RAPID COMMUNICATION

The t(10;11) Translocation in Acute Myeloid Leukemia (M5) Consistently Fuses the Leucine Zipper Motif of AF10 Onto the HRX Gene

By T. Chaplin, O. Bernard, H.B. Beverloo, V. Saha, A. Hagemeijer, R. Berger, and B.D. Young

The gene on chromosome 10 at band p12 (AF10), involved in the t(10;11) translocation in acute myeloid leukemia, has been identified and shown to contain conserved zinc finger and leucine zipper domains. These regions are highly homologous to the equivalent regions on AF17, the gene involved in the t(11;17) translocations. A series of adult, childhood, and infant leukemias with either simple or complex versions of the t(10;11) has been examined by Southern analysis and shown to involve rearrangement to the HRX locus. Reverse transcriptase-polymerase chain reaction from either bone marrow or peripheral blood cells showed that HRX sequence was fused to AF10 sequence in all 8 cases and subsequent sequence analysis showed an in-frame fusion between the HRX and AF10 sequence. A consistent feature of these fusions was the juxtaposition of the leucine dimerization motif of AF10 onto the NH2-terminal region of HRX. The published data suggest that a similar conclusion can be drawn about the t(11;17) translocation, implying a critical role for this motif in the chimeric HRX protein.

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MATERIALS AND METHODS

RNA preparation. Cryopreserved peripheral blood cells for patients A, B, and C were thawed and incubated in 10% fetal calf serum to neutralize the dimethylsulfoxide. Total RNA was extracted using the RNAzol-B method (Biogenesis, Bournemouth, UK) and stored frozen in water at -20°C. For patients D, E, F, and G, total RNA was isolated via the guanidinium isothiocyanate (GTC) method.

Reverse transcriptase-polymerase chain reaction (RT-PCR). One microgram of total RNA was placed into a reaction mixture of 50 mmol/L Tris, 75 mmol/L KCl, 3 mmol/L MgCl2, 0.2 mmol/L of each dNTP, 10 µg/µL random hexamer (Boehringer Mannheim, UK, Lewes, UK), 10 µg bovine serum albumin, and 20 U RNAsin (Promega, Madison, WI) made up to 20 µL with sterile water. Two hundred units of Moloney's Murine Leukemia Virus (M-MLV) reverse transcriptase was added and the samples were incubated at 37°C for 1 hour. On completion, 2 µL of the cDNA stock was placed into a PCR reaction mixture of 10 µL Tris, 50 mmol/L KCl, 1.5 mmol/L MgCl2, 0.2 mmol/L of each dNTP, 2 µmol/L of each appropriate primer, and 1 U of Taq polymerase (Promega). Samples were cycled in a TC-1 or 9600 Perkin Elmer PCR thermal cycler (Perkin Elmer, Warrington, UK) for 35 cycles (72°C for 2 minutes, 94°C for 30 seconds, and 57°C for 30 seconds) and the products were visualized on a 2% agarose gel.

Cloning. Fresh PCR products were cloned into plasmid via the TA cloning kit (Invitrogen, San Diego, CA). PCR reactions were performed using the original primers used in the first reactions, and clones with appropriately sized inserts were grown overnight and used to prepare "miniprep" DNA by the alkaline lysis method.

Sequence analysis. One microgram of plasmid DNA was Taq cycle sequenced using the Applied Biosystems PRISM Ready Reaction Dye-Deoxy terminator sequencing kit (Applied Biosystems, Warrington, UK), and the products were extracted with phenol/chloroform. Samples were run on an ABI 373A DNA sequencer (Applied Biosystems) and the results were analyzed using DNASTar software (DNASTar, Madison, WI).

Southern analysis. High molecular weight DNA was extracted from the patients' leukemic cells and digested with various restric...
tion enzymes using standard methods. DNA from patients B and G were digested with EcoRI, BamHI, and HindIII and hybridized with probes I5 and FA4, as previously reported. DNA from patients D, E, and F was digested with BamHI and EcoRV and hybridized with an HRX probe. The latter is a 0.85-kb BamHI cDNA fragment generated by RT-PCR from RS4;11 cDNA. Primers amplified a 1,058-bp PCR product (residues 3425-4484 of sequence8 with accession no. L04731) that was cloned using the TA cloning kit (Life Sciences, Invitrogen).

