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Multiple pathways contribute to BCR-ABL–dependent transformation of hematopoietic stem cells.

Signaling Networks Associated With BCR-ABL–Dependent Transformation

Lori A. Hazlehurst, PhD, Nadine N. Bewry, PhD, Rajesh R. Nair, PhD, and Javier Pinilla-Ibarz, MD, PhD

***Background**: The fusion protein BCR-ABL results in constitutive tyrosine kinase activity. It also affects downstream targets as well as the subcellular location of the normally tightly regulated Abl tyrosine kinase.*

***Methods:** The authors review the current knowledge concerning the signaling networks associated with BCR-ABL–dependent transformation.*

***Results:** Although BCR-ABL is considered a single genetic change, the dysregulated tyrosine kinase activates a network of signals that contributes to cytokine-independent growth, resistance to apoptosis, and genetic instability.*

***Conclusions:** The effectiveness of BCR-ABL–dependent transformation of hematopoietic stem cells is due not to a single pathway but rather to the culmination of a network of signaling pathways.*

Introduction

The cytogenetic hallmark of the presence of BCR-ABL–positive tumors is the detection of the Philadelphia chromosome. This cytogenetic abnormality is due to the reciprocal translocation of chromosomes 9 and 22 [t(9:22)].[1-4] This translocation results in the fusion of the breakpoint cluster region (BCR) gene located on chromosome 22 and c-Abl gene located on chromosome 9 (Fig 1). The corresponding protein is referred to as BCR-ABL. Three predominant products are formed depending on which breakpoint located within the BCR gene is fused with exon a2 of ABL. The corresponding fusion protein will encode either a p190, p210, or p230 molecular weight protein (Fig 2). These oncogene products are associated with a specific type of leukemia, suggesting that their respective transforming capacities are distinct.[5] In the majority of patients with chronic myelogenous leukemia (CML) and in approximately one-third of those with Ph+ acute lymphoblastic leukemia (ALL), the break occurs within the area spanning exon 12–16, often referred to as b1b5 and defined as the major breakpoint cluster region (M-BCR). The corresponding mRNA referred to as b2a2 and b3a2 is translated into a fusion protein commonly known as p210 BCR-ABL The minor breakpoint,

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Abbreviations used in this paper: CML = chronic myelogenous leukemia, ALL = acute lymphoblastic leukemia.

referred to as m-BCR, occurs between exons e1 and e2. The corresponding e1a2 mRNA is translated into a 190-kd fusion protein and is present in the remaining Ph+ ALL patients and rarely in patients with CML. More recently, a third breakpoint, μ-BCR, was identified. It is located downstream of exon e19, giving rise to the e19a2 fusion product. The corresponding protein consists of a 230-kd fusion protein and is considered a rare event found in Ph+ chronic neutrophilic leukemia.[6] Consistent with clinical findings, in vitro and transgenic studies have shown that expression of p190, p210, and p230 has distinct phenotypes. In particular, p190 had the shortest latency period, and mice developed B-cell origin leukemia exclusively. In contrast, p210 transgenic mice typically developed leukemia of B-, T-lymphoid, or myeloid origin.[7] Finally, p230 transgenic mice

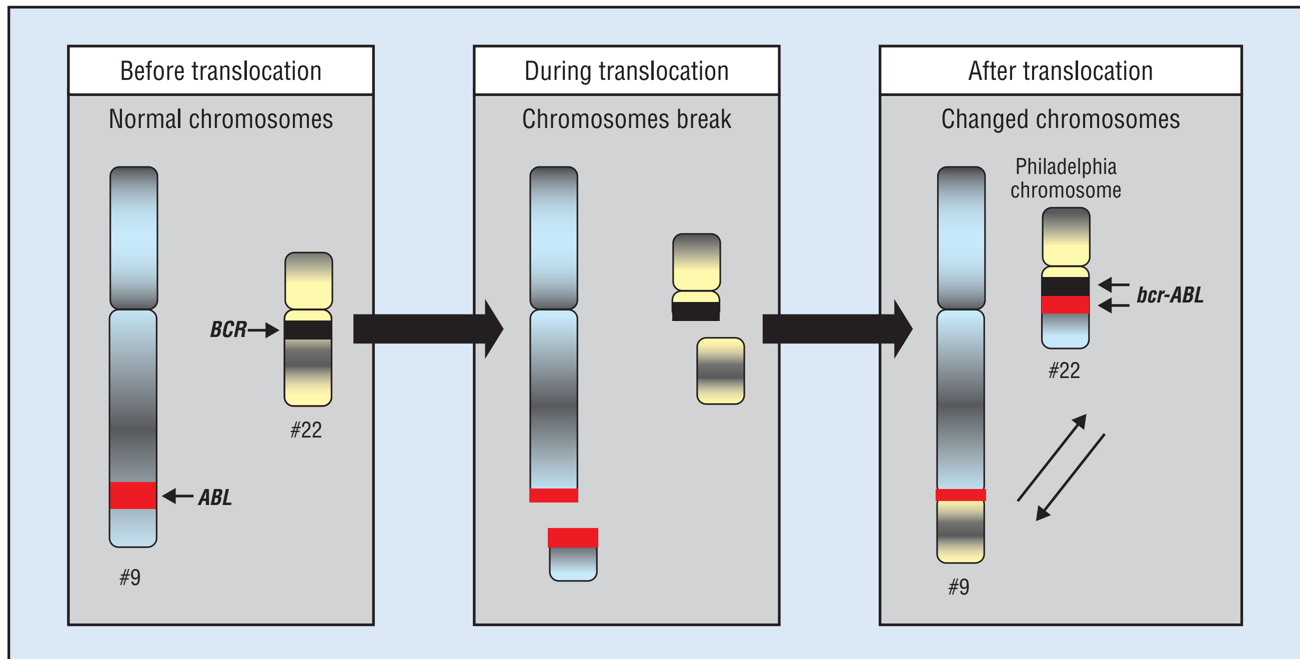

Fig 1. — The Philadelphia chromosome is the result of the reciprocal translocation of chromosomes 9 and 22. Following translocation, an extra-long chromosome 9 is formed, and an extra-short chromosome 22, harboring the BCR-ABL fusion protein, is formed.

