$ is "sequestered" and maintained away from active cyclin E/Cdk2 complexes (Bouchard et al., 1999; Perez-Roger et al., 1999). $p27^{kip1}$ levels in tumors are useful as a prognostic marker. Degradation of $p27^{kip1}$ at the proteasome requires its previous phosphorylation (at Threonine 187) by the same cyclin E/Cdk2 complexes it inhibits. Next, the SCF^{Skp2} complex recognizes the phosphorylated protein and labels it with a polyubiquitin chain leading to its degradation (Carrano et al., 1999; Montagnoli et al., 1999; Nguyen et al., 1999; Perez-Roger et al., 1999; Perez-Roger et al., 1997; Sheaff et al., 1997; Sutterluty et al., 1999; Tsvetkov et al., 1999; Vlach et al., 1997).

Skp2 expression changes along the cell cycle, being higher in S and G2 and lower in M and G1. Induction of Skp2 in the G1-S transition promotes the degradation of $p27^{kip1}$, allowing cell cycle progression (Andreu et al., 2005; Carrano et al., 1999; Marti et al., 1999; Schneider et al., 2006; Wirbelauer et al., 2000; Zhang et al., 1995). Skp2 is degraded at the proteasome in mitosis, when the APC/C complex becomes active (Wirbelauer et al., 2000). Ectopic expression of Skp2 in quiescent cells is sufficient to induce cell cycle entry, making Skp2 a potent oncoprotein. In many tumors, the levels of Skp2 and of $p27^{kip1}$ are inversely proportional: low Skp2 correlates with high $p27^{kip1}$ and slow growing tumors, whereas high Skp2 correlates with low $p27^{kip1}$ and fast growing tumors.

3.3 Proteasome inhibition: Bortezomib

The relationship between the ubiquitin-proteasome system (UPS) and the regulation of cellular proliferation, differentiation, survival and transformation led to considering the proteasome as a suitable target in cancer therapy (Adams, 2004). Proteasome inhibitors block protein degradation, causing the accumulation of damaged proteins and inducing the heat shock response and cell death (Concannon et al., 2007; Friedman & Xue, 2004).

Among the several proteasome inhibitors described, Bortezomib has been approved by the FDA for treatment of Multiple Myeloma and Mantle Cell Lymphoma (Ocio et al., 2008; Perez-Galan et al., 2006). Bortezomib is a dipeptdyl boronic acid that inhibits specifically and reversibly the chymotrypsine like activity of the proteasome, thought to be the rate limiting step of proteasome degradation (Eldridge & O'Brien, 2010; Jung et al., 2004; Richardson et al., 2006).

Surprisingly, Bortezomib appears to be quite selective against tumor cells. For some reason, normal cells seem to be more resistant to proteasome inhibition. One of the molecular effects of Bortezomib in several (but not all) cell types is the inhibition of the NF-κB pathway involved in suppressing apoptosis and increasing cell survival and resistance to cytotoxic agents (Baud & Karin, 2009). Some cases of Multiple Myeloma show an increase in NF-κB signaling making them especially sensitive to Bortezomib. Other probably important targets of Bortezomib are p53, p21, p27, Bax, Smac/Diablo and p44/42MAPK, all of them involved in the regulation of cell proliferation and/or survival (Voorhees et al., 2003).

4. Inhibition of the proteasome in CML

Proteasome activity is necessary for all cells to maintain the appropriate balance between protein synthesis and degradation. Therefore, proteasome inhibitors have a highly cytotoxic effect. However, cancer cells seem to be more sensitive to proteasome inhibition than control or normal cells.

4.1 Bcr-Abl and the proteasome

Bcr-Abl activity down-regulates the level of the cell cycle inhibitor $p27^{kip1}$ in different cell models expressing Bcr-Abl endogenously (like K562) or exogenously (like Mo7e and BaF/3) and in primary CML CD34+ cells. Moreover, exogenous $p27^{kip1}$ expression partially antagonizes the effect of Bcr-Abl on proliferation (Albero et al., 2010; Andreu et al., 2005; Bretones et al., 2011; Jonuleit et al., 2000). By inhibiting the tyrosine kinase activity of Bcr-Abl with Imatinib, the half-life of $p27^{kip1}$ is increased, meaning that Bcr-Abl induces the degradation of $p27^{kip1}$. At the same time, the expression of Skp2 decreases and when the

