Detection of Minimal Residual Disease in Cerebro-Spinal Fluid of a Patient with Acute Myelogenous Leukemia with t(16 ; 21)(p11 ; q22) Translocation by Reverse Transcriptase-Polymerase Chain Reaction

HIDEO HARIGAE, MASARU KOBAYASHI,¹ AKIO MIHARA and NORIMICHI WATANABE

Department of Internal Medicine, Suifu Hospital, Mito 310, and ¹SRL Inc., Hachioji 192

HARIGAE, H., KOBAYASHI, M., MIHARA, A. and WATANABE, N. *Detection of Minimal Residual Disease in Cerebro-Spinal Fluid of a Patient with Acute Myelogenous Leukemia with t(16 ; 21)(p11 ; q22) Translocation by Reverse Transcriptase-Polymerase Chain Reaction.* Tohoku J. Exp. Med., 1997, 183 (4), 297-302 ——— We describe a patient with acute myelogenous leukemia (AML) with t(16 ; 21)(p11 ; q22) translocation, whose minimal residual disease (MRD) both in cerebrospinal fluid (CSF) and bone marrow (BM) was monitored by reverse transcriptase-polymerase chain reaction (RT-PCR). A TLS/ERG-FUS fusion transcript, which is known to be expressed by t(16 ; 21)(p11 ; q22) translocation, was detectable by RT-PCR both in BM and CSF cells in the first complete remission, suggesting the existence of MRD. The disease relapsed 6 months after its onset and allogeneic peripheral blood stem cell transplantation (PBSCT) was undergone. A TLS/ERG-FUS fusion transcript became rapidly below the detection level after PBSCT. These findings suggest the usefulness of RT-PCR for the detection of MRD in CSF, which contains a limited number of cells, as well as BM. ——— acute myelogenous leukemia; t(16 ; 21)(p11 ; q22) translocation; TLS/FUS-ERG fusion transcript; allogeneic peripheral blood stem cell transplantation; minimal residual disease © 1997 Tohoku University Medical Press

Recently a variety of chromosomal aberrations in leukemias have been identified. The breakpoints involved in these aberrations have been cloned and shown to involve two separate genes, which generate inframe fusion proteins. The function of these fusion proteins have been investigated and shown to play important roles in developing leukemias (Nicholes and Nimer 1992; Cline 1994). In addition, the detection of these fusion transcripts, which are expressed exclusively in leukemia cells, is used clinically for diagnosis of the diseases and

Received September 17, 1997; revision accepted for publication October 27, 1997.

Address for reprints: Hideo Harigae, M.D., Department of Internal Medicine, Suifu Hospital, 2-1-40 Ohmachi, Mito 310, Ibaraki, Japan.

follow-up of residual leukemic cells after conventional chemotherapy and bone marrow transplantation (Hughes et al. 1991; Diverio et al. 1993).

t(16; 21)(p11; q22) Translocation is a chromosomal abnormality, associated with human myeloid leukemias and myelodysplastic syndrome (Shimizu et al. 1994). Earlier studies demonstrated that this translocation produces a fusion gene between the TLS/FUS gene and the ERG gene, and a TLS/FUS-ERG fusion transcript has been expressed in leukemic cells with t(16; 21)(p11; q22) translocation (Ichikawa et al. 1994).

We report here a patient with refractory AML with t(16; 21)(p11; q22) translocation who underwent allogeneic PBSCT. In order to evaluate MRD, the expression of a TLS/FUS-ERG transcript was examined by RT-PCR in not only BM but also CSF cells. While a TLS/FUS-ERG transcript was detectable both in BM and CSF in the first complete remission, it rapidly disappeared after PBSCT. These findings suggest that RT-PCR offers a powerful tool for detecting MRD in CSF as well as BM.

