Apoptosis resistance in chronic myelogenous leukemia

Mecanismos de resistência a apoptose na leucemia mielóide crônica

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ABSTRACT

The chronic myeloid leukemia (CML) is a three-phase myeloproliferative disorder, dependent on the expression of the oncoprotein Bcr-Abl, which is the product of the reciprocal translocation between chromosomes 9 and 22, resulting in the Philadelphia chromosome (Ph). Bcr-Abl protein is the constitutively activated tyrosine kinase responsible for changes in intracellular biochemical cascades, culminating into hematopoietic stem cell malignant transformation. CML leukemic cells present abnormal adhesion to medullar stroma, altered proliferation and an amazing resistance to apoptosis induced by classical chemotherapeutic drugs. Another therapy used in CML patients is imatinib mesylate (Gleevecâ), which has shown remarkable clinical activity in these patients. However, this drug does not completely eradicate BCR-ABL-expressing cells from the body, and recently some patients showed resistance to imatinib. The observation that production of Bcr-Abl is the initiating event in CML drew attention to the survival signals triggered by this oncogene. The number of altered signal transducers and transcription factors has been associated with the anti-apoptotic phenotype of CML cells, and some of them lead to the expression and/or activation of apoptosis modulators from Bcl-2 family, such as Bcl-xL, Bcl-2, Bax and Bad. In this article we review some recent data on the understanding of Bcr-Abl oncoprotein expression effect in the apoptosis machinery in CML.

Keywords: Chronic myeloid leukemia/therapy; Apoptosis; Fusion proteins, bcr-abl; Oncogene proteins

RESUMO

A leucemia mielóide crônica (LMC) é uma doença mieloproliferativa trifásica dependente da expressão da oncoproteína Bcr-Abl, produto da translocação recíproca entre os cromossomos 9 e 22, originando o cromossomo Philadelphia (Ph). A proteína Bcr-Abl é uma tirosina-quinase ativada constitutivamente que desencadeia uma cascata de reações bioquímicas intracelulares, culminando na transformação maligna da célula precursora hematopoética. A célula leucêmica apresenta alterações na adesão ao estroma medular, na proliferação celular e uma extraordinária resistência à indução da apoptose por quimioterápicos tradicionais. Outro medicamento desenhado especificamente e utilizado para o tratamento dessa doença é o mesilato de imatinibe (Gleevecâ), que promove altas taxas de remissão citogenética nos pacientes. Entretanto, recentemente alguns pacientes apresentaram refratariedade a esse medicamento, o que reforçou a necessidade de novas pesquisas e desenvolvimento de fármacos mais eficientes. A evidência de que o Bcr-Abl seria o evento inicial da transformação celular fez com que pesquisadores centralizassem seus projetos de pesquisa nas vias de sinalização que conduzem ao aumento da vida média das células leucêmicas. Numerosos sinais de transdução e diferentes fatores de transcrição têm sido associados ao fenótipo de resistência à apoptose das células leucêmicas. Esses sinais modulam a expressão e/ou ativação dos genes reguladores da apoptose, dentre os quais destacam-se alguns membros da família Bcl-2, tais como Bcl-xL, Bcl-2, Bax e Bad. Nesse artigo, revisaremos dados recentes referentes ao entendimento do efeito da expressão da oncoproteína Bcr-Abl na maquinaria apoptótica na LMC.

Descritores: Leucemia mielóide crônica/terapia; Apoptose; Proteínas de fusão bcr-abl; Proteínas de oncogene

CHRONIC MYELOID LEUKEMIA

Chronic myeloid leukemia (CML) is a myeloproliferative disease characterized by a clonal expansion of the neoplastic totipotent hematopoietic stem cell. It was the first malignant clonal disease described as resulting from a leukemogenesis process associated with a characteristic cytogenetic
abnormality, the Philadelphia chromosome (Ph). The Ph chromosome results from a reciprocal translocation between the long arms of chromosomes 9 and 22 - t(9;22)(q34;q11)(1), which promotes the appearance of a shortened chromosome 22 (Ph). This translocation originates the neogene bcr-abl, resulting from the fusion of part of the bcr (breakpoint cluster region, in chromosome 22) gene with practically the whole c-abl (Abelson leukemia virus, in chromosome 9) gene, which is present in 100% of the CML cases(3). The bcr gene codifies a protein with the same name, Bcr, whose physiological role seems to be correlated to regulation of the cellular signaling and division processes. The gene c-abl is a tyrosine-kinase protein that takes part in regulation of the cellular cycle and response to genotoxic stresses. This neogene, depending on the breakpoint location of the bcr region can codify three different Bcr-Abl oncoproteins - p190, p210 and p230. Among those, the most frequent in CML patients is the oncoprotein p210, whereas p230 is present in individuals with chronic neutrophilic leukemia, and p190, in patients with acute lymphoid leukemia and CML with poorer prognosis(3).