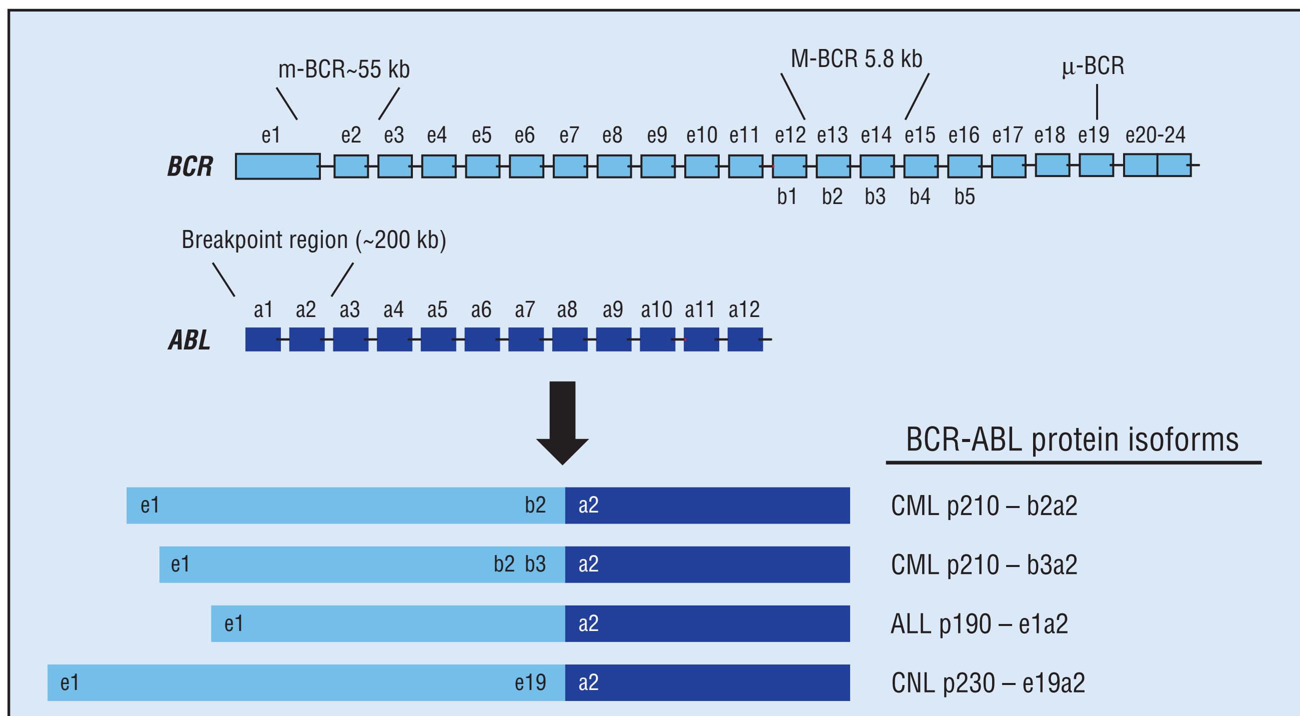

Fig 2. — Schematic of the breakpoints within the BCR and ABL genes and corresponding proteins.

showed the longest latency period and exhibited a less aggressive tumor, a finding that is consistent with the clinical disease.[8] This review focuses on the contribution of p210 BCR-ABL typically found in CML in mediating cell growth and cell survival.

Chronic Myeloid Leukemia

CML is characterized by the presence of the Ph chromosome in hematopoietic stem cells. Clinically, it is manifested in three distinct phases: chronic, accelerated, and blast. Most patients present in the chronic phase, a stage that is typically indolent in nature. Mature granulocytes are found, but patients typically have an increase in the number of myeloid progenitor cells found in the blood. Left untreated, the disease progresses to an accelerated phase followed by blast crisis, which is inevitably fatal. During blast phase, hematopoietic differentiation is blocked and blast cells accumulate in the bone marrow and peripheral blood.[9] Expression of BCR-ABL oncoproteins in hematopoietic cells induces resistance to apoptosis,[10,11] growth factor independence,[12] and leukomogenesis.[13-15]

The identification of BCR-ABL as the initial transforming event in CML provided an ideal target for drug discovery. The BCR-ABL inhibitor imatinib is considered first in class and proved to be an effective agent for treating chronic phase CML.[16-18] Although highly effective, imatinib does not eradicate the disease. Even in patients who demonstrate good response with treatment with a BCR-ABL inhibitor, minimal residual disease is detected at the molecular level using polymerase chain reaction (PCR).[17] Several hypotheses have been proposed to explain why imatinib does not eradicate the entire disease, including the possibility that the BCR-ABL–positive hematopoietic stem cell is resistant to imatinib treatment.[19,20] Further research is required to determine whether the stem cell niche, which represents a rich source of extracellular matrixes as well as cytokine and growth factors, is responsible for conferring resistance or if the stem cell is intrinsically resistant to imatinib treatment. Treatment with imatinib is less effective for treating blast cell patients, indicating that transformation to blast crisis results in BCR-ABL–independent growth.

Effects of BCR-ABL on Cell Survival

The fusion of BCR-ABL increases the diversity of protein-protein binding domains associated with the tyrosine kinase, thereby increasing the diversity of downstream targets (Fig 3). In addition, the localization of the parent tyrosine kinase c-Abl is nuclear, while the fusion protein resides in the cytoplasm. The end result is that the tyrosine kinase activity of the fusion protein is no longer tightly regulated and is available spatially to activate a multitude of survival signals (Fig 4).

