Detection of the AML1/ETO Fusion Transcript in the t(8;21) Masked Translocation in Acute Myelogenous Leukemia

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Abstract

The fusion transcript AML1/ETO was detected in the bone marrow of two t(8;21)-negative acute myelogenous leukemia (AML) patients by means of reverse transcription-polymerase chain reaction. This fusion transcript is identical to the one transcribed from the t(8;21) translocation base, as deduced from (a) the size and restriction pattern of the amplified DNA fragment and (b) the DNA sequence analysis of the fusion junction. We also showed that the ETO gene is highly expressed in these patients, much as it is in the t(8;21)-positive AML. Southern blot analysis showed rearrangement of the AML1 gene in one of the patients. Together, our results demonstrate that there is a masked t(8;21) translocation in AML that is not detectable by cytogenetic analysis but is able to transcribe an AML1/ETO fusion transcript similar to that transcribed in t(8;21)-positive AML-M2 patients.

Introduction

The nonrandom chromosomal translocation t(8;21)(q22;q22) can be found in about 18% of patients with AML3 with maturation (AML-M2 according to the French-American-British classification) (1). The AML1 and ETO genes from chromosomes 21 and 8, respectively, are involved at the breakpoint of the translocation (2-4). Rearrangement of the AML1 and the ETO genes can be detected by Southern blot analysis in samples from t(8;21)-positive AML-M2 patients (5-7), and the fusion transcript AML1/ETO can be detected consistently by RT-PCR (8). Rare cases of chromosomal translocation have been reported in t(9;22)-negative chronic myelogenous leukemia (9-12) and in t(15;17)-negative acute promyelocytic leukemia patients (13). Recently it was reported that rearrangement of the AML1 gene was detected by Southern blot analysis in 3 of 16 t(8;21)-positive AML-M2 patients (5-7), and the fusion transcript AML1/ETO was detected in the bone marrow of two patients (Fig. 1). The resulting PCR-amplified DNA fragments were sequenced by using an internal primer 821U1 according to the method as described in our previous report (14). These results demonstrate that there is a masked t(8;21) translocation in AML that is not detectable by cytogenetic analysis but is able to transcribe an AML1/ETO fusion transcript similar to that transcribed in t(8;21)-positive AML-M2 patients.

Materials and Methods

Patients. In this study, both patients had AML-M2, as diagnosed according to the French-American-British classification (1) at our institution. Bone marrow cells were obtained from each patient upon his or her informed consent. Mononuclear cells were separated and cryopreserved before DNA and RNA extraction.

DNA and RNA Isolation. Genomic DNA was isolated from bone marrow cells as described previously (14). Total RNA was isolated from patient samples by a modified method of Chirgwin et al. (15) using RNazol (Biotec Laboratories, Houston, TX) according to the suggested procedure of the manufacturer.

RT-PCR. RT-PCR was performed as previously described (8). The primer set 821U1/821D1 was purified in a Centricon-100 (Amicon, Inc., Beverly, MA). Single-stranded DNA was obtained by asymmetric PCR using the 821U primer and was sequenced by using an internal primer 821U1 according to the method as described in our previous report (14). These results demonstrate that there is a masked t(8;21) translocation in AML that is not detectable by cytogenetic analysis but is able to transcribe an AML1/ETO fusion transcript similar to that transcribed in t(8;21)-positive AML-M2 patients.

Sequencing of PCR-amplified DNA Fragments. DNA fragments obtained by RT-PCR as described above using the primer set 821U1/821D1 were purified in a Centricon-100 (Amicon, Inc., Beverly, MA). Single-stranded DNA was obtained by asymmetric PCR using the 821U primer and was sequenced by using an internal primer 821U1 according to the method as described in our previous report (14). These results demonstrate that there is a masked t(8;21) translocation in AML that is not detectable by cytogenetic analysis but is able to transcribe an AML1/ETO fusion transcript similar to that transcribed in t(8;21)-positive AML-M2 patients.