RESULTS

A series of blood and bone marrow samples was obtained from patients known to have AML and rearrangements of chromosomes 10p and 11q. Initially, Southern analysis was used to show the rearrangement of the HRX gene in samples from each patient (data not shown). Making use of the known sequence of AF10, a series of RT-PCR reactions was performed with a combination of 5' HRX and 3' AF10 oligonucleotide primers (Table 1). From each sample it was possible to amplify a unique PCR product expected to consist of HRX sequence fused to AF10 sequence; representative results are shown in Fig 1. The results from presentation and relapse samples from a patient with the t(10;11) are shown in Fig 1a. Negative controls (no DNA) were analyzed in parallel with each patient sample to detect contamination. A representative set of PCR products from this group of patients is shown in Fig 1b. To confirm the origin of all products, they were either subjected to direct sequencing or cloned into plasmid before sequence analysis. In each example it was possible to identify the precise fusion points between HRX and AF10; these results are presented in Table 2. The material from patients A and B were used in the original cloning of AF10; therefore, their fusion points were already known.14 The base positions given for AF10 correspond to the GENBANK entry for AF10 (accession no. U13948). A second minor product was derived from patient D, was shown to consist of a fusion between HRX exon 6 and AF10, and was interpreted as being due to alternative splicing that would

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Karyotype</th>
<th>HRX/AF10 Fusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>A*</td>
<td>27 yr</td>
<td>AML M5</td>
<td>46,XY,t(10;11)(p12;q23)(q23)</td>
<td>HRX ex5/AF10 2222</td>
</tr>
<tr>
<td>B†</td>
<td>27 mo</td>
<td>AML M5</td>
<td>46,XY,t(11;10)(q23;p12)</td>
<td>HRX ex6/AF10 979</td>
</tr>
<tr>
<td>C*</td>
<td>49 yr</td>
<td>AML M5</td>
<td>51,X,-1,-8,t(10;11)(p12;q23),+der(10),+19,+21</td>
<td>HRX ex6/AF10 2110</td>
</tr>
<tr>
<td>D†</td>
<td>8 mo</td>
<td>AML M5</td>
<td>46,XX,inv(11)(q13;q23)t(10;11)(p12;q23)</td>
<td>HRX ex7/AF10 979</td>
</tr>
<tr>
<td>E§</td>
<td>9 yr</td>
<td>AML M5</td>
<td>47,XY,inv(1)q13;q23)t(10;11)(p12;q23),+der(7)t(7;1q35)</td>
<td>HRX ex6/AF10 883</td>
</tr>
<tr>
<td>F‡</td>
<td>6 mo</td>
<td>AML M5a</td>
<td>46,XX,t(4;15)(q31;q22),inv(11)(q13;q23)t(10;11)(p12;q23)</td>
<td>HRX ex7/AF10 883</td>
</tr>
<tr>
<td>G‡</td>
<td>41 yr</td>
<td>AML M5</td>
<td>46,XY,inv(11)(q13;q23)t(10;11)(p12;q23)</td>
<td>HRX ex6/AF10 589</td>
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<tr>
<td>H‖</td>
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<td>AML M4</td>
<td>46,XX,inv(11)(q13;q23)t(10;11)(p12;q23)</td>
<td>HRX ex6/AF10 979</td>
</tr>
</tbody>
</table>

The AF10 base number is the first base after the fusion point.

* St Bartholomew's Hospital (London, UK).
† Hospital Saint Louis (Paris, France).
‡ Dutch Childhood Leukaemia Study Group (The Hague, The Netherlands).
§ Erasmus University Hospital (Rotterdam, The Netherlands).
‖ University College Hospital (London, UK).
The positions of AF10 primers finger and leucine zipper structures. (♀) Adult leukemias; (♂) childhood and infant leukemias. The positions of AF10 primers are indicated.

remove HRX exon 7. The five fusion points found on AF10 are indicated in relation to the conserved zinc finger and leucine zipper regions (Fig 2) and all resulted in in-frame fusions between HRX and AF10.

