Molecular Abnormalities in Chronic Myeloid Leukemia: Deregulation of Cell Growth and Apoptosis

ALESSANDRA DI BACCO, KAREN KEESHAN, SHARON L. MCKENNA, THOMAS G. COTTER

Tumour Biology Laboratory, Department of Biochemistry, University College Cork, Cork, Ireland

Key Words. Leukemia · Bcr-Abl · p53 · Apoptosis

ABSTRACT

Chronic myeloid leukemia (CML) is a disease of the hematopoietic system, characterized by the presence of the Bcr-Abl oncoprotein. The main characteristics of this disease include adhesion independence, growth factor independence, and resistance to apoptosis. Loss or mutation of the tumor suppressor gene, p53, is one of the most frequent secondary mutations in CML blast crisis. The transition between chronic phase and blast crisis is associated with increased resistance to apoptosis correlating with poor prognosis. This review focuses on the involvement of these two oncoproteins in the development and progression of the apoptotic-resistant phenotype in CML.

INTRODUCTION

The Philadelphia (Ph1) chromosome, t(9;22) (q34;q11) translocation in chronic myelocytic leukemia (CML), was the first consistent chromosomal abnormality identified in a cancer [1, 2]. The Ph1 chromosome is formed by a reciprocal translocation that fuses 5′ sequences of the bcr gene with sequences upstream of exon 2 of the c-abl proto-oncogene on chromosome 22, and it is detected in virtually all cases of CML. The Bcr-Abl gene fusion product is a protein (210 kDa) which is larger than the normal Abl protein (160 kDa), and in which the tyrosine kinase is constitutively active.

CML can be divided into three clinically distinct phases: an initial chronic phase followed by an accelerated phase which subsequently leads to blast crisis. Chronic phase CML cells have been shown to be responsive to growth factors, exhibit reduced apoptosis, demonstrate altered adhesion and activate several hematopoietic signaling pathways. After a chronic phase of variable duration, three to five years, CML almost invariably undergoes transformation leading to the blast crisis phase of the disease. The change is accompanied by the appearance of poorly differentiated myeloid or lymphoid blast cells in the bone marrow and blood. While fusion of c-abl and bcr is believed to be the primary cause of the chronic phase of CML, progression to blast crisis requires other molecular changes. Common secondary abnormalities include extra copies of Ph1 chromosome (Bcr-Abl) [3, 4], increased rate of p53, or Rb1 gene mutation [5].

The p53 and Rb1 genes are most often implicated in the transformation. Their structures are almost always normal in the chronic phase of CML, but are frequently deranged in blast crisis. Alterations in the p53 and Rb1 genes occur in about 30% and 20% of blast crisis cases, respectively, whereas abnormalities in Myc and Ras are infrequent [5]. Repeated observations during the clonal evolution of the leukemia strongly support the idea that the...
changes in p53 and Rb1 are responsible for the clonal evolution to the blast crisis stage. Mutations of the p53 gene also appear to be associated with progression rather than initiation of other types of leukemia/lymphoma. For example, p53 abnormalities are relatively uncommon in chronic lymphocytic leukemia and follicular lymphoma, but are common cases that change their clinical phenotype and become more aggressive [6]. From these observations, it is evident that loss of anti-oncogene function is a common mechanism of clonal evolution of leukemia/lymphomas. Moreover, acquisition of additional genetic and cytogenetic abnormalities can lead to growth advantage in certain cells in a diseased population, causing the outgrowth of drug resistance clones.

Over the past two decades, investigators have strived to obtain in vitro models that reflect the pathological features of the human CML disease [7]. The first concrete evidence that the p210\textsuperscript{Bcr-Abl} protein is involved in the pathogenesis of human CML came from the introduction of the p210\textsuperscript{Bcr-Abl} retrovirus into bone marrow stem cells of mice, and subsequent development of a myeloproliferative disease with the clinical and pathological features of CML [8]. The identification of the coding sequence of the human CML-specific mRNA from the erythroid leukemic K562 cell line was a major stepping stone in the study of CML [9]. This allowed investigators to assess the activity of the p210\textsuperscript{Bcr-Abl} in vitro in a variety of cellular contexts.

