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Alu and translisin recognition site sequences flanking translocation sites in a novel type of chimeric bcr-abl transcript suggest a possible general mechanism for bcr-abl breakpoints

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ABSTRACT

Background and Objectives. **We further characterized a novel type of chimeric BCR-ABL mRNA transcript detected in a patient with Philadelphia chromosome positive (Ph+) chronic myeloid leukemia (CML).**

Design and Methods. **We used reverse-transcription polymerase chain reaction (RT-PCR) and sequence analysis of the fusion region of the amplified cDNA fragment. Western analysis was performed on total protein.**

Results. **Part of exon e8 of the BCR gene was joined to an intronic sequence of ABL intron Ib spliced on exon a2 of the ABL gene, giving rise to an in-frame e8-int-a2 BCR-ABL transcript. Only part of exon 8 of the BCR gene (e8) (intra-exonic break) was retained. The consequent BCR-int-ABL transcript was translated into a BCR-ABL protein of 1804 amino acid residues with a molecular mass of 197.5 kilodaltons (kDa) called p200 BCR-ABL. The 3' part of bcr exon 8 recombined within or alongside Alu elements at the additional sites. Sequence motifs similar to consensus binding sites of the lymphoid-associated TRAX and translisin proteins were present on both participating strands at 22q11 and 9q34 recombination sites, respectively. No differences in clinical or laboratory findings at diagnosis were found between this patient and CML patients with bcr-abl fusion.**

Interpretation and Conclusions. **The presence of Alu sequences and of the translisin binding motif on both sides of the breaks in this novel translocation suggests a possible general mechanism of molecular recombination in CML patients.**

Key words: BCR-ABL, translisin, Alu, CML

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The Philadelphia (Ph) chromosome is found in 90% of patients with chronic myeloid leukemia (CML).[1] These patients generally display a reciprocal translocation between chromosomes 9 and 22 [t(9;22)(q34;q11)],[2] involving the ABL gene and BCR genes, respectively. The leukemic breaks on the BCR gene are usually located within introns positioned around exons 13 and 14 (known as e13 or b2 and e14 or b3, respectively), which constitute the breakpoint cluster or M-BCR region.[1] The resulting BCR-ABL fusion gene is transcribed as a large chimeric RNA which is spliced into an 8-Kb mRNA with b2a2 (e13a2) and/or b3a2 (e14a2) junctions (Figure 1). This mRNA produces a 210-kDa BCR-ABL protein (p210 in Figure 1), which plays a crucial role in the pathogenesis of CML.[1-3] In Ph+ CML patients, the M-BCR rearrangement can usually be detected by Southern blotting, and b2a2 or b3a2 BCR-ABL transcripts can be amplified by reverse-transcription polymerase chain reaction (RT-PCR)[4] (Figure 2a, lanes 1 and 2, respectively). The BCR-ABL fusion gene is also expressed in patients with acute lymphoblastic leukemia (ALL) as a 7-Kb mRNA, usually referred to as the e1a2 junction type (Figure 2a, lane 3), which encodes a 185-kDa hybrid protein (p185 in Figure 1).[2]

We and others have reported that a small proportion of Ph+ CML patients have breakpoints that fall outside the M-BCR.[5-8] Some of these patients may express the e1a2 BCR-ABL transcript. Another variant, which was initially described by us,[7,8] involves BCR exon 19 (e19a2) and gives rise to a p230 kDa BCR-ABL protein. We recently found a novel type of BCR-ABL transcript, detected by RT-PCR, in a patient with Ph+ CML.[9] Herein, we describe the presence of Alu sequences and Translisin or TRAX recognition sites in the region surrounding the DNA breakpoint, and their possible involvement in the generation of the translocation.

Case report

A 56-year old female patient was diagnosed as being in chronic phase CML by trephine biopsy in May 1995. At that time, her peripheral blood showed mild leukocytosis with basophilia: white blood cell (WBC) count of 15.6×10^9/L, 13% basophils, 2% promyelocytes, 72% neutrophils, 13% lymphocytes,

