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The SH2 Domain of P210<sup>B</sup>C<sup>R</sup>/<sup>ABL</sup> Is Not Required for the Transformation of Hematopoietic Factor-Dependent Cells

By Robert L. Ilaria Jr and Richard A. Van Etten

Src-homology region 2 (SH2) domains, by binding to tyrosine-phosphorylated sequences, mediate specific protein-protein interactions important in diverse signal transduction pathways. Previous studies have shown that activated forms of the Abl tyrosine kinase, including P210<sup>B</sup>C<sup>R</sup>/<sup>ABL</sup>, of human chronic myelogenous leukemia, require the SH2 domain for the transformation of fibroblasts. To determine whether SH2 is also required for Bcr/Abl to transform hematopoietic cells, we have studied two SH2 domain mutations in P210<sup>B</sup>C<sup>R</sup>/<sup>ABL</sup>: a point mutation in the conserved FLVRES motif (P210/R1053K), which interferes with phosphorytrosine-binding by SH2, and a complete deletion of SH2 (P210/ΔSH2). Despite a negative effect on intrinsic Abl kinase activity, both P210 SH2 mutants were still able to transform the hematopoietic factor-dependent cell lines Ba/F3 and FDC-P1 to growth factor independence. Unexpectedly, both mutants showed greater transforming activity than wild-type P210 in a quantitative transformation assay, probably as a consequence of increased stability of the SH2 mutant proteins in vivo. Cells transformed by both P210 SH2 mutants were leukemogenic in syngeneic mice, and P210/R1053K mice exhibited a distinct disease phenotype, reminiscent of that induced by v-Abl. These results demonstrate that while the Abl SH2 domain is essential for Bcr/Abl transformation of fibroblasts, it is dispensable for the transformation of hematopoietic factor-dependent cell lines. © 1995 by The American Society of Hematology.

THE PHILADELPHIA chromosome is the result of a reciprocal translocation in which the c-ABL gene on chromosome 9 is truncated at its 5' end by BCR sequences from chromosome 22, forming the chimeric gene BCR/ABL. BCR/ABL has been implicated in a wide range of human leukemias, including virtually all cases of chronic myelogenous leukemia (CML), approximately 20% to 30% of adult acute lymphoblastic leukemia (ALL), and less commonly acute myelogenous leukemia. The normal cellular function of the BCR gene is unknown, but c-ABL, the cellular homologue of the transforming gene of Abelson murine leukemia virus, encodes a non-receptor protein-tyrosine kinase. The normal c-Abl protein is unable to transform cells, even when over-expressed; however, the fusion protein Bcr/Abl exhibits increased tyrosine kinase activity and gains the ability to cause cellular transformation in several assays, including focus formation and soft agar colony growth in fibroblasts, transformation of hematopoietic factor-dependent cells to growth factor independence, transformation of bone marrow cells in vitro, and induction of leukemia in mice.

c-Abl, like many other proteins implicated in signal transduction, contains a Src homology region two (SH2) domain. These domains are non-catalytic regions, approximately 100 amino acids in length, that bind with high affinity to specific amino acid sequences containing phosphorylated tyrosine residues. The phosphorytrosine-binding function of SH2 is crucial for fibroblast transformation by ABL. A point mutation in the highly conserved FLVRES motif of the Abl SH2 domain, resulting in an arginine to lysine change at position 171 (R171K) in the phosphorytrosine binding site, blocks binding of SH2 to phosphorytrosine-containing proteins in vitro and greatly decreases fibroblast transformation in vivo by activated c-Abl. Complete deletion of the SH2 domain of activated c-Abl abolishes fibroblast transformation. An analogous point mutation (R552L) in the SH2 domain of P185<sup>C</sup>Bcr/<sup>ABL</sup>, the form of BCR/ABL most commonly seen in Philadelphia chromosome-positive ALL, impairs Rat-1 fibroblast transformation. These studies demonstrate that activated forms of Abl require the SH2 domain for the transformation of fibroblasts.