expression of Skp2 is silenced in Bcr-Abl expressing cells, the levels of $p27^{kip1}$ increase, showing again the inverse relationship between these two proteins. The regulation of $p27^{kip1}$ expression by Bcr-Abl depends mainly on the PI3K/Akt pathway. This can be demonstrated because when cells are treated with either the Bcr-Abl inhibitor Imatinib or the PI3K inhibitor LY294002 the effects are quite similar. These effects are threefold (Fig. 1): first, Akt phosphorylates and inactivates FoxO transcription factors, involved in the regulation of $p27^{kip1}$ gene transcription; second, Bcr-Abl induces the expression of Skp2 through the Akt pathway, which results in the proteasomal degradation of $p27^{kip1}$ (Andreu et al., 2005); third, Akt also phosphorylates $p27^{kip1}$ directly, preventing its translocation to the nucleus where it inhibits the cyclin/Cdk complexes controlling the cell cycle (Liang et al., 2002).

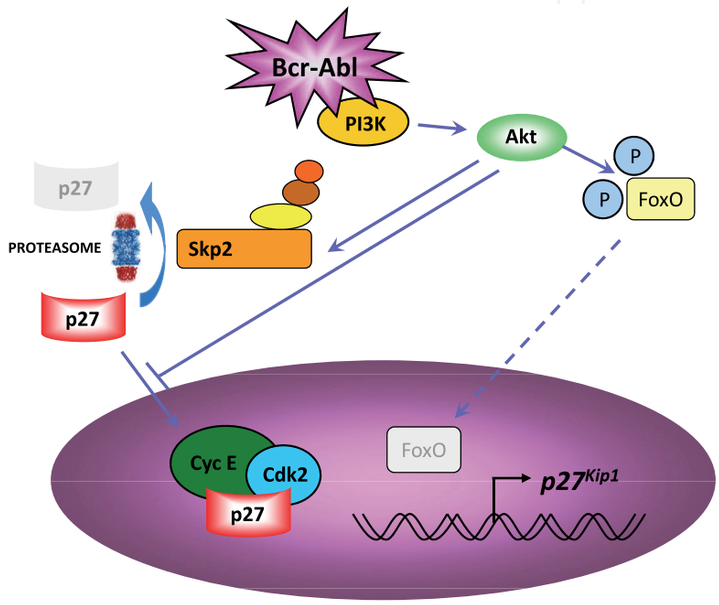

Fig. 1. Bcr-Abl regulation of the level and activity of $p27^{kip1}$ includes transcriptional inhibition, misslocalization and proteasomal degradation.

Apart from the regulation of key proteins controlling cell cycle progression, there is a direct relationship between Bcr-Abl and the proteasome. Exogenous expression of Bcr-Abl in cells directly increases the activity of the proteasome and siRNA knock down of Bcr-Abl expression in K562 cells is associated with a decrease of all three proteasome activities (Crawford et al., 2009).

As Bcr-Abl regulates the activity of the proteasome and induces the proteasomal degradation of several proteins, Bortezomib was proposed as a therapeutic alternative to Imatinib, especially in CML cases of Imatinib resistance.

4.2 Molecular changes in CML cells treated with Bortezomib

Proteasome inhibition with Bortezomib in Bcr-Abl expressing cell models or primary CD34+ cells from CML patients results in a cell cycle inhibition and induction of apoptosis (Albero et al., 2010; Gatto et al., 2003; Jagani et al., 2009). Different key regulators are affected by Bortezomib leading to the detection of apoptosis markers, such as the activation (cleavage) of caspases (3, 8 and 9), or the down-regulation of Bcl-x_L and induction of Bim. Among these

key events, Bortezomib treatment reverses the Bcr-Abl suppression of FoxO proteins and blocks the activation of NF-κB.

Although apoptosis induction is a commonplace of Bortezomib treatment, the inhibition of the cell cycle can be at the G1/S transition or at the G2/M boundary, depending on the cell models used (Albero et al., 2010; Gatto et al., 2003). The cell cycle inhibition at the G1/S transition can be explained by the accumulation of $p27^{kip1}$ in Bortezomib treated cells (Fig. 2). This accumulation inhibits the kinase activity of cyclin/Cdk complexes, which results in the dephosphorylation (activation) of Rb, blocking the cell cycle at the restriction point by the sequestration and inactivation of E2F transcription factors. The dephosphorylation of Rb is followed by its caspase-dependent processing and degradation, something associated with the induction of apoptosis. As a consequence of the impact on Rb phosphorylation, Bortezomib treatment also results in the down-regulation of cyclin A expression (Albero et al., 2010).