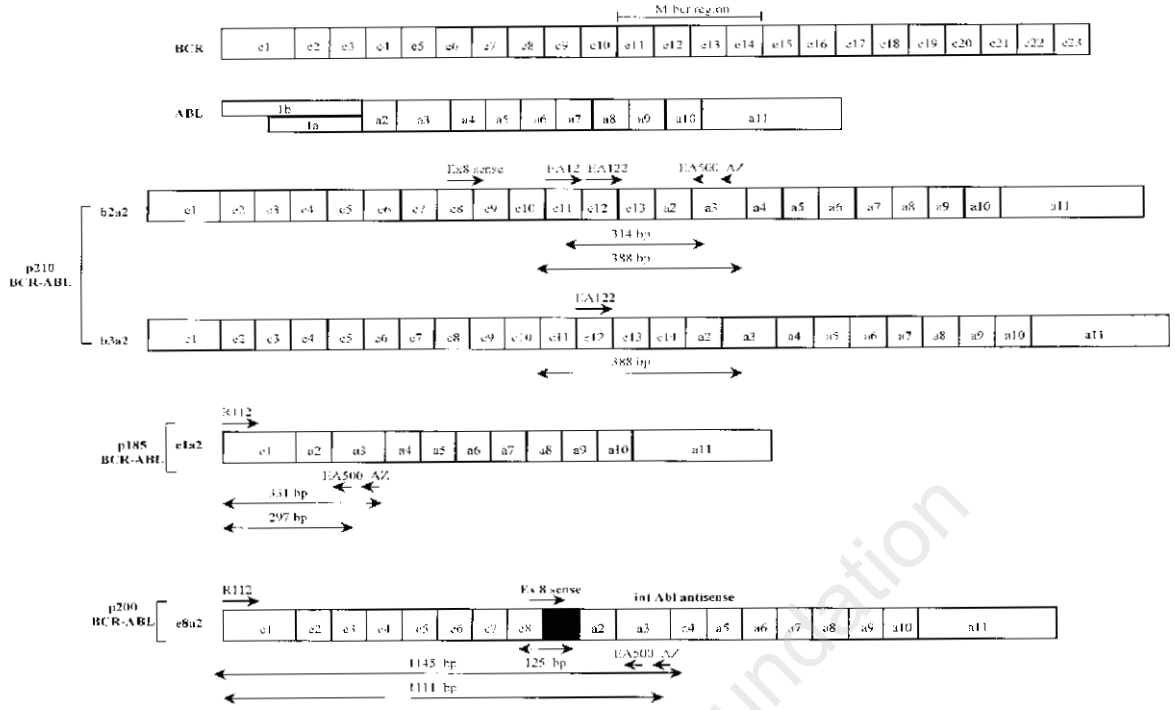

Figure 1. Schematic representation of BCR, ABL, and BCR-ABL mRNA transcripts. The most frequent and our reported BCR-ABL fusion proteins with their molecular masses (p210, p190 and p200) and their corresponding bcr-abl RNA types of junctions are indicated on the left side. White boxes = BCR exons not in scale numbered from e1 to e23, grey boxes = ABL exons not in scale numbered from a1 (alternatively spliced exons a1b and a1a, were represented) to a11. The black box represents intron Ib inserted into the BCR exon 8 (part) - ABL exon 2 transcript. BP indicates the breakpoint inside BCR exon 8. The curved line indicates that the break fell inside the BCR exon 8. Primers AZ, R112, EA12, EA122, and EA500 used for RT-PCR for BCR and BCR-ABL are designated by arrows (primers Ex8 sense, and Int ABL antisense were used for single-step PCR for BCR-ABL on DNA). The expected amplification product sizes with different combinations of primers are designated by a ↔ and the corresponding number of base pairs (bp) is given above the lines.

platelet count of 216×10^9/L, hemoglobin level of 15.0 g/dL, and an alkaline neutrophil phosphatase score of 12 (normal range, 10 to 100). The activity of lactic dehydrogenase (LDH) was 196 U/L (normal range, 120 to 240 U/L). The spleen was not palpable. Trephine biopsy showed increased bone marrow cellularity, marked eosinophilia, and decreased megakaryocytes. Cytogenetic analysis of 30 bone marrow metaphases by Giemsa banding showed a characteristic Ph karyotype [46,XX, t(9;22)(q34;q11)]. Therapy with α-interferon (α-IFN) was started, as described in our previous report:[10] the WBC count fluctuated between 2 and 5.5×10^9/L over the following 20 months.

Design and Methods

RT-PCR

After red blood cell lysis, RNA was extracted from total WBCs by standard procedures. Conditions for RT and PCR for BCR-ABL using primers AZ, EA12, EA122 and R112 have been described elsewhere.[11-13] Specific single-step PCR for BCR-ABL transcripts was performed using primers R110 (BCR exon e1) and AZ (ABL exon a2). Half-nested PCR for ABL-BCR transcripts was performed using primers EA12, EA500 and EA122 and EA500 (Figure 1).

