Alterations in the Deleted in Colorectal Carcinoma Gene in Human Primary Leukemia

By Koichi Miyake, Koiti Inokuchi, Kazuo Dan, and Takeo Nomura

To evaluate the role of the deleted in colorectal carcinoma (DCC) gene in leukemogenesis, we examined loss of heterozygosity (LOH) in the DCC gene in 64 primary human leukemias using Southern blot analysis and examined the expression of the DCC gene using reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. Allelic loss in the DCC gene was observed in two patients (6%, 2 of 35 informative cases), and expression of the DCC gene was reduced or absent in 8 of 26 (31%) patients with acute myelogenous leukemia (AML). 3 of 9 (33%) patients with acute lymphocytic leukemia (ALL), and 7 of 29 (24%) patients with chronic myelogenous leukemia (CML). Moreover, in one ALL patient with absent DCC expression at diagnosis, its expression became normal after performing chemotherapy and achieving remission. These findings suggest that inactivation of the DCC gene contributes to some instances of leukemogenesis.

© 1993 by The American Society of Hematology.

MATERIALS AND METHODS

Cell samples. Primary human leukemia cells were obtained from bone marrow (BM) aspirates or peripheral blood (PB) from 64 patients at the time of diagnosis, after obtaining informed consent. The patients consisted of 26 patients with AML, 9 with ALL, and 29 with CML (of which 23 were in chronic phase and 6 in blast crisis). Thirty-five patients with acute leukemia were classified according to the French-American-British (FAB) criteria as follows: one patient with M0 AML, 26 with M1 AML, eight with M2 AML, three with M3 AML, six with M4 AML, one with M5 AML, one with M6 AML, three with L1 ALL, five with L2 ALL, and one with L3 ALL. The diagnosis and classifications of the 29 CML patients were based on standard clinical, morphologic, cytochemical and immunologic criteria. The Philadelphia chromosome was detected in all of the patients. As normal control samples, BM mononuclear cells in the remission stage, skin biopsy, and gastric biopsy were obtained from the same patients after obtaining informed consent.

Southern blot analysis. Cell isolation and DNA extraction were performed as previously described. After the genomic DNA was digested to completion with appropriate restriction endonucleases (EcoRI or MspI; Boehringer Mannheim, Germany), 10 μg of digested DNA was size-fractionated by 0.8% standard saline citrate overnight. Hybridization was performed according to the protocol provided by New England Nuclear, as described previously. The filters were hybridized with P-labeled probes at 65°C and visualized on autoradiograms. The following probes and restriction enzymes were used in this study: pDCC1.65 (EcoRI); p515-65 (MspI); and SAM1.123 (EcoRI). For internal control of sample DNA quality, same filters were rehybridized with P-labeled N-ras probe, which was a HindIII fragment containing exon 1 of N-ras gene.

RT-PCR for detection of DCC mRNA. The total RNA of mononuclear cells was extracted by the CsCl method. The integrity of the RNA samples was determined by formaldehyde-agarose gel electrophoresis, and degraded RNA samples were rejected. RT-PCR was performed as described previously. First, cDNA synthesis was performed using an oligonucleotide primer (DCC2: 5'-AGCCTC-ATTTTCAGCCACACA-3', antisense strand). These oligonucleotide primers are the same ones as used by Fearon et al.20 They were extended 300 bases from the 30 end of the cDNA sequence (the 3' end of the cDNA sequence). The PCR products were separated by electrophoresis on 2% agarose gels, transferred onto nylon membrane filters, and hybridized with a 32P-labeled probe 1.65 kb in length (EcoRI; 5'-AGCCTC-ATTTTCAGCCACACA-3', antisense strand).

From the Third Department of Internal Medicine, Nippon Medical School, Tokyo, Japan.

Address reprint requests to Koichi Miyake, MD, The Third Department of Internal Medicine, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113, Japan.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1993 by The American Society of Hematology.
ucts were phenol-extracted, ethanol-precipitated, electrophoresed through a 4% agarose gel (NuSciene 3:1 Agarose; FMC BioProducts, Rockland, ME), and transferred onto nylon membrane filters. Hybridization was performed with the $^{32}$P-labeled pDCC1.65 probe. To show the intactness of the RNA, we examined the expression of $\beta$-actin mRNA as a control using an oligonucleotide primer (actin 1: 5'-AACGGCTCCGGCATGTGCAA-3', coding strand; and actin 2: 5'-CTTCTGACCCATGCCCAACCA-3', antisense strand) by the same RT-PCR assay.