In cell lines derived from CML patients in blast crisis, Bortezomib induces the accumulation of IκBα, correlating with a decrease in the NF-κB DNA binding activity in the nucleus at the same time (Gatto et al., 2003). However, this effect was only transient and perhaps does not explain the cellular effects of Bortezomib. In contrast, in Baf/3-derived cell lines exogenously expressing Bcr-Abl, there was no effect of Bortezomib on NF-κB activity (Albero et al., 2010). However, in this same study, the non-canonical NF-κB pathway was analyzed and the results show that Bortezomib prevents the activation of NF-κB2. This protein is activated by proteolysis from a 100 kDa precursor to yield the 52 kDa active form in a proteasome dependent manner (Barre & Perkins, 2007). As NF-κB2 is not activated, the expression of two of its regulated targets, Skp2 and Myc, is also inhibited (Albero et al., 2010; Barre & Perkins, 2007; Schneider et al., 2006). This effect, in turn, would reinforce the effect of Bortezomib on the accumulation of $p27^{kip1}$ (Fig. 2) leading to the dephosphorylation of Rb and the cell cycle inhibition at the G1/S transition (Albero et al., 2010).

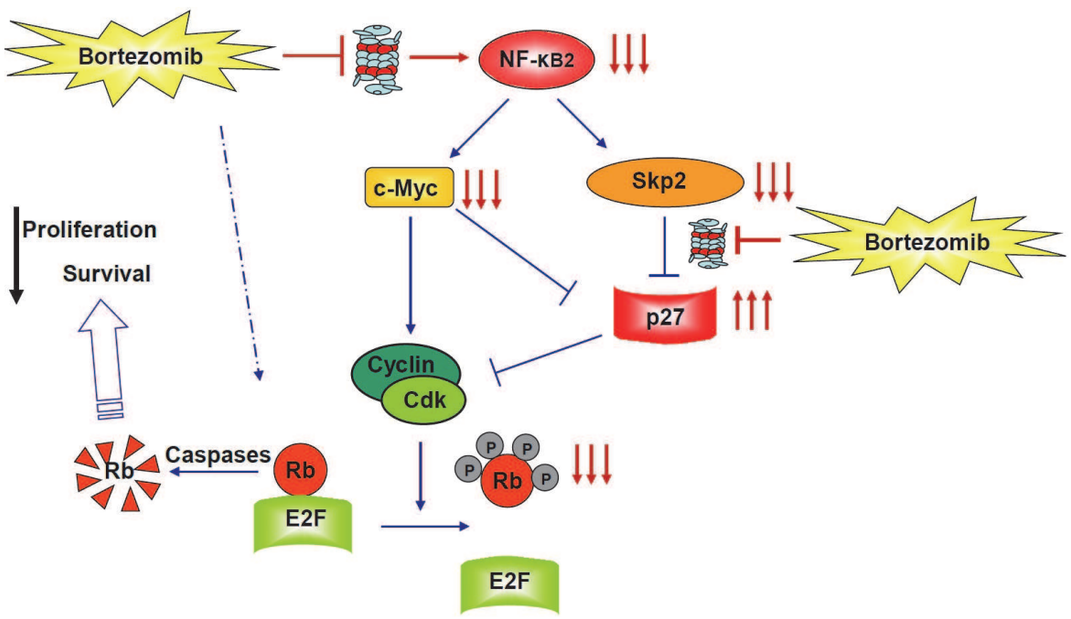

Fig. 2. Possible molecular mechanism leading to cell cycle arrest in G1 and apoptosis in CML cells treated with Bortezomib, as proposed (Albero et al., 2010).

4.3 Effect of Bortezomib on Imatinib resistant CML cells

As Bortezomib inhibition of the cell cycle and induction of apoptosis is independent of Bcr-Abl kinase activity it should be effective even in Imatinib resistant cases. Several different reports show the effect of Bortezomib on cells resistant to Imatinib, using cell lines exogenously expressing wild type and mutant forms of Bcr-Abl, primary CD34+ cells and cell lines derived from patients in blast crisis resistant to Imatinib, or CML cell lines selected for Imatinib resistance by chronic exposure to the inhibitor. In all these cases, Bortezomib induces apoptosis of Bcr-Abl expressing cells regardless of the mutational status of the protein, and at concentrations at which control cells are not sensitive (Albero et al., 2010; Colado et al., 2008; Crawford et al., 2009; Gatto et al., 2003; Heaney et al., 2010; Jagani et al., 2009). As these studies include the highly resistant gate keeper mutant T315I, it is clear that Bortezomib could be an alternative to Imatinib when this treatment is ineffective.