DNA amplification

In order to amplify the region surrounding the breakpoint, two different sets of primers were designed and synthesized on the basis of sequences located on BCR exon 8/intron 9 and BCR exon 8/ABL intron Ib, respectively. Sequences of primers were ex-8 sense (from nt 107,715 to 107,734 5'-TTGCTGAAGCACACTCCTGC-3'; BCR int-9 antisense (from nt 107,964 to 107,983 5'-CCAATCTCACCAATCCCAGA -3'; int-8 antisense (from nt 53351 to 53351 5'CAGTGAAAATCAGCACACTGG-3'. Figure 1 illustrates the primers' positions.

Cloning and sequencing

Single-step BCR-ABL PCR products were gel-separated, excised and after a purification step were sequenced in both strands as reported elsewhere.[11] Sequence comparison analysis was performed using FASTA3 software program.[14]

Western blot and protein analyses

Western blotting was performed on lysates of 1 to 10×10^6 cells from the patient's peripheral blood leukocytes, from leukocytes from a Ph^+ ALL patient, and from the K562 cell line. They were run on a 6.5% polyacrylamide gel, blotted, incubated with a 1:20,000 dilution of monoclonal anti-ABL antibody Abl (Oncogene, Italy) for 2 hours and developed by enhanced chemoluminescence (ECL) methods, similar to those described by Hochhaus.[15]

Computer analysis

The modeling procedure was performed with SWISS-Model version 2.0.[14] The prediction of molecular mass was made according to the method reported by Brendel *et al*.[16] Protein comparison analysis was obtained with Bic2 on Swiss-prot database (swall database).[17] The isoelectric point analysis was performed as previously described.[18] Repetitive elements were searched for and pictured by Findpatterns, Censor,[19] Clustalw[20] and Boxshade[21] computer programs.

Results

In the initial PCR screening, no amplification product was found with the primers for the e13a2 or e14a2 junctions, which amplify 99% of CML associated transcripts.[22] Instead, an atypical amplification product was detected using primers for the e1a2 junction. This product was considerably larger than expected: 1145 vs. 331 base pairs (bp).[11, 13] Specific half-nested RT-PCR confirmed the amplification (1,111 bp in Figure 2a, lane 5). BCR-ABL cDNA PCR products were directly sequenced. In both strands, the fragment investigated showed strong homology between the 5' part of exon 8 of the BCR gene (e8) and exon a2 of the ABL gene. The homology with exon 8 of the BCR gene ended at nucleotide (nt) 107,807 of the reported BCR sequence.[22] Thirty-one bp of the fragment, positioned between the homologs of the BCR and ABL exons, did not match either BCR exon 8 or the ABL sequences. Homology search demonstrated that this stretch of nucleotides matched an intronic sequence of ABL intron Ib[22] spanning nt 53,340 to 53,370. The homology of our BCR-ABL fragment with the second exon of the ABL gene was complete starting from nt. 49,888 of the ABL2 clone.[22]

To our knowledge, no BCR break has hitherto been reported to fall in the BCR gene exon. In order to assess whether i) the break on the BCR exon 8 fell inside the coding sequence and ii) the junction with ABL intron Ib sequences was due to a DNA link and not to a splice event, we employed genomic DNA for amplification of the 5' BCR breakpoint region and direct sequencing of the PCR product. After amplification using a set of primers located on BCR exon 8 (ex8 sense) and ABL intron Ib (int ABL antisense), the expected band of 125 bp was obtained (Figure 2b, lane 2). Sequence analysis demonstrated that the breaks were within exon 8 of the BCR gene, and at position 53,340 of ABL intron Ib (Figure 3) and were thus due to a DNA link. The sequences of ABL intron Ib 3' of the nt 53,370 revealed a typical GT(GAGT) splice donor sequence (Figure 3). We did not find any stretch of nucleotides resembling a splice donor consensus sequence on BCR exon 8.[23] Furthermore,

