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Expression of a Distinctive *BCR-ABL* Oncogene in Ph[1]-Positive Acute Lymphocytic Leukemia (ALL)

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The Philadelphia chromosome (Ph[1]) is a translocation between chromosomes 9 and 22 that is found in chronic myelogenous leukemia (CML) and a subset of acute lymphocytic leukemia patients (ALL). In CML, this results in the expression of a chimeric 8.5-kilobase *BCR-ABL* transcript that encodes the P210[BCR-ABL] tyrosine kinase. The Ph[1] chromosome in ALL expresses a distinct *ABL*-derived 7-kilobase messenger RNA that encodes the P185[ALL-ABL] protein. Since the expression of different oncogene products may play a role in the distinctive presentation of Ph[1]-positive ALL versus CML, it is necessary to understand the molecular basis for the expression of P185[ALL-ABL]. Both P210[BCR-ABL] and P185[ALL-ABL] are recognized by an antiserum directed to *BCR* determinants in the amino-terminal region of both proteins. Antisera to *BCR* determinants proximal to the *BCR-ABL* junction in CML immunoprecipitated P210[BCR-ABL] but not P185[ALL-ABL]. Nucleotide sequence analysis of complementary DNA clones made from RNA from the Ph[1]-positive ALL SUP-B15 cell line, and S1 nuclease protection analysis confirmed the presence of *BCR-ABL* chimeric transcripts in Ph[1]-positive ALL cells. In Ph[1]-positive ALL, *ABL* sequences were joined to *BCR* sequences approximately 1.5 kilobases 5′ of the CML junction. P185[ALL-ABL] represents the product of a *BCR-ABL* fusion gene in Ph[1]-positive ALL that is distinct from the *BCR-ABL* fusion gene of CML.

THE LEUKEMIC CELLS OF MORE than 95% of CML patients (*1*) and of 5 to 20% of ALL patients (*2, 3*) carry the t(9;22)(q34;q11) translocation known as the Philadelphia chromosome (Ph[1]) (*4*). In CML, the C-*ABL* gene on chromosome 9 is translocated into the middle of the *BCR* gene on chromosome 22 (*5*). Although the breakpoint on chromosome 22 is variable, it occurs within a defined 5.8-kb region of the *BCR* gene known as the breakpoint cluster region, or bcr (*6*). RNA splicing generates an 8.5-kb *BCR-ABL* chimeric transcript that is larger than the normal 6- and 7-kb C-*ABL* transcripts (*7, 8*). This results in the expression of the P210[BCR-ABL] protein (*9*) in which NH_2-terminal C-*ABL* sequences are replaced by sequences from the *BCR* gene (*10*).

Despite the fact that the Ph[1] chromosomes of CML and ALL are indistinguishable by cytogenetic analysis, cells from most Ph[1]-positive ALL patients express *ABL*-derived protein and RNA species that are distinct from the *BCR-ABL* products of CML (*11, 12*). Ph[1]-positive ALL cells display a high level of *ABL*-related tyrosine kinase activity in proteins of 180 and 185 kD, referred to collectively as P185[ALL-ABL] (*11*). Comparison of tryptic phosphopeptide maps between P210[BCR-ABL] and P185[ALL-ABL] revealed similar, but not identical, phosphorylation patterns suggesting some structural similarities (*11, 13*). The appearance of P185[ALL-ABL] correlates with the expression of a 6.5- to 7.0-kb *ABL* messenger RNA (mRNA) (*11–13*) in contrast to the 8.5-kb *BCR-ABL* transcript in CML cells. Genomic DNA analysis (*11–15*) and in situ hybridization studies (*15*) of the Ph[1] chromosome from ALL cells suggested that the breakpoint on chromosome 22 may not be in the bcr region as in the Ph[1] chromosome of CML. It is possible that a breakpoint elsewhere in the *BCR* gene, or within another gene on chromosome 22, could generate the altered *ABL* products in Ph[1]-positive ALL. Alternatively, unusual RNA splicing within the *ABL* fusion partner may also account for the expression of P185[ALL-ABL].