CASE REPORT

The patient was a 25-year-old male who was admitted to our hospital in March, 1996. He was diagnosed as having AML-M4. Chromosome analysis of BM cells revealed 46, XY, 2q⁺, 9q⁻, 11q⁺, t(16; 21)(p11; q22) in 20/20 cells. He achieved complete remission in BM after the first induction chemotherapy, however, cell counts of CSF was found to increase to 54/3/ μ l and almost all of cells were leukemic cells. Clinical symptoms of the central nervous system (CNS) involvement were not observed. After several courses of systemic chemotherapies and intrathecal injections of chemotherapeutic agents, counts and the morphology of CSF cells were normalized. At this point, total RNA from CSF and BM cells was extracted, reverse-transcribed and nested PCR was performed. A TLS/FUS-ERG fusion transcript, which is known to be expressed by t(16; 21)(p11; q22) translocation, was detected in CSF cells (Fig. 1b) as well as BM cells (Fig. 1a) despite the fact that the patient was in complete remission, suggesting the existence of MRD. Nucleotide sequence analysis showed that the PCR product of the patient had the same sequence as that of patients reported previously (Ichikawa et al. 1994). The disease relapsed 6 months after its onset with the chromosomal anomaly, t(16; 21)(p11; q22). Counts of CSF cells, which represented the morphology of leukemic cells, increased again to 46/3/ μ l. Second complete remission was not achieved in spite of intensive chemotherapies and counts of leukemic cells of his peripheral blood became to increase shortly after chemotherapies. Since allogeneic PBSCT was expected to bring more rapid hematological recovery and enhanced GVL effect than BMT, it was performed from a HLA-matched sibling after obtaining his informed consent and the approval of the institutional board. The conditioning regimen consisted of Ara-C 1 g/day $\times$ 5 and fractionated total body irradiation (6 $\times$ 2 Gy) followed by 3000 mg of cyclo-

phosphamide on each of consecutive 2 days. To mobilize stem cells, 3 $\mu\text{g/kg}$ of G-CSF was administered to the donor subcutaneously for consecutive 5 days and leukapheresis was performed for consecutive 2 days beginning on day 5 of G-CSF administration using a blood flow cell separator (AS104, Fresenius Inc., Bad Homburg, Germany). White blood cell counts of the donor increased to $35 \times 10^9/\text{liter}$ on day 5. Apheresed cells, which contained $2 \times 10^6/\text{kg}$ CD34⁺ cells total, were infused into the patient immediately on each day. For GVHD prophylaxis, a short course of MTX and cyclosporine A were used. Neutrophil counts exceed-

Fig. 1a. Expression of a TLS/FUS-ERG fusion transcript in BM cells. 1 μg of total RNA of bone marrow cells before PBSCT and after PBSCT were reverse-transcribed, and polymerase chain reaction was performed as described (Ichikawa et al. 1994). To evaluate the sensitivity of the RT-PCR, 1 μg of the total RNA isolated from leukemic cells expressing TLS/FUS-ERG fusion transcript was serially diluted in 1 μg of RNA from normal control PB cells that do not express this transcript and this preliminary experiment showed that the fusion transcript was detectable even in the solution diluted 10^{-5} . The product of 191 bp corresponds to the FUS/TLS-ERG fusion transcript. lane 1; size marker. lane 2; in complete remission before PBSCT. lane 3; after PBSCT (day 26). lane 4; after PBSCT (day 171). lane 5; negative control (HL-60).

b. Expression of a TLS/FUS-ERG fusion transcript in CSF cells. Total RNA from approximately 10×10^3 of CSF cells were reverse-transcribed, and polymerase chain reaction was performed in same condition as using BM cells. The sensitivity of the RT-PCR was evaluated as described in Fig. 1a. lane 1; size marker. lane 2; in complete remission before PBSCT. lane 3; after PBSCT (day 26). lane 4; negative control (HL-60).

c. Examination of chimerism after allogeneic PBSCT. Chimerism after PBSCT was examined by VNTRs polymorphisms using a YNH-24 probe by Southern blot hybridization (Nakamura et al. 1987). Complete chimerism was observed after PBSCT. lane 1; skin of recipient. lane 2; peripheral blood of donor. lane 3; peripheral blood of recipient after PBSCT.