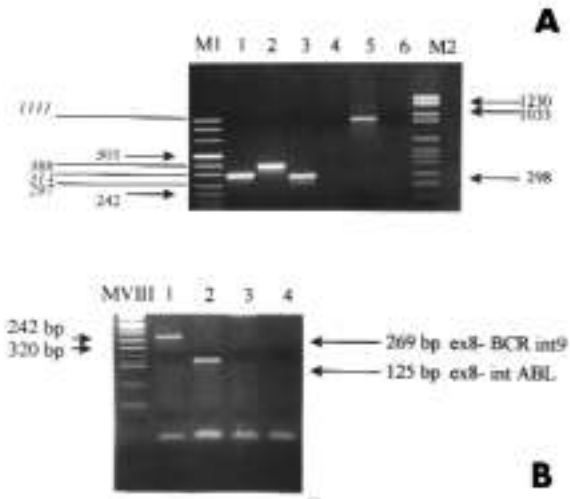

Figure 2.
A. Agarose gel electrophoresis separation of PCR for BCR-ABL transcripts. Molecular weight marker VIII (M1) and VI (M2) from Boehringer Mannheim with some sizes of DNA which are recognized by numbers and short arrows on the left and right sides of picture, respectively, lanes 1 and 2 = RT-PCR with primers EA12-EA500 from total RNA from CML Ph+ patient with b2a2 and b3a2 type of transcript, respectively. The expected sizes of PCR products (388 bp and 314 bp, respectively) are given on the left side of the picture in italic numbers and long lines, lane 3 = the RT-PCR product from acute lymphoblastic leukemia (ALL) Ph+ patients amplified with primers R112-EA500 which gave rise to our amplified product of 297 bp (e1a2 type of transcript), lane 4 = RT-PCR from patient G.V. with primers EA12-EA500: no products, lane 5 = RT-PCR products from patient G.V. with primers R112-EA500 show the presence of an amplified band of 1,111 bp corresponding to e8-int-a2 type of transcript, lane 6 = negative control.
B. Agarose gel electrophoresis separation of PCR product from genomic analysis of BCR-ABL and BCR allele, respectively. MVIII = molecular weight marker VIII from Boehringer Mannheim, lane 1 = PCR product of DNA from patient G.V. with primer ex8 (sense) and BCR int 9 (antisense). The expected band for BCR allele of 269 bp is indicated by a long arrow, lane 2 = PCR product of DNA of patient G.V. with primer ex8 (sense) and int ABL (antisense). The expected band of 129 bp for BCR-ABL allele is shown by a long arrow, lane 3 and lane 4 = negative controls.

using a second antisense primer located on BCR intron 3' of the break (BCR int9, antisense), we confirmed the presence of a 269 bp fragment corresponding to the expected normal BCR allele (Figure 2b, lane 1).

Since repeated elements such as Alu sequences are frequently located near to the CML BCR breakpoint,[24] we submitted sequences of BCR and of ABL intron Ib around the break (from nt 104,341 of BCR and from nt 53,340 of ABL gene, respectively) to the repeat identification program CENSOR.[19] A summary of the predicted location and kinds of repeated elements relative to the breakpoint sites in BCR and in ABL on both the recombinant chromosomes is presented in Figure 4. The break on 5' BCR occurred

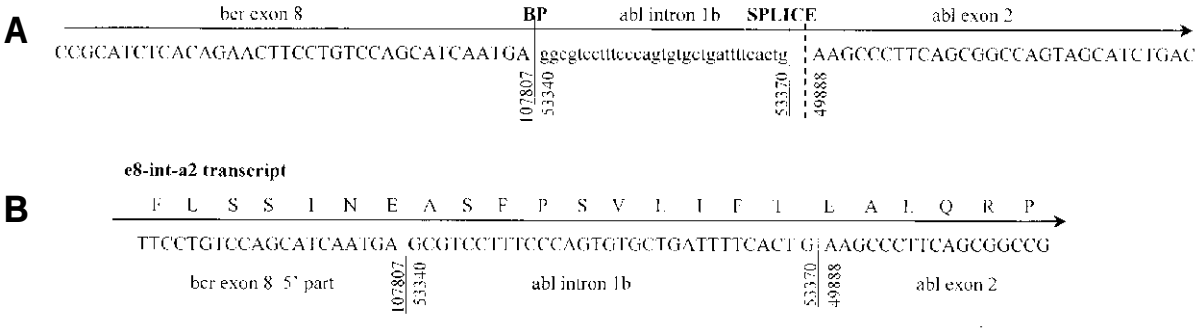

Figure 3. Proposed genomic organization of part of BCR exon 8, part of Abl intron Ib and part of Abl exon 2. Genomic organization of the human bcr gene. Sequence of bcr ex8-int-a2 transcript and its translation.

