The BCR Gene and Philadelphia Chromosome-positive Leukemogenesis

Eunice Laurent, Moshe Talpaz, Hagop Kantarjian, and Razelle Kurzrock

Departments of Bioimmunotherapy [E. L., M. T., R. K.] and Leukemia [H. K., R. K.], University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

Introduction

Recent investigations have rapidly added crucial new insights into the complex functions of the normal BCR gene and of the BCR-ABL chimera and are yielding potential therapeutic breakthroughs in the treatment of Philadelphia (Ph) chromosome-positive leukemias. The term “breakpoint cluster region (bcr)” was first applied to a 5.8-kb span of DNA on the long arm of chromosome 22 (22q11), which is disrupted in patients with CML, bearing the Ph translocation [t(9; 22)(q34;q11); Refs. 1–3]. Subsequent studies demonstrated that the 5.8-kb fragment resided within a central region of a gene designated BCR (4). It is now well established that the breakpoint within BCR can be variable, and when joined with ABL results in a hybrid M, 210,000 (p210Bcr-Ab) or a M, 190,000 (p 190Bcr-Ab) protein (p190Bcr-Ab; Fig. 1; Refs. 5–13). Of interest, p190Bcr-Ab characterizes a phenotypically acute, rather than chronic, leukemia that is often, but not always, of myeloid origin (4). Furthermore, the presence of the hybrid proteins perturbs the multiple functions of their normal counterparts. Understanding the biological sequelae of the molecular aberrations in Ph-positive disease has led to development of novel tyrosine kinase inhibitor therapy that is showing remarkable success in clinical trials. Herein, we review the current state of knowledge on the role of BCR alone or when joined with ABL in normal and leukemic pathophysiology and the implications of this knowledge for therapy.

Genomic Structure of the BCR Gene

The BCR Gene

BCR is situated in a 5′ to 3′ orientation with the 5′ end closer to the centromere (4). The entire gene spans 130 kb and contains 23 exons (Ref. 26; Table 2 and Fig. 2). The first intron separating exons 1 and 2 was initially shown to span a distance of 68 kb (26) but is now known to include two additional exons (an alternative exon 1 and an alternative exon 2; Refs. 28 and 29). Two transcripts, 4.5- and 7.0-kb long, have been found (16). The nucleotide sequence contains an open reading frame of 3818 nucleotides, which codes for a protein 1271 amino acids in length (31, 36).

BCR-related Genes

Several BCR-related pseudogenes (BCR2, BCR3, and BCR4) have also been described (34). They are not translated into proteins. All of these genes have been mapped to chromosome 22q11 by in situ hybridization. The orientation is such that BCR2 is the most centromeric, followed by BCR4, then BCR1 (the functional gene) and BCR3. BCR2 and BCR4 are retained on chromosome 22 during the t(9;22) translocation. The BCR-related genes all contain 3′ sequences identical to those encompassing the last seven exons of the BCR1 gene (34, 36). The 5′ region is highly variable, suggesting that the 3′ end of the normal BCR1 gene was inserted into another locus with subsequent duplication of the new locus (36). Another BCR pseudogene, the chromosomal location of which remains undefined, has also been described (37). It was shown to have homology with the 3′ end of exon 3 as well as the 5′ end of exon 4 (37).

There is an additional BCR-related gene located on chromosome 17p13.3 (35, 38, 39). This gene is functionally active and has been designated ABR or active BCR-related gene (35). There is 68% homology between ABR and the central and COOH-terminal regions of BCR (35, 39).

The BCR Promoter

The 5′ untranslated region of the BCR gene is important because the fusion gene created by the Ph translocation is placed under the regulation of the BCR promoter (31, 40–42). A region ~1-kb upstream of the transcription start site was demonstrated to be the principle site of promoter activity (41, 42). Within this region, a CAAT box (conserved sequence upstream of start point of transcription, which is recognized by transcription factors) at position −644 as well as an inverted CAAT sequence at position −718 have been localized. However, neither in vitro nor in vivo studies suggest that these sequences are key factors for transcriptional regulation of the BCR gene (41). There is also a TATA box [conserved sequences in the promoter that specify the position at which transcription is initiated (43)] 120 bp downstream of the CAAT box. This TATA box, TT-TAA, is also accompanied by the consensus sequence TCATCG, required for 5′ capping of the transcript (41).

DNA footprinting and gel retardation assays have also been used to discover potential sites for DNA/protein interaction, which might reflect transcription factor activity. Several sites were found including ones containing the consensus sequence GGCGCGG (as well as the inverted sequence) for the SP1 transcription factor (41, 42). A unique sequence TAGGGCGCTAGTTTCCAAAGCGA, for which no binding protein is known, was also detected (41). Deletion of that site (versus other potential binding sites) resulted in a significant decrease of promoter activity in BCR-ABL-transfected cells (41).

The 5′ untranslated region of the BCR gene is also very rich in GC content, which makes up 80% of the nucleotides with this stretch of the DNA (31, 36). Within this GC-rich region is a segment 18 nucleotides long at sites −376 to −393, containing the sequence CCCGCCGCAGGCCGCGCCGCGC with its inverted repeat 363 bp down-
stream. These sequences are apt to form secondary stem and loop structures, with a possible role in translational regulation (36), and are common to many housekeeping genes (43). Other possible start sites upstream of the normal AUG codon have been found; however, these have short open reading frames because there are stop codons downstream of these sites (31, 40). It has been suggested that these short transcripts could also play a role in regulating protein translation (40).

