The HOXD11 Gene Is Fused to the NUP98 Gene in Acute Myeloid Leukemia with t(2;11)(q31;p15)

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Abstract

The nucleoporin gene, NUP98, has been reported to be fused to seven partner genes in hematological malignancies with 11p15 translocations. We report here a novel NUP98 partner gene, HOXD11, not HOXD13, in a pediatric patient with de novo AML having t(2;11)(q31;p15), using a cDNA panhandle PCR. The HOXD11 gene is one of the HOXD cluster genes and contains 2 exons, encoding a protein of 338 amino acids with a homeodomain. Reverse transcription (RT)-PCR analysis showed that two alternatively spliced 5'-NUP98 transcripts were fused in frame to the HOXD11 gene. Both proteins consist of an NH2-terminal phenoxyamine-glycine repeat motif of NUP98 and COOH-terminal homeodomain of HOXD11. RT-PCR analysis in various leukemic cell lines showed that expression of the HOXD11 gene was significantly more frequent in BCR-ABL-positive than in BCR-ABL-negative leukemic cell lines (P = 0.028). Our results revealed that t(2;11)(q31;p15) was not a single chromosomal abnormality and that the NUP98-HOXD11 fusion genes encode similar fusion proteins, which suggests that the NUP98-HOXD11 as well as NUP98-HOXD13 fusion protein play a role in leukemogenesis through similar mechanisms.

Introduction

Chromosomal translocations are associated with specific subtypes of hematological malignancies (1–3). The AML1 (RUNX1), MLL, MOZ, and TEL (ETV6) genes are found to be fused to a variety of partner genes in AML1 (4, 5), the NUP98 gene, located on 11p15, was reported to be fused to seven different partner genes, HOX9 (7p15) (4, 5), DDX10 (11q22) (6), HOXD13 (2q31) (7), PMX1 (1q23) (8), RAP1GDS1 (4q21) (9), TOP1 (20q11) (10), and LEDGF (9p22) (11). Among the partner genes of the NUP98 gene, HOX9, HOXD13, and PMX1, are homeobox genes and part of their DNA binding homeodomain is fused in frame to a domain encoding the NH2-terminal FG repeat of the NUP98 gene (4, 5, 7, 8).

NUP98 is a Mr 98,000 component of NPC located on the nucleoplasmic side of the NPC (12). NUP98 consists of an FG repeat motif, Gle2p-binding-like motif, and ribonucleoprotein-binding motif (12). NUP98 functions as one of several docking NUPS for the transport of RNA and protein between the nucleus and cytoplasm; it is an essential component of multiple RNA export pathways (12, 13).

The homeobox genes have a conserved homeodomain sequence discovered in genes of the Drosophila melanogaster and encode transcription factors that act as morphogenetic regulatory molecules (14). The homeobox genes act in the patterning of the development of many segmental and axial structures in several organisms (14). The 39 human homeobox-containing genes (HOX genes) are organized in four clusters (HOX-A, B, C, and D). The HOX gene expression in hematopoietic cells varies, depending on differences in the lineage and maturation of the cells (15).

To our knowledge, only five patients with t(2;11)(q31;p15) have been reported previously (7, 16–19). These patients were diagnosed as having myeloid malignancies, including three with AML (7, 18, 19) and two with myelodysplastic syndrome (MDS; Refs. 16, 17). NUP98-HOXD13 fusion transcripts were found in the three AML patients with the translocation. We report here a novel partner gene of NUP98, HOXD11, in a de novo pediatric patient with t(2;11)(q31; p15)-AML. We also report a specific expression pattern of the HOXD11 and HOXD13 genes.

Materials and Methods

Patients. A 15-year-old boy was diagnosed as having acute myelomonocytic leukemia [AMMoL; French-American-British (FAB) classification, M4] that was cytogenetically characterized as 46, XY, t(2;11)(q31;p15) in all of the 20 bone marrow cells examined. The WBC count at diagnosis was 187,900/μl with 44% leukemic blasts, which expressed CD11b, CD13, CD15, CD33, CD36, CD38, CD56, and HLA-DR antigens. A complete remission was achieved 4 months after diagnosis by chemotherapy on the AML-Berlin-Frankfurt-Munster 87 protocol. His karyotype was 46, XY in all 20 bone marrow cells. Five months after diagnosis, he underwent a cord blood stem cell transplantation from an unrelated HLA two-locus mismatched donor and has been in a complete remission for 7 months. Bone marrow leukemic cells were obtained from the patient at diagnosis after informed consent was given.

