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Advances in Brief

Bcr and Abl Interaction: Oncogenic Activation of c-Abl by Sequestering Bcr[1]

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Abstract

c-Abl tyrosine kinase is under rigorous control because of an unknown cellular inhibitor that maintains c-Abl in a relatively inactive state. Because SH2 domains are positive regulators of the nonreceptor tyrosine kinases, we tested whether this putative inhibitor would bind to an Abl SH2 protein construct and thus activate the c-Abl tyrosine kinase. Expression of a M_r 10,000 Abl SH2 protein in COS-1 and Rat-1 cells activated the tyrosine kinase activity of p145 ABL and induced both morphological transformation and foci formation in Rat-1 cells. Importantly, the R to L mutant of the FLVRES sequence of the Abl SH2 protein also activated the c-Abl tyrosine kinase and induced oncogenic transformation. Addition of the Abl kinase inhibitor STI-571 to ABL SH2-transformed Rat-1 cells inhibited tyrosine phosphorylation of p145 ABL. Overexpression of Bcr has been shown to inhibit the Bcr-Abl oncoprotein, and the endogenous Bcr protein forms a complex with c-Abl in hematopoietic cells and insect cells. Therefore, we determined whether Bcr is the putative c-Abl inhibitor that interacts with the M_r 10,000 Abl SH2 protein. Bcr expression in Rat-1 cells transformed by the M_r 10,000 Abl SH2 protein reduced the activated c-Abl tyrosine kinase activity to near normal levels and reversed the oncogenic effects (morphology changes and foci formation) seen in the Abl SH2-treated cells. We additionally demonstrated that Bcr and the M_r 10,000 Abl SH2 protein are present in a complex. We conclude from these studies that Bcr is a major tyrosine kinase inhibitor of cytoplasmic c-Abl and that procedures that sequester Bcr will release the c-Abl protein from the Bcr/c-Abl complex, which leads to c-Abl oncogenic activation.

Introduction

The c-Abl protein is a nonreceptor tyrosine protein kinase that is localized in nucleus and cytoplasm (1–3). Activation of c-Abl's transforming activity is associated with cytoplasmic localization (2). Cytoplasmic c-Abl is associated with an unknown tyrosine kinase inhibitor (4). Recent studies indicate that the c-Abl protein is activated by the PDGF[3] receptor pathway (5). In these studies, binding of PDGF to the receptor induces c-Src to phosphorylate cytoplasmic c-Abl on tyrosine residues, which leads to the activation of c-Abl.

The endogenous Bcr protein forms a complex with c-Abl in hematopoietic cells (6) and insect cells (7). We have shown that phosphoserine Bcr binds to the Abl SH2 domain in a nonphosphotyrosine-dependent manner (8) and that this binding correlates with the Bcr's inhibition of Bcr-Abl oncogenic activity (8).

Because SH2 domains are positive regulators of the nonreceptor tyrosine kinases (9), we determined whether the putative inhibitor of c-Abl would bind to an Abl SH2 protein construct and thus activate the c-Abl tyrosine kinase. Our findings indicate that Bcr is the major inhibitor of cytoplasmic c-Abl.

Materials and Methods

Cell Lines and Antibodies. COS-1 and Rat-1 cells were maintained in DMEM plus 10% FBS; 32D cells in DMEM plus 10% of FBS and 10% WeHi culture medium, which contains mouse IL-3; Mo7e cells were maintained in Iscove's modified Dulbecco's medium plus 10% of FBS and human IL-3 (10 ng/ml); and anti-HA was purchased from Santa Cruz Biotechnology (San Diego, CA). The RYIRS antibody was obtained from Dr. Sue-Hwa Lin of the Department of Molecular Pathology; the anti-Abl 8E9 monoclonal antibody was prepared as described previously (10).

Plasmids Constructs. The SH2 expression plasmid for transfection studies was constructed by use of PCR to copy the human ABL SH2 domain beginning at nucleotide 379 through 648 (11). The sequence was inserted into pLXSN, which has an SV promoter and the *neo* gene. The sequence of the insert was verified by DNA sequencing. HA immuno-tag SH2 expression gene transfer plasmid for lentivirus was constructed as follows: the gene transfer vector pLOX iE was created by replacing a 848-bp *Bam*HI/*Sal*I fragment from original pLOX Dsal vector with a 1.33-Kb *Bam*HI/*Xba*I from pIRES2-EGFP vector through *Bam*HI/*Sal*I linker. The pLOX Dsal vector is a gift from Dr. Didier Trono (Geneva University, Geneva, Switzerland), and pIRES2-EGFP vector was purchased from Clontech (Palo Alto, CA). Wild-type and point mutant (R1053L) human c-ABL SH2 domains were prepared by PCR to introduce HA immuno-tag. The total amino acid of the SH2 plus the tag (8 amino acids) is 96 amino acid residues. After the PCR product was subcloned into pTOPO vector, DNA sequencing was performed to verify the sequence of the insert. Finally a 299-bp *Sma*I/*Bam*HI fragment of SH2 bearing a HA tag from pTOPO plasmid was cloned into the lentivirus gene transfer vector pLox iE at *Sma*I/*Bam*HI site. The plasmid pHR′-BCR with a HA tag was assembled as follows: the backbone vector pHR′ was linearized by *Bam*HI and blunt-ended; the HA tag sequence was introduced by PCR at the 3′ of BCR as a 300-bp fragment; it was ligated to the rest of BCR and finally inserted into the linearized pHR′ backbone by blunt-end ligation. The final lentivirus construct is similar to that shown in Fig. 1, except transcription of the BCR-GFP bicistronic mRNA was driven by a CMV promoter instead of the EF-1α promoter used for transducing the ABL SH2 domain.