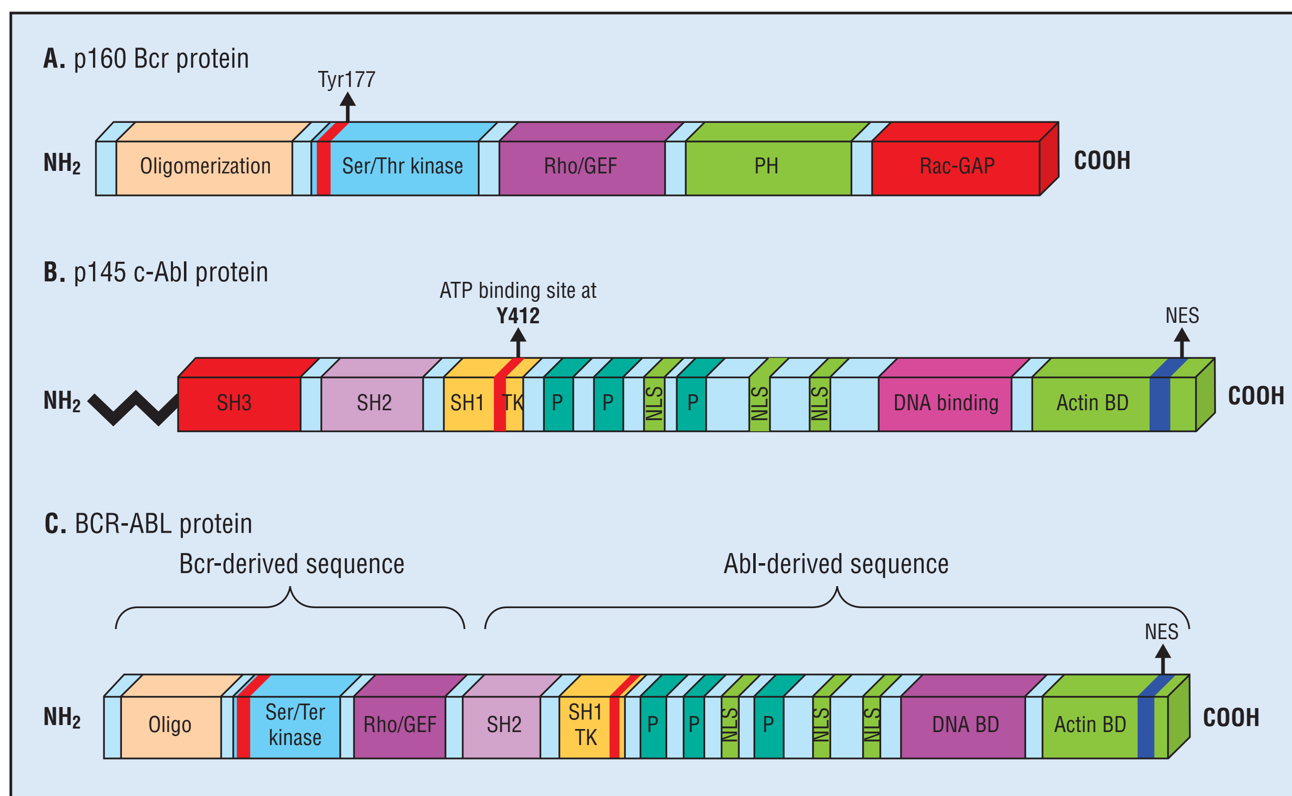

Fig 3. — (A) Schematic of the protein domains located within Bcr. The oligermization domain is critical for autophosphorylation of BCR-ABL. The Try177 residue is required for Grb2 binding. (B) Schematic of c-Abl protein. Normally the tyrosine kinase activity is tightly regulated and the protein is predominately located in the nucleus. (C) Schematic of BCR-ABL fusion protein. The fusion results in constitutive activation of the tyrosine kinase; cytoplasmic localization results in novel downstream binding partners that are now accessible to the constitutively active tyrosine kinase.

Experimental evidence indicates that BCR-ABL activation of PI3 kinase is an essential step in lymphoid transformation.[21,22] This evidence includes data showing that cells transformed with BCR-ABL have increased PI3 kinase class 1_A activity and accumulation of PIP_3. Moreover, pan PI3K inhibitors block both myeloid and lymphoid transformation.[23,24] Finally, similar to BCR-ABL inhibitors, PI3K inhibitors show selectivity towards inducing cell death in BCR-ABL leukemia cells compared with normal hematopoietic cells.[21,22] PI3 kinase is a heterodimeric protein consisting of an 85-kd regulatory subunit containing one SH3 domain, two SH2 domains, and a p110-kd catalytic subunit. P85 requires activation through the SH2 domain with phosphorylated tyrosine in YXXM motifs. Although BCR-ABL has been shown to bind the p85 subunit of PI3 kinase, it does not appear to be due to a direct interaction, as mutations in the YXXM motif contained within BCR-ABL do not attenuate PI3 kinase activity. This indicates that activation occurs through intermediates and not via a direct binding with BCR-ABL.[23] Most of the evidence indicates that a predominate pathway whereby activation of PI3 kinase occurs is via the Y177 autophosphorylation site located on the BCR region of the fusion protein.[25] This site is critical for Grb2 binding via its SH2 domain and allows for docking of Gab2 via an SH3 domain, which becomes phosphorylated within the YXXM motif. Moreover, evidence indicates that the Y177 residue is critical for activation of Ras and Akt in human hematopoietic progenitor cells.[26] However, there is also evidence that BCR-ABL–dependent pathways such as phosphorylation of Crkl and subsequent phosphorylation of c-Cbl may contribute to BCR-ABL–dependent activation of PI3 kinase.[27]

Multiple downstream targets of PI3 kinase have been identified. Akt is the downstream PI3 kinase target most consistent with providing a survival advantage capable of contributing to transformation. Experimental evidence includes the observation that forced expression of dominant-negative Akt attenuated transformation and inhibited leukemia growth in severe combined immunodeficiency (SCID) mice.[22] Akt activates a network of targets that favors cell survival. These targets include FoxO proteins, Bad, and glycogen synthase kinase 3β (GSK3β).[28] The FoxO protein family of FoxO1, FoxO3a, FoxO4, and FoxO6 transcription factors contain three Akt consensus phosphorylation sites (for FoxO3, T32, S253, and S315). Phosphorylation of the first two sites allows for binding of the 14-3-3 chaperone protein.[29,30] Binding of 14-3-3 occurs in the nucleus and allows for the efficient export of the complex. In addition, phosphorylation of the second site results in a