Another source of Imatinib resistant cells is the population of primitive, quiescent hematopoietic CML stem cells (Graham et al., 2002). During Imatinib treatment, there is an initial phase in which Bcr-Abl transcripts decrease rapidly, followed by a second phase in which this decrease is slower. This is probably due to the different sensitivity of CML cell subpopulations to Imatinib. Differentiated cells are more sensitive to Imatinib, whereas primitive CML stem cells are less sensitive to Imatinib. This difference could be due to the almost quiescent state of CML stem cells. This subpopulation of cells (Lin-CD34+) represents approximately 0.5% of the CD34+ cells but are likely to be responsible for the minimal residual disease found in Imatinib treated patients and to relapse if the treatment is interrupted. It is estimated that up to 10^6 lekemia cells may remain in patients with no detectable Bcr-Abl transcripts and therefore it is recommended that Imatinib treatment is not interrupted (Bhatia et al., 2003; Graham et al., 2002; Holyoake et al., 1999; Holyoake et al., 2001; Quintas-Cardama et al., 2009).

Bortezomib is also effective against the quiescent CML stem cells in different assays (Heaney et al., 2010). Bortezomib treatment induces apoptosis of CD34+ cells from chronic phase CML patients at diagnosis, with the accumulation of ubiquitylated proteins but with no effect on the kinase activity of Bcr-Abl. The primitive CD34+CD38- population that includes the quiescent CML stem cells is also sensitive to Bortezomib, which reduces the long-term colony formation capability of the cells. Finally, Bortezomib treatment also leads to a significant reduction in the engraftment potential of human CD34+ CML cells, although in this experiment there were still detectable Bcr-Abl positive cells. However, the effect of Bortezomib is not specific for CML cells, as it induces apoptosis also in normal CD34+CD38- hematopoietic stem cells (Heaney et al., 2010).

5. Conclusion

Imatinib is the first and so far the best example of rational design of anticancer therapeutic drugs. However, it is not able to cure and in some cases its efficacy is reduced due to Bcr-Abl dependent and independent mechanisms of resistance. In this context, the relationship between Bcr-Abl and the proteasome is worth exploiting. In fact, the proteasome inhibitor Bortezomib has shown its potential *in vitro* and in animal models. Nevertheless, proteasome inhibition is cytotoxic to all cell types. Therefore, the future of Bortezomib and other "non-specific" inhibitors or drugs may lie in the search for synergies with other "specific" drugs aimed to the molecular events characteristic of the tumor. In Chronic Myeloid Leukemia,

inhibiting the proteasome with Bortezomib and inhibiting the kinase activity of Bcr-Abl with Imatinib or one of the new compounds may prove the right combination to tackle the problems of Bcr-Abl mutations and the Imatinib resistant leukemia stem cell. This could be a good starting point for this kind of combined therapies in other tumors where the search for a "specific" therapy is still ongoing.

6. Acknowledgement

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7. References

Adams, J. (2004). The proteasome: a suitable antineoplastic target. Nat Rev Cancer, Vol.4, No.5, (May), pp. 349-360

Afar, D.E., Goga, A., McLaughlin, J., Witte, O.N. & Sawyers, C.L. (1994). Differential complementation of Bcr-Abl point mutants with c-Myc. Science, Vol.264, No.5157, (Apr 15), pp. 424-426

Albero, M.P., Vaquer, J.M., Andreu, E.J., Villanueva, J.J., Franch, L., Ivorra, C., Poch, E., Agirre, X., Prosper, F. & Perez-Roger, I. (2010). Bortezomib decreases Rb phosphorylation and induces caspase-dependent apoptosis in Imatinib-sensitive and -resistant Bcr-Abl1-expressing cells. Oncogene, Vol.29, No.22, (Jun 3), pp. 3276-3286

Almond, J.B. & Cohen, G.M. (2002). The proteasome: a novel target for cancer chemotherapy. Leukemia, Vol.16, No.4, (Apr), pp. 433-443