RESULTS

Southern blot analysis. We examined 64 DNA samples from the primary human leukemia cases by Southern blot using the pDCC1.65 cDNA probe. We found no definitive rearrangements in the DCC gene. However, we found loss of 20-kb and/or 14-kb EcoRI fragments (Fig 1Aa) in two patients. This probe detects two diallelic polymorphic EcoRI sites on Southern blot (20-kb and 14-kb EcoRI fragments), as Kikuchi-Yanoshita et al reported. To expect that two bands (20- and 14-kb) would be involved in allelic loss, we showed allelic loss using standard polymorphic probes from the DCC locus (p15-65 and SAM 1.1) in two patients. In this study, p15-65 detected an MspI polymorphism with two alleles of 10.5 and 7.8 kb, and SAM 1.1 detected an EcoRI polymorphism with two alleles of 9.2 and 1.1 kb. One case was a 29-year-old female AML (M1) patient with 98.2% blasts in her BM at diagnosis. We showed LOH using p15-65 probe. Two alleles were present in the normal DNA (skin biopsy), but only the 7.8-kb allele was present in the DNA from leukemic cells (Fig 1Ab). But unfortunately, SAM 1.1 was ‘not informative.’ The second case was a 57-year-old male ALL patient with 88% blasts in his BM at diagnosis. The karyotype of all his blast cells had an abnormality of 18q-. After performing chemotherapy, he achieved remission. Compared with the remission sample, the DNA of his blast cells showed homozygous loss by SAM 1.1 and p15-65 (Fig 1Bb). To show that approximately equal amounts of DNA were placed in the lanes, we rehybridized these membranes with N-ras probe. In addition, although we performed Southern blot analysis for five patients with CML in both the chronic phase and blast crisis, we could not find any abnormalities in the DCC gene in any of the samples (data not shown).

RT-PCR analysis. Twenty-two normal samples were obtained from BM aspirates from healthy volunteers after obtaining informed consent, and they were examined as normal controls. We examined expression of the DCC gene by the RT-PCR method in 64 human primary leukemia samples and 22 normal human samples. Whereas we found no abnormal (reduced or absent) expression of the DCC gene in any of the normal control samples, which showed a 233-bp fragment, expression of the DCC gene was absent or extremely reduced in 8 of the 26 AML samples (31%), 3 of the 9 ALL samples (33%), and 7 of the 29 CML samples (24%) (Fig 2A). These data are summarized in Table I. We also examined a remission sample of one ALL (L2) patient. She was an 18-year-old girl with 89.2% blasts in her BM. Whereas expression of the DCC gene was absent at diagnosis, its expression was detected after performing chemotherapy and achieving remission (Fig 2B). Moreover, we examined the expression of the DCC gene in five CML patients in both the chronic phase and blastic crisis. Four of the 5 patients showed normal DCC expression in both the chronic phase and blastic crisis, and the fifth patient lacked DCC gene expression in both phases (data not shown).

DISCUSSION

The DCC gene encodes a protein that is highly similar to neural cell adhesion molecules and other related cell-surface...
glycoproteins (GPs). Disruption of cell adhesion molecules (CAMs) might play a role in tumorigenesis. Neoplasia is often associated with disruption of such cell-cell contacts, and intercellular adhesion mediated by CAMs influences cellular differentiation.30 Our findings of reduced or absent expression of the DCC gene in some leukemia patients suggest that the DCC gene plays a role in leukemogenesis.

We detected allelic loss in the DCC gene in two patients. One patient showed hemizygous loss and the other showed homozygous loss. We think that homozygous loss is very uncommon evidence. We think that in the two patients who showed allelic loss, it was pure chance that one patient showed reduced or absent DCC expression, and no abnormality of the DCC gene could be detected in another 16 patients in Southern blot analysis. Thus, we speculate that a point mutation or an insertion in the DCC gene and alteration in an initiator or promoter area in the DCC gene may contribute to reduced or absent DCC expression as well as allelic loss (LOH) in the DCC gene. Further study is needed to elucidate the possible mechanisms.

The molecular mechanisms of the progression from the chronic phase to the blastic phase in CML are still unclear. Some reports have suggested that blast transformation of CML might be associated with tumor-suppressor genes such as the p53 gene30,13 or Rb gene.17 We examined DCC expression in six CML blast crisis samples. Its expression was extremely reduced in only one sample. Moreover, we examined the expression of the DCC gene in five CML patients in both the chronic phase and blastic crisis. Four of the 5 patients had normal DCC expression in both the chronic phase and blastic crisis, and the fifth patient lacked DCC gene expression in both phases (data not shown). Thus, the DCC gene might be involved in the early stage of CML, but not in blastic transformation.

In conclusion, we detected allelic loss in the DCC gene in two patients (6%, 2 of 35 informative cases) and absent or reduced expression of the DCC gene in 18 of 64 (28%) primary human leukemia cases. Although the full-length cDNA of the DCC gene and the function of the DCC protein remain unknown, our data strongly suggest that inactivation of the DCC gene is involved in some instances of leukemogenesis.

ACKNOWLEDGMENT

We thank Dr Bert Vogelstein (Johns Hopkins University School of Medicine, Baltimore, MD) for kindly providing the pDCC1.65, p15-65, and SAM1.1 probe, and helpful discussion; Dr Tamiko Shinozara (Japan Red Cross Medical Center, Tokyo, Japan) for performing the chromosome analysis; and Dr Takashi Shimada (Nippon Medical School, Tokyo, Japan) for fruitful discussion.

REFERENCES

8. Sugimoto K, Toyoshima H, Sakai R, Miyagawa K, Hagiwara...


Alterations in the deleted in colorectal carcinoma gene in human primary leukemia

K Miyake, K Inokuchi, K Dan and T Nomura