The elevated Abl tyrosine kinase activity is regarded as the “powerhouse” behind the induction of CML. The inappropriately high levels of tyrosine kinase activity appear to send an unregulated proliferation signal to the nucleus of the leukemic cell [10]. This oncoprotein is associated with enhanced expression of several major downstream effectors such as Ras [11], phosphoinositide 3-kinase (PI-3K) and protein kinase B (AKT) [12]. Another translocation resulting in constitutive Abl tyrosine kinase activity is the Tel-Abl gene fusion. This oncoprotein has little distinguishing molecular traits with respect to oncogenic substrates, e.g., rasGAP, shc, SH-PTP1, CRKL, CBL, paxillin, Stats [13].

**BCR-ABL PROTEIN: STRUCTURE AND FUNCTION**

c-Abl and Bcr-Abl can be structurally and functionally divided into separate domains. The activity of the c-Abl tyrosine kinase domain is regulated by the SH3 and SH2 regions [14]. The SH3 domain is a negative regulator of c-Abl activity, as a deletion mutant of this domain activates the kinase, whereas the SH2 domain is a positive regulator. As a result of the Bcr-Abl fusion, the N-terminus of Bcr binds to the SH2 domain of Abl in a phosphotyrosine independent manner, constitutively activating the tyrosine kinase [15] (Fig. 1). Targeted deletion of the Bcr N-terminus, or of c-Abl SH3 and SH2 domains, diminishes the activation of specific downstream signaling molecules [16, 17], and results in reduced transformation efficiency [18, 19].

The fusion of bcr and abl sequences is an early, and quite possibly the primary, event in the induction of CML and related Ph\textsuperscript{+}-positive lymphoblastic leukemias. The Bcr-Abl fusion protein alone does not allow leukemic stem cells to grow independently of hematopoietic growth factors, but it appears to initiate a stepwise process which may ultimately do so [10, 20] (Fig. 2).

**Adhesion Independence**

Retention and homing of progenitor blood cells to the marrow microenvironment are regulated by receptors and agonists of survival and proliferation. Bcr-Abl induces adhesion independence resulting in aberrant release of

---

**Figure 1. Schematic representation depicting the domain structures of c-Abl and Bcr-Abl.** Abbreviations: OD = Oligimerization domain; PH = Pleckstrin homology; DBL = DBL homology domain; NLS = Nuclear localization signals.
The net effect of Bcr-Abl activity may be the activation of signal transduction pathways downstream of adhesion receptors in the absence of ligand binding. These cells still require hematopoietic growth factors for growth and viability, but they are adhesion-independent and able to migrate from the marrow into capillaries at an immature stage of development.

Proliferation of hematopoietic progenitor cells is regulated by positive and negative signals from bone marrow stromal cells [21, 22]. Direct contact with the stromal cells is not required for proliferation, indicating that proliferation is stimulated by diffusible factors. However, excess myeloid proliferation was observed in the absence of stromal contact, indicating that the negative regulation of proliferation involves cell-cell interaction [23].

The proteins of the focal adhesion complexes, such as paxillin, talin and vinculin, in addition to connecting integrin receptors to actin microfilaments, serve as a framework for the association of signaling proteins, including protein tyrosine kinases such as Fak, c-Abl and Src and adapter proteins such as GRB2 and CRKL [24-26], thereby linking integrin receptor stimulation to various intracellular signaling pathways. Integrin stimulation is also associated with cytoskeletal translocation and activation of the PI-3K [27], which may also play an important role in modulation of integrin function. Moreover, the focal adhesion-associated proteins paxillin, Fak, vinculin, talin, and tensin are constitutively phosphorylated in Bcr-Abl transfected cell lines [28-30]. Therefore, defects in integrin function may not only be responsible for the abnormal circulation, but also the continuous proliferation of CML progenitors, leading to their growth advantage and population expansion.

Growth Factor Independence

The survival, proliferation and differentiation of hematopoietic cells are controlled by growth factors e.g., erythropoietin, thrombopoietin, interleukin 3 (IL-3), granulocyte-macrophage-colony-stimulating factor (GM-CSF), G-CSF, IL-6, stem cell factor (steel factor, c-kit). These growth factors activate signaling pathways by binding to cell surface receptors resulting in a phosphorylation cascade. However, leukemic cells in vitro and in vivo display growth factor-independent characteristics.

The mechanism by which Bcr-Abl-positive cells achieve growth factor independence has not yet been fully elucidated. The possible mechanisms include the activation of cytokine signal transduction pathways by Bcr-Abl and/or aberrant expression of cell cycle control genes, cytokine receptors, and the autocrine production of the growth factor by the cell itself.