To determine whether P185[ALL-ABL] contains *BCR* sequences, we compared the immunoreactivity of P185[ALL-ABL] and P210[BCR-ABL] with a panel of site-directed *BCR* antisera. Normal rabbit sera (NRS) did not recognize either protein (Fig. 1), while antisera directed against NH_2-terminal *BCR* sequences (antisera A) immunopre-

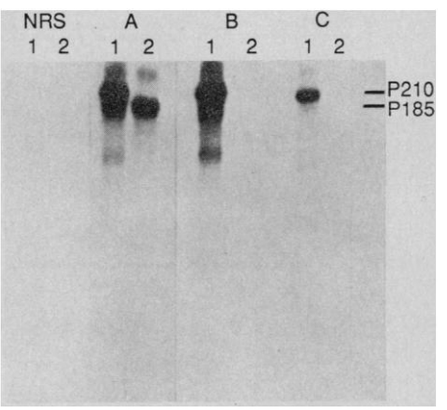

Fig. 1. P185[ALL-ABL] displays *BCR* homology limited to the NH_2-terminal region of *BCR*. K562 cells (*23*) (lanes 1) or the Ph[1]-positive ALL cell line, ALL-1 (lanes 2) (*15, 24*) were immunoprecipitated with either normal rabbit serum (NRS) or with a panel of rabbit antisera raised against specific *BCR* determinants as illustrated in Fig. 2. Antiserum A was raised by immunizing rabbits with a *trpE-BCR* fusion protein expressed in the pATH-11 expression vector as described (*26*). Antigenic *BCR* sequences were encoded by a 1.4-kb Bam HI fragment (*8*). Antiserum B corresponds to the *BCR* 558 antiserum as reported (*10*). Rabbit antiserum C (*27*) was raised against a peptide sequence, IKSDIQREKRANKGSY, beginning 134 amino acids and 403 base pairs upstream of the *BCR-ABL* junction of K562 cells. The COOH-terminal tyrosine is not found in this position in the *BCR* sequence. Cell lysates were immunoprecipitated with the indicated antisera and prepared for the autokinase labeling reaction as described (*11*). The proteins were then reprecipitated with the same antisera used for the first cycle immunoprecipitation, separated by 8% SDS–polyacrylamide gel electrophoresis, and detected by autoradiography.

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cipitated P210$^{BCR-ABL}$ from K562 cells and P185$^{ALL-ABL}$ from the Ph^1-positive ALL cell line ALL-1 (Fig. 1A). This antiserum has also been shown to immunoprecipitate a 185-kD protein from three independent Ph^1-positive ALL patients and from the Ph^1-positive ALL cell line SUP-B13 that comigrates with P185$^{ALL-ABL}$ precipitated with anti-*ABL* sera (*13*). *BCR* antisera A does not recognize P145^{C-ABL} or P160^{v-abl} proteins (*13*), indicating that it does not cross-react with *ABL* antigenic determinants. Faint phosphoproteins of about 150 to 160 kD are detectable in K562 (Fig. 1, lanes 1) but not in ALL-1 lysates (Fig. 1, lanes 2) precipitated with the *BCR* antisera. This may represent the C-*BCR* gene product, which is reported to co-precipitate with a serine-threonine–specific kinase activity (*16*). The fact that this phosphoprotein is not observed in ALL-1 cells suggests that it is not expressed at detectable levels in these cells.

In contrast to the data obtained with *BCR* antisera A, antisera B and C, directed against *BCR* determinants closer to the *BCR-ABL* junction in K562 cells (see lower panel of Fig. 2), recognized P210$^{BCR-ABL}$ but not P185$^{ALL-ABL}$ (Fig. 1, B and C). Together, these data demonstrate that the P185$^{ALL-ABL}$ protein retains antigenic determinants that are cross-reactive with the *BCR* NH_2-terminal determinants of P210$^{BCR-ABL}$. However, P185$^{ALL-ABL}$ does not share cross-reactive *BCR* antigenic determinants near the *BCR-ABL* junction of the P210$^{BCR-ABL}$ protein (Fig. 2, lower panel). It is possible that the two proteins share NH_2-terminal homology, but express divergent sequences near the breakpoint. Alternatively, P185$^{ALL-ABL}$ may represent a fusion protein in which only a limited NH_2-terminal portion of *BCR*, or a *BCR*-like gene, is spliced to C-*ABL* exon 2. Either possibility is consistent with the difference in apparent molecular weight between the two proteins.