The fusion protein Bcr-Abl is found in the cytoplasm, usually associated with actin filaments, and presents an elevated basal tyrosine-kinase activity as compared to c-Abl.

The bcr-abl neogene expression determines the transformation of normal hematopoietic progenitor cells into malignant cells, presenting a constant myeloproliferation generated by three main mechanisms: change in the progenitor cell adhesion to stromal cells and to the extracellular matrix, maintenance of a constant mitogenic signal and resistance to cellular apoptosis. In this context, the progenitor cell escapes the regulation of its proliferative process, since adhesion to the extracellular matrix and to stromal cells downregulates proliferation. On the other hand, the cell receives a constant mitogenic activation signal through the RAS and MAP kinases, and its susceptibility to apoptotic mechanisms is diminished. These changes in regulating mechanisms of proliferation and death of the progenitor cell result in the accumulation of positive Bcr-Abl leukemic cells found in CML(6-5).

Chronic myeloid leukemia is a disease whose clinical course involves three stages: the chronic, accelerated and blastic phases. The chronic phase of CML lasts several years and is characterized by the accumulation of precursor and mature myeloid cells in peripheral blood (leukocytosis usually above 25,000/mm$^3$), bone marrow (granulocytic series hypercellularity) and extramedullary sites (splenomegaly and hepatomegaly).

In the accelerated phase of CML, the laboratory tests show an even higher increase in circulating leucocytes and basophils and the treatment used in the chronic phase is no longer effective. The disease progresses fastly to the blastic phase, in which the bone marrow presents more than 20% of leukemic blastic cells and most of the circulating cells in the peripheral blood are immature blasts. The total of basophils can increase to 20% and patients develop thrombocytopenia(6). In this phase, patients are refractory to most treatments and can die in a period from three to six months(8). The cellular and molecular mechanisms responsible for CML progression have not been elucidated yet, but it seems that genetic and epigenetic events are involved in the process(7).

Chronic myeloid leukemia can be treated by chemotherapy (hydroxyurea), interferon-a (IFN-a), IFN-a associated to cytarabine, tyrosine-kinase inhibitors (imatinib mesylate, Gleevec®) and bone marrow transplantation (BMT). Although imatinib mesylate has significantly increased the number of complete and molecular cytogenic remissions, the follow-up of patients that use it is still short to determine its potential to cure.

Allogeneic BMT is still the only therapy to achieve cure for CML. However, due to its high morbidity and mortality, it is limited to patients aged under 55 years and that have compatible HLA donors (revised in Goldman 2001). Allogeneic BMT necessarily includes inducing the recipient bone marrow aplasia with the use of chemotherapeutical agents and posterior stem cell infusion. Chemotherapy eradicates the patient leukemic clones and the recipient bone marrow is repopulated by the donor’s bone marrow. For a successful BMT, the donor’s new bone marrow must induce the “graft versus leukemia” reaction in the recipient, a process in which leukemic cells are actively eliminated by the healthy immune cells from the donor. This procedure implies in keeping patients in partial isolation and submitting them to periodic platelet infusions, among other therapies. The complete treatment stands a good chance of success, but involves an extremely high cost, which restricts its adoption by the public health system, in addition to a very high risk for the patients, since it involves eliminating immune cells for a period that may last several weeks, during which the patient is subject to infections and other complications(8).

Treating CML with IFN-a, cytarabine and a tyrosine-kinase inhibitor (STI571, imatinib mesylate or Gleevec®) aims to re-establish the physiological processes of progenitor cell proliferation and maturation(5,9-10). These drugs seem to normalize the mechanisms that regulate cellular proliferation, decrease mitogenic activity and directly or indirectly induce progenitor cell apoptosis. In other words, it enables proliferation of normal
progenitor cells and promotes eradication of malignant progenitor cells.

Imatinib mesylate demonstrated to be more potent than IFN-α(11) to control the disease. This compound is synthesized based on the tertiary structure of the catalytic site of the SRC family kinases and mainly tested in oncogenic kinases, together with several other small compounds with the similar structure.

Imatinib mesylate presents high specificity for Abl tyrosine-kinase(12) and competitively inhibits ATP binding to of Abl-kinase domain at micromolar concentrations(12). Consequently, the enzyme activity is suppressed and the leukemic cell dies.