Because BCR/ABL is associated with hematopoietic malignancies, it is important to determine whether the SH2 domain is also required for hematopoietic cell transformation and leukemogenesis by P210<sup>B</sup>C<sup>R</sup>/<sup>ABL</sup>. A previous report suggested that the Abl SH2 domain may be dispensable for the transformation of a hematopoietic cell line. We have generated P210<sup>B</sup>C<sup>R</sup>/<sup>ABL</sup> mutants containing either a FLVRES point mutation (P210/R1053K), or a complete deletion of the SH2 domain (P210/ΔSH2), and have examined their ability to transform the hematopoietic factor-dependent cell lines Ba/F3 (a murine pro-B lymphoid cell line) and FDC-P1 (a murine early myeloid cell line). Although P210/R1053K and particularly P210/ΔSH2 exhibit significantly decreased intrinsic kinase activity relative to P210<sup>B</sup>C<sup>R</sup>/<sup>ABL</sup>, they were still able to transform Ba/F3 and FDC-P1 cells to growth factor independence. Unexpectedly, both P210 SH2 mutants exhibited a higher transforming activity than wild-type P210 in a quantitative transformation assay, and retained the ability to induce leukemia in syngeneic mice, although P210/R1053K mice exhibited a distinct disease phenotype.

MATERIALS AND METHODS

Construction of BCR/ABL SH2 Domain Mutants

The P210/R1053K mutant was constructed using site-specific mutagenesis using the Mutagen MutaGene Phagemid system (Bio-Rad Laboratories).
tories, Melville, NY), based on the method of Kunkel et al. The mutagenic primer (5'-GCACTCTTTGAGAAGAGATGAGATTGAGCCGAGATCTC-3') was hybridized to a single-stranded, uracil-containing, P210BCR/ABL HindIII fragment contained in the vector pTZ/19U. The primer contained a new Hpa II site to identify mutant clones. The P210/R1053K mutant was confirmed byideoxy DNA sequencing, and the HindIII fragment was subcloned into the vector pGDA11, which is identical to the vector pGD, but lacks the EcoR1 site in the neomycin resistance gene cassette.

The P210/D8SH2 mutant was generated through a series of restriction digests in which the HindI site at nucleotide #804 and the Kpn I site at #527 were blunt-ended and fused in-frame, resulting in the deletion of the SH2 domain from amino acid #1030 to #1120 in the K562 b3a2 cDNA. The mutation was verified byideoxy DNA sequencing and restriction mapping was performed to confirm preservation of the Kpn I site. The mutant cDNA was subcloned into pGDA11 for transfection.

Cells and Cell Culture
Ba/F3 and FDC-P1 cells were grown in liquid culture at 37°C and 5% CO2 in Dulbecco’s modified Eagle’s medium (DME-high) containing 10% heat-inactivated fetal calf serum, penicillin/streptomycin, and 2 mmol/L glutamine. Where indicated, the medium was supplemented with growth factor in the form of either 5% to 10% (vol/vol) WEHI-3B-conditioned medium as a source of interleukin 3 (IL-3) (Ba/F3 and FDC-P1 cells), or murine recombiant granulocyte macrophage colony-stimulating factor (GM-CSF; Immunex, Seattle, WA) at a concentration of 30 U/mL. BOSC-23 cells were grown in DME-high containing 10% heat-inactivated fetal calf serum, penicillin/streptomycin, 2 mmol/L glutamine, and nonessential amino acids.

Gene Transfer
Retroviral infection. Ten micrograms of P210 or SH2 domain mutant DNA in the retroviral expression vector pGDA11, which contains a gene encoding resistance to neomycin, was introduced into the retroviral packaging cell line BOSC-23 by calcium phosphate transfection, as described. Twelve to 16 hours later, 6 x 106 Ba/F3 cells were gently added to each 10 cm BOSC-23 plate in the nonessential amino acids.