**Bcr Protein(s)**

Currently, the normal BCR gene is known to code for two major proteins that are \( M_r 160,000 \) and \( M_r 130,000 \) in size (17, 25, 30). It is possible that these proteins are derived from the 7.0- and 4.5-kb BCR transcripts, respectively (15, 17, 26). In addition, Li et al. (44) have described putative Bcr proteins of \( M_r 160,000/M_r 185,000, M_r 155,000, M_r 135,000, M_r 125,000, \) and \( M_r 108,000 \).

**Bcr-related Protein(s)**

The ABR gene encodes for a \( M_r 98,000 \) protein that contains both the GEF and GAP domains of Bcr (respectively, the central and COOH-terminal regions) but lacks the serine/threonine kinase domain found at...
the NH₂-terminus (39, 45). Analysis of GAP activity showed that both Bcr and Abr acted similarly toward different G proteins, especially members of the Rho subfamily, which includes Cdc42 and Rac (39, 46).

**BCR Expression**

The *BCR* gene is expressed ubiquitously (4, 31, 32). In chick embryos, as well as in mouse and human tissues, mRNA levels were found to be highest in the brain and hematopoietic cells (32, 41). Similarly, *ABR* transcripts are also most prominent in hematopoietic tissue and brain, with the highest levels in the latter (39, 45). To date, however, the aberrant *BCR-ABL* gene appears to affect hematopoietic cells. Of interest, Bcr protein is expressed primarily in the early stages of myeloid differentiation, and levels are reduced significantly as cells mature to polymorphonuclear leukocytes (33). Because p210Bcr-Abl is expressed off the *BCR* promoter, it is not surprising that this protein shows a similar correlation between expression pattern and myeloid differentiation (33).

**Bcr Subcellular Localization**

The Bcr-Abl protein is known to be cytoplasmic (36). In addition, the normal product of the *BCR* gene was initially demonstrated to be a cytoplasmic protein by immunofluorescence staining and cell fractionation studies (17, 33, 47). A wealth of studies implicate this protein in cellular signal transduction pathways, especially those regulated by G proteins (discussed below; Ref. 48). Subsequent investigations have, however, shown that the Bcr product can also associate with condensed DNA as well as the highly condensed heterochromatin in interphase cells (49). In some cell lines, the M₁, 130,000 Bcr protein predominates in the nucleus, and the M₁, 160,000 form predominates in the cytoplasm (50). Of interest in this regard, Bcr was found recently to be associated with the XPB protein (51, 52). XPB plays a role in DNA repair, general transcription initiation, and cell cycle regulation (53).

**Functional Domains of BCR**

The *BCR* gene is now known to be a complex molecule with many different functional domains (Ref. 48; Table 3; Fig. 2). Within the first exon of *BCR* resides a structurally novel protein kinase domain (25, 54). At the COOH-terminus, a GAP domain, which demonstrates activity for the Ras-related GTP-binding protein p21 Rac, has been described (65). In addition, the central part of the *BCR* gene contains sequences homologous to various GEFs (63, 64, 68). These data...
implicate the participation of BCR in two major intracellular signaling mechanisms in eukaryotic cells (phosphorylation and GTP-binding).

First Exon

The first exon of the BCR gene is of critical significance, because it is the one exon of BCR included in all known Bcr-Abl fusion proteins (e.g., the p190BCR-Abl in acute leukemias as well as the p210BCR-Abl in CML; Refs. 1, 4, 8–10, 22, 60, 69, and 70; Fig. 1). Within this region of BCR resides the kinase domain, two serine-rich boxes containing SH2-binding domains, and an oligomerization domain.

Serine/Threonine Kinase Domain. The Bcr protein has serine/threonine kinase activity within its first exon (25, 30, 54, 71). A consensus sequence homologous to the ATP-binding site of other kinases as well as a likely phosphotransferase site of other kinases as well as a likely phosphotransferase domain has been identified (36, 44). Bcr can autophosphorylate on serine and threonine residues, as well as transphosphorylate casein and histones in vitro (54). (The latter are known substrates for serine/threonine kinases.)

In Ph-positive disease, phosphotyrosines are present on both Bcr and Bcr-Abl (mutually p210BCR-ABL and p190BCR-ABL) because of the tyrosine kinase activity of Bcr-Abl (72). The first exon of the Bcr component of Bcr-Abl is also phosphorylated on serine/threonine residues (54, 72). Indeed, the majority of autophosphorylation of Bcr-Abl occurs within the Bcr rather than the Abl portion of the protein (72).