Lentivirus Preparation. Lentiviruses were prepared by transfecting three plasmids into 293T cells as described previously (12). The plasmids are: pCMVΔ8.2 (GAG-POL DNA); the vesicular stomatitis virus envelope plasmid pMD.G; and gene transfer plasmid pLOX-ABL SH2/IRES/GFP). The gene transfer vector used in this study is the self-inactivating vector (13). The gene transfer plasmid contains a Psi packaging signal, whereas the pHR′ and the vesicular stomatitis virus envelope plasmid lacked the Psi packaging signal. In brief, 1×10^6 293T cells were seeded in a 100-mm culture dish 1 day before the transfection. The promoter for the ABL SH2 domain/GFP is EF-1α; CMV was used for the BCR-GFP construct. The culture medium was collected at the second and third day after the transfection. Usually two dish collections were done to harvest a virus preparation. The collected medium containing the virus was filtered with 45 μM filter and stored at −80°C for later usage.

Transfection and Western Blotting. COS-1 and Rat-1 fibroblasts were transfected as described previously (14). Western blotting was performed as described previously (14).

Lentivirus Infection. Cells were infected by the spin-inoculation method (15). In brief, cells were seeded at 1×10^5 cells/well (6-well-plate). Two ml of viral supernatant were added to each well and centrifuged at 2500 rpm for 90 min at 30°C. After spin-inoculation, the cells were incubated at 37°C, 5% CO overnight, and culture medium was replaced with fresh medium. At

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[3] The abbreviations used are: PDGF, platelet-derived growth factor; FBS, fetal bovine serum; IL, interleukin; CMV, cytomegalovirus; GFP, green fluorescent protein; IRES, internal ribosomal entry site; FLVRES, Phe, Leu, Val, Arg, Glu, Ser; RYIRS, Arg, Tyr, Iso, Arg, Ser; EF-1α, elongation factor-1α.