Analysis of Clones for Growth Factor Independence
Neomycin-resistant populations of Ba/F3 or FDC-P1 cells, containing P210 or one of the SH2 domain mutants, were cloned by limiting dilution into 96-well plates. Clones were isolated in the presence of growth factor and neomycin, with fresh medium added every 3 days to prevent any possibility of growth factor depletion. After approximately 10 days the wells were sufficiently confluent to be harvested. The cells were gently pelleted in 1.6 mL eppendorf tubes, washed twice in PBS, and distributed equally into parallel 24-well plates containing medium with or without supplemental growth factor. After 48 to 72 hours, the 24-well plates were scored by counting the number of clones capable of factor-independent growth. A growth factor-independent clone was defined as a clone that was able to proliferate both in the presence and absence of supplemental growth factor. Analysis of individual growth factor-independent clones by Southern blot revealed that the majority possessed a single copy of the BCR/ABL gene. The data were expressed as a ratio of the number of factor-independent clones divided by the total number of clones analyzed. The proportions obtained from hematopoietic factor-dependent cell lines containing the BCR/ABL SH2 domain mutants were compared with those containing wild-type P210, and analyzed using a chi-squared test for significance.

Autocrine Growth Analysis
(A) Conditioned media obtained from growth factor-independent populations of Ba/F3 and FDC-P1 cells expressing P210 or one of the SH2 mutants was concentrated 25- to 30-fold using centrprep-10 ultrafiltration (Amicon, Beverly, MA), and added to duplicate populations of Ba/F3 or FDC-P1 cells expressing P210 or one of the SH2 mutants at a concentration (10 pg/mL) 10-fold higher than was required to cause growth arrest of Ba/F3 cells and death of FDC-P1 cells growing in 1% WEHI-3B-conditioned medium. Viable cells were counted daily and their growth rate compared with cells grown in the absence of neutralizing antibody.

RNA Analysis
Total RNA was prepared from populations of neomycin-resistant Ba/F3 cells transfected with P210 or the SH2 mutants, before and after selection for the growth factor-independent phenotype, using the guanidinium isocyanate method. Equal amounts of total RNA were subjected to denaturing agarose gel electrophoresis, transferred to a nylon membrane, and hybridized with a 32P-labeled DNA probe generated from a 1,100-bp Cla I fragment from the neomycin gene cassette contained in the pGDA11 vector.

In Vitro Kinase Assay
P210, P210/R1053K, or P210/D8SH2, contained in the vector P31I(23) were introduced into 293T cells by transient transfection; alternatively, growth factor-independent Ba/F3 cells were used as the source of Bcr/Abl protein. Lysates were made and normalized by western blot for the amount of Bcr/Abl protein using the anti-Abl monoclonal antibody 19-84. Equal amounts of Bcr/Abl protein were subjected to immunoprecipitation with anti-GE4X antisera (recognizing COOH-terminal Abl sequences(25), and an immunocomplex kinase assay performed as previously described, with enolase added as an exogenous substrate. The proteins were then separated on a 5% to 9% gradient sodium dodecyl sulfate (SDS)-polyacrylamide gel, detected by autoradiography, and quantitated using a phosphorimagex (GS-250; Bio-Rad Laboratories).
TRANSFORMATION BY BCR/ABL SH2 MUTANTS

Leukemogenesis Assay

Polyclonal populations of Ba/F3 cells transformed by wild-type P210, P210/R1053K, or P210/ΔSH2 were propagated in liquid culture as described above, without a source of IL-3. Cells were washed twice in PBS, counted, and resuspended at a density of 10^6 cells per 0.5 mL PBS. Recipient BALB/c BYJ female mice (Jackson Laboratories, Bar Harbor, ME), between 6 and 8 weeks of age and having 17 to 20 g body weight, received 10^6 cells intravenously by tail vein injection. Injected mice were subsequently evaluated on a daily basis for weight loss, failure to thrive, splenomegaly, or hind-limb paralysis. Mice that appeared pre-morbid were killed. Hind-limb paralysis was scored if mice exhibited an inability to use hind limbs for ambulation on a smooth surface such as a countertop.