SH2-binding Domain. There are several SH2-binding domains in the first exon of BCR (60). SH2 domains are highly conserved, noncatalytic regions of ~100 amino acids that bind SH2-binding sites consisting of three to five amino acids including a phosphotyrosine. This interaction is important in the assembly of signal transduction complexes (73–75.) Bcr is unique in that SH2 domains of other proteins can bind to the phosphoamino acid serine, threonine, or tyrosine (rather than only tyrosine) in two of its SH2-binding sites (60, 61). One SH2 domain that binds to Bcr is that of Abl, and this interaction is mediated via phosphorylated serines and threonines (60). Bcr sequences involved in this interaction lie between amino acids 192–242 and 298–413 and are essential for the oncogenic activation of Bcr-Abl (60). A third Bcr SH2-binding region has also been found. It interacts through a phosphorylated tyrosine with the adaptor protein Grb2 (59). The latter is an essential protein in the Ras signal transduction pathway (Fig. 2).

Oligomerization Domain. A third functional domain found within exon 1 of BCR is an oligomerization domain that is characterized by a heptad repeat of hydrophobic residues between amino acids 28 and 68 (57). Bcr and Bcr-Abl have been found to coimmunoprecipitate through this domain (76, 77). Mutations within the oligomerization domain attenuate the tyrosine kinase enzymatic activity of Bcr-Abl and abolish the interaction between Bcr and Bcr-Abl (57). Of interest, TEL (an ETS family gene) can also partner with ABL (t(9;12) translocation of acute leukemia) and activate Abl tyrosine kinase activity (78). It has been postulated that, similar to the situation with Bcr, the Tel helix-loop-helix domain facilitates dimerization or oligomerization of the fusion partner to activate its function.

The oligomerization domain also affects Bcr-Abl localization. Abl contains both a nuclear localization and an F-actin-binding (cytoplasmic) motif (70, 79). Although normal Abl can be found both in the nucleus and in the cytoplasm (Refs. 33 and 80; Table 4), Bcr-Abl is found to be cytoplasmic and partially associated with the cytoskeleton (33, 70, 106). Deletion of the oligomerization domain results in decreased binding of Bcr-Abl to F-actin, suggesting that this domain of Bcr enhances the F-actin binding capacity of Bcr-Abl and is at least partially responsible for the cytoplasmic localization of Bcr-Abl (57).

Table 4 ABL: Molecular features and function

<table>
<thead>
<tr>
<th>Functions</th>
<th>Comments</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-receptor tyrosine kinase (not constitutively active)</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Nuclear translocation signal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interacts with meiotic chromosome</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>DNA-binding domain at COOH-terminus:</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>binds to A4C motif</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F-Actin-binding domain at COOH-terminus</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>Ab1 inhibited through SH3 domain</td>
<td>87–89</td>
<td></td>
</tr>
<tr>
<td>Associates with cell cycle proteins Rh, p53, p73, Atm, and cyclin D</td>
<td>90–97</td>
<td></td>
</tr>
<tr>
<td>Cyclically phosphorylated during cell cycle</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>Associates with SH2/SH3 adaptor protein Crkl</td>
<td>99, 100</td>
<td></td>
</tr>
<tr>
<td>Overexpression of Abl leads to G arrest</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>Kinase activity regulated by Abelson interacting protein</td>
<td>102–105</td>
<td></td>
</tr>
</tbody>
</table>

Central Domain

Central (and COOH-terminal) regions of Bcr appear to interact with G proteins at multiple levels. These proteins are essential players in intracellular signaling, cytoskeletal organization, cell growth, and normal development (107, 108). G proteins cycle between an inactive GDP-bound state and an active GTP-bound state, a process that is regulated by GAPs and GEFs (107). GEFs activate G proteins by exchanging GDP for GTP. In contrast, GAPs inactivate G proteins by activating the GTPase of the protein, which in turn down-regulates the protein by hydrolysis of GTP (107).

GEF-related Domain. The central region of the BCR gene shows homology to the hematopoietically expressed VAV proto-oncogene (a GEF) as well as with a CDC25 mouse homologue termed CDC25Mm (64, 109). [CDC25 Mm is homologous to the Ras activator CDC25 of Saccharomyces cerevisiae (64, 109).] In addition, central BCR sequences demonstrate homology to the yeast cell division cycle gene CDC42, as well as its human counterpart, the Dbl oncogene, which codes for a GEF (63, 68). The CDC24 gene codes for a cell division cycle control protein and also plays a role in cytoskeletal and cytoplasmic organization, bud formation in yeast, cell polarity, and acts as a GDP-GTP exchange factor for Ras-like G proteins (68, 110–112). Cdc24 interacts with the product (a GTP-binding protein) of the bud assembly gene CDC42 and is a member of the Ras superfamily of GTPases (113, 114). To add to the complexity of the BCR gene, the GAP domain at the COOH-terminus contains activity toward the product of CDC42Hs, the human homologue of CDC42 (68). Taken together, these observations indicate that Bcr has both GAP and GEF functions and hence suggests a dual role for this molecule in G protein-associated signaling pathways.