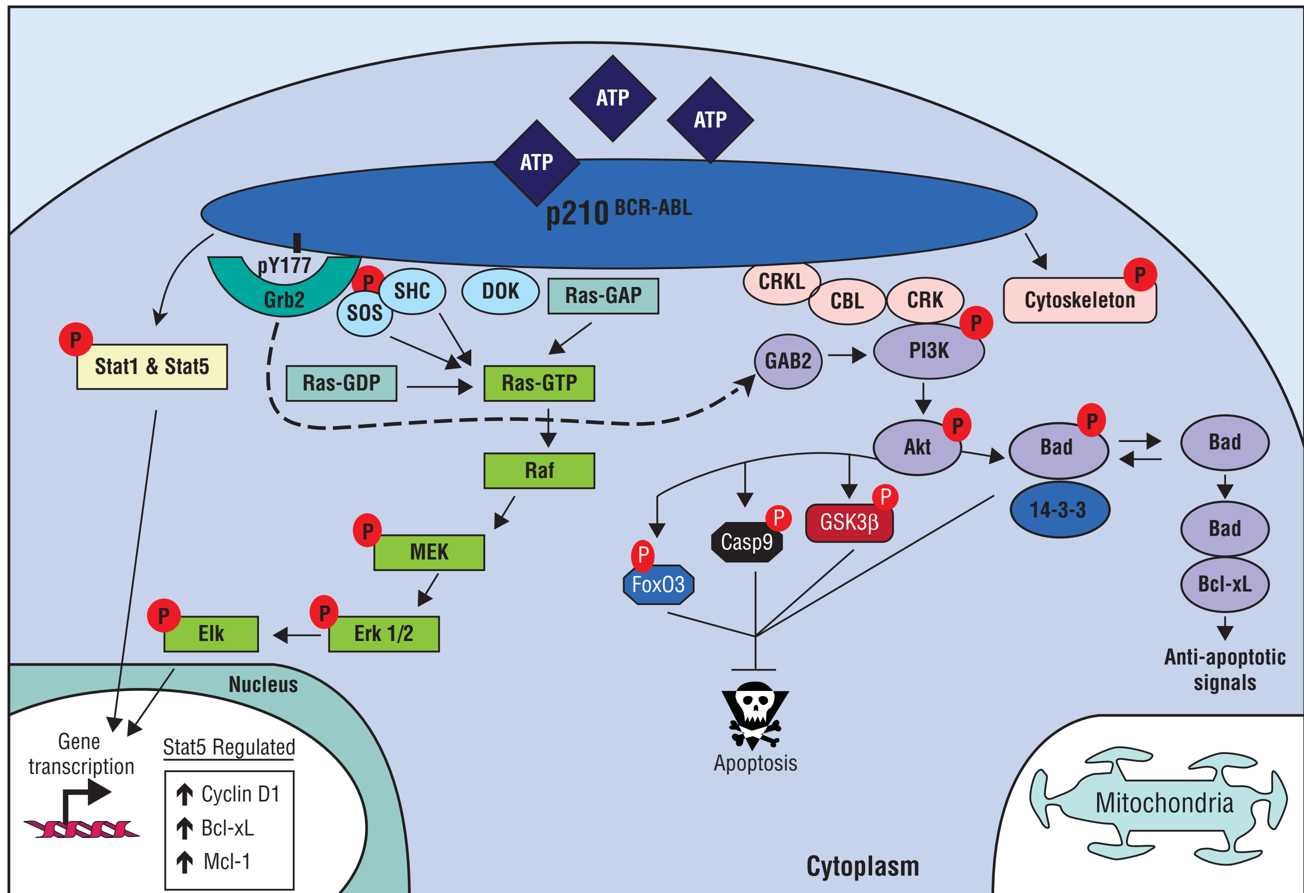

Fig 4. — BCR-ABL activates multiple downstream signaling pathways that contribute to growth and survival.

negative charge in the basic stretch of the nuclear localization signal, preventing re-entry into the nucleus.[31] Cytoplasmic sequestration effectively inhibits the transcriptional activation of several proapoptotic molecules, including Bim and Trail.[32,33] The promoter region of Bim contains a Forkhead responsive element, and ChIP assays have determined that FoxO3a binds to the promoter region of Bim.[34] Moreover, several investigators have shown that treatment with BCR-ABL inhibitors causes an increase in Bim levels and that siRNA directed at Bim induces resistance to BCR-ABL inhibitors.[35-38] Additionally, Akt can lead to phosphorylation of the proapoptotic Bcl-2 family member Bad at S136.[39,40] Non-phosphorylated Bad is free to bind antiapoptotic Bcl-2 family members, replacing Bax. Release of Bax allows for oligomerization to occur in the mitochondria membrane and contributes to the induction of the outer mitochondria membrane permeabilization, leading to the eventual activation of caspases and apoptosis.[41] In contrast, phosphorylated Bad is sequestered by 14-3-3 in the cytosol, thereby increasing the levels of Bcl-2 antiapoptotic members to sequester Bax and reducing the threshold for apoptosis to occur.[42] BCR-ABL has been shown to result in the phosphorylation of Bad, contributing to cell survival.[43] However, Neshat et al[43] noted that BCR-ABL–dependent phosphorylation of Bad does not completely reconstitute the survival advantage associated with BCR-ABL expression, again suggesting that the transforming capabilities of BCR-ABL are not the result of the activation of a single pathway but rather the culmination of the activation of a complex signaling network. GSK3β is a downstream target of Akt, and becomes inactivated following phosphorylation.[44,45] Two downstream targets of GSK3β include cyclin D1 and β-catenin. Phosphorylation of these proteins by GSK3β targets both of these proteins for proteasome-mediated degradation.[46] In addition, Coluccia et al[47] showed that BCR-ABL physically interacts with β-catenin and is required for phosphorylation of β-catenin at Y86 and Y654.[47] They also showed that BCR-ABL–dependent tyrosine phosphorylation of β-catenin can increase the half-life of the protein independent of GSK3β activity. Finally, siRNA targeted at β-catenin inhibited cell growth of BCR-ABL–positive cells and sensitized them to imatinib-induced cell death.[47] β-catenin may play an especially important role in the maintenance of the stem cell population in both normal and CML stem cells. Zhao et al[48] recently showed that β-catenin–deficient hematopoietic stem cell population demonstrated a reduction in long-term self renewal as demonstrated by in vivo transplant studies. Interestingly, BCR-ABL–transduced cells deficient in β-catenin failed to develop CML, a finding that was associated with reduced self-renewal in the stem cell population. However, β-catenin –/– mice did develop ALL, suggesting that the dependence on β-catenin may be dependent on cell origin.