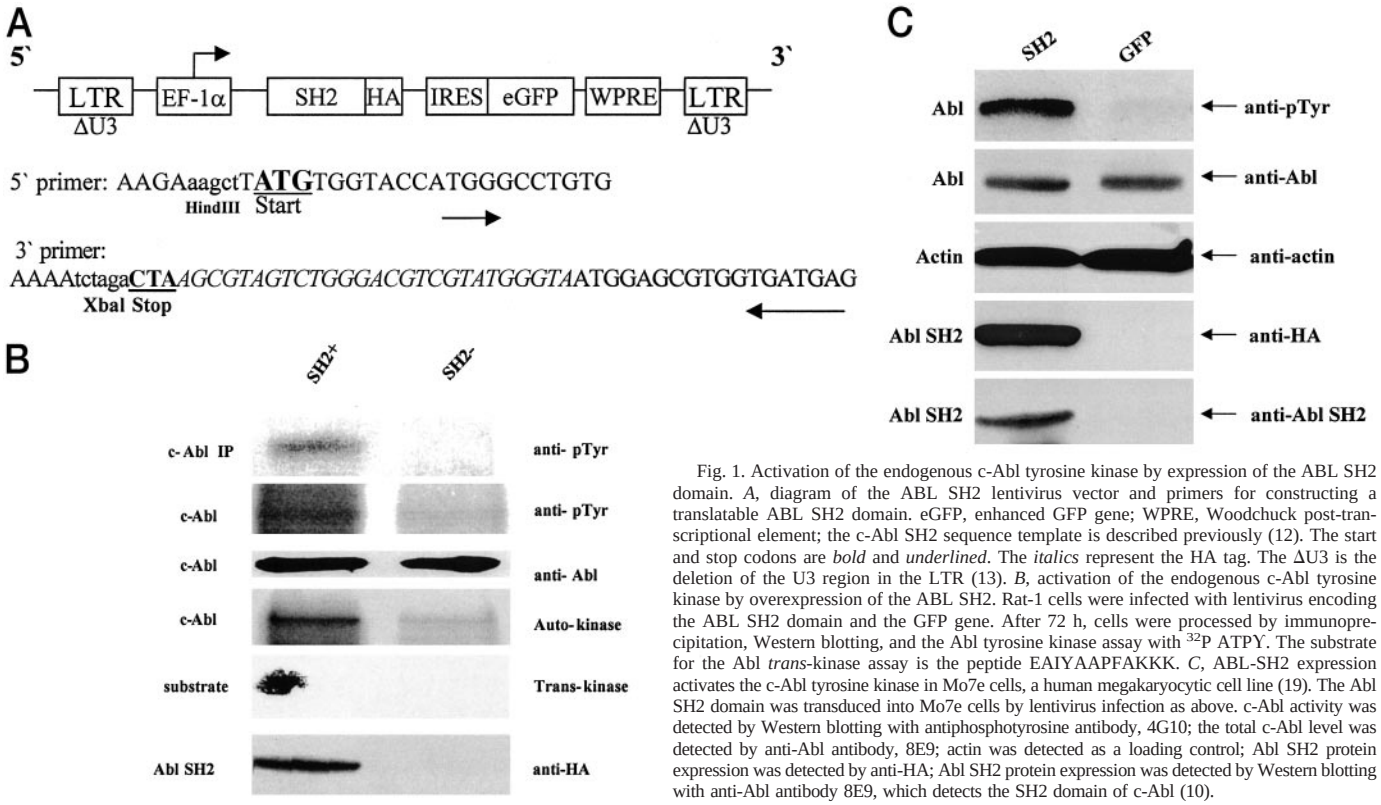

Fig. 1. Activation of the endogenous c-Abl tyrosine kinase by expression of the ABL SH2 domain. *A*, diagram of the ABL SH2 lentivirus vector and primers for constructing a translatable ABL SH2 domain. eGFP, enhanced GFP gene; WPRE, Woodchuck post-transcriptional element; the c-Abl SH2 sequence template is described previously (12). The start and stop codons are *bold* and *underlined*. The *italics* represent the HA tag. The ΔU3 is the deletion of the U3 region in the LTR (13). *B*, activation of the endogenous c-Abl tyrosine kinase by overexpression of the ABL SH2. Rat-1 cells were infected with lentivirus encoding the ABL SH2 domain and the GFP gene. After 72 h, cells were processed by immunoprecipitation, Western blotting, and the Abl tyrosine kinase assay with ^{32}P ATPY. The substrate for the Abl *trans*-kinase assay is the peptide EAIYAAPFAKKK. *C*, ABL-SH2 expression activates the c-Abl tyrosine kinase in Mo7e cells, a human megakaryocytic cell line (19). The Abl SH2 domain was transduced into Mo7e cells by lentivirus infection as above. c-Abl activity was detected by Western blotting with antiphosphotyrosine antibody, 4G10; the total c-Abl level was detected by anti-Abl antibody, 8E9; actin was detected as a loading control; Abl SH2 protein expression was detected by anti-HA; Abl SH2 protein expression was detected by Western blotting with anti-Abl antibody 8E9, which detects the SH2 domain of c-Abl (10).

72 h after infection, the culture was monitored/sorted for GFP fluorescence by flow cytometry. The cells expressing GFP also expressed the M_r 10,000 Abl SH2-HA-tagged protein.

Abl Protein Kinase Assay. The protein kinase assay for c-Abl was as described using radioactive ATP (16).

Immunoprecipitation Experiments. Cells were lysed in buffer containing 20 mM Na_2HPO_4 (pH 7.4), 150 mM NaCl, 1% Triton in the presence of a mixture of protease and phosphatase inhibitors, including AEBSF (Sigma Chemical Co., St. Louis, MO), a mixture of protease inhibitors (catalogue no. 1-836-153; Mannheim Boehringer, Indianapolis, IN). Anti-HA antibody and anti-Bcr (p5C; Ref. 17) were used to immunoprecipitate the Abl SH2 M_r 10,000 protein and p160 BCR, respectively. Immune complexes were analyzed by Western Blotting with appropriate antibodies, including the above antibodies and antiphosphotyrosine 4G10 monoclonal antibody (catalogue no. 05-321; Upstate Biotech, Lake Placid, NY). Anti-Abl 8E9 was used to detect the M_r 10,000 Abl SH2 domain because the 8E9 antibody was produced by immunization with the mouse Abl SH2 domain sequences. This antibody is a conformational/sequence-specific antibody, which does not recognize the R to L mutant of the conserved FLVRES sequence.

Results

Abl SH2 Expression Activates c-Abl Tyrosine Kinase and Induces Oncogenic Transformation. Our previous studies have shown that Bcr and Abl modulate each others' kinase activity (16–18), and binding of a phosphoserine form of Bcr to the Abl SH2 domain inhibits the tyrosine kinase of the c-Abl and Bcr-Abl proteins (8). These results prompted experiments to test whether forced expression of a translatable Abl SH2 sequence would activate the endogenous c-Abl tyrosine kinase. Initial experiments were performed with an ABL SH2 construct prepared by PCR from the human c-ABL 1b gene using primers that produced the ABL SH2 domain from nucleotides 379 to 648. We added a consensus translational start codon in frame to the upstream primer, and a tag and a stop codon to the downstream primer. The construct was inserted into pLXSN vector (allows *neo* selection) at the *Eco*RI site. Compared with vector-transfected COS-1/Rat-1 cells, ABL SH2-expressing COS-1/Rat-1 cells (*neo* selected) had a strong pTyr-containing p145 ABL protein and activated Abl kinase *in vitro*; ABL SH2-transduced Rat-1 cells formed foci, whereas vector-transfected cells did not (data not shown).

We devised a lentivirus strategy for efficient transduction of the ABL SH2 DNA construct into various cell types. We used a lentivirus vector system (12) that we modified to increase expression of the HA-tagged ABL SH2 domain by use of an EF-1α promoter. The lentivirus also encoded an IRES to allow GFP synthesis from the same transcript (Fig. 1*A*). This lentivirus was able to transduce efficient GFP expression in 55% to 85% of cells of various types with 3–4 days after infection (data not shown). Expression of the HA-tagged SH2 domain protein was detected as a M_r 10,000 protein by Western blotting in ABL SH2/GFP lentivirus-infected cells but not in GFP lentivirus-infected cells (Fig. 1*B*, bottom panel). Rat-1 cells showed a strong phosphotyrosine-containing M_r 145,000 c-Abl protein within 72 h, whereas GFP-transduced cells lacked the activated c-Abl protein (Fig. 1*B*). The level of c-Abl was not increased (Fig. 1*B*), indicating that the activation event increased the specific activity of the c-Abl kinase. *In vitro* kinase assays performed on these cells detected an activated c-Abl tyrosine kinase as measured by autophosphorylation and transphosphorylation of an added substrate (Fig. 1*B*). Western blotting of Rat-1 cells and 293T cells with anti-Abl 8E9 after infection with the ABL SH2/GFP lentivirus detected the M_r 10,000 HA-tagged protein (data not shown); the 8E9 antibody is a monoclonal antibody derived from the mouse Abl SH2 domain. Similar experiments were performed with the human Mo7e cell line, which is a hematopoietic cell line requiring IL-3 for growth (19). The c-Abl protein was activated by ABL SH2 expression in Mo7e cells as measured by phosphotyrosine Western blotting and kinase assays (Fig. 1*C*). Recent experiments indicated that activation of c-Abl in Mo7e cells resulted in a reduced requirement for IL-3 to maintain cell survival (data not shown).