Cytokine receptors function by recruiting kinases involved in signal transduction cascades and ultimately converge upon transcription factors that regulate various cellular functions [31]. Ras proteins are critical components of signaling pathways that link the activation of cell surface receptors with transcriptional events leading to the control of proliferation, differentiation and apoptosis [32]. Autocrine growth factor synthesis may be a consequence of dysregulation of these signaling pathways and indeed has been well documented in CML. In addition, aberrant IL-3 production has been linked to chromosomal translocations resulting in factor-independent proliferation in leukemic cells [33].

Transformed factor-independent FDC-P1 myeloid cells and MO7e megakaryocytic cells have been shown to release growth factors into their culture media, suggesting that an autocrine loop may be involved in their transformation [34, 35]. However, a later study using Bcr-Abl SH2, kinase domain mutants and a temperature sensitive v-abl mutant, found that although growth factors are produced by transformed cells, they are not required for the cells to become factor-independent. These investigators suggest
that the presence of an activated tyrosine kinase alone is necessary and may be sufficient for growth factor-independent proliferation [36], and that the production of growth factors may further support the proliferation of leukemic and/or non-leukemic cells by autocrine and/or paracrine mechanisms, respectively [37].

The role of autocrine production of growth factors has also been investigated in vivo. A mouse model exhibiting a myeloproliferative disease was found to produce excess IL-3 and GM-CSF, and this was reported to contribute to the disease progression [38]. In contrast, another study has shown that insertion of growth factor cDNA into normal hematopoietic cells (including GM-CSF, IL-3, and G-CSF) resulted in proliferation and increased expression of the receptors involved [39-41]. However, a true leukemic syndrome was not generated when such cells were transplanted into mice.

A major mechanism which Bcr-Abl may exploit for autonomous growth is the constitutive activation of growth factor-induced downstream signaling events [42, 43]. Indeed, the expression of Bcr-Abl in cytokine-dependent hematopoietic cells permits proliferation in the absence of autocrine production of growth factors [17, 44, 45]. For instance, Ba/F3 transformed cells, relieved of their IL-3-dependence, did not reveal any detectable levels of autocrine growth factor production, excluding this mechanism of autonomous growth and providing evidence that Bcr-Abl has an intrinsic signaling capability that renders IL-3 receptor ligation unnecessary [46]. It was then shown that Bcr-Abl substitutes for IL-3-induced growth via tyrosine kinase-mediated signals in a lymphoid cell line leading to increased expression of proto-oncogene transcription factors involved in the regulation of cell cycling, i.e., c-fos, c-jun and c-myc [47].

In the past few years, the Jak/Stat pathway has emerged as a key pathway in human CML [48]. This pathway directly links cytokine receptors to gene transcription. Stats were the first and remain the only transcription factors known to be regulated by tyrosine phosphorylation [49]. In addition, this pathway is regulated by growth factors but has been found to be constitutively activated in megakaryocytic leukemic cell lines that display growth factor independence [50, 51]. However, antibody-blocking studies show that the activation of Stat5 in Bcr-Abl-expressing cell lines cannot be attributed to the activation of an IL-3/GM-CSF-driven autocrine loop, thereby suggesting that Bcr-Abl employs an alternative route to induce Stat activation [52].

Recently it was elucidated that there is a physical association between Bcr-Abl and the IL-3 receptor, concomitant with activation of Jak2, possibly rendering the IL-3 cytokine redundant [53]. A tetracycline-dependent expression system in Ba/F3 cells and tumorigenicity studies in mice demonstrated that the constitutive tyrosine phosphorylation of Stat proteins by Bcr-Abl is necessary for complete transformation [54]. A study devoted to the elucidation of signal transduction pathways activated by Bcr-Abl and IL-3 revealed that Bcr-Abl is not just mimicking IL-3 effects, nor is it inducing the endogenous expression of IL-3.

**Bcr-Abl and Cell Cycle**

Although c-Abl protein functions have not been completely elucidated, it is, however, known to be involved in the regulation of cell cycle, gene transcription, stress responses and integrin signaling [55]. Growth suppression by c-Abl requires tyrosine kinase activity, nuclear localization and an intact SH2 domain. On the other hand, Bcr-Abl is predominantly cytoplasmic and has elevated tyrosine kinase activity. This disruption of normal c-Abl function will undoubtedly contribute to aberrant cell cycle progression [56].