RESULTS

Abl SH2 Mutations Attenuate Bcr/Abl Kinase Activity In Vitro

To examine the effect of mutations in SH2 on the tyrosine kinase activity of P210, we expressed P210 and SH2 mutant proteins in 293T cells and performed an immune complex kinase assay. Addition of Bcr sequences to the NH2-terminus of Ab1 increases the intrinsic tyrosine kinase activity of the protein. Relative to c-Ab1, P210 kinase activity was increased approximately six-fold, while P190, the form of Bcr/Ab1 most commonly associated with Philadelphia chromosome-positive ALL, was elevated almost ten-fold, consistent with previous observations (Fig 1). The mutation disrupting the phosphotyrosine-binding function of P210, P210/R1053K, resulted in a modest decrease in kinase activity relative to P210, to 4.4-fold over c-Ab1. However, complete deletion of the SH2 domain abrogated the elevated kinase activity of P210, essentially to the level of c-Ab1 (0.7-fold). The impact of these mutations on Bcr/Ab1 autophosphorylation paralleled their effects on the phosphorylation of the exogenous substrate enolase. Similar results were obtained when these proteins were immunoprecipitated from Ba/F3 cells (data not shown).

Neither Phosphotyrosine-Binding nor Other Functions of SH2 Are Required for the Transformation of Factor-Dependent Hematopoietic Cell Lines by BCR/ABL

Quantitation of differences between wild-type and mutant forms of BCR/ABL in fibroblast transformation have relied on comparisons in focus formation or the number of colonies formed in soft agar. Because both Ba/F3 and FDC-P1 cells grow in liquid culture, we compared the transforming ability of the P210/R1053K and P210/SH2 mutations to wild-type P210 by the analysis of individual transfected clones. The neomycin-containing vector pGDAIRI, containing either wild-type P210, P210/R1053K, or P210/ΔSH2, was introduced into the factor-dependent cell lines Ba/F3 and FDC-P1 by helper-free retroviral infection or electroporation. After neomycin selection in the presence of growth factor, individual clones were isolated by limiting dilution and assessed for growth in medium with or without growth factor (WEHI-3B conditioned medium as source of IL-3 for Ba/F3 cells, and WEHI-3B or recombinant murine GM-CSF for FDC-P1 cells). Analysis of the site of chromosomal integration of proviral or plasmid DNA confirmed that independent clones were analyzed (data not shown). In parallel experiments where transfected cells were cloned directly in neomycin, the efficiency of recovery of neomycin-resistant clones was similar with either parental pGDAIRI vector alone, P210, or the SH2 mutants, suggesting there was no significant lethal or cytotoxic effect of transfection of Bcr/Ab1 in these cells. Cells incapable of growth in the absence of IL-3 or GM-CSF generally died within 24 hours of growth factor deprivation. No Ba/F3 or FDC-P1 cells spontaneously acquired the ability to grow without growth factor (data not shown). Further, neither pGDAIRI vector alone nor a kinase-inactive mutant of Ab1 supported any factor-independent growth (data not shown), confirming that the tyrosine kinase activity of Ab1 is required for the transformation of factor-dependent hematopoietic cells.

The ratio of growth factor-independent clones to total number of clones was calculated for each of the P210 mutants, and compared with wild-type P210 (Table 1). P210/R1053K conferred growth factor independence on approximately 25% and 23% of transfected Ba/F3 and FDC-P1 clones, respectively. Surprisingly, both SH2 domain mutants not only retained the ability to confer growth factor independence on these cells, but also exhibited approximately twice the transforming activity of wild-type P210;
factor-independent growth. The differences between P210 of the parental BaF3 and FDC-P1 cells, and the effect of case of the FDC-P1 cells, there was no significant difference by P210 or the SH2 mutant, we tested the ability of conditioned medium from transformed cells to support the growth of factor-independent cells expressing wild-type P210 or the P210 ASH2 mutant. How-
vGM-CSF (data not shown). We were unable to detect similar growth-promoting activity in conditioned medium from cells expressing wild-type P210 or the P210 ΔSH2 mutant. However, neutralizing anti-IL-3 antibodies, added at levels ten-
fold higher than necessary to induce apoptosis of parental Ba/F3 or FDC-P1 cells in 1% WEHI-3B conditioned medium, did not affect the proliferation of factor-independent cells transformed by P210, P210/R1053K, or P210/ΔSH2 (data not shown). Therefore, autocrine production of IL-3 does not play a role in the induction of growth factor independence by P210 or the SH2 mutants, consistent with previous observations.

