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REVIEW

Chronic myelogenous leukemia: mechanisms underlying disease progression

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Chronic myelogenous leukemia (CML), characterized by the BCR-ABL gene rearrangement, has been extensively studied. Significant progress has been made in the area of BCR-ABL-mediated intracellular signaling, which has led to a better understanding of BCR-ABL-mediated clinical features in chronic phase CML. Disease progression and blast crisis CML is associated with characteristic non-random cytogenetic and molecular events. These can be viewed as increased oncogenic activity or loss of tumor suppressor activity. However, what causes transformation and disease progression to blast crisis is only poorly understood. This is in part due to the lack of a good *in vivo* model of chronic phase CML even though animal models developed over the last few years have started to provide insights into blast crisis development. Thus, additional *in vitro* and *in vivo* studies will be needed to provide a complete understanding of the contribution of BCR-ABL and other genes to disease progression and to improve therapeutic approaches for blast crisis CML.
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Introduction

Chronic myelogenous leukemia (CML) is a clonal myeloproliferative disorder of the hematopoeitic stem cell (HSC). In the initial chronic phase, myeloid progenitors and mature cells accumulate in the blood and extramedullary tissues.[1] After 3–4 years, the disease transforms and terminates in a blast crisis characterized by a maturation arrest in the myeloid or lymphoid lineage.[2–4] The only curative therapy for this disorder is allogeneic stem cell transplantation performed during chronic phase.[5–7] Treatment with interferon α delays progression of the disease to blast crisis and in 10–20% of patients results in complete remission.[6,8] The median survival of patients with blast crisis without treatment is 3 months.[3]

CML is a malignancy that is consistently associated with an acquired genetic abnormality, the Philadelphia chromosome. Ph is present in >90% of patients and is the result of a rearrangement between the BCR and Abelson genes. The BCR-ABL fusion gene, is seen in up to 95% of CML patients and gets translated into an oncoprotein, $p210^{BCR/ABL}$.[9] The presence of $p210^{BCR/ABL}$ is necessary and sufficient for malignant transformation as demonstrated in animal models[10–13] and *in vitro* systems.[14,15] Although the molecular mechanisms that underlie BCR-ABL-mediated transformation have been extensively studied, progression of the disease from chronic phase to blast crisis is incompletely understood. In this review we will summarize the patho-physiology of chronic phase CML as it is currently understood and then discuss the molecular mechanisms thought to contribute to the progression of chronic phase to blast crisis CML.

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Molecular biology of BCR-ABL

Approximately 90% of patients with CML have an acquired genetic abnormality, the Philadelphia chromosome (Ph).[16] The Ph is a shortened chromosome 22 resulting from a reciprocal translocation between the long arms of chromosomes 9 and 22 t(9; 22q34;q11).[16,17] In this translocation, the c-ABL proto-oncogene is transposed from its normal position on chromosome 9 to a 5.8 kb major breakpoint cluster region M-BCR on chromosome 22, forming a BCR-ABL fusion gene.[17,18] The new gene encodes $p210^{BCR/ABL}$, an oncoprotein that has increased tyrosine kinase (TK) activity[19,20] and increased binding to the actin cytoskeleton[21] compared with the p145 Abelson protein, both of which contribute to transformation. The presence of $p210^{BCR/ABL}$ causes growth factor independence and leukemic cell growth in hematopoietic cell lines.[14,15,22] Transplantation of BCR-ABL-transduced hematopoietic stem cells or transgenic expression of $p210^{BCR/ABL}$ induces leukemia, lymphomas and CML-like syndromes[10–13,23–27] proving the direct causal relationship to CML.

Prior to discussing the biology of the BCR-ABL gene we will review the functions of the gene products of the normal Abelson and BCR genes. The $p145^{ABL}$ protein, the product of the Abelson gene, is a TK whose function is not totally known. There is evidence that $p145^{ABL}$ is important for cell growth,[28–30] induction of apoptosis[31–33] and is involved in DNA repair.[34–36] Although $p145^{ABL}$ is found mainly in the cell nucleus, there is mounting evidence that it plays a role in cell signaling from integrins[37] as well as other cell surface receptors such as the B cell receptor and CD19.[38] $Abelson^{-/-}$ mice die early after birth due to severe runting and impaired lymphoid development[39] that may be related to the recently discovered role of $p145^{ABL}$ in B cell function.[38] $ABL^{+/-}$ mice, however, are normal indicating that it is unlikely that loss of one normal Abelson allele in CML plays a role in the disease. Even less is known about the function of the normal BCR gene.