Although STI571 has selective action for Bcr-Abl, it also acts on other tyrosine-kinase, such as PDGF-R (platelet-derived growth factor receptor) and c-kit(13).

Therefore, it prevents progression of leukemia by blocking advance to the accelerated or blastic phase. Imatinib mesylate can induce hematological remission in 98% of patients and complete cytogenetic remission in 76% of cases after a 12-month treatment(14).

Despite the advances obtained with the use of imatinib mesylate in treating CML patients, several resistance mechanisms of leukemic cells in this therapy were described and positive Bcr-Abl cells persist in the bone marrow and peripheral blood even after therapy. The main causes of resistance are presence of mutations in Bcr-Abl catalytic site; duplication of Philadelphia chromosomes, overexpression of the gene bcr-abl(15) and reduced uptake of the compound due to overexpression of glycoprotein P (Pgp) or to excessive metabolization(12).

The persistence of positive Bcr-abl cells in patients under treatment with imatinib mesylate indicates that inhibition of abl tyrosine-kinase activity may not be enough to eradicate leukemic cells. Hence, identifying other cellular components or signal paths related to pathogenesis of the disease will be necessary to develop new drugs which could be combined with Gleevecα to cure patients suffering from CML.

CML, Bcr-Abl and apoptosis

Apoptosis is a physiological process of programmed cell death. It plays an important role in homeostasis of different tissues and also controls the hematopoiesis(16). The abnormal resistance to apoptosis may lead to neoplasms and auto-immune diseases(17) due to persistent mutant or altered cells, whereas its exacerbation results in the onset of acute neurodegenerative diseases(18).

When the phenomenon of cellular apoptosis initiates, it triggers diverse molecular events that culminate in activation of members of a protease family that are called caspases. In the context of cell death, these proteases participate in different manners: initiating the death program; as regulating elements and effectors; in charge of morphological and biochemical changes in the nucleus, cytoplasm, organelles and plasma membrane of cells in apoptosis.

Caspases are in the cytoplasm and endoplasmatic reticulum of cells as zymogens and are activated after cleavage change in arrangement.

The activation cascade of caspases may be triggered by the extrinsic or by the intrinsic pathways(19). The intrinsic pathway is initiated by the action of several intracellular stress signs, such as irradiation, chemotherapeutical agents, virus, bacteria infection and absence of cellular growth factors, which converge to mitochondria. The activation of this path depends on release of certain mitochondrial factors for cytosol, such as cytochrome c. This molecule, together with the APAF-1, promotes activation of caspase-9, which, in turn, activates caspases -3, -6 and –7 and result in an apoptotic phenotype. In addition to cytochrome c, another mitochondrial protein called SMAC/DIABLO is released for cytosol and contributes to activation of caspases, by means of blocking its endogenous inhibitors – the IAP family molecules. Some molecules that act in mitochondrias are essential to control apoptosis through the intrinsic path, such as the Bcl-2 family proteins. Some members of this family (Bcl-xL, Mcl-1, A1 Bcl-w and Bcl-2) inhibit apoptosis, regulating translocation of apoptotic factors, such as cytochrome-c, SMAC and AIF in cytosol, where they bind to other molecules that take part in the effector phase of apoptosis. Other members (Bad, Bax, Bak, Bid,etc) promote or sensitize the cell for cell death(20). The extrinsic path is activated by interaction of receptors, which are located in the cell surface and called death receptors (DR), with their specific ligands. These receptors include Fas (CD95), TNFR1, DR3, DR4, DR5 and DR6(21). This path is initiated by trimerization of the DR, and in the case of interaction with Fas-FasL, it enables recruiting two signaling proteins, FADD and pro-caspase-8, making the complex DISC (death-inducing signaling complex). After being recruited, procaspase-8 becomes active caspase-8 and is released to the cytoplasm, where it may directly act in the executing phase of the process, by directly activating caspase-3, or by triggering the path intrinsic through cleavage of molecule Bid; this, in turn, goes to the mitochondria and induces release of cytochrome c and SMAC.

These recruitment and activation of procaspase-8 are targets of anti-apoptotic proteins, such as the viral protein v-FLIP (FADD-like ICE inhibitory proteins), which has a similar structure to that of caspase-8, but with no catalytic domain. This molecule, likewise its cellular homologue c-FLIP, can bind to the complex
Fas-FADD, inhibiting recruitment and consequent cleavage and activation of procaspase-8, thus preventing triggering of apoptosis\textsuperscript{(22)}.