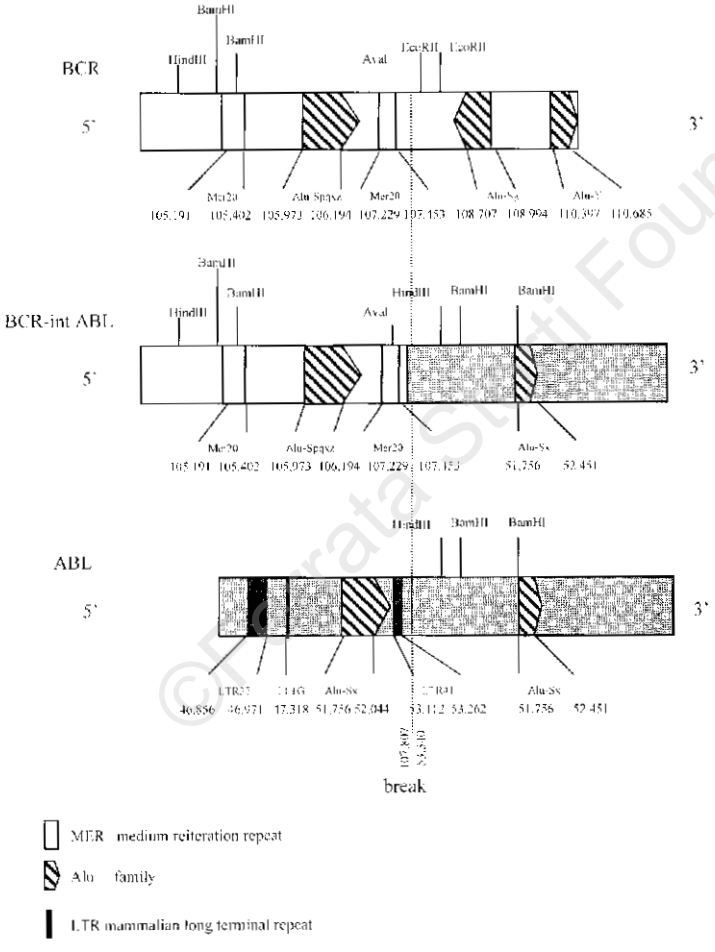

Figure 4. Schematic representation of repeat element features in the regions of BCR exon 8 in 5' bcr exon 8 -3' ABL int Ib fragment and in Abl intron Ib.
Schematic representation of repeat element features surrounding the breaks in the regions of BCR exon 8 (a), in 5' bcr exon 8 -3' ABL int Ib fragment (b) and in Abl intron Ib (c). Breakpoint sites in our patient G.V. are shown by dotted lines on the map, and are indicated by the vertical number at the bottom.
Expanded maps showing repeat element features and different gray scales correspond to different chromosomal origins: the white block represents BCR sequences and the grey one represents ABL intron Ib sequences. Restriction enzyme sites (*EcoR*II, *Hind*III, *Ava*I and *Bam*HI) marked above the maps are intended as guides only and not all sites are shown. Different repeat element families identified by the CENSOR program are designated by symbols as in the key. Arrowhead marks the direction 5'-3' of the different repeat elements. The subfamily of repeat element features and their presumed portions on BCR and ABL are given under the maps.

3' of two repetitive elements: the Alu-Spqxz and the MER20 (human medium reiteration frequency) elements located at position 105,973 and 107,229, respectively (Figure 4). The break on 3' BCR occurred 5' of the Alu-Sg element located at position 108,707. The break on ABL intron Ib part occurred immediately downstream (10 bp), 3' of the human endogenous-retrovirus related element, namely the LTR41@1 element (mammalian long terminal repeat retrotransposon) located on the ABL gene at position 53,112, and 5' of the Alu-Sx element located on ABL gene at position 51,756 (Figure 4), thereby confirming the presence of reported elements[24] on both sides of the recombinant genes.