$BCR^{-/-}$ mice develop septic shock when challenged with lipopolysaccharide due to a significantly increased and dysregulated neutrophilic oxidative burst.[40] $BCR^{+/-}$ mice are normal, again suggesting that loss of one normal BCR allele does not play a role in the pathogenesis of CML.

Signal transduction in chronic phase CML

The biology of the BCR-ABL oncogene and its intracellular signal transduction pathways have been extensively

reviewed[41–44] and only the key parts will be discussed here (Figure 1). The BCR-ABL oncoprotein has constitutively expressed tyrosine kinase activity as a result of oligomerization of the coiled coil region of $p210^{BCR/ABL}$ [45] and deletion of the inhibitory SH3 domain of ABL.[46] This results in phosphorylation of $p210^{BCR/ABL}$ itself on the Y-177 tyrosine residue[47] and leads to recruitment of GRB2,[48] a small adapter molecule that can activate the RAS pathway.[49–52] $p210^{BCR/ABL}$ kinase also phosphorylates JAK2 and STAT1/STAT5,[53–56] proteins that transfer signals from non-TK cytokine receptors to the nucleus. RAS and STAT activation contributes to growth factor independence of cell lines containing the BCR-ABL gene.

Furthermore, $p210^{BCR/ABL}$ activates the PI(3)K/Akt pathway,[57–60] increases expression of BCL-2,[61] and phosphorylates STAT5[62,63] leading to the increased resistance of CML progenitors to apoptosis.

Finally, $p210^{BCR/ABL}$ is localized almost exclusively in the cytosol due to loss of a nuclear localizing signal and has increased binding to actin compared with $p145^{ABL}$.[21,64,65] This results in phosphorylation of a number of neighboring cytoskeletal proteins including FAK[66] and paxillin,[67] all of which contribute to aberrant adhesion receptor function and may explain the premature circulation of progenitors and precursors in the blood.[68,69]

The features of chronic phase CML, expansion and premature circulation of the malignant myeloid population can therefore be explained by activation of mutagenic pathways, antiapoptotic pathways and abnormal cytoskeletal function (Figure 1). These same characteristics, increased mutagenicity and decreased susceptibility to apoptosis may also be responsible for disease progression.[70] Moreover, the continuously proliferating malignant cell population is prone to secondary genetic abnormalities, which may be better tolerated due to the antiapoptotic phenotype conferred by the presence of $p210^{BCR/ABL}$. Subsequently, multiple malignant clones with new genetic errors emerge that can ultimately lead to disease progression and blast crisis.

Mechanisms of disease progression

Although the BCR-ABL gene plays a central role in the pathogenesis of chronic phase CML and its continued expression is required for the proliferation of cells in the acute phase, the molecular events leading to the evolution to blast crisis are not fully understood. CML cells develop additional cytogenetic or molecular defects that commonly precede blast crisis (Table 1). The significance of these cytogenetic and molecular defects and their impact on disease transformation will be discussed in this section. We will also describe the underlying pathways that are considered important in disease progression.

Cellular events

Decreased apoptosis

Apoptosis or programmed cell death can be considered a protective mechanism against cancer and its derangement has significance in many malignancies. Cells harboring or acquiring chromosomal errors by DNA damaging agents undergo apoptosis. Signals instructing a cell to undergo apoptosis are multiple, complex and highly redundant. The final decision for a cell to initiate apoptosis rather than cell cycle arrest, or a failure to respond by either method may be dependent on the magnitude and duration of the damage stimulus.

