Detection of the AML1/ETO Fusion Transcript in the (t8;21) Masked Translocation in Acute Myelogenous Leukemia

Fumio Maruyama, Peirong Yang, Sanford A. Stass, Ann Cork, Emil J Freireich, Ming-Sheng Lee, and Kun-Sang Chang

Hematopathology Program, Division of Laboratory Medicine, The University of Texas, M. D. Anderson Cancer Center, Houston, Texas 77030

Abstract

The fusion transcript AML1/ETO was detected in the bone marrow of two (t8;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 the 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 AML, 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)-negative AML-M2 patients (7). We demonstrated in the study reported here that this type of masked t(8;21) translocation transcribes an AML1/ETO fusion transcript identical to the one transcribed from the t(8;21) translocation.

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 sequences of primers 821U (5’-AGCTGACCTGACATCACCACG-3’), 821U1 (5’-TTACAAAATCCCACCGCAAGTC-3’), and 821D1 (5’-TGAACTGTCTTTGAGCTCTT-3’) were derived from the AML1 and AML1/ETO complementary DNA sequences as previously reported (5, 8). In all PCR, all reagents were added except the Taq polymerase (Promega Corp., Madison, WI), the reaction mixture was heated to 75°C, and PCR was started by adding Taq polymerase (2.5 units in 20 μl). Thirty-five cycles of PCR were performed; each cycle was carried out at 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min.

Possible contamination in all reactions was strictly monitored by additional measures, i.e., in all experiments, a negative control reaction was carried out with normal RNA sample and H2O.

Sequencing of PCR-amplified DNA Fragments. DNA fragments obtained by RT-PCR as described above using the primer set 821U/821D1 were purified in a Centricron-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).

Southern Blot Analysis. Five μg of total genomic DNA was restriction-digested to completion and electrophoresed in a 0.7% agarose gel. Southern blotting and hybridization was then performed using the 263 base pairs of the AML1 complementary DNA probe (nucleotides 1201–1463) as previously described (3).

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. By means of RT-PCR designed to amplify the fusion junction of AML1/ETO, a single DNA fragment of 198 base pairs was detected by primer set 821U1/821D1 in the samples isolated from these two patients (Fig. 1). The resulting PCR-amplified DNA fragments were then digested with either restriction endonuclease Rsal or TaqI, and two DNA fragments of predicted size (80 and 118 base pairs for Rsal; or 128 and 70 base pairs for TaqI) were obtained. The amplified DNA

Received 6/14/93; accepted 8/19/93.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

1 Supported in part by a grant from the Physician Referral Service, The University of Texas, M. D. Anderson Cancer Center to K. S. C.

2 To whom requests for reprints should be addressed.

3 The abbreviations used are: AML, acute myelogenous leukemia; RT-PCR, reverse transcription-polymerase chain reaction.

4 J. C. Liang and L. Zhao, unpublished results.
AML1/ETO FUSION TRANSCRIPT IN (8;21)-NEGATIVE AML

Fig. 1. Amplification of the fusion junction of AML1/ETO by RT-PCR. RNA samples from AML-M2 patients were subjected to 35 cycles of RT-PCR using primer set 821U1/821D1. A DNA fragment of 198 base pairs was amplified in all samples (Lanes 1–3). These DNA fragments were digested with restriction endonuclease RsaI (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 SaullIA-digested pUC18 plasmid.

Fig. 2. Sequence analysis of the PCR-amplified DNA fragments. Internal primer 821U1 was used to sequence the fusion junction of AML1/ETO as described in "Materials and Methods." Arrows, the exact sites of the fusion junctions.

Fig. 3. RNA slot-blot analysis of ETO gene expression in AML-M2 and other types of leukemia. Different quantities of total RNA were blotted on each lane (1, 3, and 5 µg). Filters were hybridized with a complementary DNA probe of ETO (A) and with a complementary DNA probe of β2-microglobulin (B). RNA loaded on each lane was isolated from the following leukemia cells: Kasumi-1 cell line (17) (A); a t(8;21)-positive AML (B); acute lymphoblastic leukemia (C); chronic lymphocytic leukemia (D); acute promyelocytic leukemia with a t(15;17) translocation (E); acute lymphoblastic leukemia (F); chronic lymphocytic leukemia (G); acute promyelocytic leukemia with a t(15;17) translocation (H). Filters were hybridized with a complementary DNA probe of /3z-microglobulin (B). RNA loaded on each lane was isolated from the following leukemia cells: Kasumi-1 cell line (17) (A); a t(8;21)-positive AML (B); acute lymphoblastic leukemia (C); chronic lymphocytic leukemia (D); acute promyelocytic leukemia with a t(15;17) translocation (E); acute lymphoblastic leukemia (F); chronic lymphocytic leukemia (G); acute promyelocytic leukemia with a t(15;17) translocation (H). Filters were hybridized with a complementary DNA probe of /3z-microglobulin (B). RNA loaded on each lane was isolated from the following leukemia cells: Kasumi-1 cell line (17) (A); a t(8;21)-positive AML (B); acute lymphoblastic leukemia (C); chronic lymphocytic leukemia (D); acute promyelocytic leukemia with a t(15;17) translocation (E); acute lymphoblastic leukemia (F); chronic lymphocytic leukemia (G); acute promyelocytic leukemia with a t(15;17) translocation (H).

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 t(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 was detected 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 expression of the proline-rich domain can be predicted. Such a domain is similar to the mislabeled is remote.

The biological function of the ETO gene in Philadelphia chromosome-negative chronic myeloid leukemia is currently unknown; however, according to the DNA sequence of 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.

Acknowledgments

We are grateful to Garland Yee for synthesizing the oligonucleotide primers and to Dr. Nanao Kamada, Hiroshima University, Japan, for providing the Kasumi-1 cell line.

References

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

Fumio Maruyama, Peirong Yang, Sanford A. Stass, et al.


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/53/19/4449

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.