The absent or reduced expression of proapoptotic proteins is associated with recurrent infections, hematological diseases (lymphomas and leukemias), auto-immune diseases and drug resistance. In contrast, their exacerbated expression by means of radiotherapy, and use of antineoplastic and immunotherapeutic agents in the treatment of solid tumors and leukemias, results in cure of diseases. For example, in B-cell chronic lymphoid leukemias (CLL-B), the antigen Fas is expressed at levels that are lower than those of Bel-2, whereas in M2 and M3 acute myeloid leukemias (AML-M2 and AML-M3), the antigen Bel-2 is reduced in relation to Fas. These data may partly explain why AML-M2 and AML-M3 patients have a high remission rate when treated\textsuperscript{(23)}.

Another fact that should be emphasized is that cytotoxicity mediated by T- and NK cells against tumor cells may also be performed by the Fas-FasL system activation path. However, it is important to mention that tumor cells may not give an immune response by FasL expression\textsuperscript{(23)}. Hence, some studies have suggested that tumor cells secreting or expressing the antigen FasL could promote apoptosis of effector cells, preventing them from acting in eradication of the tumoral clone\textsuperscript{(23)}. The antigen FasL is present in tumor cells and, when bound to the Fas antigen of effector cells, would lead to apoptosis of these cells, reducing the circulating pool of immune response cells. Therefore, it is necessary to observe the expression level of Fas antigens in immune response cells and FasL in malignant precursor cells. The increased expression of FasL in tumor and leukemic cells is one of the escape mechanisms of leukemic clones for anti-tumor immune response.

It is known that in CML, the malignant hematopoietic precursor cells are more resistant to apoptosis than normal cells due to expression of Bcr-Abl tyrosine-kinase that prolongs cellular survival and seems to be necessary for cell transformation mediated by Bcr-Abl. However, this phenotype seems to involve the ERK and JNK paths. Other Bcr-Abl-activated molecules are c-myc and cycline D1, suggesting that several components of the cellular cycle act below the signaling paths that depend on Bcr-Abl SH2 domain to induce malignant transformation\textsuperscript{(26-28)}. The Bcr-Abl-mediated activation of JNK requires Ras, MEK kinase and is important for the changing activity of Bcr-Abl.

The fosfatidylinositol-3 kinase (PI3-K) path acts in several processes, such as mitosis, cell transformation, apoptosis and other events involving tyrosine-kinases. One of the target molecules of PI3-K is Akt, a serine-threonine kinase, which acts in signal regulation of survival cells. The survival cell mediated by Akt comprises inactivation of cell death components, including the inhibition of the proapoptotic protein Bad and blocking of the caspase-dependent apoptotic path. The non-phosphorylated molecule Bad binds to Bcl-x\textsubscript{L} inhibiting its anti-apoptotic function and promoting cell death. Survival signals (cytokines, growth factors, oncogenes) promote phosphorylation of Bad through the PI3K/Akt-dependent path, which seems to be scavenged in cytosol, remaining associated to protein 14-3-3. This phenomenon prevents its binding to Bcl-x\textsubscript{L} in the case of hematopoietic cells expressing Bcr-Abl, Bad is constitutively phosphorylated\textsuperscript{(29-32)}.

Apart from the effects on Bad, Akt suppresses apoptosis by upregulating the transactivation potential of NF\textsubscript{eB}, which raises the expression of apoptotic inhibitors, such as Bcl-x\textsubscript{L} and A1\textsuperscript{(29-32)}. The activation of NF\textsubscript{eB} by Bcr-Abl promotes the expression of IAPs, and seems to be necessary for cell transformation through Bcr-Abl\textsuperscript{(33)}.

Another Bcr-Abl-activated signaling path that inhibits apoptosis is Jak/STATs path. STATs are latent cytoplasmatic proteins that are activated by phosphorylation after recruitment for a receptor complex. Active STATs are translocated to the nucleus, where they bind to elements that respond specifically to DNA and induce the expression some genes. Bcr-Abl induces the activation of STAT5 in CML and such activation induces the expression of Bcl-x\textsubscript{L}\textsuperscript{(32)}. Ras is a small protein that binds to GTP and conveys signals to some molecules, such as Raf1 and mitogen-activated protein kinases (MAPKs). The MAPK family comprises three serine-threonine kinases: ERK, JNK/SAPK and p38. ERK is activated by mitotic stimuli leading to cell transformation and differentiation, whereas the other two are activated by cellular stress\textsuperscript{(26-27)}.
Among the signaling molecules that are activated by Bcr-Abl, ERK and PI3K seem to be the key molecules for cellular transformation mediated by this oncoprotein. The inhibition of Abl tyrosine-kinase significantly decreased activation of the kinases ERK and Akt. Thus, Bad and Bcl-xL are apparently the two major apoptosis regulators under Bcr-Abl control.