Interaction with XBP Gene Product. Recent studies using the yeast two-hybrid system were performed to identify proteins that could interact with the central Cdc24 homologous region of Bcr (51). The results yielded one surprising candidate, the DNA repair XBP protein (51). Xeroderma pigmentosum is an inherited disorder characterized by increased sensitivity to sunlight, coupled with a defect in the DNA repair machinery. The XBP protein is also a component of the transcription factor IIH core complex, which plays a role in initiation of transcription as well as in DNA repair (53). The XBP protein was found to interact with both normal Bcr as well as with p210BCR-ABL (51, 52). Importantly, the Bcr Cdc24 homologous domain is present in p210BCR-ABL but absent in p190BCR-ABL. When XBP is

Downloaded from cancerres.aacrjournals.org on October 23, 2017. © 2001 American Association for Cancer Research.
coexpressed with p210Bcr-Abl, XPB becomes phosphorylated and is unable to correct a DNA repair defect (51). This phenomenon is not observed in the presence of p190Bcr-Abl (51). It is therefore conceivable that the presence of p210Bcr-Abl interferes with the normal interaction between Bcr and XPB, thus leading to defective DNA repair and subsequent genomic instability in Bcr-Abl-positive CML. This mechanism may explain the clonal evolution that occurs in CML and drives the inevitable progression of this disease from an easily controlled “benign” phase to a fatal blast crisis.

COOH-Terminal Domain

COOH-terminal sequences are also involved in G protein regulation. However, whereas the central sequences have GEF homology (activates G proteins), the COOH-terminal sequences have GAP homology (inactivates G proteins). One of the best-studied G proteins is the oncogene p21RAS (115). The RAS superfamily is known to have multiple members (116). Members of the RHO subfamily are critical components of signaling pathways that link extracellular signals with reorganization of the cytoskeleton as well as with membrane ruffling (117). A GAP protein specific for Rh family GTPases, termed RhoGAP, has structural similarity to the COOH-terminus of Bcr (118). The BcrGAP domain accelerates the GTPase activity of Rac in vitro and specifically inhibits Rac-induced membrane ruffling in vivo (65, 117). In BCR knockout mice, Bcr regulates Rac-mediated superoxide production during neutrophil priming and activation (66).

A human brain protein, chimaerin, with GAP activity also has homology to COOH-terminal Bcr sequences (119). Interestingly, another protein designated 3BP-1, which also has sequence homology to the COOH-terminus of Bcr as well as to Rhogap, binds the SH3 region of Abl with high specificity (67). The Abl SH3 region has an important negative regulatory function in transformation because its deletion activates the transforming potential of Abl.

Bcr-associated Proteins

The Bcr protein has been found to complex with several proteins, which, as discussed previously, include Abl, Bcr-Abl, the adaptor protein Grb2, and the XBP protein (51, 52, 59, 60, 76, 77). Included among the Bcr-associated proteins are members of a family of acidic proteins termed 14-3-3. These proteins play a role in intracellular trafficking of the mitotic inducer Cdc25C from the nucleus to the cytoplasm (120).

Structure of the BCR-ABL Gene in CML and Acute Leukemia

BCR-ABL Genes and Proteins

The hallmark of CML is the Ph chromosome, which is a shortened chromosome 22 resulting from a translocation, t(9;22)(q34;q11), between chromosomes 9 and 22 (2, 27). A cytogenetically identical translocation also occurs in about 20% of adult ALL, 5% of pediatric ALL, and rare cases of AML (125; Table 5). The involvement of chromosome 9 is now known to disrupt the ABL gene (15, 20, 81, 144). The actual breakpoint on chromosome 9 may span a large distance. In some cases, the break is 5’ to the two alternative first exons of the ABL gene (exons 1a and 1b), whereas in others, the break occurs within the first intron (1, 126, 145, 146), which extends over 200 kb (Ref. 82; Fig. 1). [Regarding, the fusion transcript almost always includes exon 2 of ABL (a2)]. In contrast, in CML, the break on chromosome 22 is restricted in most patients to an area of 5.8-kb termed the M-bcr (1). M-bcr consists of five exons termed M-bcr exons b1–b5. These exons are actually located within the central region of the BCR gene and are equivalent to exons 11–15 (e11–e15) of this gene. Most breaks occur immediately downstream of exon 2 or 3 of the M-bcr region (4) and result in b2a2 or b3a2 fusion transcripts. In acute leukemia, however, the breakage can also occur outside M-bcr in about half the cases (8, 9, 22, 135, 136, 139), usually within the 3’ end of intron 1 of the BCR gene (26, 141), resulting in an e1a2 fusion transcript. The breakpoint sites in both BCR and ABL appear to involve Alu sequences (transposable repeat elements found throughout the human genome; Refs. 141 and 147–150).

The fusion of BCR and ABL on the Ph chromosome occurs in a head-to-tail manner, with the 3’ end of ABL joined to the 5’ end of BCR (4). This configuration places the fusion gene under the control of the BCR promoter. When the break occurs in the M-bcr region, the transcript is 8.5-kb long. However, when the break occurs in the first intron of BCR, the transcript is 7.0-kb long (4, 10, 15, 16, 20, 126). Although it was initially felt that BCR-ABL transcripts are specific for leukemia, surprisingly, recent experiments suggest that small amounts of such transcripts can be found in normal individuals if very sensitive PCR techniques are used (151, 152). It is possible that these transcripts represent “errors” that occur because of the close proximity of chromosomes 9 and 22 during the S to G2 transition of the cell cycle (153–155) and that immune surveillance prevents the emergence of CML in individuals who remain healthy.

The protein encoded by the 8.5-kb BCR-ABL mRNA is M, 210,000 and designated p210Bcr-Abl (5–7). The two most common molecular variants of p210Bcr-Abl are generated depending on whether the break in M-bcr is centromeric or telomeric to exon 3 of M-bcr (b2a2 versus b3a2; Ref. 8). Therefore, p210Bcr-Abl may or may not contain the 25-amino acid sequence encoded by exon 3.