ed 0.5×10^9 /liter on day 11 and platelet counts exceeded 20×10^9 /liter on day 14. Acute GVHD was not observed. To evaluate MRD, CSF and BM cells were collected and RT-PCR was repeated on day 26. The TLS/FUS-ERG fusion transcript was not detectable in both BM and CSF cells (Fig. 1a, b). On day 171, MRD was evaluated again by same RT-PCR method and it was confirmed that TLS/FUS-ERG fusion transcript was still kept undetectable (Fig. 1a). Complete chimerism was confirmed by variable numbers of tandem repeats (VNTRs) polymorphisms on day 33 (Fig. 1c). He had an extensive chronic GVHD from day 70, however, it has been controlled by the administration of prednisolone and dose-up of cyclosporine A. He has been in complete remission since then (~ 200 days post PBSCT).

DISCUSSION

The TLS/FUS-ERG fusion gene is known to be generated by t(16;21)(p11;q22) translocation. The TLS/FUS gene is a RNA binding protein which was originally found to be fused to the CHOP gene in human myxoid liposarcoma (Crozat et al. 1993; Rabbitts et al. 1993) and the ERG gene is a member of the ets oncogene superfamily functioning as transcriptional activators (Reddy and Rao 1991). Although the function of TLS/FUS-ERG fusion protein has been investigated and shown to have transcriptional activation properties (Prasad et al. 1994), the role of this fusion protein on developing leukemias still remains unclear. To date, many characteristic fusion genes, like the TLS/FUS-ERG, resulting from chromosome aberrations, have been identified and the expression of their transcripts were examined by RT-PCR to detect residual leukemic cells. However, the detection of fusion transcripts by this method do not always predict the clinical relapse of the diseases, because of the sensitivity of the method and difference of the functions of fusion proteins. It has been reported that hematopoietic cells which have a capacity of normal differentiation and express the AML1/ETO fusion transcript exist in long term remission patients with t(8;21) translocation (Miyamoto et al. 1996). This result suggests that some of these fusion proteins may not be responsible for leukemogenesis by themselves. If the TLS/FUS-ERG fusion protein has a strong transforming activity, as suggested by the short remission period of our case, the disappearance of the expression of this protein would be essential for the continuous remission of the disease. In that case, RT-PCR is a useful method to predict a relapse and the undetectable expression level of the fusion transcript by RT-PCR may be a criteria of the remission.

It should be noted that our study demonstrated the usefulness of RT-PCR in detecting MRD in CSF cells. Therapeutic effects of the treatment for CNS involvement have been evaluated by cell counts and morphology of cells obtained by spinal taps. However, the diagnosis of MRD using CSF cells is often difficult, because only few cells can be recovered, particularly after chemotherapy, and

morphological diagnosis is clearly inadequate in sensitivity and specificity. In contrast, as shown in our study, RT-PCR is extremely sensitive, highly specific and can be readily performed using a limited number of cells ($\sim 10\,000$ cells), such as those obtained from CSF. In our knowledge, evaluation of MRD by RT-PCR has been performed only in BM cells. The ability to detect a fusion gene transcript in CSF by RT-PCR should provide an important means of detecting MRD.

In this case, the dose of G-CSF administered to the donor was reduced compared to the dose in a previous review (Russel et al. 1996) in which G-CSF is administered more than $10\,\mu\text{g/kg/day}$, because (1) collected PBSC was infused immediately in our case without freezing which impairs the viability of cells, (2) dose reduction of G-CSF was expected to avoid side-effects of donor. In second allogeneic PBSCT undergone in our hospital, hematopoiesis was also recovered by collected PBSCs from the donor received $3\,\mu\text{g/kg/day}$ of G-CSF administration. It has been, however, reported that counts of collected PBSCs fluctuate among individuals (Bensinger et al. 1996). Further studies are needed to determine if this dose of G-CSF administration is acceptable as a standard dose for the safe PBSCT.

Acknowledgments

We thank Drs. K. Iitake, T. Kimura & T. Gomibuchi for their fruitful discussions, and also thank Dr. S. Sassa for preparing the manuscript.