To identify sequence patterns which might disclose a common site-specific mechanism for DNA breakage and rejoining, we performed exhaustive computer-based pairwise and pile-up comparisons of about 100 bp of sequence (from nt 107,701 of the BCR gene) spanning the breakpoint sites in 5' BCR exon 8 and 3'ABL intron Ib (Figure 5). None of the translocation features,[25] was detected at either of the BCR-exon 8 int Ib ABL breakpoints. Sequence comparisons using the CLUSTALW and BOXSHADE programs identified a pentameric motif 5'-CCCAG-3' near the breaks. As reported by Jeffs *et al*.[24] these motifs show similarities to parts of a number of recombination-prone sequences, including human hypervariable minisatellites, various DNA polymerase framshift hotspots and the 26 bp recombinogenic Alu core.[26] Most importantly, a >80% homology with the consistent consensus sequence (A,C)TGCAG-N(0-4bp)-GCCC (A/T) (G/C) (G/C) (A/T) for translisin[27,28] was found in both sites of the breaks: on 5' BCR exon 8, 48 bp before the breaks, and on 3' ABL intron Ib at 56 bp after the break. Furthermore, on the 3' ABL fragment, sequences with >80% homology with the motif GCCC (A/T)(G/C) (A/T/G)(A/T) were found five times at 23 bp, and the following four spliced by 70 bp, 15 bp, 46 bp, and 76 bp respectively after the first translisin binding motif. Similarly, the same translisin consensus sequences[28] were found on the breaks of 5' ABL intron Ib and the 3' BCR fragment: at 226 bp before the breaks on ABL gene (not shown) and at 84 bp from the break on BCR gene, respectively. These findings are similar to those reported in 5 out of 5 cases of *classical* BCR-ABL translocation occurring in the M-BCR region.[24]

A BCR-ABL protein signal was detected on Western blots of our patient's leukocytes (data not shown). The patient's protein was slightly larger than the p185 BCR-ABL protein. The BCR-ABL translated protein of 1,804 amino acid residues has a predicted molecular mass of 197.5 kDa.[28,29] We called this protein p200 BCR-ABL. Protein alignment findings showed alignment scores of AA segment 1-690 with the BCR protein and of AA segment 700-1,804 with the ABL protein, thus confirming the BCR-ABL nature of the protein. Furthermore, sequence analysis allowed us to predict that due to insertion of sequences from intron ABL Ib the P200 BCR-ABL protein contained a stretch of 10 amino acids (aa) ASFPSVLIFT not present in any reported BCR-ABL fusion protein. Prediction of our BCR-ABL protein localization site, as obtained with PSORT version 6.4, showed that this protein could be 84% nuclear.[29]

Discussion

The specific recombination mechanism of myeloid leukemia is currently unknown. The CML patient studied by us displayed recombinant features that could shed some light on this mechanism.

Although the novel BCR-ABL transcript under study is an isolated finding, some of its features have been reported in other forms of leukemia.

In particular, the introduction of an intronic sequence and the existence of intra-exonic DNA breaks have both been reported in AML translocations,[30,31] particularly in acute promyelocytic leukemia (APL). In two reported cases of intra-exonic DNA breaks in AML patients,[31] the PML and RAR sequences involved in APL were interrupted by a few intervening nucleotides lacking homology with either PML or RAR cDNA sequences. The resulting PML-RAR proteins contained residues not homologous with either PML or RAR. Both these two breaks were very similar to the one recorded in our patient. This suggests that the apparently unique case analyzed by us may actually disclose features of more general relevance.

The study supports the concept that Alu sequences may have a role in the BCR-ABL recombination that initiates CML. Our anomalous case is in keeping with the concept that although Alu sequences are often located around the breaks and always close to recombination sites, they are generally not directly involved. The role of Alu sequences may simply keep chromosome regions close together, making recombination more likely in their vicinity.[32] The mechanisms that determine chromosome geography in the interphase nucleus remain controversial, but a potential role for Alu elements as structural modifiers of chromatin organization is recognized.[24] The role of the translisin protein or translisin-related protein (TRAX) in the generation of the reciprocal translocation found in our patient is strongly supported by the fact that homology sequences were present on both side of the genes involved in the rearrangement. This finding has also been reported in four other different CML cases.[24] These observations support the hypothesis that the role of translisin could be more important in CML than in other non-lymphoid leukemia-associated rearrangements.

A second point which should be discussed is the involvement in the point of translocation of open reading frame sequences (i.e. BCR exon 8) and another possible one (the 31 bp stretch of the ABL intron which recombined with BCR exon 8 showed 100% homology with the nucleotide sequences of several anonymous human cDNA fragments in DBEST obtained with BLASTN). Such transcriptionally active chromatin is more open in structure, has a high number of matrix attachment regions and is prone to recombine more frequently than inactive chromatin.[32-35] Other authors[24,36] reported that the 3' M-BCR recombination site disrupted coding regions of two genes (IGL and GSTPI), and suggested that the breaks which occurred within the coding domains of actively transcribed genes are a common feature of BCR-ABL translocation.