As mentioned above, in Ph-positive acute leukemia patients with breakpoints in the first intron of BCR, an abnormal BCR-ABL transcript of about 7.0-kb in size is found (10–12). This encodes a protein M, 190,000 in size (9–13, 133). p190Bcr-Abl (also designated p185Bcr-Abl by some investigators) was initially felt to be involved only in lymphoid lineage leukemia but was eventually also demon-
Breakpoint sites have been found. These include a microregion of BCR (respectively termed M-bcr and m-bcr), other unique breakpoint sites have been found. Include a b3a3, an e6a2, an e2/a1a, and more recently, e8/a2 and e19a2 fusion transcript (158–161). Reports of other patients with classic CML and AML phenotypes who possess the BCR-ABL gene occur upstream of exon 1 (15, 16, 20). (An ABL-BCR fusion transcript of uncertain significance is also expressed) (21).

In addition to the classic breakpoints found within the major (central 5.8-kb region) and minor (first intron of BCR) breakpoint cluster regions of BCR (respectively termed M-bcr and m-bcr), other unique breakpoint sites have been found. These include a micro 3' site termed μ-bcr [BCR exon 19 (c3) to BCR exon 20 (c4)], which can yield a Mf230,000 protein (24, 156, 157). Patients with the e19a2 fusion between BCR exon 19 (e19) and ABL exon 2 (a2) were classified as having neutrophilic CML (157). Because these cases were viewed as being less aggressive than typical CML, it has been suggested that the amount of BCR sequences present in the fusion transcripts correlate with disease phenotype (23, 157). However, there have been reports of patients with classic CML and AML phenotypes who possess the e19a2 fusion transcript (158–161). Reports of other BCR-ABL variants include a b3a3, an e6a2, an e2/a1a, and more recently, e8/a2 and e13a2 fusion transcripts (Refs. 162–165; Fig. 1; Table 5).

The differences in size of BCR-ABL transcripts is not only a reflection of variation in the sites of breakage/fusion but is also a result of alternative splicing between BCR and ABL and within BCR itself (19, 166). The normal ABL gene is composed of 11 exons, which include exons 1a and the more proximal exon 1b. These exons can be alternatively spliced, yielding two different mRNAs of 6- and 7-kb in size (19). The distance between exon 2 and exon 1b is greater than 200 kb, demonstrating the ability of ABL exon 2 to skip over the vast expanse of intron 1 to join to the splice donor site of exon 1b (7, 19, 82). This ability is maintained in the fusion gene, and the end results are the joining of ABL splice acceptor site of exon 2 to the splice donor sites of various BCR exons and the excision of ABL exons 1a and 1b. (ABL exons 1a and 1b do not have splice acceptor sites.) Indeed, even in CML containing breaks within M-bcr, splicing can occasionally result in a transcript with an e1a2 junction in addition to transcripts with a b2a2 or b3a2 junction (143, 166).

**Bcr-Abl Functional Characteristics**

**Kinase Activation.** Tyrosine kinase activity is essential to cellular signaling and growth, and constitutively elevated kinase activity has been associated with oncogenic changes in several systems. The Abl protein is a nonreceptor tyrosine kinase, which normally has very little constitutive kinase activity (5). Both the p210BCR-ABL and the p190BCR-ABL proteins have constitutively activated tyrosine kinase enzymatic activity, with higher levels of activity in the p190 protein (Tables 6 and 7). Interestingly, most of the autophosphorylated tyrosines occur within the Bcr segment of Bcr-Abl (72). The tyrosine kinase activity is attributable to the kinase domain found within the Abl segment of the fusion proteins (5, 6, 8–10, 200). Indeed, it

---

### Table 6 Characteristics of p210BCR-ABL

<table>
<thead>
<tr>
<th>Comments</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location of gene encoding p210BCR-ABL</td>
<td>Chromosome 22q11</td>
</tr>
<tr>
<td>Size of gene</td>
<td>Varies according to site of breakpoint</td>
</tr>
<tr>
<td>Break is in central region of BCR gene known as 5.8-kb (M-bcr); [Breaks occur between exons 2 and 3 or exons 3 and 4 of M-bcr (b2-a2 or b3-a2 junction)]</td>
<td></td>
</tr>
<tr>
<td>Breaks in ABL gene occur upstream of exon 1</td>
<td>15</td>
</tr>
<tr>
<td>Size of transcript</td>
<td>8.5-kb BCR-ABL transcript;</td>
</tr>
<tr>
<td>(An ABL-BCR fusion transcript of uncertain significance is also expressed)</td>
<td>15, 16, 20</td>
</tr>
<tr>
<td>Localization</td>
<td>Cytoplasmic protein</td>
</tr>
<tr>
<td>Functions</td>
<td>Constitutively activated tyrosine kinase</td>
</tr>
<tr>
<td>Can bind cytoketoskeletal actin and microfilaments</td>
<td>5, 6</td>
</tr>
<tr>
<td>Expression decreases with myeloid differentiation</td>
<td>70</td>
</tr>
<tr>
<td>Substrates may include Ras-related signal transduction molecules</td>
<td>33</td>
</tr>
<tr>
<td>Complexes with tyrosine phosphoprotein Crkl, an SH2/SH3 adaptor protein</td>
<td>59, 168–170</td>
</tr>
<tr>
<td>Interacts with docking protein p62Dok</td>
<td>99, 100</td>
</tr>
<tr>
<td>May prevent apoptosis</td>
<td>171–174</td>
</tr>
<tr>
<td>Linked to Jak/Stat pathway (especially Stat5)</td>
<td>175–181</td>
</tr>
<tr>
<td>Linked to phosphotyrosylinositol 3-kinase/Akt pathway</td>
<td>182–188</td>
</tr>
<tr>
<td>Activates Jnk</td>
<td>189–192</td>
</tr>
<tr>
<td>Interacts with and regulates the XPB protein</td>
<td>193</td>
</tr>
<tr>
<td>May induce alterations in adhesion properties</td>
<td>51</td>
</tr>
<tr>
<td>Down-regulates expression of Shp protein</td>
<td>194, 195</td>
</tr>
<tr>
<td>Comments Refs.</td>
<td></td>
</tr>
</tbody>
</table>