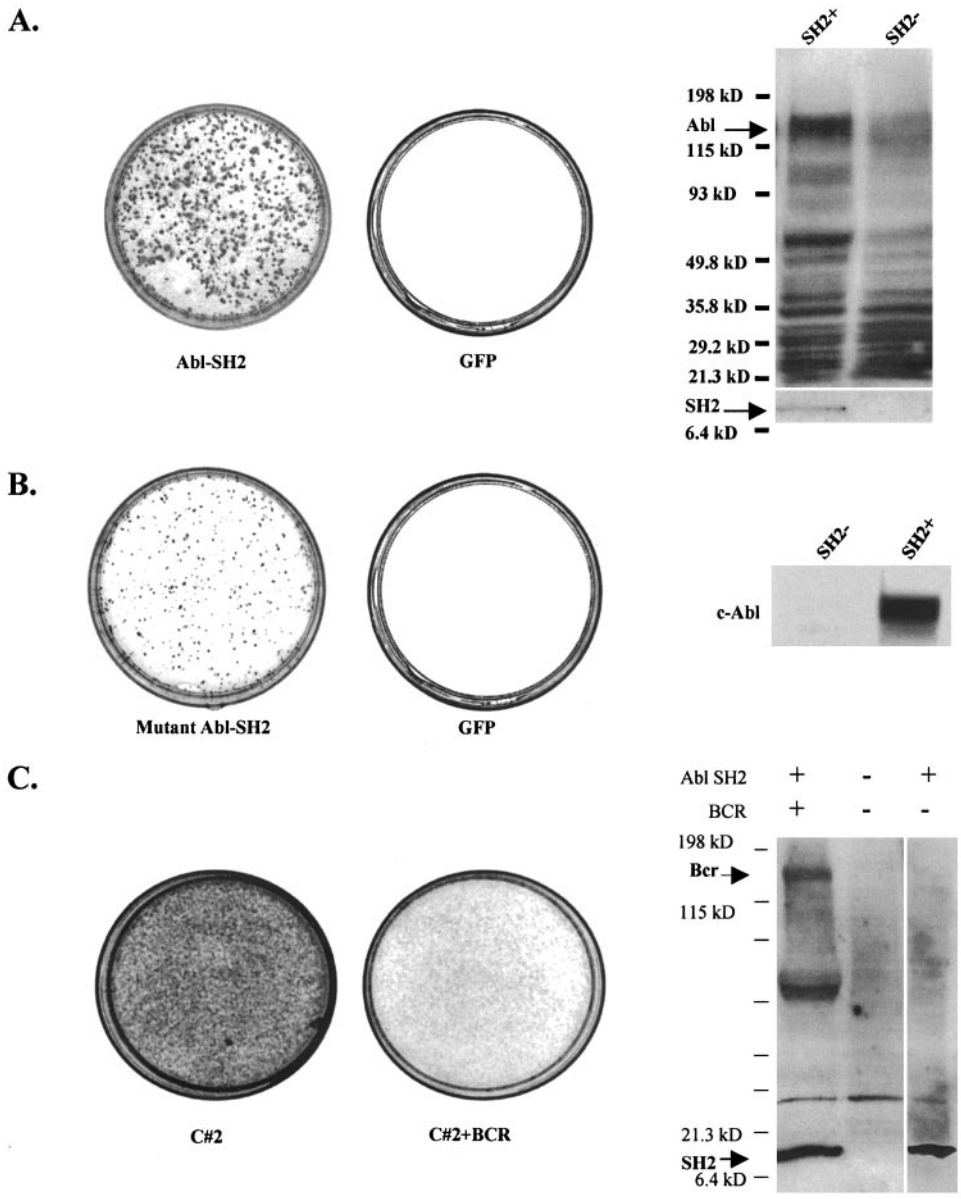

Fig. 2. BCR expression reverses ABL SH2-induced oncogenic transformation of Rat-1 cells. *A*, foci formation in Rat-1 cells infected with the ABL SH2/GFP lentivirus. Rat-1 cells were transduced by recombinant lentivirus encoding HA c-ABL SH2 and GFP (293T cell titer, 1×10^5GFP/ml; left). Control cells were infected with virus encoding GFP only, and experimental cells were infected with virus encoding both ABL SH2-HA and GFP. Foci were stained with crystal violet. Foci were formed after cells became confluent after 2 weeks (*left*). Control cells infected with GFP virus did not form foci (*middle*). Abl SH2-mediated activation of tyrosine phosphorylation of cellular proteins in transformed Rat-1 cells (*right*). Protein tyrosine phosphorylation was detected by anti-pTyr (*right, top panel*); Abl SH2-HA was detected by anti-HA (*right, bottom panel*). SDS-PAGE was done with a 4–20% gel. *B*, expression of the FLVRES mutant (R to L) of ABL SH2 in Rat-1 cells induces foci formation. Rat-1 cells were transduced by recombinant lentivirus encoding the HA-tagged c-ABL SH2 domain with a R/L mutation and GFP (*left*). Control cells were infected with virus containing GFP only (*middle*). Foci formation was performed as in *A*. FLVRES (R 1053 to L) mutant ABL-SH2 activates c-Abl tyrosine kinase in Rat-1 cells (*right*). A total of 88 μg of total protein was loaded on each lane. *C*, effect of Bcr on transformed ABL SH2 Rat-1 cells. A colony foci (C#2) was picked, cells expanded, and BCR cDNA was transduced by lentivirus encoding either BCR-GFP (*middle*) or GFP only (*left*) infection for 72 h. Cells were seeded in 60-mm dishes. Bcr expression in Rat-1 Abl SH2 protein positive cells (*right*) was determined by Western blotting with anti-Bcr (1–16); Abl SH2 protein was detected by anti-HA. A total of 66 μg of total protein was loaded on each lane.

Rat-1 cells have been used to assess the transformation effects of the Bcr-Abl oncoprotein (20). Therefore, we assessed the ability of Rat-1 cells to be transformed after infection with the recombinant ABL SH2/GFP lentivirus as above. Confluent cultures of Rat-1 cells expressing the M_r 10,000 Abl SH2 protein formed foci within 7 days in culture (~55 foci/60-cm dish; data not shown) and larger numbers of foci after 15 days in culture (Fig. 2*A*, left), whereas GFP-transduced cells did not form foci under the same conditions (Fig. 2*A*, middle). Several foci were selected and tested for their ability to form foci; selected foci formed very high numbers of foci within 15 days after the cell sheet became confluent (Fig. 2*C*, left). The overall tyrosine-phosphorylation of cellular proteins was also increased by expression of the ABL SH2 domain (Fig. 2*A*, right). Western blotting of anti-c-Src immunoprecipitates obtained from Rat-1 cells, 32D mouse myeloid cells, and Mo7e human cells, either expressing Abl SH2 protein or not, failed to detect increased tyrosine-phosphorylated c-Src in Abl SH2-expressing cells, indicating that the Abl SH2 M_r 10,000 protein does not cause activation of c-Src (data not shown).