Bar/F3 Cells Transformed by P210 SH2 Mutants Express Higher Levels of Bcr/Abl Protein, But Exhibit Similar Levels of Phosphoryrosine on Bcr/Abl

Polyclonal populations and individual clones of factor-independent cells expressing wild-type and mutant forms of P210BCWABL were analyzed by Western blot for levels of Bcr/Abl expression and pattern of protein tyrosine phosphorylation. Most of the clones that were incapable of factor-independent growth failed to express detectable Bcr/Abl protein. Some factor-dependent clones expressed low levels of Bcr/Abl, generally less than 5% of the amount of Bcr/Abl protein found in cells capable of factor-independent growth, and at about the same level as endogenous c-Abl (data not shown). This suggests that a threshold level of Bcr/Abl is necessary to overcome the requirement for growth factor, analogous to an effect documented for v-Src transformation of fibroblasts. In factor-independent cells, the total level of Bcr/Abl protein was approximately twofold higher for both P210/R1053K and P210/ΔSH2 compared with wild-type P210 (Fig 2A); however, the level of phosphoryrosine on Bcr/Abl was more similar, perhaps due to the decreased kinase activity of the SH2 mutants (Fig 2B). Interestingly, the increased level of Bcr/Abl protein expression by the SH2 mutants was evident even before selection for growth factor independence (Fig 2C), while the relative levels of BCR/ABL mRNA were similar between the SH2 mutants and wild-type P210 (Fig 2D) before and after selection for growth factor independence. There was a general increase in expression of both Bcr/Abl mRNA and protein following selection for growth factor independence, consistent with a threshold requirement for transformation. These results suggest that the elevated expression of the Bcr/Abl SH2 mutant proteins may be due to increased protein stability. We could not demonstrate any significant decrease in the level of mutant or wild-type Bcr/ Abl protein even after over 24 hours of treatment with cyclo-

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* P210BCWABL, P210 containing an FLVRES mutation (P210/R1053K), or P210 containing an in-frame deletion of the entire SH2 domain (P210/ΔSH2) were introduced into the hematopoietic factor-dependent cell lines Ba/F3 and FDC-P1 by retroviral gene transfer or electro-

approximately 46% to 50% of clones containing the point that severely impairs phosphoryrosine binding (P210/R1053K), and 47% to 49% of clones lacking the Abl SH2 domain entirely (P210/ΔSH2) were capable of growth factor-independent growth. The differences between P210 and the SH2 domain mutants were statistically significant (P < .02, using a χ² test comparing two proportions). In the case of the FDC-P1 cells, there was no significant difference in the ability of clones to grow in the absence of either IL-3 (WEHI-3B-conditioned medium) or GM-CSF (data not shown). Therefore, in contrast to fibroblast transformation, neither the phosphoryrosine-binding function of SH2 nor the SH2 domain itself was required for BCR/ABL to transform these hematopoietic factor-dependent cell lines to growth factor-independent proliferation.

To investigate the possibility that autocrine secretion of IL-3 was responsible for induction of factor-independence by P210 or the SH2 mutants, we tested the ability of conditioned medium from transformed cells to support the growth of the parental Ba/F3 and FDC-P1 cells, and the effect of neutralizing anti-IL-3 antibodies on their growth. High concentrations of conditioned medium from Ba/F3 or FDC-P1 cells expressing P210/R1053K supported the growth of parental FDC-P1 cells, consistent with secretion of IL-3 or GM-CSF (data not shown). We were unable to detect similar growth-promoting activity in conditioned medium from cells expressing wild-type P210 or the P210 ΔSH2 mutant. However, neutralizing anti-IL-3 antibodies, added at levels tenfold higher than necessary to induce apoptosis of parental Ba/F3 or FDC-P1 cells in 1% WEHI-3B conditioned medium, did not affect the proliferation of factor-independent cells transformed by P210, P210/R1053K, or P210/ΔSH2 (data not shown). Therefore, autocrine production of IL-3 does not play a role in the induction of growth factor independence by P210 or the SH2 mutants, consistent with previous observations.