To confirm that P185$^{ALL-ABL}$ is a *BCR-ABL* fusion product, we examined the molecular structure of the P185$^{ALL-ABL}$ transcript. Clones from a λgt10 complementary DNA (cDNA) library made from polyadenylated RNA (*17*) from the Ph^1-positive ALL SUP-B15 cell line (*18, 19*) were screened with human C-*ABL* and *BCR* oligonucleotide probes. We selected two recombinant phage clones that hybridized to a fragment 20 nucleotides long (20mer) corresponding to a C-*ABL* exon 2 sequence found near the junction in P210$^{BCR-ABL}$ mRNA, and which failed to hybridize to two BCR oligonucleotides (30mer and 31mer) also found near the *BCR-ABL* junction in K562 cells (*8*). Sequence analysis (*17*) of both phage inserts revealed that the same 5′ *BCR* sequences are joined to C-*ABL* exon 2 sequences. The sequence of the longer clone, A27 (Fig. 2), shows that the 416 bases from *BCR* that join to C-*ABL* exon 2 are nearly identical to *BCR* sequences 5′ of nucleotide 1749 from the *BCR* sequence reported by Mes-Masson *et al.* (*8*). The divergence of this sequence from the reported *BCR* sequence occurs at nucleotides 33 and 34 in Fig. 2 [or nucleotides 1366 and 1367 in Mes-Masson *et al.* (*8*)]. The previously reported sequence for these bases is GC, while we report the sequence as CG. This change occurs in the last two bases of a codon and results in the change of a serine for a threonine. This may represent a polymorphic site in *BCR*.

We used S1 nuclease protection to confirm the expression of *BCR-ABL* transcripts in the steady-state RNA pools from Ph^1-

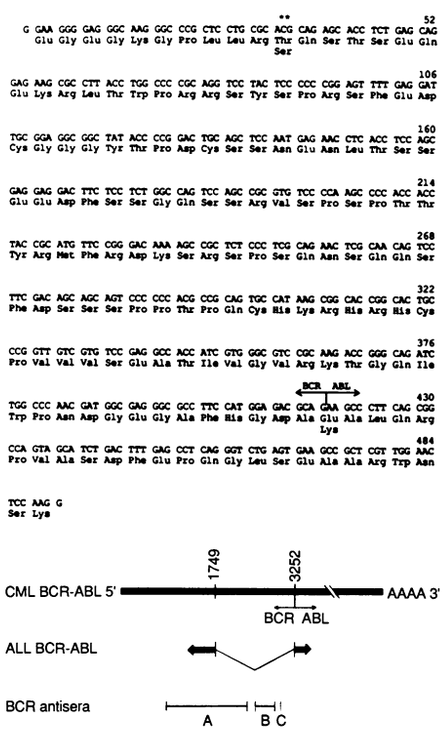

Fig. 2. Nucleotide and amino acid sequence (top) is shown for the 491-bp insert from recombinant phage clone A27. Numbering of the nucleotide sequence begins with the first 5′ nucleotide not part of the M13 sequence and proceeds through the 3′ end of the cloned fragment. In comparison to the published *BCR-ABL* sequence from CML (*21*), position number 1 shown here corresponds to nucleotide 1335 as previously published, and the *BCR-ABL* junction shown at nucleotide 415 corresponds to *BCR* position 1749 in CML. The two bases that are different from the previously reported sequence for K562 (*21*) occur at nucleotides 33 and 34, and involve a change from GC to CG (shown as **). The amino acid sequence, shown beneath the nucleotide sequence, is identical to that previously reported from K562 cells with two exceptions which are listed under the sequence we report for the SUP-B15 cell line. A comparison of the CML and ALL *BCR-ABL* transcripts is shown in the lower panel (the portion of the figure that is 5′ of the CML junction is drawn to scale). The positions of the antigenic determinants recognized by site-specific *BCR* antisera A, B, and C are also shown drawn to scale.