Our previous findings indicate the R to L mutation within the conserved FLVRES sequence of the Abl SH2 domain does not interfere with the binding of phosphoserine Bcr (8). The FLVRES R to L mutant is known not to bind phosphotyrosine sequences (21). Therefore, we determined whether the R to L FLVRES ABL SH2 mutant domain would also activate c-Abl. The FLVRES (R to L) ABL SH2 mutant maintained its ability to activate the c-Abl tyrosine kinase (Fig. 2*B*, right) and caused foci formation similar to that observed with wild-type ABL SH2 (Fig. 2*B*, left). However, the size and number of foci induced by the mutant Abl SH2 protein were reduced slightly compared with wild type. The number of foci induced by the mutant Abl SH2 protein was ~10% less than wild type in our standard assay, and the size of the foci was reduced as well (compare Fig. 2, *A* and *B*, right panels). These results suggests that phosphotyrosine binding by the added M_r 10,000 Abl SH2 protein has only a minor role in the cell transformation effects induced by the Abl SH2 protein. However, it is also possible that the structural alteration of the Abl SH2 domain as a result of this mutation changes the confirmation of the Abl SH2 protein, thereby causing indirect effects that reduce Bcr protein binding. The latter is supported by our previous observation that the mutant Abl SH2 protein is very poorly recognized by the anti-Abl 8E9 monoclonal antibody in Western blotting experiments, whereas the

wild-type Abl SH2 protein is efficiently recognized by the 8E9 antibody (8, 10).

BCR Expression Reverses the c-Abl Kinase Activation and the Transformation Phenotype Induced by the Abl SH2 Domain Protein. Our previous findings also showed that reduction of endogenous p160 BCR by antisense 3′ BCR oligos, which were specific for BCR, enhanced the growth and survival of human chronic myelogenous leukemia cells (14). In contrast, forced expression of the BCR gene reduced the level of phosphotyrosine-containing proteins and colony formation in soft agar of Rat-1 cells transformed by P185 BCR-ABL (14). Similarly, induction of BCR expression in K562 cells, a cell line derived from a blast crisis chronic myelogenous leukemia patient (22), prevented a lethal leukemia in 80% of mice (18). Therefore, we determined whether BCR expression would reverse c-Abl induced oncogenic effects caused by expression of the M_r 10,000 Abl SH2 protein. We reinfected a SH2 GFP-positive transformed foci cell clone (clone 2) with either the BCR/GFP-HA lentivirus or the GFP lentivirus and measured foci formation (Fig. 2*C*). BCR expression and Abl SH2-HA was validated by Western blotting (Fig. 2*C*, right). In three separate experiments, BCR expression strongly reduced foci formation (Fig. 2*C*, middle) compared with the GFP control (Fig. 2*C*, left). As shown in Fig. 3*A*, Bcr expression within the Abl SH2-transformed Rat-1 cells sharply reduced c-Abl tyrosine kinase activation. The incomplete reversal of the activation of c-Abl by BCR transduction is likely because of either the lack of Bcr expression or reduced Bcr expression in some of the transformed Rat-1 cells. Importantly, the c-Abl kinase inhibitor, STI-571 (1 μM) (23), also sharply inhibited the activation of the c-Abl tyrosine kinase by the M_r 10,000 Abl SH2 protein (Fig. 3*B*). Importantly, BCR expression also restored the normal morphological pattern of ABL SH2-expressing Rat-1 cells (Fig. 3*C*).