Andreu, E.J., Lledo, E., Poch, E., Ivorra, C., Albero, M.P., Martinez-Climent, J.A., Montiel-Duarte, C., Rifon, J., Perez-Calvo, J., Arbona, C., Prosper, F. & Perez-Roger, I. (2005). BCR-ABL induces the expression of Skp2 through the PI3K pathway to promote p27Kip1 degradation and proliferation of chronic myelogenous leukemia cells. Cancer Res, Vol.65, No.8, (Apr 15), pp. 3264-3272

Barre, B. & Perkins, N.D. (2007). A cell cycle regulatory network controlling NF-kappaB subunit activity and function. Embo J, Vol.26, No.23, (Nov 28), pp. 4841-4855

Baud, V. & Karin, M. (2009). Is NF-kappaB a good target for cancer therapy? Hopes and pitfalls. Nat Rev Drug Discov, Vol.8, No.1, (Jan), pp. 33-40

Baumeister, W., Cejka, Z., Kania, M. & Seemuller, E. (1997). The proteasome: a macromolecular assembly designed to confine proteolysis to a nanocompartment. Biol Chem, Vol.378, No.3-4, (Mar-Apr), pp. 121-130

Baumeister, W., Walz, J., Zuhl, F. & Seemuller, E. (1998). The proteasome: paradigm of a self-compartmentalizing protease. Cell, Vol.92, No.3, (Feb 6), pp. 367-380

Bedi, A., Zehnbauer, B.A., Barber, J.P., Sharkis, S.J. & Jones, R.J. (1994). Inhibition of apoptosis by BCR-ABL in chronic myeloid leukemia. Blood, Vol.83, No.8, (Apr 15), pp. 2038-2044

Ben-Neriah, Y., Daley, G.Q., Mes-Masson, A.M., Witte, O.N. & Baltimore, D. (1986). The chronic myelogenous leukemia-specific P210 protein is the product of the bcr/abl hybrid gene. Science, Vol.233, No.4760, (Jul 11), pp. 212-214

Bhatia, R., Holtz, M., Niu, N., Gray, R., Snyder, D.S., Sawyers, C.L., Arber, D.A., Slovak, M.L. & Forman, S.J. (2003). Persistence of malignant hematopoietic progenitors in chronic myelogenous leukemia patients in complete cytogenetic remission following imatinib mesylate treatment. Blood, Vol.101, No.12, (Jun 15), pp. 4701-4707

Bouchard, C., Thieke, K., Maier, A., Saffrich, R., Hanley-Hyde, J., Ansorge, W., Reed, S., Sicinski, P., Bartek, J. & Eilers, M. (1999). Direct induction of cyclin D2 by Myc contributes to cell cycle progression and sequestration of p27. Embo J, Vol.18, No.19, pp. 5321-5333.

Bretones, G., Acosta, J.C., Caraballo, J.M., Ferrandiz, N., Gomez-Casares, M.T., Albajar, M., Blanco, R., Ruiz, P., Hung, W.C., Albero, M.P., Perez-Roger, I. & Leon, J. (2011). SKP2 oncogene is a direct MYC target gene and MYC down-regulates p27(KIP1) through SKP2 in human leukemia cells. J Biol Chem, Vol.286, No.11, (Mar 18), pp. 9815-9825

Carrano, A.C., Eytan, E., Hershko, A. & Pagano, M. (1999). SKP2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27. Nat Cell Biol, Vol.1, No.4, (Aug), pp. 193-199

Colado, E., Alvarez-Fernandez, S., Maiso, P., Martin-Sanchez, J., Vidriales, M.B., Garayoa, M., Ocio, E.M., Montero, J.C., Pandiella, A. & San Miguel, J.F. (2008). The effect of the proteasome inhibitor bortezomib on acute myeloid leukemia cells and drug resistance associated with the CD34+ immature phenotype. Haematologica, Vol.93, No.1, (Jan), pp. 57-66

Concannon, C.G., Koehler, B.F., Reimertz, C., Murphy, B.M., Bonner, C., Thurow, N., Ward, M.W., Villunger, A., Strasser, A., Kogel, D. & Prehn, J.H. (2007). Apoptosis induced by proteasome inhibition in cancer cells: predominant role of the p53/PUMA pathway. Oncogene, Vol.26, No.12, (Mar 15), pp. 1681-1692