Signal transducer and activator of transcription (Stat) proteins are known to be regulated by cytokine receptors and are critical for driving transcription necessary for growth, survival, and differentiation of hematopoietic cells. Experimental evidence indicates that BCR-ABL activates predominately Stat5 and to a lesser extent Stat3 and Stat1.[49-52] BCR-ABL expression results in constitutive activation of Stat5 and essentially bypasses cytokine or growth factor-dependent activation of Stat5. Activation of Stat5 contributes to increased expression of the antiapoptotic Bcl-2 family members MCL-1 and BCL-x_L as well as increased expression of cyclin D1, a critical molecule that facilitates G_1 to S phase cell cycle progression.[53-57] Experimental evidence indicates that both the SH3 and SH2 domains are required for the BCR-ABL–dependent activation of Stat5.[58] Moreover, these same investigators showed that expression of a dominant negative Stat5 in BCR-ABL–positive murine myeloid precursor 32Dcl3 cells inhibited resistance to apoptosis, growth factor independent proliferation, and leukemogenesis in vivo.[58] In contrast, bone marrow cells from STAT5-deficient mice were not limited in BCR-ABL–dependent transformation in vitro or in vivo, suggesting that alternative pathways can be activated in this model system.[59] Although BCR-ABL is known to activate Jak2 kinase, cells expressing kinase-inactive Jak2 fail to reduce phosphorylated Stat5 levels, indicating that BCR-ABL activates Stat5 independent of Jak2.[60]

In summary, the survival signals initiated by BCR-ABL are critical for normal hematopoiesis. However, the presence of the fusion protein has now resulted in constitutive activation of a network of survival pathways and contributes to transformation and resistance to apoptosis. Interestingly, because these pathways can be activated by extracellular signals, the role of the bone marrow microenvironment (which contains locally rich areas of cytokines and extracellular matrixes) in mediating resistance to BCR-ABL inhibitors is an active area of investigation. For example, granulocyte-macrophage colony-stimulating factor (GM-CSF) autocrine secretion can result in BCR-ABL–independent activation of Stat5 and resistance to BCR-ABL inhibitors.[61] Furthermore, our laboratory recently showed that conditioned media derived from a bone marrow stroma cell line referred to as HS-5 protect CML cell lines from apoptosis induced by BCR-ABL inhibitors. These findings were associated with activation of Stat3 and sustained expression of Stat3/Stat5 target genes Bcl-x_L, Mcl-1, and survivin following treatment with BCR-ABL inhibitors.[62]

Effects of BCR-ABL on Cell Growth

In addition to promoting cell survival, BCR-ABL has pronounced effects on cell cycle progression. Cortez et al[63] showed that BCR-ABL–transformed 32D cells proliferated in low serum conditions, whereas control cells

became arrested in G_0 when cultured in low serum.[63] Furthermore, they demonstrated that inducible expression of BCR-ABL was sufficient to initiate cell cycle progression in cells arrested due to culturing in low serum conditions. This finding correlated with activation of CDK2, which is required for G_1/S progression. BCR-ABL–dependent activation of CDK2 can be attributed in part to reduced nuclear accumulation of the cyclin-dependent kinase inhibitor $p27^{kip1}$. Reports indicate that the cytoplasmic nuclear ratio of $p27^{kip1}$ is increased by BCR-ABL.[64] Conversely, treatment of BCR-ABL–positive cells with a BCR-ABL inhibitor caused increased $p27^{kip1}$ levels and decreased cyclin D and E levels.[65] The regulation of $p27^{kip1}$ by BCR-ABL appears to be multifactorial since BCR-ABL inhibits transcription and increases proteasome-mediated degradation of the protein.[65] These findings are associated with decreased CDK2 activity, a kinase required for G_1 to S progression. BCR-ABL–dependent transcriptional regulation of $p27^{kip1}$ may be mediated by Akt-dependent inhibition of FoxO transcription factors that can drive $p27^{kip1}$ expression.[33] In addition to transcriptional regulation, BCR-ABL was shown to mediate degradation of $p27^{kip1}$ via a skp2-dependent manner.[65] Finally, recent evidence indicates that survival was prolonged in mice transplanted with BCR-ABL transfected SKP2–/– marrow compared with recipients of BCR-ABL–expressing SKP2+/+ marrow.[66] Taken together, these data indicate that BCR-ABL–dependent pathways that contribute to cell cycle progression and survival contribute to the leukogenic potential of this oncogene.