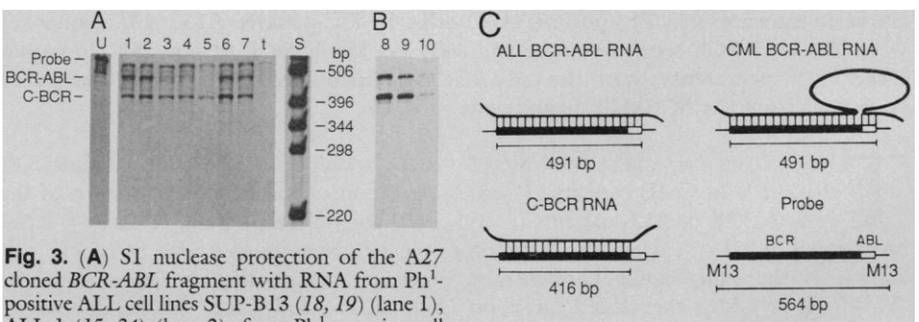

Fig. 3. (**A**) S1 nuclease protection of the A27 cloned *BCR-ABL* fragment with RNA from Ph^1-positive ALL cell lines SUP-B13 (*18, 19*) (lane 1), ALL-1 (*15, 24*) (lane 2); from Ph^1-negative cell lines L697 (lane 3), SMS-SB (lane 4), HL60 (lane 5); and from Ph^1-positive CML cells K562 (lane 6), and Bv173 (lane 7). U, undigested; t, transfer RNA; S, standards. The probe was made by primer extension from the universal primer of M13mp19 containing the A27-positive strand and contained all 491 nucleotides of the *BCR-ABL* insert as well as 73 nucleotides from M13. Total RNA (25 μg) was heated at 80°C for 3 minutes and allowed to anneal with the probe at 57°C overnight. S1 nuclease (50 units) was used to digest each sample for 1 hour at 37°C. The digested samples were then separated on a 5% urea-polyacrylamide sequencing gel with size standards from Hinf I–digested pBR322 labeled with Klenow polymerase. Controls included undigested probe and probe mixed with yeast transfer RNA prior to digestion. The C-*BCR* transcript protects a 416-bp fragment in all cells. A putative 75-bp fragment which could be protected by C-*ABL* mRNA was run off the gel and not detected. Both the Ph^1-positive ALL 7.0-kb *BCR-ABL* transcript as well as the CML 8.5-kb transcript can protect the full-length 491-bp fragment as described in the text. (**B**) S1 nuclease protection of the A27 clone was done with *BCR-ABL* RNA generated in vitro from the CML-specific 8.5-kb *BCR-ABL* cDNA 172/215 (*28*) cloned into the Eco RI site of pT7-1 containing the T7 RNA polymerase promoter (*29*) (U.S. Biochemicals). The plasmid was linearized and RNA was generated from 3 μg of DNA with T7 polymerase (U.S. Biochemicals). DNA was digested with ribonuclease-free deoxyribonuclease and serial tenfold dilutions of RNA (lanes 8, 9, and 10) were used in an S1 protection assay as described above. *BCR-ABL* RNA that had been generated in vitro protected both the 491-bp full-length fragment and the 416-bp *BCR* fragment as noted in the text. (**C**) Diagrammatic representation of S1 nuclease protection model.

positive ALL cell lines. Total RNA was used to protect a 564-bp single-stranded M13mp19 fragment containing the 491-bp *BCR-ABL* insert of clone A27. RNAs from all cell lines tested protect a common 416-bp fragment representing C-*BCR* mRNA (Fig. 3A, lanes 1 to 7). With RNA from two Ph^1-positive ALL cell lines, we observed protection of a full length 491-bp fragment (Fig. 3A, lanes 1 and 2), which is consistent with the presence of *BCR-ABL* transcripts in which C-*ABL* exon 2 sequences are contiguous with *BCR* sequences 5′ of nucleotide 1749. Both Ph^1-positive ALL cell lines expressed the 7.0-kb *BCR-ABL* transcript, but not the 8.5-kb *BCR-ABL* message (*13*). We also observed a similar 491-bp protected fragment with RNA from two Ph^1-positive CML cell lines (Fig. 3, lanes 6 and 7), both of which made the 8.5-kb *BCR-ABL* message but not the 7.0-kb *BCR-ABL* transcript (*13*). In the 8.5-kb transcripts, *BCR* sequences upstream of nucleotide 1749 are brought adjacent to *ABL* sequences while *BCR* sequences that are not included within the probe (between position 1749 and the junction) would be looped out (*20*) (Fig. 3C), explaining why we see protection of the full-length 491-bp probe.

The full 491-bp *BCR-ABL* probe is also protected by in vitro transcribed RNA generated with T7 polymerase from an 8.5-kb clone of the CML *BCR-ABL* message (*13*) (Fig. 3B, lanes 8 to 10). In addition, some hybrids are formed between the in vitro transcribed RNA and M13 probe which stably protect only the *BCR* segment (band size of 416). We believe this may account for the increased protection of this fragment when natural mRNA preparations from Ph^1-positive CML lines like K562 and Bv173 (Fig. 3A, lanes 6 and 7) are compared to Ph^1-negative lines like SMS-SB and HL60 (Fig. 3A, lanes 4 and 5).