In CML, multiple mechanisms contribute toward resistance against apoptosis. Phosphorylation and activation of the PI(3)-K/Akt pathway[59,71] is a major pathway by which BCR-ABL exerts its antiapoptotic effect. PI(3) kinase activation also via protein kinase B, results in the phosphorylation and inactivation of the pro-apoptotic protein BAD a member of the Bcl-2 family.[60] Increased expression of BCL-2 the prototype member of the BCL family of antiapoptotic proteins[72] has been described in cell types harboring the BCR-ABL oncogene and contributes to decreased apoptosis.[61] BCR-ABL-mediated activation of STAT5 and subsequent increases in BCLxl levels may also increase resistance to apoptosis.[62,63,73]

Most but not all studies using $p210^{BCR/ABL}$ expressing cell lines have demonstrated that BCR-ABL expression protects from apoptosis induced by physical and chemical stresses.[74–78] Additionally, chronic phase CML $CD34^+$ cells undergo delayed apoptotic death upon cytokine withdrawal when compared with normal progenitors.[79–81] The use of antisense oligonucleotides against BCR-ABL could reverse the delay in apoptosis in CML cell lines.[80,81] $P210^{BCR/ABL}$ may protect cells from cytotoxic agent-induced apoptosis by preventing the release of cytochrome-c[82] and preventing the activation of caspases especially caspase 3.[83]

The redox-sensitive transcription factor NF-κB translocates to the nucleus upon cellular activation and its activity is generally associated with protection from apoptosis. There is evidence for activation of NF-κB in transgenic models of CML which is yet another mechanism that may protect against apoptosis.[84] Finally, the RAS pathway may be involved in BCR-ABL-mediated inhibition of apoptosis[75,85,86] and elevated cytokine production[87,88] may have an inhibitory effect on apoptosis.

Interestingly, c-ABL is thought to have a pro-apoptotic function in the cytoplasm by inhibiting the survival pathway mediated by PI(3)K and as discussed later, methylation of the only normal ABL allele seen during disease progression may contribute towards resistance against apoptosis. Nuclear ABL is also believed to have a role in cell death. More evidence to support this theory has emerged with the demonstration that nuclear import of BCR-ABL in a CML cell line by treatment with STI-571 (gleevec, a new specific tyrosine kinase inhibitor that inhibits the BCR-ABL TK at micromolar concentrations[89]) and its subsequent nuclear entrapment by leptomycin B induced apoptosis.[90] Therefore, the abnormal cytoplasmic location of BCR-ABL and the reduction in nuclear ABL protein may enhance resistance to apoptosis.

Protection against apoptosis is relatively minimal in chronic phase CML but there is evidence that BCR-ABL mediates resistance to apoptosis in a dose-dependent fashion.[91] Increased expression of the BCR-ABL mRNA, often associated with disease progression[92] may therefore lead to increased resistance to apoptosis in accelerated phase and blast crisis.[91] Despite being a characteristic feature of CML decreased apoptosis alone is not sufficient for disease transformation and disease progression likely requires additional abnormalities such as for instance, loss of a tumor suppressor gene.

Differentiation block

During the process of hematopoeitic differentiation, pluripotent stem cells become lineage committed and eventually dif-