Cortez and coworkers have shown that 32D cells stably expressing Bcr-Abl protein fail to induce cell cycle arrest following growth factor starvation [57]. The ability of these Bcr-Abl-expressing cells to continue to cycle under low growth factor conditions suggested that Bcr-Abl signaling may maintain the activation of components of the cell cycle machinery which control the G2-to-S phase transition. It has been shown that the activity of cyclin-dependent kinases (CDks) including D2- and D3-associated Cdk4 and Cdk6 is high in Bcr-Abl-expressing cells under reduced serum and cytokine conditions and, thus, permits G1-to-S phase progression.

A recent study has shown that Bcr-Abl delays cell cycle progression at the G2/M transition point. This delay, induced by Bcr-Abl expression, may allow time to repair damage induced by cytotoxic drugs and segregation of an abnormal karyotype, thereby preventing a mitotic catastrophe [58]. In addition, Nishii and coworkers have shown, using BaF3 cells transfected with Bcr-Abl, that enhanced phosphorylation of cdc2, a cdk, by Bcr-Abl may be responsible for the prolonged G2 arrest following radiation [59]. Because mitotic catastrophe shares several features with apoptosis, including dissolution of the nuclear lamina, H1 kinase activity, chromosomal condensation and DNA fragmentation, it is conceivable that apoptosis may result from a similar failure of coupling the G2/M transition to completion of DNA repair or replication.

**Bcr-Abl and Apoptosis**

Normal hematopoiesis involves proliferation, differentiation and apoptotic cell death regulated by the concentration and composition of cytokines. Apoptosis follows terminal differentiation, negative selection of autoreactive lymphocytes...
and the removal of activated lymphocytes at the end of an immune response. Apoptosis is a genetically controlled cell suicide program that may be activated by a cell upon detection of oncogene deregulation, stress-inducing stimuli and DNA damaging agents [60, 61]. Oncogenic Bcr-Abl has been attributed antiapoptotic activity, which appears to promote the development and drug resistance of CML.

It is currently unknown whether the signaling pathways leading to growth also cause resistance to apoptosis. However, several lines of evidence suggest that these are separable pathways. A study demonstrated that Bcr-Abl has a stronger antiapoptotic function than cytokines and this corresponds with selective activation of Stats [62]. The increased kinase activity in Bcr-Abl-positive cells leads to activation of Stat proteins which subsequently enter the nucleus and activate the transcription of genes involved in apoptosis [63]. Some of these genes have been shown to be involved in Bcr-Abl-mediated oncogenesis, e.g., c-myc, Bcl-x, cyclin D1, and p21waf.

As the disease progresses to the aggressive acute phase, the expression level of Bcr-Abl frequently increases. This phase exhibits profound antiapoptotic features as is reflected in the difficulty of treatment. Indeed, a duplication of the Ph1 chromosome represents the most frequent karyotypic abnormality in acute phase CML [3]. Also, in CML immortal cell lines, multiple copies or amplification of the Bcr-Abl gene have been observed [4]. Recently, a dose-dependent hierarchy of Bcr-Abl-induced biological effects has been established [64]. Evidence suggests that low levels of Bcr-Abl mimic growth factor survival signaling (Ras-dependent survival signals inducing growth factor independence) whereas additional survival pathways are activated by high levels of Bcr-Abl, i.e., PI-3K and Shc to Myc pathway [65-67].

Inhibition of apoptosis is, however, not considered to be the primary mechanism Bcr-Abl employs in CML clonogenesis [68]. As seen with cells from CML patients, at early stages of the disease, Bcr-Abl expression alone does not prevent drug-induced apoptosis nor that induced by cytokine withdrawal, emphasizing that cellular context and disease stage is instrumental in Bcr-Abl-mediated effects [17, 69].

Resistance to apoptosis in Bcr-Abl-positive cells has been reported to occur via Bcl-2-dependent and independent pathways [70, 71]. It is of interest that overexpression of Bcl-2 in the presence of IL-3 does not replace the antiapoptotic activity induced by Bcr-Abl [64]. Bcl-2 expression has been shown to function in preventing the pro-apoptotic activity of Myc, thereby allowing cells to proliferate in the absence of apoptosis [72]. Bcr-Abl drives up expression of another antiapoptotic gene, Bcl-xL and this seems to be a powerful participant in Bcr-Abl-mediated resistance to apoptosis. This antiapoptotic molecule is also upregulated by IL-3, and its function appears to be dependent on the Map kinase pathway. Levels of Bcl-xL decrease preceding apoptosis induced by cytokine withdrawal [73].

