Persistence of the 8;21 Translocation in Patients With Acute Myeloid Leukemia Type M2 in Long-Term Remission

By Giuseppina Nucifora, Richard A. Larson, and Janet D. Rowley

The translocation between chromosomes 8 and 21, t(8;21) (q22;q22), is the most frequent abnormality in acute myeloid leukemia (AML) with French-American-British type M2 (FAB-M2) morphology. The breakpoints in this translocation have been characterized at the molecular level, and the genes involved are AML1 on chromosome 21 and ETO on chromosome 8. The rearrangement of the two chromosomes results in a fusion gene and in the production of a consistent fusion transcript on the der(8) chromosome. We have used oligonucleotide primers derived from both sides of the fusion cDNA junction and reverse transcription-polymerase chain reaction (RT-PCR) to analyze six AML-M2 patients with a t(8;21) during various stages of their disease. Two patients studied at diagnosis and one studied at first relapse are alive off therapy and in continuous complete remission for 83 to 94 months. We have detected the AML1/ETO fusion transcript in recent peripheral blood samples from each of them. Three other patients also had a fusion transcript detected after 1 to 4 months in remission. Two of these patients subsequently relapsed and died whereas the third patient is alive and in continuous complete remission 70 months later. Thus, our preliminary data suggest that cells with the translocation are still circulating in (t;21) patients in long-term remission. This finding raises serious questions regarding the interpretation of positive results obtained only with this technique that may not be suitable to decide appropriate further treatment for patients in clinical remission.

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ONE OF THE central tenets of the management of patients with acute leukemia is that the cytogenetically abnormal leukemic clone, present at diagnosis, disappears during remission. Its reappearance is often used to confirm a relapse. This view has now been called into question by our ability to detect a very small number of abnormal cells, in the range of 1 in 10^5 or 10^6, with the use of the polymerase chain reaction (PCR). This technique is especially powerful when used to detect the product of fusion genes that result from chromosome translocations.

The 8;21 translocation with breaks at 8q22 and 21q22.3 is a recurring translocation observed in approximately 20% of patients with acute myeloid leukemia type M2 (AML-M2). This translocation results in the fusion of two genes, AML1 on chromosome 21, and ETO on chromosome 8, with the formation of a chimeric gene AML1/ETO on the derivative 8 (der(8)) chromosome. Molecular cloning of the 8;21 translocation has provided genomic probes from either side of the breakpoint junction; in several studies, DNA rearrangements can be detected by Southern blot analysis in 80% to 100% of the patients. More importantly, appropriate primers derived from the chimeric cDNA sequence from both sides of the junction can detect an identical size chimeric transcript by reverse transcriptase-PCR (RT-PCR) in all of the patients tested. We used RT-PCR to analyze peripheral blood (PB) samples recently obtained from patients who had been diagnosed with AML-M2 and the t(8;21) and treated as long ago as 8 years. Our analysis shows that the chimeric transcript is still detectable in these samples, indicating that cells with the 8;21 chromosomal translocation are still present in patients with AML-M2 in long-term remission.

MATERIALS AND METHODS

Patients' and normal control samples. PB samples were recently obtained with informed consent from three patients in long-term remission (patient nos. 4, 5, and 6, Table 1). The red blood cells (RBCs) were separated by centrifugation, and the leukocytes were removed and used for RT-PCR analysis. Although bone marrow (BM) samples were not evaluated, there was no clinical evidence of recurrent disease in these patients. In addition, we had frozen leukocytes from BM samples taken at diagnosis and in early remission for these patients, for another patient in long-term remission (no. 3, Table 1), and for two other t(8;21) AML patients who later relapsed (patient nos. 1 and 2, Table 1). The karyotypes of the leukemia cells at diagnosis or at relapse of all of the patients analyzed have been reported previously. The cell line Kasumi-1 established from a patient with AML-M2 and the t(8;21) was used as a positive control. The TK-6 (American Type Culture Collection, Rockville, MD) and the ML-1 cell lines and PB samples from two individuals who did not have AML were used as negative controls. These cells do not contain the t(8;21). The cell lines were grown in RPMI 1640 with 10% fetal bovine serum FBS and were maintained under standard conditions.