Taken together, these observations prompt us to postulate a possible common mechanism of translocation whereby some actively transcribed genes, such as BCR and ABL, which are open in structure and prone to recombination may remain close together because of the vicinity of similar Alu sequences. When the breaks occur the presence of binding sites of recombinatory proteins, such as translisin, could mediate illegitimate conjunction single-strand breaks. We think that such a mechanism might favor breaks and reciprocal recombinations on the BCR and ABL genes in both common and variant CML translocations, and could even represent a general feature of

the specific molecular recombinations leading to myeloid leukemias as a whole.

Contributions and Acknowledgments

GM was the principal investigator: he designed the study and was responsible for ethical approval of the program, was responsible for funding and direct supervision. CT and MA set up PCR procedures and drafted the paper. VM developed and carried out cryopreservation procedures and flow cytometric assays. NT, EO, AdV and AM were responsible for cytogenetics. ST and GS critically revised the manuscript and gave the final approval for publication. The order of authorship has been made according to the contribution given to the study. The authors are grateful to Prof. Pier Giuseppe Pelicci for helpful discussion on the results and to Mr. Robin M.T. Cooke for helping work up the manuscript.

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Disclosures

Conflict of interest: none.

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Potential implications for clinical practice

- The clinical relevance of our study should be the identification of rare types of BCR-ABL transcript and their possible responsiveness to a-interferon therapy similar to more common types.

References

1. Heisterkamp N, Stephenson JR, Groffen J, et al. Localization of the c-abl oncogene adjacent to a translocation break point in chronic myelocytic leukaemia. Nature 1983; 306:239-42.
2. Hermans A, Heisterkamp N, von Linden M, et al. Unique fusion of bcr and c-abl genes in Philadelphia chromosome positive acute lymphoblastic leukemia. Cell 1987; 51:33-40.
3. Grosveld G, Verwoerd T, van Agthoven T, et al. The chronic myelocytic cell line K562 contains a breakpoint in bcr and produces a chimeric bcr/c-abl transcript. Mol Cell Biol 1986; 6: 607-16.
4. Martinelli G, Gasparini P, Ambrosetti A, Battista R, Perona G, Pignatti PF. Molecular genetic analysis of bcr breakpoint in Philadelphia chromosome rearrangements and correlation with prognosis and age of chronic myelogenous leukaemia patients. In: Bartsocas CS, Loukopoulos D, eds. Genetics of hematological disorders. Washington: Hemisphere Publishing Corp.; 1990. p. 185-93.
5. Melo JV. The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype. Blood 1996; 88:2375-84.
6. Saglio G, Guerrasio A, Tassinari A, et al. Variability of the molecular defects corresponding to the presence of a Philadelphia chromosome in human hematologic malignancies. Blood 1988; 72:1203-8.
7. Saglio G, Guerrasio A, Rosso C, et al. New type of Bcr/Abl junction in Philadelphia chromosome-positive chronic myelogenous leukemia. Blood 1990; 76: 1819-24.
8. Pane F, Frigeri F, Sindona M, et al. Neutrophilic-chronic myeloid leukemia: a distinct disease with a specific molecular marker. Blood 1996; 88:2410-4.
9. Martinelli G, Terragna C, Amabile M, et al. Translisin recognition site sequences flank translocation breakpoints in a Philadelphia chromosome positive chronic myeloid leukemia patient expressing a novel type of chimeric bcr-abl transcript (e8-int-a2). Leukemia 1999; 13:1635-7.
10. The Italian Cooperative Study Group on Chronic Myeloid Leukemia. Interferon alpha-2a as compared with conventional chemotherapy for the treatment of chronic myeloid leukemia. N Engl J Med 1994; 330: 820-5.
11. Testoni N, Martinelli G, Farabegoli P, et al. A new method of "in cell reverse transcriptase-polymerase chain reaction" for the detection of BCR-ABL transcript in chronic myeloid leukemia patients. Blood 1996; 87:3822-7.
12. Saglio G, Pane F, Gottardi E, et al. Consistent amounts of acute leukemia-associated p190BCR/ABL transcripts are expressed by chronic myelogenous leukemia patients at diagnosis. Blood 1996; 87:1075-80.
13. Martinelli G, Testoni N, Montefusco V, et al. Detection of bcr-abl transcript in chronic myelogenous leukemia patients by reverse-transcription-polymerase chain reaction and capillary electrophoresis. Haematologica 1998; 83:593-601.
14. Peitsch MC, Wilkins MR, Tonella L, Sanchez JC, Appel RD, Hochstrasser DF. Large-scale protein modelling and integration with the SWISS-PROT and SWISS-2DPAGE databases: the example of Escherichia coli. Electrophoresis 1997; 18:498-501.
15. Hochhaus A, Reiter A, Skladny H, et al. A novel BCR-ABL fusion gene (e6a2) in a patient with Philadelphia chromosome-negative chronic myelogenous leukemia. Blood 1996; 88:2236-40.
16. Brendel V, Bucher P, Nourbakhsh IR, Blaisdell BE, Karlin S. Methods and algorithms for statistical analysis of protein sequences. Proc Natl Acad Sci USA 1992; 89:2002-6.
17. Smith TF, Waterman MS. Identification of common molecular subsequences. J Mol Biol 1981; 147:195-7.
18. Bjellqvist B, Basse B, Olsen E, Celis JE. Reference points for comparisons of two-dimensional maps of proteins from different human cell types defined in a pH scale where isoelectric points correlate with polypeptide compositions. Electrophoresis 1994; 15: 529-39.
19. Jurka J, Klonowski P, Dagman V, Pelton P. CENSOR-a program for identification and elimination of repetitive elements from DNA sequences. Comput Chem 1996; 20:119-21.
20. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994; 22:4673-80.
21. http://www.isrec.isb-sib.ch/software/BOX_faq.html
22. Chissoe SL, Bodenteich A, Wang YF, et al. Sequence and analysis of the human ABL gene, the BCR gene, and regions involved in the Philadelphia chromosomal translocation. Genomics 1995; 27:67-82.
23. Green MR. Pre-mRNA splicing. Annu Rev Genet 1986;