Protein kinase C (PKC) has also been implicated as a critical downstream target of Bcr-Abl in the mediation of apoptosis suppression. Selective inhibition of Bcr-Abl and PKC has demonstrated the requirement of sustained PKC activation to suppress apoptosis in response to chemotherapeutic agents [74, 75].

Bcr-Abl and Differentiation
The characteristic features of the transition from chronic phase to blast crisis include not only resistance to apoptosis but also inhibition of terminal differentiation of hematopoietic cells. However, the role of Bcr-Abl in differentiation pathways has yet to be fully elucidated. During the process of hematopoietic differentiation, pluripotent stem cells become lineage-committed and eventually differentiate into functional, morphologically distinct end-stage cells. This process is accompanied by the coordinate expression of numerous genes. Little data exist regarding the role of Bcr and Abl proteins in hematopoiesis. M. Wetzler and coworkers reported that the expression of both Bcr and Abl proteins among myeloid cells is inversely related to maturation; thus, myeloblasts and promyelocytes are highly positive for Bcr and Abl proteins, whereas polymorphonuclear cells are weakly positive [76].

Bcr-Abl is expressed at high levels in immature myeloid CML cells compared to more mature cells, but the signal appears to be stronger than that of c-Abi in normal bone marrow myeloid precursors, and does not disappear completely with full myeloid maturation. Interestingly, the same drop in Bcr-Abl expression occurs upon differentiation to the metamyelocyte and neutrophil stages in CML mice, where Bcr-Abl is expressed from a retroviral promoter, so this phenomenon might be of a more general nature. The mechanism by which both Bcr-Abl, Bcr, and Abl proteins are downregulated upon differentiation of myeloid cells and cell lines is not known. This might occur at the transcriptional or post-transcriptional level. Previous experiments demonstrating a decrease in Bcr-Abl after hemin-induced erythroid differentiation of K562 cells [77, 78] suggest a translational mechanism since Bcr-Abl mRNA remains unchanged. However, the relationship between erythroid differentiation of K562 cells and Bcr-Abl expression may be more complex, since the use of certain alternative differentiation agents such as cytosine arabinoside does not alter Bcr-Abl levels [77].

**Tumor Suppressor Activity of P53**
Eukaryotic cells respond to DNA damage by activating signal transduction pathways that lead to cell cycle arrest,
DNA repair, and/or apoptosis. Evidently, these choices are designed to maximize cellular survival while minimizing the chance of carcinogenesis. Despite their importance, however, many of the steps in these signaling cascades are not completely understood. Perhaps the most prominent among the early responses induced by DNA damage is the activation of the transcription factor \( p53 \) \([79, 80]\). In normal cells, under physiological conditions, the tumor suppressor protein \( p53 \) is expressed at low levels and has a short half-life due to rapid turnover mediated by ubiquitination and proteolysis. However, \( p53 \) is stabilized in response to DNA damage, survival factor withdrawal and oncogene deregulation. It manifests its tumor suppressive effects via apoptosis, cell cycle arrest and differentiation (Fig. 3). \( p53 \) knockout mice appear in most cases to undergo normal development and maturation \([81]\), but later they are more susceptible to tumor development. This suggests that \( p53 \) is not essential for normal cellular function, but is essential for maintaining cellular integrity later in life.

The ability of \( p53 \) to promote growth arrest and apoptosis is influenced by several determinants such as the oncogenic composition of the cell, and the nature and extent of the extra-cellular stimuli. In addition, pathways leading to growth arrest and apoptosis are mutually exclusive and can be functionally separated by the action of cytokines \([82]\). Growth arrest induced by \( p53 \) is insensitive to the addition of cytokines \([83]\). Growth factor-dependent cell lines undergo \( p53 \)-mediated apoptosis in response to growth factor withdrawal \([84]\). IL-3 can protect hematopoietic cells from irradiation-induced apoptosis \([85, 86]\), but only up to a certain threshold of radiation. Above this threshold IL-3 is no longer capable of allowing genomic instability.