---

### Table 7 Characteristics of p190BCR-ABL

<table>
<thead>
<tr>
<th>Comments</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location of gene encoding p190Bcr-Ab</td>
<td>Chromosome 22q11</td>
</tr>
<tr>
<td>Size of gene</td>
<td>Contains NH₂-terminal BCR sequences (exon 1 of BCR) and COOH-terminal ABL sequences</td>
</tr>
<tr>
<td>Breaks is in proximal 5' region of BCR gene (in the first intron)</td>
<td>8, 10–12, 26, 135, 136, 139, 141, 199</td>
</tr>
<tr>
<td>Size of transcript</td>
<td>7.0-kb</td>
</tr>
<tr>
<td>Localization</td>
<td>Cytoplasmic protein</td>
</tr>
<tr>
<td>Functions</td>
<td>Constitutively activated tyrosine kinase</td>
</tr>
<tr>
<td>May prevent apoptosis</td>
<td>10, 200</td>
</tr>
<tr>
<td>Linked to Jak/Stat pathway (especially Stat5)</td>
<td>181, 195</td>
</tr>
<tr>
<td>Linked to phosphotyrosylinositol 3-kinase pathway</td>
<td>179, 185</td>
</tr>
<tr>
<td>Interacts with and regulates the XPB protein</td>
<td>191</td>
</tr>
<tr>
<td>Activates Jnk</td>
<td>193</td>
</tr>
<tr>
<td>Interacts with and regulates the XPB protein</td>
<td>51</td>
</tr>
<tr>
<td>May induce alterations in adhesion properties</td>
<td>196, 197</td>
</tr>
<tr>
<td>Comments Refs.</td>
<td>198</td>
</tr>
</tbody>
</table>
appears that the degree of transforming activity of Bcr-Abl correlates with the degree of tyrosine kinase activity (200), and this activity has been implicated in the growth factor independence that Bcr-Abl confers on cells (202). Furthermore, Bcr-Abl induces the tyrosine phosphorylation of many cellular proteins including Crkl, Shc, Syp, Fes, Vav, and paxillin (99, 100, 170, 203–208).

**Interaction with Ras-related Pathways.** The importance of Ras pathways in Bcr-Abl oncogenesis is underscored by experiments demonstrating that Ras activation is necessary for transformation by both p210BCR-ABL and p190BCR-ABL (201, 209). The mechanisms by which Bcr-Abl interacts with the Ras pathway are complex. Implicated molecules include the adapter protein Grb2, the Ras activator SOS (son of sevenless), RasGAP, Crkl (an SH2/SH3 adapter protein), and the docking protein, p62Nck (59, 99, 100, 168, 169, 171–174). Crkl has also been found to interact with normal Abl (100). It was shown recently that p210 BCR-ABL mutants with deletion of the Grb2-binding site, the SH2 domain, as well as the tetramerization domain within the Bcr portion do not have diminished transforming properties in hematopoietic cells, although these mutations drastically reduce transforming activity of BCR-ABL in fibroblasts (210). It has been suggested that Ras activation in cells bearing these BCR-ABL mutants was mediated by Shc proteins and was critical to transformation of hematopoietic cells (210). Finally, p110Ras is linked to pathways that activate downstream Jun kinase, and it appears that this molecule is also a requisite for Bcr-Abl transforming activity (193).

**Interaction with Ship Proteins.** The Ship proteins (Ship1 and Ship2) are also downstream targets of Bcr-Abl (211–215). Ship1 has been shown to regulate hematopoiesis in mice, and disruption or murine Ship results in a myeloproliferative syndrome (216). p210BCR-ABL negatively regulates the expression of Ship1, in part, by reducing its half-life (198). The Ship proteins have also been found to interact with the adapter proteins Shc and Grb2, which, as discussed above, play a role in Ras-mediated signal transduction pathways (211, 212, 214, 217). Finally, the Bcr-Abl-specific kinase inhibitor STI 571 (CGP 57148B) causes reexpression of Ship, further supporting the implication that p210BCR-ABL directly, but reversibly, regulates Ship expression (198).