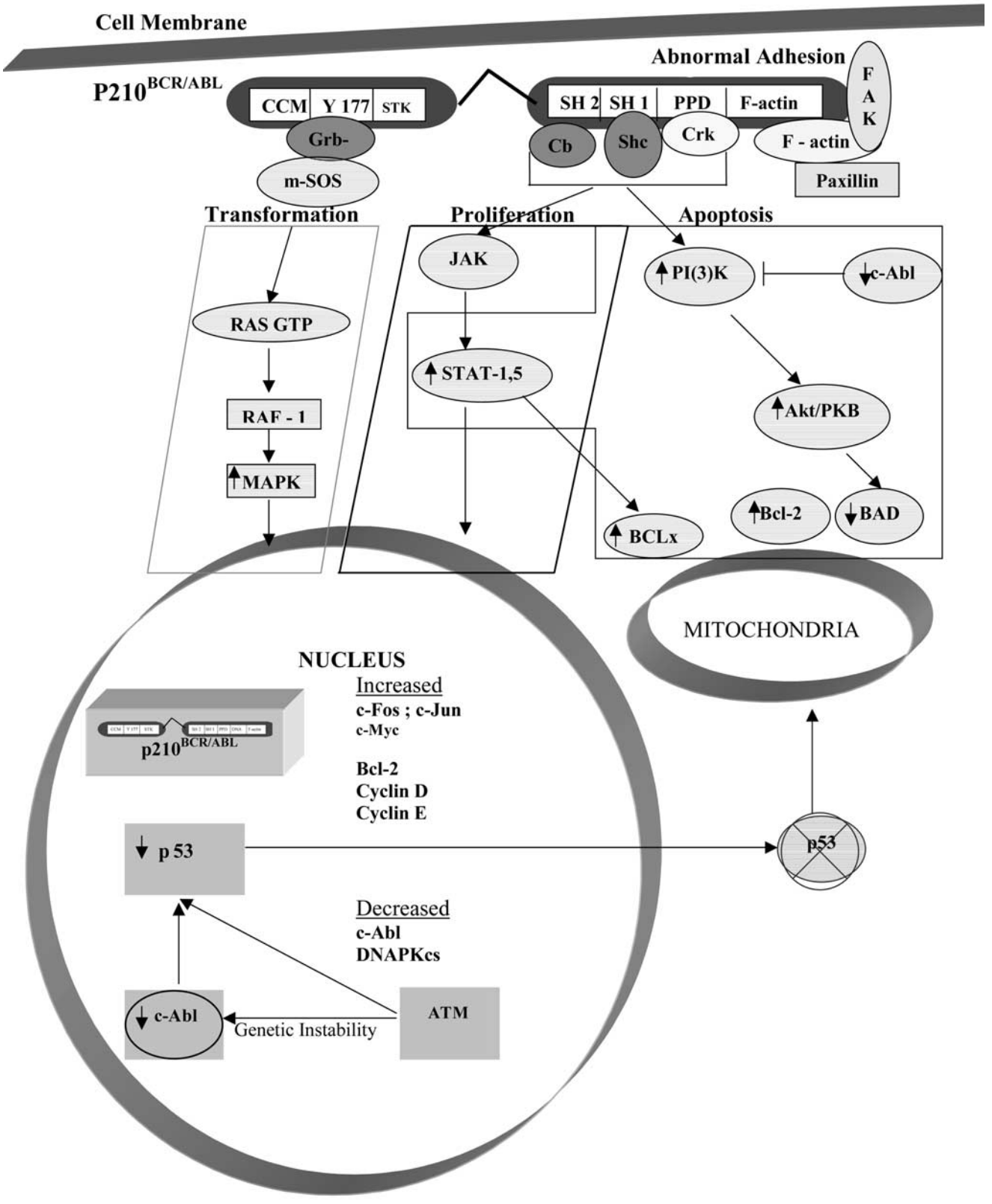

Figure 1 CCM, coiled coil motif; Y-177, tyrosine residue; STK, serine-threonine kinase; SH1 and 2, Src homology domain; PPD, polyproline domain; F-actin, actin binding domain; FAK, focal adhesion kinase. Signal transduction pathways affected by $p210^{BCR-ABL}$. Activation of the RAS, Jak/Stat and PI-3 kinase pathways results in increased proliferation, differentiation and decreased apoptosis of CML progenitors. F-actin binding via integrins results in impaired adhesion and premature release of CML progenitors into the circulation. Loss of p53 results in diminished apoptosis and increases the likelihood of secondary genetic abnormalities. Decreased DNAPKcs can result in inadequate DNA repair, as may decreased amounts of nuclear ABL. Shuttling of ABL occurs between the nucleus and cytoplasm and cytoplasmic ABL has a role in cell surface signaling through integrins. Cytoplasmic ABL is also believed to inhibit the PI(3)K pathway, a function that may be impaired due to reduced amounts of ABL.

Table 1 Cellular and molecular events associated with disease progression

Mechanisms of disease progression	*Ref.*
Cellular events	
Increased proliferation	53, 55, 70, 174
Drug resistance	103–106
Decreased apoptosis	58–63, 71, 73, 75, 79–84, 86, 91
Differentiation block	93–96
Abnormal immune surveillance	98–99
Genetic and molecular events	
Oncogene formation/activation	
Ph duplication	108, 109, 151, 173
c-MYC	115–118, 152–155, 157
RB	114, 166
AML-EV1	119, 120, 159–161
Tumor suppressor gene inactivation	
p53 loss	109–111, 129–136
$p16^{INK4a}$	112, 138–139
Miscellaneous	
c-ABL hypermethylation	141–144
Genomic instability	169–170
Impaired DNA repair	172
Loss of imprinting	150