Both the expression and activity of \( p53 \) are tightly regulated by multiple positive and negative feedback loops. Key players in this regulation are the Murine Double Minute-2 (Mdm2) proto-oncprotein \([89, 90]\), the JUN-NH2-terminal kinase-1 (JNK1) \([91]\), the c-Abl nonreceptor tyrosine kinase \([92]\), the tumor suppressor 19ARF \([93, 94]\), WT1 \([95]\), hypoxia-inducible factor \([96]\), p14ARF \([97]\), and retinoblastoma protein (Rb) \([98]\). \( p53 \) has many functional domains and is regulated at different levels including transcription, translation, post-translation modifications and protein turnover. Phosphorylation is one of the major mechanisms of regulation of \( p53 \) activity. However, the role it plays in the tumor suppressor activity of \( p53 \) remains unclear.

Cell cycle arrest requires \( p53 \)-dependent transcription. Transcription of p21 mediates \( p53 \)-dependent G\(_1\) arrest by inhibiting the activity of Cdk, in particular cyclin D-Cdk4/6 and cyclin A,E-Cdk2, which phosphorylate the retinoblastoma protein (Rb) \([99]\). p21 can also bind to PCNA (proliferation cellular nuclear antigen), blocking PCNA from activating DNA polymerase \( \delta \) which is essential for DNA replication, thereby preventing cell cycle replication \([100]\). \( p53 \) can also trigger growth arrest in a p21-independent manner. By binding to cyclin H and p36Mat1, \( p53 \) inhibits the protein kinase complex Cdk7/cyclinH1/Mat1 (a Cdk-activating kinase termed CAK kinase) which activates the Cdk2/cyclin A kinase required for the G\(_2\)/S transition \([101]\). G\(_2\) arrest requires further \( p53 \)-mediated transcription of 14-3-3 \( \sigma \) and GADD45 \([102]\). The overexpression of the GADD45 gene (a \( p53 \)-responsive gene) in some cells in culture can also induce arrest at G\(_1\).
DNA damage-induced phosphorylation of either p53 or Mdm2 prevents the two proteins from interacting, thus stabilizing and activating p53-mediated apoptosis. In addition, the degradation of p53 is prevented by caspase-3 cleavage of Mdm2 [103]. p53 stimulates the expression of a number of gene products that are known to participate in the apoptotic pathway. These include Bax [104], insulin-like growth factor-binding protein 3 [105, 106], GADD45 [86], oxidative stress genes [107], and Cathepsin D [108].

p53 has been shown to activate Fas and FasL-induced apoptosis via a cell surface trafficking mechanism [109, 110]. The Fas death receptor contains a p53-responsive element which is bound by p53 and acts as a p53-dependent enhancer of Fas transcription. Apoptosis does not necessarily follow activation of this response element, but rather a cell is “primed” for apoptosis [111].

p53 has also been implicated in cell differentiation. This was concluded from the observation that in some cases, transfecting wild-type p53 into cells did not cause an immediate cell growth arrest, but rather permitted cellular events associated with the appearance of cell differentiation characteristics. Evidence suggests that p21 has been implicated in the coupling of differentiation signals to growth arrest. It is consistent with the finding that an indirect target of p21, Rb, becomes hypophosphorylated on differentiation induction, thereby preventing entry into the cell cycle [112]. Recent reports have shown that the function of the p21 gene as an inhibitor of cell cycle progression or apoptosis is determined by its subcellular localization. p21 ectopically expressed in immature monocytes is localized in the nucleus and induces cell cycle arrest. The differentiation of immature monocytes is also associated with relocalization of nuclear p21 to the cytoplasm. Thus, the nuclear expression of p21 and subsequent G1 cell cycle arrest may allow the differentiation program already present in precursor cells to proceed. While this differentiation program takes place, p21 relocates to the cytoplasm. An apoptosis-resistant phenotype appears concomitantly with the expression of cytoplasmic p21 [113]. Aberrant responsiveness of p21 to differentiation signals could be a component of the oncogenic blockade in differentiation. p21 mRNA modulation in human bone marrow by G-CSF underscores the physiologic significance of normal regulators of p21 gene expression.

p53 is a multifunctional cellular tumor suppressor protein, whose importance is highlighted by its loss during disease progression. p53 loss or mutation renders a cell insensitive to oncogenes, unable to arrest and repair damaged DNA and induce apoptosis. This further contributes to cellular instability, drug resistance and acquisition of further mutations.