**Effect on Adhesion Molecules.** Other substrates of the tyrosine kinase activity of Bcr-Abl include the focal adhesion proteins Fak (focal adhesion kinase) and paxillin (208, 218). Clinically, an increased expansion of committed myeloid progenitors and precursors, elevated levels of mature granulocytes, and premature release of the progenitor/precursor cells characterize CML. This is postulated to be attributable to defects in the adhesion properties of these cells, perhaps because of inside-to-outside disruption of focal adhesion molecule function and subsequent perturbation of adhesion molecules such as integrin β1 (196). [Normally, hematopoietic cells can attach to extra-cellular matrix such as fibronectin and stroma via integrins (i.e., α4β1 and α5β1), which in turn interacts with cytoskeletal proteins at focal adhesion points (196).] The salutary effect of IFN-α in a subset of CML patients may be attributable to restoration of normal β1 integrin function (196, 219, 220). In addition, inhibition of either the levels or the kinase activity of Bcr-Abl can restore β1 integrin-mediated adhesion (221, 222). However, other investigators have disputed these results with experiments that demonstrate that expression of Bcr-Abl in certain cell lines leads to increased adhesion of these cells to fibronectin-coated surfaces mediated through expression of α5β1 integrin (223). It has also been shown that overexpression of Crkl, the Bcr-Abl substrate, which can act as a mediator between Bcr-Abl and paxillin (224), increases cell adhesion to fibronectin-coated plates (225). At least some of these experimental disparities may be attributed to differences in cell type used and/or the use of established cell lines versus bone marrow samples from CML patients.

**Effect on Apoptosis.** One proposed mechanism through which the activity of Bcr-Abl is mediated is suppression of apoptosis, or programmed cell death. Hematopoietic growth factors are known to suppress apoptosis, and BCR/ABL can abrogate growth factor dependence in a variety of hematopoietic cell lines (226). Suppression of apoptosis by Bcr-Abl may be mediated by inducing the expression of the antiapoptotic protein, Bcl-2 (perhaps through Stat pathways), by phosphorylation of the proapoptotic protein Bad or through Ras-dependent pathways (176, 195, 227).

**Effect on Stat5.** The growth factor independence demonstrated by Bcr-Abl-positive cells is, to some extent, mediated through constitutive activation of the growth factor-dependent signal transducer and activator of transcription (Stat) proteins, especially Stat5 (182, 184–186, 188, 228, 229). Hematopoiesis is strongly regulated by growth factors and, in myeloid cells, cytokines such as interleukin 3 and granulocyte/macrophage-colony stimulating factor, upon binding to their respective receptors, can elicit the Jak/Stat signaling pathway. [Phosphorylation on its tyrosine residues can activate the Jak protein, and they in turn can activate the Stat proteins also through tyrosine phosphorylation. Further downstream, activated Stats are then able to increase the transcriptional expression of cell survival genes (230).] In cells transformed by p210BCR-ABL, Bcr-Abl not only constitutively phosphorylates the shared β subunit of the interleukin 3 and granulocyte/macrophage-colony stimulating factor receptor in the absence of ligand but also coimmunoprecipitates with it (183). Bcr-Abl may also work upstream of the Stat proteins by constitutively phosphorylating, and thus activating, the Jaks, which in turn activate the Stat proteins (183, 186, 228). However, because Bcr-Abl can directly activate the Stat proteins, the need for the Jaks as a mediator could be bypassed (185).

Although both p210BCR-ABL and p190BCR-ABL phosphorylate several members of the Stat proteins in BCR-ABL-positive or BCR-ABL-transformed cells and cell lines, Stat5 appears to be the most prominent substate (182, 184–186, 228). In addition, phospho-Stat5 is required for the growth factor-independent growth and contributed to the transformation, as well as the antiapoptotic activity, of BCR-ABL cell lines developed from CML patients (186–188).

**Negative Regulation of Bcr-Abl by Bcr.** Bcr can form heterotetramers with Bcr-Abl through the NH2-terminal oligomerization domains of the two proteins (76, 77). In addition, Bcr binds to SH2 domains of normal Abl and coprecipitates with normal Abl (60). The result of interaction between Bcr and Bcr-Abl may be functional feedback regulation (71). Indeed, phosphorylation of the tyrosine 360 of Bcr by Bcr-Abl inhibits the kinase activity of Bcr, whereas serine phosphorylation within the first exon of Bcr inhibits the kinase activity of Abl (71, 231, 232).

**Therapeutic Implications and Future Directions**

Recently, molecules that specifically target the BCR-ABL gene product have been developed. One such molecule that has aroused tremendous interest is the Bcr-Abl-specific kinase inhibitor STI 571 (CGP 57148B). STI 571 is a derivative of 2-phenylaminopyrimidine, a class of inhibitors believed to function by competing with ATP for the ATP-binding site of the kinases (233). This compound was shown to be a potent inhibitor of oncogenic forms of Abl. It suppresses tumor growth in v-Abl-transformed cells as well as in primary cells from CML patients (233–236). In addition, long-term (2–8 weeks) exposure of the CML progenitor cells to STI 571 results in a sustained inhibition of these cells (236). In addition to its antiproliferative attributes, STI 571 also induces apoptosis in Bcr-Abl-positive cells (235, 237). Subsequent studies demonstrated that the apoptotic effect of STI 571 may be mediated by decreased levels of phosphorylated...
Stat-5 (238, 239). This in turn leads to down-regulation of Stat-5-dependent expression of the antiapoptotic protein, Bcl-xL (238, 239). Several investigators are also examining the in vitro effect of STI 571 when used in conjunction with other established therapies for BCR-ABL-positive leukemias (240–242). These studies demonstrate, for example, that the combination of STI 571 and IFN-α additively inhibited proliferation of CML cells (240, 241).