**DISCUSSION**

All 11q23 translocations so far investigated result in in-frame fusions between the 5' HRX and 3' elements of the involved partner gene. The molecular cloning of the partner genes has not identified any consistent features in the 3' elements donated to HRX. Indeed, the partner genes seem to constitute a diverse group with only partial homology between certain members. The genes AF9 and ENL are related to each other through 5' and 3' regions of homology, and AF10 and AF17 are related through the presence of zinc finger and leucine zipper structures. The remainder (AF4, AF6, AF1-p, ELL, and AF1q) are unrelated at the sequence level. Several carry nuclear localisation signals and are rich in serines and prolines, suggesting a role as transcriptional activators. The gene previously named AF1p is the human homolog of the murine eps15 (epidermal growth factor [EGF] receptor pathway substrate 15). eps15 encodes a cytoplasmic protein that is a target for the tyrosine kinase activity of the EGF receptor, eps15, like the AF6 protein, exhibits similarities with the rod-like region of various myosin chains, structures known to be involved in protein-protein interactions. It was thus suggested that the partner protein contributes a dimerization domain. To understand the role of the partner gene in these events, it will be necessary to determine if any elements are consistently translocated to HRX in a range of translocations.

Our data show that a consistent feature of the t(10;11) translocation is the creation of HRX/AF10 chimeric protein containing the AF10 leucine zipper motif. No breakpoints 3' of the leucine zipper were found. The AF17 gene also encodes a leucine zipper region 77% identical to that of AF10; the limited data available for t(11;17) translocations (2 patients) suggest that a similar structure of chimeric HRX/AF17 protein would result. These results indicate an important role for the leucine zipper motif in the chimeric protein. Sequence comparison of AF10 with AF17 and with a related C elegans gene CEZF indicates considerable divergence outside the zinc finger and leucine zipper regions. Thus, there has been selective pressure to maintain the leucine zipper, suggesting that it has an important functional role. There is a precedent for the involvement of a leucine zipper motif in chimeric proteins. The t(17;19)(q22;p13) translocation has been shown to result in chimeric transcripts that contain sequences from the E2A basic helix-loop-helix gene fused to the leucine zipper sequence of the HLF gene. This translocation, which occurs in pre-B–cell acute lymphocytic leukemia (ALL), particularly with disseminated intravascular coagulation, is related to the more frequent t(1;19)(q23;p13) that also occurs in pre-B ALL. However, in this case, the translocation fuses the homeobox domain of the PBX1 gene on chromosome 1 to the E2A basic helix loop-helix gene on chromosome 19. Thus, as with the 11q23 translocations, the E2A family of translocations includes events that involve the transfer of a leucine zipper motif.

The five infant and childhood leukemias studied had only two fusion points on AF10, whereas the three adult leukemias appeared to have more widely scattered fusion points on AF10. More leukemias will have to be examined before any significance can be attached to the distribution of AF10 fusion points. In patient A, the translocation joined exon 5 HRX sequence to AF10 sequence. This was the first observation of such breakpoint position on HRX for any of the 11q23 abnormalities. This study confirms that it is also unusual in the t(10;11) translocation.

The cytogenetic evidence suggests that there may be heterogeneity in the breakpoints on 10p with 10p11, 10p12, 10p13, 10p14, and 10p15, all of which are implicated in rearrangements with 11q23. In addition, a complex insertion ins(10;11) has been noted involving 10p, 11q, and is observed in four of our acute leukemias. Despite the cytogenetic complexity of some of these rearrangements, we have been able to show that consistent fusions between HRX and AF10 result. RT-PCR can therefore be used as a valuable diagnostic test in cases in which the t(10;11) translocation is suspected. The full elucidation of these complex rearrangements will require further investigation using the DNA probes generated in this study.

**ACKNOWLEDGMENT**

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