Our previously published results indicate that Bcr binds to the Abl SH2 domain and that this binding inhibits the tyrosine kinase activity of Abl catalytic domain (8, 17). The results presented here suggest that forced expression of the Abl SH2 domain would compete for Bcr's binding to the c-Abl kinase, thereby releasing an activated c-Abl. We searched for complexes of Bcr and the M_r 10,000 HA-tagged Abl SH2 protein in SMS-SB cells, which are human pre-B leukemia cells (Bcr-Abl negative) that express Bcr protein (6). The cells were transduced with ABL SH2-HA by lentivirus infection. Immunoprecipitation of SMS-SB cells with antibody to Bcr immunoprecipitated the p160 BCR protein; the anti-Bcr immunoprecipitate also contained the M_r 10,000 Abl SH2-HA protein (Fig. 4*A*). We also found that immunoprecipitation of the M_r 10,000 Abl SH2-HA protein coimmunoprecipitated p160 BCR (Fig. 4*B*, *Lanes 1* and *2*). A similar experiment was performed with Abl SH2-transformed Rat-1 cells expressing both human Bcr and the M_r 10,000 Abl SH2 protein; the HA-tagged Abl SH2 protein and p160 BCR were coprecipitated by the HA antibody (Fig. 4*B*, *Lane 3*). Because rodent Bcr cannot be detected by several different human Bcr antibodies (unpublished data), Rat-1 cells not expressing human Bcr (GFP only) as expected did not detect a Rat Bcr protein (Fig. 4*B*, *Lane 4*). These results show that human Bcr is associated with the M_r 10,000 Abl SH2 protein in ABL SH2-transformed Rat-1 cells.