**P53 and Disease Progression**

p53 loss of function is a common abnormality in the transition from chronic phase CML to blast crisis, and results in a more aggressive, drug-resistant and less differentiated phenotype. Chronic phase CML retains p53 functions, suggesting that its tumor suppressor activity delays progression of the disease [114].

This type of tumor suppressor activity has been documented with other oncogenes. Accumulation of p53 has been reported in cells expressing c-Myc, EIA and E2F-1 [115, 116]. Overexpression of these oncogenes rapidly induces p14ARF expression which binds to the Mdm2-p53 complex preventing p53 degradation, and therefore facilitating p53-dependent apoptosis [93, 117]. In addition, overexpression of p53 in a variety of leukemic cells has been shown to elicit several features of p53-mediated responses, including the induction of apoptosis [118], and enhanced dependence upon survival factors [119]. Such studies have directly implicated p53 loss as an important factor in disease progression.

Evidence suggests that in Bcr-Abl-expressing cells, the ability of p53 to exert its tumor suppressor activity may depend upon the relative expression levels of Bcr-Abl and p53. A recent study has established that the biological effects of Bcr-Abl are dictated by its level of expression. High levels of Bcr-Abl expression were shown to block the induction of apoptosis (see previous section), whereas cells expressing low levels of Bcr-Abl, could not protect against apoptotic stimuli [64]. Moreover, a recent study has shown that in a Bcr-Abl-expressing cell line (K562), the stabilization of exogenously expressed p53 could be achieved by inhibition of Mdm2. Despite the expression of high levels of Bcr-Abl in these cells, the concomitant high expression of p53 exerted tumor suppressor activity and induced differentiation and apoptosis (analogous to chronic phase CML). Such studies have demonstrated the importance of the relative ratio of the oncogene and tumor suppressor [120]. The mechanisms underlying this relationship between Bcr-Abl and p53 remain to be established. Undoubtedly such mechanisms play a key role in determining the nature of a leukemia. The very existence of the chronic phase of CML provides a unique model, and a valuable insight into the role of tumor suppressor function in the development of malignancy (Fig. 4).

**Future Perspectives**

The design of more representative models of CML and increasing availability of protein inhibitors have enabled us to gain a better understanding of the signaling pathways involved in the pathogenesis of CML. It is clear that the loss of p53 function results in a much more aggressive
Further understanding of Bcr-Abl activated-signaling pathways, leading to the abrogation of apoptotic resistance, is crucial to the development of new therapeutic strategies.

Figure 4. p53 and Bcr-Abl cooperation. p53 tumor suppressor activity delays progression of the disease as shown in pathways 1 and 2, depending upon the relative expression level of Bcr-Abl protein as shown in pathway 3.

REFERENCES


37 Anderson SM, Mladenovic J. The BCR-ABL oncogene requires both kinase activity and src-homology 2 domain to induce cytokine secretion. Blood 1996;87:238-244.


44 Carlesso N, Griffin JD, Druker BJ. Use of a temperature-sensitive mutant to define the biological effects of the p210BCR-ABL tyrosine kinase on proliferation of a factor-dependent murine myeloid cell line. Oncogene 1994;9:149-156.


50 Frank DA, Varticovski L. BCR/abl leads to the constitutive activation of Stat proteins, and shares an epitope with tyrosine phosphorylated Stats. Leukemia 1996;10:1724-1730.

52 Chai SK, Nichols GL, Rothman P. Constitutive activation of JAKs and STATs in BCR-ABL-expressing cell lines and peripheral blood cells derived from leukemic patients. J Immunol 1997;159:4720-4728.
72 Leverrier Y, Thomas J, Perkins GR et al. In bone marrow derived BaF-3 cells, inhibition of apoptosis by IL-3 is mediated by two independent pathways. Oncogene 1997;14:425-430.
88 Lane DP. Cancer. p53: guardian of the genome [news; comment] [see comments]. Nature 1992;358:15-16.
97 Ichimura K, Bolin MB, Goike HM et al. Deregulation of the p14ARF/MDM2/p53 pathway is a prerequisite for human astrocytic gliomas with G2-M transition control gene abnormalities [In Process Citation]. Cancer Res 2000;60:417-424
100 Waga S, Hannon GI, Beach D et al. The p21 inhibitor of cyclin-dependent kinases controls DNA replication by interaction with PCNA [see comments]. Nature 1994;369:574-578.
104 Miyashita T, Reed JC. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell 1995;80:293-299.