Results

In the studies reported here, the hematological features of patient 2 but not patient 1 were typical of t(8;21)-positive AML-M2 (data not shown). Cytogenetic analysis of the metaphase chromosomes did not reveal a t(8;21) translocation. In both patients, a normal diploid karyotype was shown in all 25 cells analyzed (data not shown). Fluorescence in situ hybridization of metaphase chromosomes of the two patients using total chromosomes 8 and 21 specific DNA probes also failed to detect any obvious chromosome abnormality.4 By means of RT-PCR, the fusion junction of AML1/ETO was demonstrated.

4 J. C. Liang and L. Zhao, unpublished results.
AML1/ETO fusion transcript in (8;21)-negative AML

821D1. A DNA fragment of 198 base pairs was amplified in all samples. These DNA fragments were digested with restriction endonuclease TaqI (Lanes 1-3). These DNA fragments were digested with restriction endonuclease Aul (Lanes 4-6) or TaqI (Lanes 7-9). Lane 3 contained RNA isolated from a t(8;21)-positive AML as a positive control. Lanes 1 and 2 contained samples isolated from patients 1 and 2. DNA fragment sizes are indicated on the right or left in base pairs. Lane M contained DNA size markers from a Sau96I-digested pUC18 plasmid.

To confirm that this amplified DNA fragment was indeed derived from the AML1/ETO fusion transcript, the sequences of PCR-amplified DNA fragments were analyzed. Our subsequent results demonstrated that the DNA sequence was identical to that of the AML1/ETO fusion transcript transcribed from t(8;21)-positive patients as previously reported (Fig. 2) (4, 8). Therefore, our results confirmed that the t(8;21) masked translocation in AML-M2 patients transcribes the AML1/ETO fusion transcript that is expected to encode a fusion protein.

We had previously reported that expression of the ETO gene in t(8;21)-positive AML-M2 patients is activated by the translocation (8). Therefore, in this study, we also analyzed the expression of the ETO gene in our two patients by RNA slot-blot hybridization (Fig. 3) and demonstrated that expression of the ETO gene in patient 1 was comparable to that in the t(8;21)-positive samples. In patient 2, though only a small quantity of RNA was left for this analysis, a weak positive signal was observed (Fig. 3).

Genomic DNA from patient 2 was isolated and Southern blot analysis of the AML1 gene was performed as described in “Materials and Methods.” As shown in Fig. 4, a rearranged band of about 9 kilobases was clearly detected in the DNA sample of patient 2 by means of restriction endonuclease HindIII digestion. Unfortunately, no DNA sample from patient 1 was available for this analysis.

Discussion

In RT-PCR studies using RNA samples isolated from a series of t(8;21)-positive and -negative AML-M2 patients (8) we have consistently detected the AML1/ETO fusion transcript in patients with the t(8;21) translocation. In the study reported here, we demonstrated that the AML1/ETO fusion transcript was also detected in AML patients without the t(8;21) translocation.

Recently, Maseki et al. (7) reported finding a variant form of the t(8;21) translocation, which they termed t(8;V;21), in 5 of 33 AML patients. Another form of masked translocation was also found in three (8;21)-negative AML (7). Southern blot analysis of both the variant form and the masked t(8;21) translocation revealed rearrangement of the AML1 gene. In the present study, we demonstrated by means of RT-PCR that two t(8;21)-negative AML-M2 patients with the masked translocation expressed the AML1/ETO fusion transcript. We also found that the AML1/ETO fusion transcript transcribed from the masked translocation and the t(8;21) translocation are identical, as demonstrated by sequence analysis of the PCR-amplified DNA fragment. This finding could not be the result of contamination since 30 cycles of PCR (data not shown) was sufficient to amplify the 198-base pair DNA fragment under our reaction conditions. Furthermore, no
t(8;21)-positive AML patient samples were collected on the day that this sample was received. Thus, the possibility that a sample was mislabeled is remote.

We further demonstrated here, by RNA slot-blot hybridization, that expression of the ETO gene in our two t(8;21)-negative AML patients was activated, much as it is in t(8;21)-positive AML patients, as a result of the translocation (8). The biological function of the ETO gene is currently unknown; however, according to the DNA sequence of the fusion junction of AML1/ETO, production of an ETO peptide with a proline-rich domain can be predicted.6 Such a domain is similar to the transcription-activating domain of transcription factors (16). It is therefore possible to infer from our results that activation of ETO gene expression in these patients may contribute to the development of leukemia.

* Unpublished observation.

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References

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