Crawford, L.J., Windrum, P., Magill, L., Melo, J.V., McCallum, L., McMullin, M.F., Ovaa, H., Walker, B. & Irvine, A.E. (2009). Proteasome proteolytic profile is linked to Bcr-Abl expression. Exp Hematol, Vol.37, No.3, (Mar), pp. 357-366

DeSalle, L.M. & Pagano, M. (2001). Regulation of the G1 to S transition by the ubiquitin pathway. FEBS Lett, Vol.490, No.3, (Feb 16), pp. 179-189

Druker, B.J. (2008). Translation of the Philadelphia chromosome into therapy for CML. Blood, Vol.112, No.13, (Dec 15), pp. 4808-4817

Eldridge, A.G. & O'Brien, T. (2010). Therapeutic strategies within the ubiquitin proteasome system. Cell Death Differ, Vol.17, No.1, (Jan), pp. 4-13

Friedman, J. & Xue, D. (2004). To live or die by the sword: the regulation of apoptosis by the proteasome. Dev Cell, Vol.6, No.4, (Apr), pp. 460-461

Gatto, S., Scappini, B., Pham, L., Onida, F., Milella, M., Ball, G., Ricci, C., Divoky, V., Verstovsek, S., Kantarjian, H.M., Keating, M.J., Cortes-Franco, J.E. & Beran, M. (2003). The proteasome inhibitor PS-341 inhibits growth and induces apoptosis in

Bcr/Abl-positive cell lines sensitive and resistant to imatinib mesylate. Haematologica, Vol.88, No.8, (Aug), pp. 853-863

Gordon, M.Y., Dowding, C.R., Riley, G.P., Goldman, J.M. & Greaves, M.F. (1987). Altered adhesive interactions with marrow stroma of haematopoietic progenitor cells in chronic myeloid leukaemia. Nature, Vol.328, No.6128, (Jul 23-29), pp. 342-344

Graham, S.M., Jorgensen, H.G., Allan, E., Pearson, C., Alcorn, M.J., Richmond, L. & Holyoake, T.L. (2002). Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. Blood, Vol.99, No.1, (Jan 1), pp. 319-325

Heaney, N.B., Pellicano, F., Zhang, B., Crawford, L., Chu, S., Kazmi, S.M., Allan, E.K., Jorgensen, H.G., Irvine, A.E., Bhatia, R. & Holyoake, T.L. (2010). Bortezomib induces apoptosis in primitive chronic myeloid leukemia cells including LTC-IC and NOD/SCID repopulating cells. Blood, Vol.115, No.11, (Mar 18), pp. 2241-2250

Hershko, A. & Ciechanover, A. (1998). The ubiquitin system. Annu Rev Biochem, Vol.67, pp. 425-479

Holyoake, T., Jiang, X., Eaves, C. & Eaves, A. (1999). Isolation of a highly quiescent subpopulation of primitive leukemic cells in chronic myeloid leukemia. Blood, Vol.94, No.6, (Sep 15), pp. 2056-2064

Holyoake, T.L., Jiang, X., Jorgensen, H.G., Graham, S., Alcorn, M.J., Laird, C., Eaves, A.C. & Eaves, C.J. (2001). Primitive quiescent leukemic cells from patients with chronic myeloid leukemia spontaneously initiate factor-independent growth in vitro in association with up-regulation of expression of interleukin-3. Blood, Vol.97, No.3, (Feb 1), pp. 720-728

Horita, M., Andreu, E.J., Benito, A., Arbona, C., Sanz, C., Benet, I., Prosper, F. & Fernandez-Luna, J.L. (2000). Blockade of the Bcr-Abl kinase activity induces apoptosis of chronic myelogenous leukemia cells by suppressing signal transducer and activator of transcription 5-dependent expression of Bcl-xL. J Exp Med, Vol.191, No.6, (Mar 20), pp. 977-984

Jagani, Z., Song, K., Kutok, J.L., Dewar, M.R., Melet, A., Santos, T., Grassian, A., Ghaffari, S., Wu, C., Yeckes-Rodin, H., Ren, R., Miller, K. & Khosravi-Far, R. (2009). Proteasome inhibition causes regression of leukemia and abrogates BCR-ABL-induced evasion of apoptosis in part through regulation of forkhead tumor suppressors. Cancer Res, Vol.69, No.16, (Aug 15), pp. 6546-6555