20:671-708.
24. Jeffs AR, Benjes SM, Smith TL, Sowerby SJ, Morris CM. The BCR gene recombines preferentially with Alu elements in complex BCR-ABL translocations of chronic myeloid leukaemia. Hum Mol Gen 1998; 7:767-76.
25. Mills FC, Brooker JS, Camerini-Otero RD. Sequences of human immunoglobulin switch regions: implications for recombination and transcription. Nucleic Acids Res 1990; 18:7305-16.
26. Jurka J, Smith T. A fundamental division in the Alu family of repeated sequences Proc Natl Acad Sci USA 1988; 85:4775-8.
27. Aoki K, Ishida R, Kasai M. Isolation and characterization of a cDNA encoding a Translin-like protein, TRAX. FEBS Lett 1997; 401:109-12.
28. Aoki K, Suzuki K, Sugano T, et al A novel gene, Translin, encodes a recombination hotspot binding protein associated with chromosomal translocations. Nat Genet 1995; 10:167-74.
29. Nakai K, Kanehisa M. A knowledge base for predicting protein localization sites in eukaryotic cells. Genomics 1992; 14:897-911.
30. Gallagher RE, Li YP, Rao S, et al. Characterization of acute promyelocytic leukemia cases with PML/RAR alpha break/fusion sites in PML exon 6: identification of a subgroup with decreased in vitro responsiveness to all-trans retinoic acid. Blood 1995; 86:1540-7.
31. Pandolfi PP, Alcalay M, Fagioli M, et al. Genomic variability and alternative splicing generate multiple PML/RAR alpha transcripts that encode aberrant PML proteins and PML/RAR alpha isoforms in acute promyelocytic leukaemia. EMBO J 1992; 11:1397-407.
32. Chen SJ, Chen Z, Font MP, d'Auriol L, Larsen CJ, Berger R. Structural alterations of the BCR and ABL genes in Ph1 positive acute leukemias with rearrangements in the BCR gene first intron: further evidence implicating Alu sequences in the chromosome translocation. Nucleic Acids Res 1989; 17:7631-42.
33. Datta A, Jinks-Robertson S. Association of increased spontaneous mutation rates with high levels of transcription in yeast. Science 1995; 268:1616-9.
34. Jackson DA, Bartlett J, Cook PR. Sequences attaching loops of nuclear and mitochondrial DNA to underlying structures in human cells: the role of transcription units. Nucleic Acids Res 1996; 24:1212-9.
35. Liu WM, Chu WM, Choudary PV, Schmid CW. Cell stress and translational inhibitors transiently increase the abundance of mammalian SINE transcripts. Nucleic Acids Res 1995; 23:1758-65.
36. Koduru PR, Goh JC, Pergolizzi RG, Lichtman SM, Broome JD. Molecular characterization of a variant Ph1 translocation t(9;22;11) (q34;q11;q13) in chronic myelogenous leukemia (CML) reveals the translocation of the 3'-part of BCR gene to the chromosome band 11q13. Oncogene 1993; 8:3239-47.