RNA preparation. PolyA RNA was prepared from 0.3 to 3 X 10^7 PB leukocytes or BM cells or cell lines with the Fast Track mRNA isolation kit (Invitrogen, San Diego, CA) according to the manufacturer's instructions. All of the polyA RNA isolated (0.1 to 1 µg) was used for the synthesis of the first-strand cDNA necessary.
Table 1. Clinical Characteristics of Patients With AML-M2 and the t(8;21), and Results of the Cytogenetics, Southern Blots, and PCR Analyses

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Months in CR1</th>
<th>% of t(8;21) cells</th>
<th>Southern blot/PCR</th>
<th>Source</th>
<th>Months in CR2</th>
<th>% of t(8;21) cells</th>
<th>Southern blot/PCR</th>
<th>Source</th>
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<tr>
<td>1</td>
<td>07/05/85</td>
<td>4</td>
<td>42</td>
<td>n.d./n.d.</td>
<td>BM</td>
<td>1</td>
<td>50</td>
<td>+/n.d.†</td>
<td>BM</td>
</tr>
<tr>
<td>2</td>
<td>04/03/85</td>
<td>12</td>
<td>100</td>
<td>+/n.d.†</td>
<td>BM</td>
<td>5</td>
<td>91</td>
<td>+/n.d.†</td>
<td>BM</td>
</tr>
<tr>
<td>3</td>
<td>11/05/81</td>
<td>0</td>
<td>94</td>
<td>n.d./n.d.</td>
<td>BM</td>
<td>1</td>
<td>91</td>
<td>+/n.d.†</td>
<td>BM</td>
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<tr>
<td>4</td>
<td>05/19/83</td>
<td>12/11/85</td>
<td>94</td>
<td>n.d./n.d.</td>
<td>BM</td>
<td>38</td>
<td>n.d.†</td>
<td>n.d./n.d.†</td>
<td>BM</td>
</tr>
<tr>
<td>5</td>
<td>10/22/85</td>
<td>06/03/85</td>
<td>100</td>
<td>+/n.d.†</td>
<td>BM</td>
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<td>BM</td>
</tr>
</tbody>
</table>

Abbreviations: NED, no evidence of disease; n.d., not determined; CR1, first complete remission; CR2, second complete remission.

* Cells analyzed by cytogenetics.
† Data from reference 6.

for PCR amplification. The synthesis was performed with the cDNA First Strand Synthesis kit (Pharmacia LKB Biotechnology, Piscataway, NJ) in a total reaction volume of 15 μL.

PCR amplification and Southern blot analysis. For each PCR amplification, 4 μL of the cDNA reaction volume was used. Primers I and II and the conditions for the PCR reactions have been described. The amplification produces a DNA fragment of 339 bp. The probe used for the Southern blot analysis of the electrophoresed PCR reactions was a 0.77-kb fragment obtained by PCR amplification of an AML1/ETO cDNA as previously described.

RESULTS

Clinical analysis. All patients had received intensive remission induction and consolidation chemotherapy, usually with cytarabine and an anthracycline and/or with high-dose cytarabine regimens. No maintenance chemotherapy was administered. One of the patients (no. 5) received a syngenic BM transplant. Four of the patients (nos. 1 through 4) had a relapse. Patient 2 was studied by PCR after 4 months in first clinical and cytogenetic remission (CR1), and the patient relapsed 3 months after the marrow sample was taken. Patients 1 and 3 were studied by PCR only in second remission and patient 4 only at first relapse and second remission. In none of the remission marrow samples were metaphase cells with the t(8;21) observed. Four of the patients (nos. 3 through 6) currently remain in complete remission more than 5 to 8 years after last chemotherapy.