Transformation to Blast-Stage Crisis

The clinical transition from chronic to blast phase is associated with a more clinically aggressive phenotype that is typically resistant to BCR-ABL inhibitors. The mechanism of transition remains poorly understood. The blast phase of the disease is characterized by the addition of secondary molecular or chromosomal changes that presumably allow for BCR-ABL independent growth and resistance to BCR-ABL inhibitors.[67] However, BCR-ABL expression is not decreased but rather increased at blast stage.[68] Thus, it is feasible that BCR-ABL promotes secondary changes that allow for the transition to blast phase. In support of this premise are data showing that BCR-ABL kinase activity increases the levels of endogenous reactive oxygen species (ROS) and increases the amount of ROS-induced DNA damage as measured by the comet assay.[69] Furthermore, the addition of n-acetylcysteine, a free-radical scavenger, decreased the mutagenesis rate and frequency of resistance to imatinib.[69]

In addition to increasing DNA damage due to greater ROS levels, BCR-ABL has been shown to attenuate DNA repair pathways. Two major DNA pathways operative in mammalian cells are classified as homologous recombination (HR) and nonhomologous end joining (NHEJ). NHEJ repairs DNA double-strand breaks by joining ends together. Ku70 and Ku80 recognize and bind DNA double-strand breaks and recruit a repair complex that includes DNA-PK to the damaged DNA. Deutsch at al[70] showed that BCR-ABL expression results in decreased levels of DNA-PK, a finding that could be reversed by treatment with a proteasome inhibitor. Thus, reduced levels of DNA-PK could attenuate NHEJ-mediated repair and contribute to genomic instability in BCR-ABL–positive cells. These investigators more recently showed that expression of BCR-ABL attenuates the levels of BRCA1 and that inhibition of the BCR-ABL kinase activity with imatinib restored the levels of BRCA1.[71] Following DNA damage, BRCA1 is localized to DNA damage foci containing FANCD2 and RD51 and is thought to facilitate HR-mediated DNA repair.[72] Moreover, ectopic expression of BCR-ABL in UT-7 cells increased the frequency of sister chromatid exchange following gamma radiation.[71] Supporting the role of BCR-ABL in increasing genomic instability is recent information showing that ectopic expression of BCR-ABL resulted in greater levels of single-strand breaks induced by etoposide.[73] This finding corresponded to defective S-phase checkpoint following DNA damage. Furthermore, following DNA damage, BCR-ABL was shown to translocate to the nucleus, physically interact with ataxia-telangiectasia and rad 3-related protein, and inhibit ATR-dependent phosphorylation of CHK1.[73] It is currently unclear whether continued suppression of BCR-ABL kinase activity with inhibitors will prevent the secondary genetic abnormalities associated with transformation to blast stage.

Conclusions

Although CML is linked to the transformation capabilities of one oncoprotein, it is clear that BCR-ABL has pleiotropic effects on multiple pathways that contribute to cell survival, disease progression, and genomic stability.

Disclosures

No significant relationship exists between the authors and the companies/organizations whose products or services may be referenced in this article.

References

1. Bartram CR, de Klein A, Hagemeijer A, et al. Translocation of c-ab1 oncogene correlates with the presence of a Philadelphia chromosome in chronic myelocytic leukaemia. *Nature*. 1983;306(5940):277-280.
2. Groffen J, Stephenson JR, Heisterkamp N, et al. Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. *Cell*. 1984;36(1):93-99.
3. Nowell PC, Hungerford DA. A minute chromosome in human chronic granulocytic leukemia. *Science*. 1960;132:1497.
4. Rowley JD. Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature*. 1973;243(5405):290-293.
5. Melo JV. The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype. *Blood*. 1996; 88(7):2375-2384.

6. Pane F, Frigeri F, Sindona M, et al. Neutrophilic-chronic myeloid leukemia: a distinct disease with a specific molecular marker (BCR/ABL with C3/A2 junction). *Blood*. 1996;88(7):2410-2414. Erratum in: *Blood*. 1997; 89(11):4244.

7. Voncken JW, Kaartinen V, Pattengale PK, et al. BCR/ABL P210 and P190 cause distinct leukemia in transgenic mice. *Blood*. 1995;86(12):4603-4611.

8. Inokuchi K, Dan K, Takatori M, et al. Myeloproliferative disease in transgenic mice expressing P230 Bcr/Abl: longer disease latency, thrombocytosis, and mild leukocytosis. *Blood*. 2003;102(1):320-323. Epub 2003 Mar 6.

9. Melo JV, Barnes DJ. Chronic myeloid leukaemia as a model of disease evolution in human cancer. *Nature Rev Cancer*. 2007;7(6):441-453.

10. Bedi A, Zehnbauer BA, Barber JP, et al. Inhibition of apoptosis by BCR-ABL in chronic myeloid leukemia. *Blood*. 1994;83(8):2038-2044.

11. McGahon A, Bissonnette R, Schmitt M, et al. BCR-ABL maintains resistance of chronic myelogenous leukemia cells to apoptotic cell death. *Blood*. 1994;83(5):1179-1187. Erratum in: *Blood*. 1994;83(12):3835.

12. Sirard C, Laneuville P, Dick JE. Expression of bcr-abl abrogates factor-dependent growth of human hematopoietic M07E cells by an autocrine mechanism. *Blood*. 1994;83(6):1575-1585.

13. Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. *Science*. 1990;247(4944):824-830.

14. Heisterkamp N, Jenster G, ten Hoeve J, et al. Acute leukaemia in bcr/abl transgenic mice. *Nature*. 1990;344(6263):251-253.

15. Kelliher MA, McLaughlin J, Witte ON, et al. Induction of a chronic myelogenous leukemia-like syndrome in mice with v-abl and BCR/ABL. *Proc Natl Acad Sci U S A*. 1990; 87(17):6649-6653. Erratum in: *Proc Natl Acad Sci U S A*. 1990;87(22):9072.

16. Buchdunger E, Zimmermann J, Mett H, et al. Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylaminopyrimidine derivative. *Cancer Res*. 1996;56(1):100-104.

17. Druker BJ, Guilhot F, O'Brien SG, et al. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N Engl J Med*. 2006;355(23):2408-2417.

18. Druker BJ, Tamura S, Buchdunger E, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat Med*. 1996;2(5):561-566.

19. Cortes J, O'Brien S, Kantarjian H. Discontinuation of imatinib therapy after achieving a molecular response. *Blood*. 2004;104(7):2204-2205.