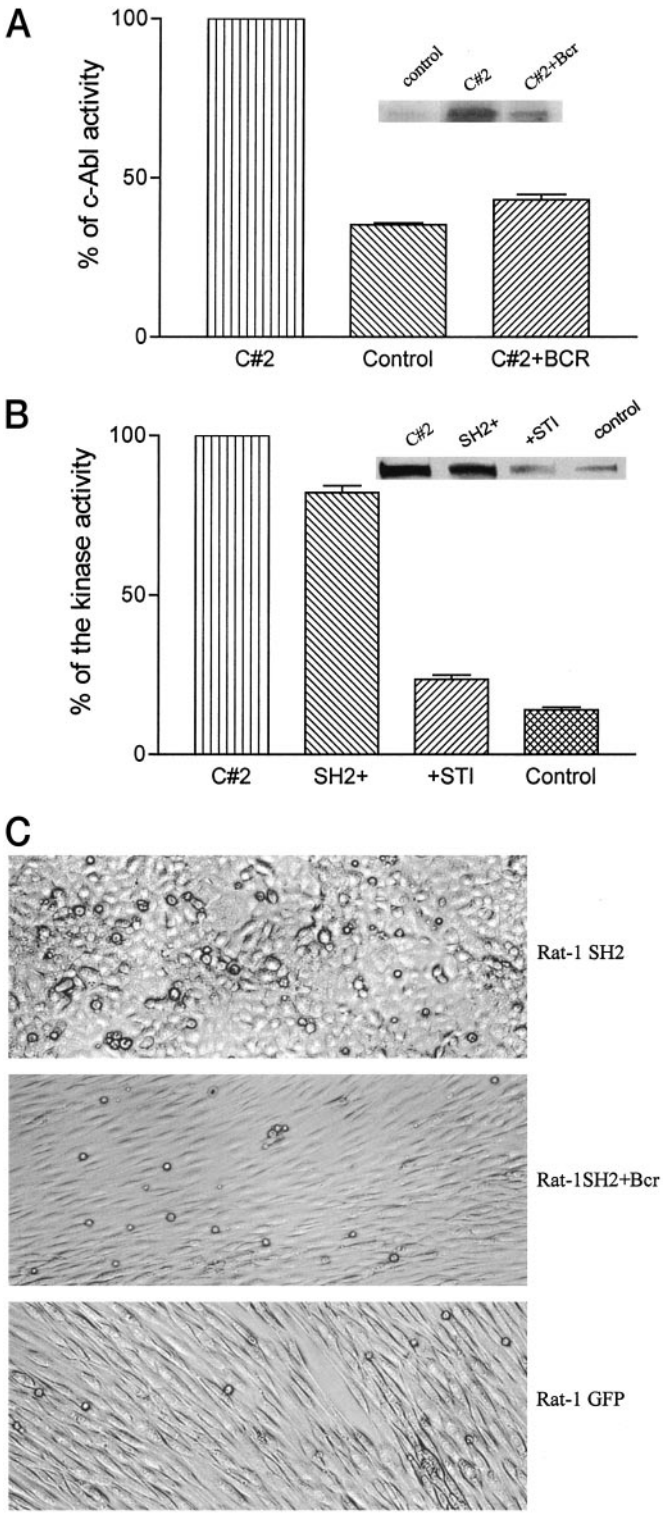

Fig. 3. BCR expression inhibits the endogenous c-Abl tyrosine kinase activated by ABL SH2 expression. *A*, activation of c-Abl tyrosine kinase by Abl SH2 is reversed by Bcr expression. c-Abl activity was detected by anti-pTyr. The intensities of bands were analyzed by volume densitometry. C#2: Rat-1 Abl SH2-positive foci no. 2; control: Rat-1 GFP cells, which are negative for the Abl SH2 protein; C#2 + BCR: C#2 cells were transduced with BCR cDNA by lentivirus infection. *B*, STI-571 inhibits c-Abl tyrosine phosphorylation in Rat-1 ABL SH2 cells. The C#2 cells were incubated with STI-571 at 1 μM for 4 h before harvesting. Cells were processed as in *A*. C#2: Rat-1 ABL SH2 C#2 foci; SH2: uncloned ABL SH2 Rat-1 cells; +STI: C#2 cells treated with STI-571; control: ABL SH2-negative Rat-1cells. *C*, BCR expression reverses transformation of Rat-1 cells caused by the Abl SH2. ABL SH2 Rat-1 cells (*top panel*); BCR-transduced Rat-1 ABL SH2-positive cells (*middle panel*). Rat-1 cells transduced with GFP lentivirus (*bottom panel*).

Discussion

The results presented here indicate that forced expression of the M_r 10,000 Abl SH2 protein activated the c-Abl tyrosine protein kinase, induced morphological transformation and foci formation in Rat-1 fibroblastic cells (Figs. 1 and 2). In addition, increased tyrosine phosphorylation of several proteins was observed in Rat-1 cells transformed by the ABL SH2 domain (Fig. 2*A*, right). Bcr expression in

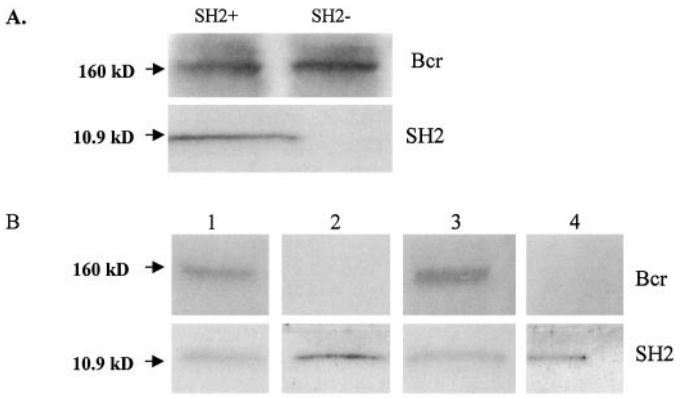

Fig. 4. Bcr forms complex with the M_r 10,000 Abl SH2 protein. *A*, Bcr-Abl negative human B cell leukemia cell line, SMS-SB, was infected with HA-tagged c-ABL SH2 domain/GFP lentivirus or the GFP lentivirus, and cells were sorted by flow cytometry. The sorted cells were lysed and immunoprecipitated with anti-Bcr (1–16). Western blotting was carried out with anti-HA (for Abl SH2-HA) and anti-Bcr (298–310) to detect the endogenous Bcr protein and the associated Abl SH2-HA protein. *B*, SMS-SB cells were processed as in *A*, except immunoprecipitates were performed with anti-HA, and Western blotting was performed with either anti-Bcr or anti-HA. *Lane 1*: SMS-SB cells expressing Abl SH2 as in *A*. The cells lysate was immunoprecipitated with anti-HA, then Western blotting with anti-Bcr (1–16) (*top panel*) or anti-HA (*bottom panel*) to detect the precipitated Abl SH2 protein and the complexed endogenous Bcr protein; *Lane 2*, GFP lentivirus-infected SMS-SB cells were immunoprecipitated with anti-HA and analyzed by Western blotting with either anti-Bcr (1–16) (*top panel*) or anti-HA (*bottom panel*); *Lane 3*: Rat-1 cells expressing the M_r 10,000 Abl SH2 protein were transfected with human BCR cDNA. Immunoprecipitation was carried out with anti-HA, then Western blotting with either anti-Bcr 1–16 (*top panel*) or anti-HA (*bottom panel*); *Lane 4*: as in *Lane 3*, except Western blotting was done with anti-HA in both *top and bottom panels*. The Bcr protein in SMS-SB cells or the transfected human BCR in Rat-1 cells do not have a HA tag.

cells transformed by the M_r 10,000 Abl SH2 protein sharply reduced the level of tyrosine phosphorylation of p145 ABL (Fig. 3*A*), inhibited foci formation (Fig. 2*C*) and blocked the morphological transformed pattern of growth induced with the M_r 10,000 Abl SH2 protein (Fig. 3*C*). Similarly, incubation of Rat-1 cells transformed cells with the Abl kinase inhibitor STI-571 strongly reduced the tyrosine kinase activity of c-Abl (Fig. 3*B*). Of importance, p160 BCR and the M_r 10,000 Abl SH2 protein formed stable complexes (Fig. 4). These effects of Bcr suggest that the tyrosine kinase activity of the endogenous cytoplasmic form of c-Abl is persistently down-regulated by Bcr, which we propose is bound to c-Abl through the SH2 domain by a process involving a serine-phosphorylated form of Bcr (see Ref. 8).