ferentiate morphologically and functionally into distinct blood cell types. Although the $p210^{BCR/ABL}$ itself does not significantly affect terminal cell differentiation in chronic phase, differentiation is blocked in blast phase CML.[93–95] The role of BCR-ABL itself or other events in this phenomenon is not fully elucidated. Additional mutations, formation of new oncogenes, elevated cytokine levels, inactivation of tumor suppressor genes are hypothetical reasons for this phenomenon. As exemplified in acute promyelocytic leukemia, tumor progression can be suppressed by inducing differentiation with cytokines or factors that regulate normal hematopoiesis.[96,97] Further studies elucidating the mechanisms underlying the differentiation block seen in blast crisis CML are warranted to help define a role for differentiating agents in the therapeutic armamentarium against blast crisis.

Decreased immune surveillance

MHC-restricted and MHC-unrestricted mechanisms play an *important* role in the natural control of the Ph clone in chronic phase as well as during progression of CML.[98] Among the MHC-restricted mechanisms, T lymphocyte-mediated killing of target cells via Fas-receptor triggering plays an important role in elimination of malignant CML cells. CML progenitor cells also express functional Fas-ligand, which may be an important immune surveillance escape factor. In comparison to the chronic phase, CML cells derived from patients in blast crisis are refractory to Fas-mediated apoptosis, regardless of the expression levels of Fas, suggesting that an immune-mediated selection pressure could result in acquisition of Fas resistance.[99,100] Natural killer (NK) and activated killer (AK) cells mediate MHC-unrestricted cytotoxicity. There is evidence for declining NK cell function in blast crisis CML but it is unclear if this is a cause rather than an effect of disease progression.[101,102]

Drug resistance

CML progenitors obtained from patients during chronic phase and blast crisis are equally sensitive to STI-571 by *in vitro* assay methods.[103] Despite this observation there is a lower response rate in accelerated phase and blast crisis compared with chronic phase CML indicating development of drug resistance *in vivo*. The other possibility to consider is the presence of additional genetic errors that can stimulate the malignant clone in the absence of BCR-ABL. The observation that some patients who initially respond to STI-571 redevelop Ph-positive hematopoiesis may also indicate development of drug resistance. Additionally, resistance to interferon α or hydroxyurea is thought to be a herald of disease transformation. Drug resistance may be contributory to the change in character of chronic phase CML. There are multiple mechanisms involved in resistance to therapy, which could impact on disease progression. These include expression of the MDR-1 gene,[104,105] AGP-1,[106] reduplication of BCR-ABL or its overexpression,[104,107] decreased apoptosis[74] and possibly defective drug transport.

Genetic events

Cytogenetic and molecular changes occur in the majority of the patients during evolution to blast crisis. Approximately 70–80% of patients with classical Ph-positive CML show additional non-random chromosomal changes[108,109,172] involving chromosomes 8, 17, 19 and 22 with duplication of the Ph chromosome[108] or trisomy 8[3] being the most frequent. In about 15% of patients, progression of the disease is associated with –7, –17, +17, +21 and –Y.[172]

At the molecular level, the most frequent abnormality is a deletion of p53, a tumor suppressor gene located on the short arm of chromosome 17,[110–112] leading chiefly to myeloid blast crisis. About 50% of patients with lymphoid blast crisis have a homozygous deletion of the $p16^{INK4a}$ gene located on chromosome 9.[113] Other less frequent acquired genetic abnormalities are deletions of the retinoblastoma gene,[114] overexpression of c-MYC[115–118] or N-RAS[119] and generation of an oncogene AML-EVI-1.[120,121]

Inactivation of tumor suppressor genes

DNA damage in eukaryotic organisms leads to activation of DNA damage sensor proteins such as ATM.[122] These proteins when activated phosphorylate p53, which in turn upregulates expression of proteins such as BAX,[123], CD95[124] and DR5,[125] all members of core apoptotic pathways. In addition, activated p53 causes increased expression of cell cycle blocking genes such as $p21^{cip1}$,[126] leading to cell cycle arrest at the G_1–S phase to allow cell repair. Thus, the p53 gene manifests its tumor suppressive effects via apoptotic cell death or cell cycle arrest.[126] The tumor suppressor role of p53 has been demonstrated in p53 knock out mice that develop normally but are prone to developing spontaneous tumors.[127]