Currently, STI 571 is in early clinical trials aimed at chronic phase CML patients who have failed IFN therapy and at blast crisis patients (243–246). Twenty-three of 24 (96%) IFN-refractory chronic phase patients treated with 300–500 mg of STI 571 p.o. on a daily basis attained complete hematological response (243). Even more striking, many patients had cytogenetic responses, and there was little in the way of side effects. The durability of these responses is not yet clear. Preliminary results of a trial in 234 patients with accelerated phase showed that 78% attained hematological responses at 4 weeks (246). In addition, 82% of patients with Ph-positive ALL or lymphoid blast crisis have responded. Fifty-five % of the responses were complete, albeit short-lived. Patients with myeloid blast crisis of CML also responded. Indeed, 55% of a group of 33 such patients attained response, with 22% of the responses being complete (244, 245). The latter observations are surprising because by definition, blast crisis patients are presumed to have additional molecular defects beyond Bcr-Abl that drive evolution of the disease and growth of the leukemic cells. These findings therefore suggest that inactivation of Bcr-AbI nevertheless interferes with a growth or survival signal in advanced disease. These striking results indicate that gene-targeted therapies have the potential to control malignant cell proliferation without host toxicities.

Another innovative approach is to target BCR-ABL at the RNA level. This can be accomplished by the use of antisense oligonucleotides or of catalytic RNAs called ribozymes (247, 248). One requirement for the success of these two strategies is specificity for the target gene. In the case of BCR-ABL, the majority of antisense/ribozyme constructed is targeted toward the junction of BCR-ABL (e.g., b3a2 junction). Theoretically, this would increase the selective binding of the aberrant RNA and lessen the occurrence of nonspecific binding toward normal BCR or ABL RNA. However, the incidence of non-specific cleavage of other RNAs as well as poor uptake of the nucleotides are some of the problems encountered with these kind of strategies. These difficulties have led to the exploration of modified oligonucleotides and ribozymes (248–251), as well as alternative forms of catalytic RNA. This includes the use of M1 RNA (the catalytic RNA subunit of RNase P from Escherichia coli), a ribozyme that can be easily modified to target specific mRNA sequence, through the addition of a guide sequence against a specific site of the target mRNA (e.g., BCR-ABL junction; Ref. 252). Targeting BCR-ABL at the RNA level may be best applicable to ex vivo purging of bone marrow cells from patients with CML (253).

Another potentially unique direction using Bcr fragments as therapy is based on the observation that high levels of Bcr in Bcr-AbI-transformed cells attenuate the kinase activity of Bcr-AbI (254). In addition, a Bcr peptide fragment containing a mutated (S354 to E354) or phosphorylated serine 354 greatly inhibits the tyrosine kinase activity of both AbI and Bcr-AbI (231, 255). Similarly, a peptide containing the Bcr oligomerization domain (amino acids 1–160) has been demonstrated to reverse the transformed phenotype of p210Bcr-AbI-positive 32D myeloid leukemia cells (58). On the basis of these results, it has been hypothesized that these peptide fragments, or even a truncated Bcr containing exon 1, could be used as a therapeutic agent for Bcr-AbI-positive leukemias (58, 71).

Finally, direct suppression of Bcr-AbI by interference with downstream pathways critical to transformation merits exploration. Because of the interaction between Bcr and G proteins and the crucial role of Ras-dependent pathways in Bcr-AbI-mediated oncogenesis (201, 209), Ras is an apt target (256). Activation of Ras depends on the addition of a prenyl group (generally a farnesyl), which allows it to attach to the cell membrane. Molecules that inhibit farnesyl transferase, one of the key enzymes responsible for this reaction, are now entering clinical trials in CML.

In summary, understanding the molecular genetics of BCR-ABL leukemogenesis is leading rapidly toward molecular targeting as a treatment. Indeed, several vital lessons have been learned from the development of the Bcr-AbI tyrosine kinase inhibitor (257). These include the importance of identifying molecular targets and the feasibility of using novel drug design technology to discover specific and selective inhibitors, even for enzymes such as kinases that have widespread biological impact. The success of STI 571 in the clinic indicates that this approach can and should be used as a paradigm for applying molecular therapeutics in other cancers.

References


THE BCR GENE AND LEUKEMOGENESIS

2354


The *BCR* Gene and Philadelphia Chromosome-positive Leukemogenesis

Eunice Laurent, Moshe Talpaz, Hagop Kantarjian, et al.

*Cancer Res* 2001;61:2343-2355.

Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/6/2343

Cited articles

This article cites 248 articles, 110 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/6/2343.full#ref-list-1

Citing articles

This article has been cited by 8 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/61/6/2343.full#related-urls

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.