Jiang, Y., Zhao, R.C. & Verfaillie, C.M. (2000). Abnormal integrin-mediated regulation of chronic myelogenous leukemia CD34+ cell proliferation: BCR/ABL up-regulates the cyclin-dependent kinase inhibitor, p27Kip, which is relocated to the cell cytoplasm and incapable of regulating cdk2 activity. Proc Natl Acad Sci U S A, Vol.97, No.19, (Sep 12), pp. 10538-10543

Jonuleit, T., van der Kuip, H., Miething, C., Michels, H., Hallek, M., Duyster, J. & Aulitzky, W.E. (2000). Bcr-Abl kinase down-regulates cyclin-dependent kinase inhibitor p27 in human and murine cell lines. Blood, Vol.96, No.5, pp. 1933-1939.

Jung, L., Holle, L. & Dalton, W.S. (2004). Discovery, Development, and clinical applications of bortezomib. Oncology (Williston Park), Vol.18, No.14 Suppl 11, (Dec), pp. 4-13

Liang, J., Zubovitz, J., Petrocelli, T., Kotchetkov, R., Connor, M.K., Han, K., Lee, J.H., Ciarallo, S., Catzavelos, C., Beniston, R., Franssen, E. & Slingerland, J.M. (2002). PKB/Akt phosphorylates p27, impairs nuclear import of p27 and opposes p27-mediated G1 arrest. Nat Med, Vol.8, No.10, pp. 1153-1160.

Manning, B.D. & Cantley, L.C. (2007). AKT/PKB signaling: navigating downstream. Cell, Vol.129, No.7, (Jun 29), pp. 1261-1274

Marti, A., Wirbelauer, C., Scheffner, M. & Krek, W. (1999). Interaction between ubiquitin-protein ligase SCFSKP2 and E2F-1 underlies the regulation of E2F-1 degradation. Nat Cell Biol, Vol.1, No.1, (May), pp. 14-19

Medema, R.H., Kops, G.J., Bos, J.L. & Burgering, B.M. (2000). AFX-like Forkhead transcription factors mediate cell-cycle regulation by Ras and PKB through p27kip1. Nature, Vol.404, No.6779, (Apr 13), pp. 782-787

Montagnoli, A., Fiore, F., Eytan, E., Carrano, A.C., Draetta, G.F., Hershko, A. & Pagano, M. (1999). Ubiquitination of p27 is regulated by Cdk-dependent phosphorylation and trimeric complex formation. Genes Dev, Vol.13, No.9, (May 1), pp. 1181-1189

Murray, A.W. (2004). Recycling the cell cycle: cyclins revisited. Cell, Vol.116, No.2, (Jan 23), pp. 221-234

Nguyen, H., Gitig, D.M. & Koff, A. (1999). Cell-free degradation of p27(kip1), a G1 cyclin-dependent kinase inhibitor, is dependent on CDK2 activity and the proteasome. Mol Cell Biol, Vol.19, No.2, (Feb), pp. 1190-1201

Ocio, E.M., Mateos, M.V., Maiso, P., Pandiella, A. & San-Miguel, J.F. (2008). New drugs in multiple myeloma: mechanisms of action and phase I/II clinical findings. Lancet Oncol, Vol.9, No.12, (Dec), pp. 1157-1165

Perez-Galan, P., Roue, G., Villamor, N., Montserrat, E., Campo, E. & Colomer, D. (2006). The proteasome inhibitor bortezomib induces apoptosis in mantle-cell lymphoma through generation of ROS and Noxa activation independent of p53 status. Blood, Vol.107, No.1, (Jan 1), pp. 257-264

Perez-Roger, I., Kim, S.H., Griffiths, B., Sewing, A. & Land, H. (1999). Cyclins D1 and D2 mediate myc-induced proliferation via sequestration of p27(Kip1) and p21(Cip1). Embo J, Vol.18, No.19, pp. 5310-5320.

Perez-Roger, I., Solomon, D.L., Sewing, A. & Land, H. (1997). Myc activation of cyclin E/Cdk2 kinase involves induction of cyclin E gene transcription and inhibition of p27(Kip1) binding to newly formed complexes. Oncogene, Vol.14, No.20, pp. 2373-2381.