20. Michor F, Hughes TP, Iwasa Y, et al. Dynamics of chronic myeloid leukaemia. *Nature*. 2005;435(7046):1267-1270.

21. Kharas MG, Deane JA, Wong S, et al. Phosphoinositide 3-kinase signaling is essential for ABL oncogene-mediated transformation of B-lineage cells. *Blood*. 2004;103(11):4268-4275. Epub 2004 Feb 19.

22. Skorski T, Bellacosa A, Nieborowska-Skorska M, et al. Transformation of hematopoietic cells by BCR/ABL requires activation of a PI-3k/Akt-dependent pathway. *EMBO J*. 1997;16(20):6151-6161.

23. Jain SK, Susa M, Keeler ML, et al. PI 3-kinase activation in BCR/abl-transformed hematopoietic cells does not require interaction of p85 SH2 domains with p210 BCR/abl. *Blood*. 1996;88(5):1542-1550.

24. Varticovski L, Daley GQ, Jackson P, et al. Activation of phosphatidylinositol 3-kinase in cells expressing abl oncogene variants. *Mol Cell Biol*. 1991;11(2):1107-1113.

25. Sattler M, Mohi MG, Pride YB, et al. Critical role for Gab2 in transformation by BCR/ABL. *Cancer Cell*. 2002;1(5):479-492.

26. Chu S, Li L, Singh H, et al. BCR-tyrosine 177 plays an essential role in Ras and Akt activation and in human hematopoietic progenitor transformation in chronic myelogenous leukemia. *Cancer Res*. 2007;67(14):7045-7053.

27. Sattler M, Salgia R, Okuda K, et al. The proto-oncogene product p120CBL and the adaptor proteins CRKL and c-CRK link c-ABL, p190BCR/ABL and p210BCR/ABL to the phosphatidylinositol-3´ kinase pathway. *Oncogene*. 1996;12(4):839-846.

28. Brunet A, Bonni A, Zigmond MJ, et al. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell*. 1999;96(6):857-868.

29. Brunet A, Kanai F, Stehn J, et al. 14-3-3 transits to the nucleus and participates in dynamic nucleocytoplasmic transport. *J Cell Biol*. 2002;156(5):817-828. Epub 2002 Feb 25.

30. Obsilova V, Vecer J, Herman P, et al. 14-3-3 protein interacts with nuclear localization sequence of forkhead transcription factor FoxO4. *Biochemistry*. 2005;44(34):11608-11617.

31. Rena G, Woods YL, Prescott AR, et al. Two novel phosphorylation sites on FKHR that are critical for its nuclear exclusion. *EMBO J*. 2002; 21(9):2263-2271.

32. Modur V, Nagarajan R, Evers BM, et al. FOXO proteins regulate tumor necrosis factor-related apoptosis inducing ligand expression. Implications for PTEN mutation in prostate cancer. *J Biol Chem*. 2002;277(49): 47928-47937. Epub 2002 Sep 25.

33. Stahl M, Dijkers PF, Kops GJ, et al. The forkhead transcription factor FoxO regulates transcription of p27Kip1 and Bim in response to IL-2. *J Immunol*. 2002;168(10):5024-5031.

34. Essafi A, Fernández de Mattos S, Hassen YA, et al. Direct transcriptional regulation of Bim by FoxO3a mediates STI571-induced apoptosis in Bcr-Abl-expressing cells. *Oncogene*. 2005;24(14):2317-2329.

35. Hazlehurst LA, Argilagos RF, Dalton WS. Beta 1 integrin adhesion increases Bim protein degradation and contributes to drug resistance in leukemia cells. *Br J Haematol*. 2007;136(2):269-275.

36. Kuribara R, Honda H, Matsui H, et al. Roles of Bim in apoptosis of normal and Bcr-Abl-expressing hematopoietic progenitors. *Mol Cell Biol*. 2004; 24(14):6172-6183.

37. Aichberger KJ, Mayerhofer M, Krauth MT, et al. Low-level expression of proapoptotic Bcl-2-interacting mediator in leukemic cells in patients with chronic myeloid leukemia: role of BCR/ABL, characterization of underlying signaling pathways, and reexpression by novel pharmacologic compounds. *Cancer Res*. 2005;65(20):9436-9444.

38. Kuroda J, Puthalakath H, Cragg MS, et al. Bim and Bad mediate imatinib-induced killing of Bcr/Abl+ leukemic cells, and resistance due to their loss is overcome by a BH3 mimetic. *Proc Natl Acad Sci U S A*. 2006; 103(40):14907-14912. Epub 2006 Sep 22.

39. Datta SR, Dudek H, Tao X, et al. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell*. 1997;91(2): 231-241.

40. del Peso L, González-García M, Page C, et al. Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. *Science*. 1997;278 (5338):687-689.

41. Reed JC. Bcl-2-family proteins and hematologic malignancies: history and future prospects. *Blood*. 2008;111(7):3322-3330.

42. Zha J, Harada H, Yang E, et al. Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCL-X(L). *Cell*. 1996;87(4):619-628.

43. Neshat MS, Raitano AB, Wang HG, et al. The survival function of the Bcr-Abl oncogene is mediated by Bad-dependent and -independent pathways: roles for phosphatidylinositol 3-kinase and Raf. *Mol Cell Biol*. 2000;20(4):1179-1186.

44. Pap M, Cooper GM. Role of glycogen synthase kinase-3 in the phosphatidylinositol 3-Kinase/Akt cell survival pathway. *J Biol Chem*. 1998;273(32): 19929-19932.

45. Cross DA, Alessi DR, Cohen P, et al. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature*. 1995;378(6559): 785-789.

46. Diehl JA, Cheng M, Roussel MF, et al. Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. *Genes Dev*. 1998;12(22):3499-3511.

47. Coluccia AM, Vacca A, Duñach M, et al. Bcr-Abl stabilizes beta-catenin in chronic myeloid leukemia through its tyrosine phosphorylation. *EMBO J*. 2007;26(5):1456-1466. Epub 2007 Feb 22.