References

- 1) Bensinger, W.I., Clift, R., Anasetti, C., Appelbaum, F.R., Demirer, T., Rowley, S., Sandmaier, B.M., Storb, T.B., Storb, R. & Buckener, C.D. (1996) Transplantation of allogeneic peripheral blood stem cells mobilized by recombinant human granulocyte colony stimulating factor. *Stem Cells*, **14**, 90–105.
- 2) Cline, M.J. (1994) The molecular basis of leukemia. *N. Engl. J. Med.*, **330**, 328–336.
- 3) Crozat, A., Aman, P., Mandahl, N. & Ron, D. (1993) Fusion of CHOP to a novel RNA-binding protein in human myxoid liosarcoma. *Nature*, **363**, 640–643.
- 4) Diverio, D., Pandolfi, P.P., Biondi, A., Avvisati, G., Petti, M.C., Mandelli, F., Pelicci, P.G. & Coco, F.L. (1993) Absence of reverse transcriptase-polymerase chain reaction detectable residual disease in patients with acute promyelocytic leukemia in long-term remission. *Blood*, **82**, 3556–3559.
- 5) Hughes, T.P., Morgan, G.J., Martiat, P. & Goldman, J.M. (1991) Detection of residual leukemia after bone marrow transplant for chronic myeloid leukemia: Role of polymerase chain reaction in predicting relapse. *Blood*, **77**, 874–878.
- 6) Ichikawa, H., Shimizu, K., Hayashi, Y. & Ohki, M. (1994) An RNA-binding protein gene, TLS/FUS, is fused to ERG in human myeloid leukemia with t(16;21) chromosomal translocation. *Cancer Res.*, **54**, 2865–2868.
- 7) Miyamoto, T., Nagafuji, K., Akashi, K., Harada, M., Kyo, T., Akashi, T., Takenaka, K., Gondo, H., Okamura, T., Dohy, H. & Niho, Y. (1996) Persistence of multipotent progenitors expressing AML/ETO transcripts in long-term remission patients with t(8;21) acute myelogenous leukemia. *Blood*, **87**, 4789–4796.
- 8) Nakamura, Y., Leppert, M., O'Connell, P., Wolff, R., Holm, T., Culver, M., Martin, C.,

- Fujimoto, E., Hoff, M., Kumlin, E. & White, R. (1987) Variable number of tandem repeat (VNTR) markers for human gene mapping. *Science*, **235**, 1616-1622.
- 9) Nichols, J. & Nimer, S.D. (1992) Transcription factors, translocations, and leukemia. *Blood*, **80**, 2953-2963.
- 10) Prasad, D.D.K., Ouchida, M., Lee, L., Rao, V.N. & Reddy, E.S.P. (1994) TLS/FUS fusion domain of TLS/FUS-erg chimeric protein resulting from the t(16;21) chromosomal translocation in human myeloid leukemia functions as transcriptional activation domain. *Oncogene*, **9**, 3717-3729.
- 11) Rabbitts, T.H., Forster, R., Larson, R. & Nathan, P. (1993) Fusion of the dominant negative transcription regulator CHOP with a novel gene FUS by translocation t(12;16) in malignant liposarcoma. *Nat. Genet.*, **4**, 175-180.
- 12) Reddy, E.S.P. & Rao, V.N. (1991) Erg, an ets-related gene, codes for sequence-specific transcriptional activators. *Oncogene*, **6**, 2285-2289.
- 13) Russel, N., Gratwohl, A. & Schmitz, N. (1996) The place of blood stem cells in allogeneic transplantation. *Br. J. Haematol.*, **93**, 747-753.
- 14) Shimizu, K., Ichikawa, H., Tojo, A., Kaneko, Y., Masaki, N., Hayashi, Y., Ohira, M., Asano, S. & Ohki, M. (1994) An ets-related gene, ERG, is rearranged in human myeloid leukemia with t(16;21) chromosomal translocation. *Proc. Natl. Acad. Sci. USA*, **90**, 10280-10284.
-