Analysis of the PCR amplification reactions. We had previously analyzed leukemia samples from all of the patients at diagnosis or relapse and detected either a rearrangement in the AML1 gene by Southern blot analysis or a fusion transcript by RT-PCR. Using RT-PCR, we analyzed all of the early and long-term remission samples. We consistently observed a faint band of 339 bp in etidium bromide-stained gels representing the chimeric AML1/ETO mRNA junction only in samples from the six patients and from the Kasumi-1 cell line but not from the TK-6 and ML-1 cell lines or the control individuals (not shown). Figure 1A shows the autoradiogram of the Southern blot for the six patients during early remission. Figure 1B shows the results...
Fig 1. (A) Autoradiogram of the PCR-amplified products from the cell line Kasumi-1, carrying the t(8;21) (lane 1), the t(8;21) patients early in hematologic and cytogenetic remission (patients 1 to 6, lanes 2 to 7), and the myeloid cell line ML-1, (lane 8). The karyotypes of the patients at diagnosis have been described, corresponding numbers of patients 1 to 6 in reference 6 are: patients 5, 9, 12, 14, 16, and 20. (B) Autoradiogram of the PCR-amplified products from the cell line Kasumi-1, carrying the t(8;21) (lane 1), the PB of the patients in long-term remissions (patients 4, 5, and 6; lanes 2, 3, and 4), the cell line TK-6 (lane 5), and two control individuals (lanes 6 and 7). The probe used was a 0.77-kb chimeric fragment obtained from a t(8;21) chimeric cDNA.

from recent PB samples for 3 of the 4 long-term patients (nos. 4, 5, and 6); we were not able to obtain a recent blood sample from patient 3.

DISCUSSION

We have used RT-PCR with primers mapping on both sides of the AML1/ETO chimeric cDNA junction to detect the fusion transcript in PB samples obtained recently from three patients who were diagnosed and successfully treated for AML-M2 with the t(8;21) more than 6 to 8 years ago (patients 4 through 6, Table 1). Our results show that these patients still have circulating leukocytes with the t(8;21) producing the chimeric AML1/ETO transcript. We studied samples from two of these patients at initial diagnosis and during remission (nos. 5 and 6) and one of them during first relapse and subsequent remission (no. 4). All of the patients at diagnosis or relapse had DNA rearrangements on standard Southern blot analysis, and they amplified the same size chimeric AML1/ETO mRNA junction on RT-PCR during early or long-term remission. So far we have only studied three patients who had sequential samples from diagnosis or relapse through long-term remission, and thus we cannot extrapolate our results to all patients. We have studied one additional patient after 1 month and 4.5 years in remission whose cells had the t(8;21) at diagnosis. Several analyses on this patient’s early remission BM and more recent blood sample failed to detect an amplified chimeric message on RT-PCR. We did not have any sample of this patient at diagnosis to analyze for the presence of the chimeric transcript, but it is possible that some long-term survivors may actually be cured.

The t(8;21) is a recurring chromosomal abnormality that is closely associated with a characteristic morphologic subtype of AML and a favorable response to chemotherapy. The great majority of these patients (in some series 100%) will achieve complete hematologic remission, and the translocation is then no longer detectable in BM metaphase cells by cytogenetic methods. After additional remission-consolidation chemotherapy, these remissions are generally durable, and relapses after more than 2 years in remission are uncommon. Indeed, AML-M2 with t(8;21) is generally felt to be among the most “favorable” subtypes of AML because of its predictably high rate of cure (about 60%). Characteristically, the arrest in maturation of granulocytic differentiation is not complete in this subtype of leukemia, and at diagnosis it is likely that many circulating mature granulocytes are in fact the progeny of the neoplastic clone. It is remarkable that even after 8 years in clinical remission (and in one case after BM transplantation), cells that appear to be derived from the leukemic lineage continue to circulate in the blood.

The role of molecular methods of detection of subclinical leukemia is currently being explored. PCR methods that can amplify a unique nucleotide sequence from 1 in 10⁵ cells can detect residual disease with a sensitivity that far exceeds standard light microscopy or routine cytogenetics. The t(8;21) AML is not the only malignancy in which minimal residual disease has been detected by PCR methods during long-term remission. As in these other cases, the
biologic and clinical significance of the presence of cells carrying specific abnormalities associated with cancer are still unknown. The expression of the chimeric protein could represent only one of the modifications necessary for the development of cancer and leukemia, and the affected clone would require additional mutations to express the transformed phenotype. Alternatively, it is possible that these t(8;21) cells are in fact completely transformed, but that cell proliferation is repressed by an unknown mechanism. However, as illustrated by our patients who have remained in continuous remission for over 5 years, further cytotoxic chemotherapy or BM transplantation is not an appropriate response to a positive test result. Longer follow-up is required to gain the appropriate perspective on our relatively disquieting observations. However, they suggest that a new paradigm regarding the implications of translocation-carrying cells may be required.

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