The p53 gene is located on chromosome 17 and its inactivation has been shown to be important in the evolution of CML to blast crisis.[128] Loss of p53 function can be due to mutations,[129] deletions and rearrangements[130] and is seen in 25% of myeloid blast crisis.[111,129,131,132] In addition to loss of function mutations, the production of mutant p53 proteins can

function as a dominant negative isoform or can act co-operatively with BCR-ABL to stimulate proliferation.[133]

The best proof for a role of p53 in disease progression is derived from a murine model that closely resembles chronic phase CML in which BCR-ABL cDNA regulated by the *Tec* promoter was introduced.[23] Mice expressing BCR-ABL from the *Tec* promoter were crossed with $p53^{+/-}$ mice. In contrast to BCR-ABL transgenic animals, BCR/ABL-$p53^{+/-}$ animals developed rapid progression to blast crisis.[134] This rapid progression of chronic phase to blast crisis was due to loss of function of the remaining p53 allele. Similarly, transplantation of p53-deficient bone marrow cells transduced with BCR-ABL cDNA led to rapid blast crisis.[135]

In one report, marrow from a CML patient in blast crisis showed a point mutation in the coding region of the p53 gene that was absent in chronic phase CML and upon return to second chronic phase the mutation was undetectable.[136] A number of studies using sequential evaluation of bone marrow of patients in chronic phase that evolved to blast crisis have revealed frequent acquisition of p53 mutations.[111,112,130,137] Additionally, the blasts of some of these patients displayed decreased apoptosis emphasizing the role of p53-mediated apoptotic cell death that appears to be deficient in blast phase.[111]

Another tumor suppressor gene $p16^{INK4a}$ located on chromosome 9 has been associated with progression of CML to blast crisis.[113,138] $P16^{INK4a}$ is an inhibitor of cyclin D. Cyclin D kinase complexes phosphorylate Rb and prevent cell cycle arrest in G1.[139] $P16^{-/-}$ mice are prone to developing cancer and many human tumors carry transcriptionally silent p16 genes due to aberrant methylation of upstream regulatory sequences.[140] Sequential studies of CML patients demonstrate homozygous deletions of $p16^{INK4a}$ acquired in associated with progression to lymphoid blast crisis in approximately 50% cases.[113]

Alterations in methylation of the proximal promoter of c-ABL appear to be specifically and consistently associated with progression of CML.[141–144] In chronic phase the promoter of ABL is seldom methylated while progressive hypermethylation is observed with late chronic phase, accelerated phase and blast crisis CML. As mentioned above, nuclear $p145^{ABL}$ is important for DNA repair and can induce apoptosis. Thus, silencing of the c-ABL promoter by methylation will further decrease nuclear $p145^{ABL}$ levels, further decreasing apoptosis and perhaps enhancing genomic instability, features that are related to disease progression. Another gene that is hypermethylated during acceleration of CML is the calcitonin gene, although its importance in disease progression is unclear.[145]

Deletions associated with acquired somatic mutations have been described previously in other leukemias, and small deletions of chromosome 22 sequences adjacent to the Ph chromosome translocation have been identified in CML.[146,147] In the past these deletions were thought too small and without pathological significance. Recently, however, sequential analysis of bone marrow of 56 patients with CML revealed that 16 patients had large acquired genomic deletions resulting in loss of chromosome 9 or 22 sequences flanking the translocation breakpoint on the derivative 9, on additional partner chromosomes, or on both.[148] These results suggest that the loss of a gene or genes adjacent to the translocation breakpoint may influence the progression of CML.

Other rare forms of genetic or molecular defects such as deletions in the retinoblastoma gene,[114] decreased Ikaros activity[149] and loss of imprinting[150] have been described in progression from chronic phase to blast crisis CML but their contribution to disease progression remains speculative.