48. Zhao C, Blum J, Chen A, et al. Loss of beta-catenin impairs the renewal of normal and CML stem cells in vivo. *Cancer Cell*. 2007;12(6):528-541.

49. Ilaria RL Jr, Van Etten RA. P210 and P190(BCR/ABL) induce the tyrosine phosphorylation and DNA binding activity of multiple specific STAT family members. *J Biol Chem*. 1996;271(49):31704-31710.

50. Carlesso N, Frank DA, Griffin JD. Tyrosyl phosphorylation and DNA binding activity of signal transducers and activators of transcription (STAT) proteins in hematopoietic cell lines transformed by Bcr/Abl. *J Exp Med*. 1996;183(3):811-820.

51. 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(11):1724-1730.

52. Shuai K, Halpern J, ten Hoeve J, et al. Constitutive activation of STAT5 by the BCR-ABL oncogene in chronic myelogenous leukemia. *Oncogene*. 1996;13(2):247-254.

53. Gesbert F, Griffin JD. Bcr/Abl activates transcription of the Bcl-X gene through STAT5. *Blood*. 2000;96(6):2269-2276.

54. Huang M, Dorsey JF, Epling-Burnette PK, et al. Inhibition of Bcr-Abl kinase activity by PD180970 blocks constitutive activation of Stat5 and growth of CML cells. *Oncogene*. 2002;21(57):8804-8816.

55. Magné S, Caron S, Charon M, et al. STAT5 and Oct-1 form a stable complex that modulates cyclin D1 expression. *Mol Cell Biol*. 2003;23(24): 8934-8945.

56. Sillaber C, Gesbert F, Frank DA, et al. STAT5 activation contributes to growth and viability in Bcr/Abl-transformed cells. *Blood*. 2000;95(6):2118-2125.

57. de Groot RP, Raaijmakers JA, Lammers JW, et al. STAT5-Dependent CyclinD1 and Bcl-xL expression in Bcr-Abl-transformed cells. *Mol Cell Biol Res Commun*. 2000;3(5):299-305.

58. Nieborowska-Skorska M, Wasik MA, Slupianek A, et al. Signal transducer and activator of transcription (STAT)5 activation by BCR/ABL is dependent on intact Src homology (SH)3 and SH2 domains of BCR/ABL and is required for leukemogenesis. *J Exp Med*. 1999;189(8):1229-1242.

59. Sexl V, Piekorz R, Moriggl R, et al. Stat5a/b contribute to interleukin 7-induced B-cell precursor expansion, but abl- and bcr/abl-induced transformation are independent of stat5. *Blood*. 2000;96(6):2277-2283.

60. Xie S, Wang Y, Liu J, et al. Involvement of Jak2 tyrosine phosphorylation in Bcr-Abl transformation. *Oncogene*. 2001;20(43):6188-6195.

61. Wang Y, Cai D, Brendel C, et al. Adaptive secretion of granulocyte-

macrophage colony-stimulating factor (GM-CSF) mediates imatinib and nilotinib resistance in BCR/ABL+ progenitors via JAK-2/STAT-5 pathway activation. *Blood*. 2007;109(5):2147-2155. Epub 2006 Nov 7.

62. Bewry N, Nair RR, Emmons M, et al. Stat3 contributes to resistance towards BCR-ABL inhibitors in a bone marrow microenvironment model of drug resistance. *Mol Cancer Ther*. 2008;7(10):3169-3175.

63. Cortez D, Reuther G, Pendergast AM. The Bcr-Abl tyrosine kinase activates mitogenic signaling pathways and stimulates G1-to-S phase transition in hematopoietic cells. *Oncogene*. 1997;15(19):2333-2342.

64. Jiang Y, Zhao RC, Verfaillie CM. Abnormal integrin-mediated regulation of chronic myelogenous leukemia CD34+ cell proliferation: BCR/ABL up-regulates the cyclin-dependent kinase inhibitor, $p27^{Kip}$, which is relocated to the cell cytoplasm and incapable of regulating cdk2 activity. *Proc Natl Acad Sci U S A*. 2000;97(19):10538-10543.

65. Andreu EJ, Lledó E, Poch E, et al. BCR-ABL induces the expression of Skp2 through the PI3K pathway to promote $p27^{Kip1}$ degradation and proliferation of chronic myelogenous leukemia cells. *Cancer Res*. 2005;65(8):3264-3272.

66. Agarwal A, Bumm TG, Corbin AS, et al. Absence of SKP2 expression attenuates BCR-ABL-induced myeloproliferative disease. *Blood*. 2008;112(5):1960-1970. Epub 2008 Jun 17.

67. Shet AS, Jahagirdar BN, Verfaillie CM. Chronic myelogenous leukemia: mechanisms underlying disease progression. *Leukemia*. 2002;16(8):1402-1411.

68. Gaiger A, Henn T, Hörth E, et al. Increase of bcr-abl chimeric mRNA expression in tumor cells of patients with chronic myeloid leukemia precedes disease progression. *Blood*. 1995;86(6):2371-2378.

69. Koptyra M, Falinski R, Nowicki MO, et al. BCR/ABL kinase induces self-mutagenesis via reactive oxygen species to encode imatinib resistance. *Blood*. 2006;108(1):319-327. Epub 2006 Mar 9.

70. Deutsch E, Dugray A, AbdulKarim B, et al. BCR-ABL down-regulates the DNA repair protein DNA-PKcs. *Blood*. 2001;97(7):2084-2090.

71. Deutsch E, Jarrousse S, Buet D, et al. Down-regulation of BRCA1 in BCR-ABL-expressing hematopoietic cells. *Blood*. 2003;101(11):4583-4588. Epub 2003 Feb 6.

72. Kennedy RD, D'Andrea AD. The Fanconi Anemia/BRCA pathway: new faces in the crowd. *Genes Dev*. 2005;19(24):2925-2940.

73. Dierov J, Dierova R, Carroll M. BCR/ABL translocates to the nucleus and disrupts an ATR-dependent intra-S phase checkpoint. *Cancer Cell*. 2004;5(3):275-285.