The overall pattern of phosphorrosinated proteins was quite similar between P210 and the SH2 domain mutants, except that R1053K-expressing clones frequently demonstrated decreased tyrosine phosphorylation of proteins migrating at 120 and 60 kd, which are likely p120 rasGAP (GTPase-activating protein) and p62 rasGAP-associated protein, respectively. These proteins have been shown to be tyrosine-phosphorylated in cells transformed by various tyrosine kinase oncogenes, including BCR/ABL. Decreased tyrosine phosphorylation of p62 has also been reported in fibroblasts transformed by a BCR/ABL SH2 point mutant. The overall pattern of Bcr/Abl expression and protein tyrosine phosphorylation seen in P210 and the SH2 domain mutations did not differ significantly between Ba/F3 and FDC-P1 cells (data not shown), suggesting that these findings are not peculiar to one hematopoietic lineage.

The P210/R1053K Mutant Exhibits a Distinct Disease Phenotype in an In Vivo Leukemogenesis Assay

The transformation of Ba/F3 cells to growth factor independence by P210 has been previously shown to correlate with their ability to form tumors after subcutaneous inocula-

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Fig 2. Analysis of protein and mRNA expression of P210 SH2 domain mutants. Protein lysates from populations (p) or individual clones (numbers) of factor-independent Ba/F3 cells transformed by P210, P210/ΔSH2, or P210/R1053K were studied by either anti-Ab1 (A) or anti-phosphotyrosine (B) antibody. The position of Bcr/Abl is indicated by the solid arrow; the presumptive p62 Ras-GAP-associated protein is indicated by the open arrow. Molecular weight standards are shown at right. Populations of transfected Ba/F3 cells were analyzed for expression of P210 protein (C) or mRNA (D) after selection for neomycin resistance in the presence of IL-3 (neo^R), or following selection for growth factor-independence by withdrawal of IL-3 (~IL-3). M indicates P210 protein size marker. Antibody used in C was anti-Ab1 monoclonal antibody 19-84. Probe used in the northern blot in D was a neomycin gene fragment, which hybridizes to the BCR/ABL mRNA produced from the pGD210 provirus.

BALB/c mice within 16 days (Van Etten et al, in preparation). Autopsy of diseased animals reveals an overwhelming leukemia, with diffuse replacement of bone marrow, liver, and spleen with cells that morphologically resemble the input Ba/F3 cells (data not shown). Injections of factor-dependent parental Ba/F3 cells have yielded no disease in recipient mice followed for up to 3 months.

The clinical manifestations or latency of BCR/ABL-associated illness did not differ significantly between mice that received Ba/F3 cells transformed by P210 or P210/ΔSH2, with all animals dying secondary to a diffuse leukemic process between days 15 and 16. In contrast, mice that received cells transformed by the SH2 point mutant P210/R1053K demonstrated a significantly longer latency, with all animals succumbing by day 26 (Fig 3). This was unlikely to be due to a difference in growth rate, because all three cell populations grew at similar rates in culture (data not shown). In addition to the longer latency period, mice receiving Ba/F3 cells transformed by P210/R1053K exhibited a distinct disease phenotype, with approximately two-thirds of animals developing a hind-limb paralysis syndrome before death. This condition, which is characteristic of adult BALB/c mice infected with Abelson murine leukemia virus and reflects compression of the lumbar spinal cord nerve roots due to leukemic involvement of the paraspinal lymph node chain, was never observed in recipients of P210- or P210/ΔSH2-transformed Ba/F3 cells.