Activation of oncogenes

Increased levels of BCR-ABL mRNA and protein are associated with disease progression.[92] This can be due to duplication of the Philadelphia chromosome, the most frequent cytogenetic change preceding disease transformation.[108,151] In other patients increased levels of BCR-ABL mRNA may represent increased translational activity of the existing BCR-ABL gene. *In vitro* studies have shown that resistance to STI-571 can be induced by amplification of the BCR-ABL gene or by reduplication.[104] This correlates with poor response rates seen in blast crisis as well as loss of response to STI-571 in responding patients.

Activation of oncogenes in addition to the Ph chromosome has been investigated in CML blast crisis. c-MYC appears to play a role in BCR-ABL-mediated transformation[30,152–155] and may mediate its effects either by antagonising the function of p53[156,157] or by acting as a co-operative oncogene with the BCR-ABL.[154] MYC expression is normal in chronic phase CML but is increased in patients with blast crisis.[116,155] Overexpression of c-MYC occurs as a result of increased transcription[156] or trisomy 8[158] frequently observed during disease progression or stabilization of c-MYC m-RNA due to polyadenylation.[117]

Additional translocations like t(3;21) have been reported in high frequency in blast crisis.[120,159–161] This results in the formation of the chimeric AML/EVI-1 fusion protein that blocks differentiation and stimulates proliferation. It can therefore assist the progression of CML through interference with cell growth and differentiation.[162] Although RAS plays a central role in the signal transduction activity of BCR-ABL,[85,86,163–165] the role of RAS mutations in CML blast crisis seems unclear. In one study, only two of 22 samples of patients in blast crisis showed mutations for K-RAS[166] and no mutations for N-RAS were detected in another analysis of 121 patients in blast crisis.[167] Despite evidence that many oncogenes are involved in the progression of CML, currently there is no single oncogene in addition to BCR-ABL that has been shown to definitively cause this phenomenon.

Impaired DNA repair

Genetic instability due to dysfunction of DNA repair has long been considered a cause of the non-random chromosomal abnormalities that contributes to disease progression. However, not all the studies support the notion of genomic instability in CML.[105,168,169] Additionally, whether genomic instability is a cause or effect of disease progression is also not known.[170] Although c-ABL has a role in DNA repair after damage[34,171] and can interact with DNA repair proteins such as DNA protein kinases (DNA-PKcs),[35] how loss of one ABL allele and presence of BCR-ABL affects DNA repair is not understood. In an interesting recent study it was shown in murine and human $p210^{BCR/ABL}$ containing cells that BCR-ABL might down-regulate the DNA repair protein DNA-PKcs.[172] These findings suggest that increasing levels of BCR-ABL protein lead to inhibition of DNA repair that can lead to accumulation of other genetic defects and disease progression. It is possible that instability of the genome may be a feature of the

CML, which becomes significant at a certain threshold level of BCR-ABL protein or signaling.

Conclusions

In summary, expression of the BCR-ABL oncoprotein leads to an inherently unstable genome in the hematopoeitic stem cell. There is a resultant increase in proliferation, which may increase the likelihood of non-random mutations. Impaired DNA repair contributes to the accumulation of non-random chromosomal abnormalities in addition to BCR-ABL and multiple different cytogenetic and molecular abnormalities are commonly seen with disease progression. Increased $p210^{BCR-ABL}$ protein levels in advanced disease further increases the resistance of the leukemic clone to apoptosis, resulting in tolerance to the genetic errors accumulating in the malignant clone. Subsequent dominance of one or more clones finally leads to culmination in fatal blast crisis. Currently, there is no well-characterized genetic or cellular event that overwhelmingly contributes to disease progression, emphasizing the complexity and redundant nature of the multiple signaling pathways in this disease. The most frequent abnormalities seen are loss of p53 and reduplication of the Ph chromosome, which lead to rapid development of blast crisis. These abnormalities need further characterization to determine their precise role and exact contribution to disease progression.

Ideally, disease progression should be studied in an animal model of chronic phase CML. Despite the existence of many different murine models of CML only one model replicates relatively faithfully the extended chronic phase of CML.[23] This model has been successfully used to demonstrate a role of p53 in disease progression.[134] However, loss of p53 alone does not fully explain this complicated event and the search for other co-operative genes must continue. Development of better animal models can delineate mechanisms for disease progression and may facilitate development of novel methods that would treat this fatal event.

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