Sequence analysis of two Ph^1-positive ALL cDNA clones demonstrates that C-*ABL* exon 2 sequences are joined to *BCR* sequences upstream of position 1749, and S1 nuclease protection confirms the expression of *BCR-ABL* chimeric mRNA in Ph^1-positive ALL cells. A breakpoint in this region of *BCR* would occur within the region against which *BCR* antiserum A was made and would occur upstream of the regions recognized by anti-*BCR* sera B and C (Fig. 2, lower panel). Thus, the molecular data are consistent with the serological results. Since the *BCR-ABL* junction in K562 cells occurs at *BCR* position 3252 (*21*) and this junction in Ph^1-positive ALL cells occurs at *BCR* position 1749, it is predicted that Ph^1-positive ALL *BCR-ABL* mRNA would lack about 1.5 kb of coding sequences relative to CML *BCR-ABL* mRNA. This is consistent with the presence of a 7.0-kb *BCR-ABL* transcript in Ph^1-positive ALL which is about 1.5 kb smaller than the 8.5-kb *BCR-ALL* transcript in CML.

In Ph^1-positive CML and ALL, expression of different *BCR-ABL* gene products correlates with the presence of Ph^1 chromosomes that are indistinguishable by cytogenetic analysis (*3*). Molecular analysis of the genomic structure of the *BCR* gene in Ph^1-positive CML reveals consistent rearrangement within the 5.8-kb bcr region (*6*). In Ph^1-positive ALL, however, this region is not rearranged (*11–15*); rather, in situ hybridization (*15*) reveals that the translocation breakpoint occurs 5′ (centromeric) of the bcr. After submission of this manuscript, serological (*22*) and molecular (*23*) studies reported that the unique *BCR-ABL* fusion in Ph^1-positive ALL cells is similar to that described here. These observations suggest that similar mechanisms are responsible for the formation of the $P185^{ALL\text{-}ABL}$ and $P210^{BCR\text{-}ABL}$ products. That is, chromosomal translocation joins C-*ABL* with different regions of the *BCR* gene in Ph^1-positive ALL and CML. RNA splicing then is responsible for the expression of *BCR-ABL* chimeric messages in which *BCR* coding sequences 5′ of the *BCR* breakpoint are joined in frame with C-*ABL* exon 2 sequences. Thus, different breakpoints within the same gene on chromosome 22 result in the expression of distinctive *BCR-ABL* gene products.

In different leukemias, chromosomal translocation creates Ph^1 chromosomes with reproducible differences in structure. The precise mechanisms that give rise to the two Ph^1 chromosomes are not known, but we speculate that the resultant distinctive oncogene products may relate to the specific pathologies of Ph^1-positive CML and ALL.

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17. The cDNA cloning was as previously reported (*8*). Briefly, polyadenylated RNA was obtained from the SUP-B15 Ph^1-positive ALL cell line (*18, 19*). First-strand synthesis was primed simultaneously with a C-*ABL* 21mer (*8*), a C-*ABL* exon 2 27mer (5′-AGCGAGAAGGTTTTCCTTGGAGTTCCA-3′), as well as oligo(dT). After second-strand synthesis, the cDNA was methylated at internal Eco RI sites and ligated with Eco RI linkers. Double-stranded cDNA was ligated to the Eco RI cloned arms of λ gt10 (Vector Cloning Systems) and packaged in vitro. From a library of approximately 10^6 recombinant phage we selected two clones that hybridized with a 20mer from C-*ABL* exon 2 (5′-TGAGGCTCAAAGTCAGATGC-3′) and not with a 30mer or 31mer from *BCR* sequences near the cDNA junction in K562 cells (*8*). The inserts were recloned into M13mp18 and M13mp19 and sequencing was done by the dideoxy chain termination method (Sequenase Kit, U.S. Biochemicals). The reaction was primed with either the C-*ABL* exon 2 20mer or M13 universal primers.
18. SUP-B13 was isolated from a Ph^1-positive ALL patient at diagnosis, SUP-B15 from the same patient at relapse (*19*). Both cell lines carry the t(9;22)(q34;q11) (*19*) and express the $P185^{ALL\text{-}ABL}$ protein (*13*).
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