The results obtained by Sonoyama et al.\(^\text{(30)}\) show that the Ras-, STAT5- and PI3K paths individually take part in the survival and growth that are Bcr-Abl-dependent; nevertheless, cooperation among these signaling paths is required for the complete leukemic activity of Bcr-Abl.

Due to the effects of Bcr-Abl expression already mentioned, this molecule is able to change fibroblasts and hematopoietic cells, as well as to block apoptosis that is induced by removing growth factors through ultraviolet light, by means of several chemotherapeutic agents and of Fas-FasL binding.

The pharmacological inhibition of Bcr-Abl tyrosine-kinase with imatinib mesylate partially avoids its anti-apoptotic role and its changing capacity\(^\text{(31-34)}\), indicating that both cellular transformation and apoptosis resistance induced by Bcr-Abl present elements that depend or not of its tyrosine-kinase activity. Other studies, however, pointed out that a malignant transformation and protection against apoptosis are separate events, since mutations in other structural domains of the molecule distinctly affect these two functions. Double mutations in the isoform p185 affect the main site of autophosphorylation and the SH2 domain (involved in binding phosphotyrosine residues in other molecules) and result in loss of the changing capacity in fibroblasts and hematopoietic cells; however, they have no effect at all in the anti-apoptotic proprieties of this molecule in HL-60 cells.\(^\text{(35)}\)

Amarante-Mendes et al.\(^\text{(35)}\) described that resistance to apoptosis in positive Bcr-Abl cell lineages was related to increased expression of the protein Bcl-x\(_L\) and not of Bcl-2. On the other hand, HANDA et al.\(^\text{(36)}\) reported that, in CML, the expression of the protein Bcl-2 raises as the disease progresses, which corroborates the hypothesis that the expression of these proteins in high levels would prolong the survival of leukemic cells, making them resistant to apoptosis. Thus, data about expression of Bcl-2 in positive Bcr-abl cells are controversial.

The Fas antigen seems to be expressed at normal levels in malignant cells of CML patients in the chronic phase; nonetheless, due to high expression of Bcl-2, malignant cells present apoptosis resistance \(^\text{(37)}\).

In an oral communication in the Brazilian Congress of Hematology, in 2004, Castro et al.\(^\text{(38)}\) reported a raised expression of the anti-apoptotic genes \(bcl-x_L\) and flip in CML patients using the semi-quantitative RT-PCR technique. This study also demonstrated that the expression of these genes increases as the disease progresses, what would contribute to exacerbated resistance of leukemic cells to apoptosis, as well as to refractoriness of patients to treatment in the most advanced phases of the CML.

Other studies used cell lineages from patients with CML and also demonstrated that the oncoprotein Bcr-Abl increases survival of leukemic cells by deregulating molecules involved in the process of cell apoptosis, reducing the expression of the proapoptotic proteins Bim and FasL, and raising the expression of some proteins, such as Survivin, Mcl-1, Bax and p53\(^\text{(39-40)}\).

The cellular and molecular mechanisms associated with the property of Bcr-Abl to induce raised expression of anti-apoptotic genes and reduced expression of proapoptotic genes, thus contributing to progression of CML, have not been elucidated yet.

The increased expression of anti-apoptotic genes could partly contribute to selective advantage of these malignant cells in relation to normal cells. These literature data demonstrate not only the role of the neogene \(bcr-abl\) in different signaling paths that lead to cell apoptosis, but also its association with pathophysiology and prognosis of the CML.

In this context, some treatments for the CML, such as immune therapies (infusion of cytotoxic T cell specific for Bcr-Abl, dendritic cells \(vacci\)nation and DLI), IFN-á, cytarabine, tyrosine-kinase inhibitors (imatinib mesylate and BMS-354825), seem to seek greater susceptibility of leukemic cells to apoptosis by different mechanisms of action. Hence, a better understanding of the mechanisms by which the protein Bcr-Abl plays its anti-apoptotic role could also enhance the understanding of the etiopathogenesis of the CML, resulting in greater efficacy some therapeutic options (chemotherapeutic agents, tyrosine-kinase inhibitors, etc) for this disease.

**REFERENCES**