Puil, L., Liu, J., Gish, G., Mbamalu, G., Bowtell, D., Pelicci, P.G., Arlinghaus, R. & Pawson, T. (1994). Bcr-Abl oncoproteins bind directly to activators of the Ras signalling pathway. Embo J, Vol.13, No.4, (Feb 15), pp. 764-773

Quintas-Cardama, A., Kantarjian, H.M. & Cortes, J.E. (2009). Mechanisms of primary and secondary resistance to imatinib in chronic myeloid leukemia. Cancer Control, Vol.16, No.2, (Apr), pp. 122-131

Richardson, P.G., Mitsiades, C., Hideshima, T. & Anderson, K.C. (2006). Bortezomib: proteasome inhibition as an effective anticancer therapy. Annu Rev Med, Vol.57, pp. 33-47

Salesse, S. & Verfaillie, C.M. (2002). BCR/ABL: from molecular mechanisms of leukemia induction to treatment of chronic myelogenous leukemia. Oncogene, Vol.21, No.56, (Dec 9), pp. 8547-8559

Salesse, S. & Verfaillie, C.M. (2003). BCR/ABL-mediated Increased Expression of Multiple Known and Novel Genes That May Contribute to the Pathogenesis of Chronic Myelogenous Leukemia. Mol Cancer Ther, Vol.2, No.2, (February 1, 2003), pp. 173-182

Sawyers, C.L. (1993). Molecular consequences of the BCR-ABL translocation in chronic myelogenous leukemia. Leuk Lymphoma, Vol.11 Suppl 2, pp. 101-103

Schneider, G., Saur, D., Siveke, J.T., Fritsch, R., Greten, F.R. & Schmid, R.M. (2006). IKKalpha controls p52/RelB at the skp2 gene promoter to regulate G1- to S-phase progression. Embo J, Vol.25, No.16, (Aug 23), pp. 3801-3812

Sheaff, R.J., Groudine, M., Gordon, M., Roberts, J.M. & Clurman, B.E. (1997). Cyclin E-CDK2 is a regulator of p27Kip1. Genes Dev, Vol.11, No.11, (Jun 1), pp. 1464-1478

Sutterluty, H., Chatelain, E., Marti, A., Wirbelauer, C., Senften, M., Muller, U. & Krek, W. (1999). p45SKP2 promotes p27Kip1 degradation and induces S phase in quiescent cells. Nat Cell Biol, Vol.1, No.4, (Aug), pp. 207-214

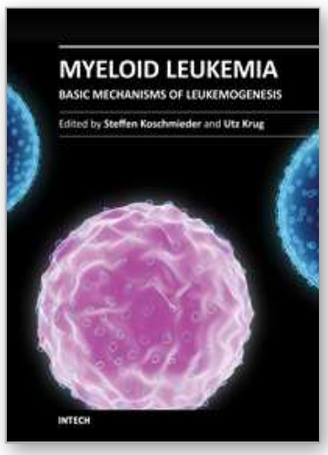
Tsvetkov, L.M., Yeh, K.H., Lee, S.J., Sun, H. & Zhang, H. (1999). p27(Kip1) ubiquitination and degradation is regulated by the SCF(Skp2) complex through phosphorylated Thr187 in p27. Curr Biol, Vol.9, No.12, (Jun 17), pp. 661-664

Vlach, J., Hennecke, S. & Amati, B. (1997). Phosphorylation-dependent degradation of the cyclin-dependent kinase inhibitor p27. Embo J, Vol.16, No.17, (Sep 1), pp. 5334-5344

Voorhees, P.M., Dees, E.C., O'Neil, B. & Orlowski, R.Z. (2003). The proteasome as a target for cancer therapy. Clin Cancer Res, Vol.9, No.17, (Dec 15), pp. 6316-6325

Wirbelauer, C., Sutterluty, H., Blondel, M., Gstaiger, M., Peter, M., Reymond, F. & Krek, W. (2000). The F-box protein Skp2 is a ubiquitylation target of a Cul1-based core ubiquitin ligase complex: evidence for a role of Cul1 in the suppression of Skp2 expression in quiescent fibroblasts. Embo J, Vol.19, No.20, (Oct 16), pp. 5362-5375

Zhang, H., Kobayashi, R., Galaktionov, K. & Beach, D. (1995). p19Skp1 and p45Skp2 are essential elements of the cyclin A-CDK2 S phase kinase. Cell, Vol.82, No.6, (Sep 22), pp. 915-925



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