Our findings suggest that the M_r 10,000 Abl SH2 protein sequesters the c-Abl inhibitor (4), and thus activates the c-Abl tyrosine kinase. Furthermore, our findings suggest that this inhibitor is Bcr (Figs. 2 and 3). Importantly, Bcr and the M_r 10,000 Abl SH2 protein are present in a complex (Fig. 4). In support of these findings, we have previously shown that decreasing the level of p160 BCR by antisense 3′ BCR oligos increases cell proliferation and survival of Bcr-Abl positive hematopoietic cells (14). Therefore, sequestration of Bcr by the Abl SH2 protein releases c-Abl from the complex with Bcr, which sets in motion a signaling process that induces oncogenic transformation as a result of c-Abl activation.

It is known that the endogenous Bcr and c-Abl proteins are present in a complex under normal physiological conditions (6, 7). Specifically, our studies have previously shown that endogenous Bcr is associated with c-Abl in SMS-SB cells, a pre-B leukemia cell line lacking the BCR-ABL fusion (7). The c-Abl/Bcr complex is proposed to be formed, in part, by binding phosphoserine Bcr to the SH2 domain of c-Abl (8). We cannot eliminate the possibility that another factor(s) is also bound to the Abl SH2 domain besides Bcr and that overexpression of the M_r 10,000 Abl SH2 protein binds or sequesters both factors. Nevertheless, Bcr overexpression is sufficient to re-establish the inhibited state of c-Abl (Figs. 2 and 3). We note that BCR transduction did not completely reverse c-Abl tyrosine kinase activation (Fig. 3*A*). We believe that the incomplete reversal is related to the efficiency of BCR cDNA transduction. Thus, some transformed cells may either lack Bcr expression or express insufficient levels of Bcr. Thus at this time, we cannot eliminate the possibility that activation of c-Abl by the Abl SH2 protein involves other effects, albeit minor, in addition to sequestration of endogenous Bcr.

It is known that PDGF is involved in activating the c-Abl tyrosine kinase in fibroblasts by a series of reactions involving the activation of the PDGF receptor, activation of c-Src, and then activation of c-Abl by Src phosphorylation of tyrosine residues within c-Abl (5). How the Bcr protein fits into this scenario, if at all, is unknown. One possible scenario that would involve Bcr in the PDGF activation of c-Abl would be as follows: inhibition of c-Abl within the Bcr/c-Abl complex would be overridden by activation of c-Abl by c-Src as a result of PDGF signaling (5). Abl activation would then cause tyrosine phosphorylation of Bcr and thus inactivate the serine kinase activity of Bcr (16, 24), which in turn would lead to loss of Bcr serine autophosphorylation and subsequent dissociation of c-Abl from the complex.

The Gag-Crk avian sarcoma retrovirus provided the first evidence of the oncogenic activity of SH2/SH3 motifs (25). These viruses cause tumors and induce tyrosine phosphorylation of a variety of cellular proteins typically seen in retroviruses that transduce a tyrosine kinase (*e.g.*, avian sarcoma viruses encoding the v-Src tyrosine kinase). The tyrosine kinases activated by Gag-Crk expression lead to development of tumors in birds (26). To our knowledge, the findings presented here are the first report of the expression of a SH2 domain in the absence of the SH3 domain and that the ABL SH2 domain is critically involved in the steady-state suppression of the oncogenic potential of c-Abl. It is of interest to test the effects of Bcr on other SH2 domains (*e.g.*, c-Src SH2 domain) to determine whether similar oncogenic effects are observed. However, we observed no effects of BCR expression on Src-transformed cells (14, 17), nor did we detect any activation of c-Src by expression of the Abl SH2 protein in Rat-1 cells (data not shown).

The mechanism by which Bcr protein expression neutralizes the effects of the M_r 10,000 Abl SH2 protein probably relates to the ability of phosphoserine Bcr to bind to the Abl SH2 domain in a nonphosphotyrosine-dependent manner (8). Our studies indicate that a unique structural form of phosphoserine Bcr is required for binding to the Abl SH2 domain (8). The structure of this altered phosphoserine Bcr protein is as yet unknown, but hyperserine phosphorylation appears to be involved (8). The binding of phosphoserine Bcr to the Abl SH2 is not affected by mutating the SH2 domain in a way that inhibits the binding of phosphotyrosine sequences because the FLVRES mutant (R to L) and wild-type of the Abl SH2 domain showed no differences in their ability to bind to Bcr (8). In this current study, the R to L FLVRES mutant ABL SH2 protein maintained the ability, albeit at a somewhat reduced level (10% less activity), to activate the c-Abl kinase and induce oncogenic transformation, consistent with the involvement of phosphoserine Bcr as the major factor involved with the inhibition of the c-Abl kinase. Other possible effects of the Abl SH2 protein must also be considered based on the finding that the NH_2-terminal 80 amino acids of c-Abl is autoinhibitory to the c-Abl kinase activity (27). In this scenario, expression of the Abl SH2 M_r 10,000 protein would relieve the kinase block by interaction with kinase inhibited c-Abl protein. However, the reversal of Abl SH2 protein induced activation of c-Abl by Bcr observed in our studies (Fig. 2*C* and Fig. 3, *A* and *C*) argues that c-Abl interaction with the Abl SH2 protein is a minor effect at best. Nevertheless, this experimental system establishes a model to provide useful information about

the molecular signals involved in oncogenic transformation of cells by the c-Abl protein and its mutant forms (Gag-Abl and Bcr-Abl). In addition, our data raise the possibility that other tyrosine kinases besides c-Abl are also down-regulated by SH2 binding proteins.

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