DISCUSSION

The interaction of SH2 domain-containing proteins with specific phosphotyrosinated sequences has been shown to be important for signal transduction involving both receptor and nonreceptor protein-tyrosine kinases. Previous studies have shown that the SH2 domain of activated forms of Abl is required for fibroblast transformation. We have studied two P210^{BCR/ABL}, SH2 domain mutants, P210/R1053K, which contains a point mutation interfering with phosphotyrosine-binding by SH2, and P210/ΔSH2, lacking the entire domain. In
Others have also recently found FDC-P1 cells to be transphosphotyrosine-independent kinase, while complete deletion of SH2 abrogated the activation of the ASH2 mutant was more similar to that formed by a 1.1 wild-type P210, despite decreased intrinsic kinase activity. These results confirm a previous report that noted that a population of Ba/F3 cells transfected with an SH2-deleted form of BCR/ABL was able to grow in the absence of growth factor. Others have also recently found FDC-P1 cells to be transformed by BCR/ABL lacking an SH2 domain.

The tyrosine kinase function of Abl is required for Bcr/Abl transformation of hematopoietic factor-dependent cell lines Ba/F3 and FDC-P1, despite decreased intrinsic kinase activity. These results confirm a previous report that noted that a population of Ba/F3 cells transfected with an SH2-deleted form of BCR/ABL was able to grow in the absence of growth factor. Others have also recently found FDC-P1 cells to be transformed by BCR/ABL lacking an SH2 domain.

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![Fig 3. P210 SH2 mutant leukemogenesis assay. 10^6 Ba/F3 cells transformed either by wild-type P210, (Δ) the SH2 domain point mutation P210/R1053K, or (Δ) the SH2 deletion mutant P210/ΔSH2 were injected into BALB/c BYJ female mice intravenously by tail vein injection. Survival was assessed from time of injection. The curve depicted represents one of three independent experiments, all of which obtained similar results.](image)

Fig 3. P210 SH2 mutant leukemogenesis assay. 10^6 Ba/F3 cells transformed either by wild-type P210, (Δ) the SH2 domain point mutation P210/R1053K, or (Δ) the SH2 deletion mutant P210/ΔSH2 were injected into BALB/c BYJ female mice intravenously by tail vein injection. Survival was assessed from time of injection. The curve depicted represents one of three independent experiments, all of which obtained similar results.
mutant die from an overwhelming leukemia by day 16, with infiltration of bone marrow, liver, and spleen. Interestingly, the SH2 domain point mutant P210/R1053K demonstrated a distinct disease phenotype, manifested by a greater latency of illness (death by day 25) and the frequent development of a syndrome of lower extremity paraparesis due to paraspinal lymph node infiltration, not seen in wild-type or P210/ΔSH2 disease, but reminiscent of classic disease induced by the Abelson virus. These findings raise the possibility that interference with the phosphotyrosine-binding function of P210BCR/ABL may affect cell homing in vivo. The possible mechanisms for this remain speculative, but may reflect alternative signal transduction pathways used by P210/R1053K, perhaps related to the differences in protein tyrosine phosphorylation of p120 rasGAP and p62 rasGAP-associated proteins seen on Western blot.

In summary, while the Abl SH2 domain is essential for BCR/ABL transformation of fibroblasts, it is dispensable for the transformation of hematopoietic factor-dependent cell lines. Transformation of fibroblasts may require a specific signal mediated by the Abl SH2 domain, which is not required for induction of growth factor independence in hematopoietic factor-dependent cell lines. Compared with wild-type P210, the P210/R1053K mutant demonstrated differences in both the pattern of protein tyrosine phosphorylation and leukemic phenotype in vivo. Whether these two phenomena are related, or reflect a physiologic difference between phosphotyrosine binding and other functions of Abl SH2, remains to be investigated. It will be important to determine whether the Abl SH2 domain is required for BCR/ABL-induced leukemia in humans. This issue is particularly relevant if the Abl SH2 domain is to be considered a target for rational drug design for the therapy of patients afflicted with these diseases. Experimental models that permit the analysis of P210 SH2 domain mutants in the context of early hematopoietic stem cells may help resolve this issue.

**ACKNOWLEDGMENT**

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