cDNA Panhandle PCR. To detect the 3’ unknown gene fused to the 5’ NUP98 gene, we adapted the cDNA panhandle PCR strategy with some modification, which was reported as a method to detect MLL fusion partner genes (Ref. 20; Fig. 2A). Total RNA was extracted from the patient’s leukemic cells using oligonucleotides with the known NUP98 coding sequence from the predicted breakpoint region at the 5’ ends and random hexamers at the 3’ ends (5’-CTGGACAGGCATCTTTGTTTGGGAACAACCNNNNNN-3’), using a cDNA synthesis kit (Amersham Pharmacia Biotech, Buckinghamshire, England). To test for the presence of amplifiable RNA, β-actin was amplified using the same cDNA by RT-PCR. To generate sense second-strand templates for panhandle PCR, 2 μl of the cDNA solution was added to a 98-μl mixture containing 2 units of T7 DNA polymerase, 200 μM each: dNTP, 3.3X buffer, MgCl2, 1 mm (Applied Biosystems, Foster, CA), and 40 pmol of NUP98–1S (5’-GGGACTCTTGGGAACCTGCGT-3’). PCR amplification was performed with this mixture using a DNA thermal-cycler (Applied Biosystems) under the following conditions: preheating at 94°C for 1 min, 25 cycles of denaturation

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3 The abbreviations used are: AML, acute myeloid leukemia; RT, reverse transcription; NUP, nucleoporin; ALL, acute lymphoblastic leukemia; FG, phenylalanine-glycine; NPC, nucleoporin complex; MNC, mononuclear cell; CML, chronic myelogenous leukemia; AMKL, acute megakaryoblastic leukemia; AMOL, acute monocytic leukemia; EBV-B, EBV-transformed B lymphocyte; HOX, homeobox.
for 15 s at 94°C, and annealing and extension for 10 min at 68°C, with a final extension of 10 min at 72°C. To make intrastrand annealing and polymerase extension, NUP98–1S extension was achieved by denaturing at 94°C for 9 min followed by 1 cycle at 94°C for 1 min and 72°C for 8 min. As a result, stem-loop templates contained the fusion point of the chimeric transcript in the loop. Using NUP98–2S (5′-CGCAGCTTCAGGAGCCCTTTT-3′) between the NUP98 sequence that was complementary to the ligated oligonucleotide and the breakpoint region, RT-PCR was performed with mixtures (50 µl) containing 12.5 pmol of NUP98 primer 1 and 2, 200 µM each: dNTP and 10× buffer. After initial denaturation at 94°C for 9 min, 40 cycles at 94°C for 1 min, at 55°C for 1 min, and at 72°C for 3 min, were used, followed by a final elongation at 72°C for 7 min. Nested RT-PCR was performed with the same conditions and 1 µl of the initial RT-PCR. The primers for nested RT-PCR were NUP98–3S (5′-GGGAGAGTTTGGACACCTCTTGT-3′) and NUP98–4S (5′-ACTACGACGACATTGGCCG-3′).

Nucleotide Sequencing. PCR products were cloned into the TA cloning vector (Ref. 21; Invitrogen, Carlsbad, CA). Nucleotide sequences were determined by the fluorometric method (Dye Terminator Cycle sequencing kit; Applied Biosystems).

Southern Blot Analysis. High-molecular-weight DNA was extracted from bone marrow cells of the patients by proteinase K digestion and phenol/chloroform extraction (22). Ten µg of DNA were digested with EcoRI, subjected to electrophoresis on 0.7% agarose gels, and transferred to cDNA probes that were 3P-labeled by the random hexamer method (21). Probes used were 837 bp NUP98 cDNA fragments [nucleotide (nt)1213 to 2049; GenBank accession no. U41815] and 316 bp HOXD11 cDNA fragments (nt 527 to nt 842; GenBank accession no. XM002543).

RT-PCR. The same conditions for the panhandle PCR were used for RNA extraction and cDNA synthesis. One µl of the cDNA solution was amplified by PCR in a total volume of 20 µl with 10 µM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.001% (w/v) gelatin, 200 µM each dNTP, 2.5 units of Taq polymerase (Applied Biosystems), and 20 pmol of each primer (21). NUP98–5S (5′-TTGTTGCTGTTGTTGCAGC-3′) and HOXD11–1AS (5′-GGAAACAGATTTGTGCTGCGG-3′) were used to confirm the NUP98-HOXD11 fusion transcripts. HOXD13–1AS (5′-ACTCCTCTTTCCCTTCCTTC-3′) was used to confirm the NUP98-HOXD11 fusion transcripts. PCR amplification was performed under the following conditions: preheating at 94°C for 9 min plus 40 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 55°C, and extension for 1 min at 72°C, with a final extension of 7 min at 72°C. Also, expression of the HOXD11 and HOXD13 genes was examined by RT-PCR. The primers used for RT-PCR were HOXD11–1S (5′-TCTTCTCTCTCTCTCCTGAG-3′). How-ever, no reciprocal fusion transcripts (HOXD11-NUP98) were detected (Fig. 2B).

We performed RT-PCR analysis in 61 leukemic cell lines, 5 EBV-B cell lines and 5 normal MNCs. Expression of the HOXD11 gene was found in 4 (80.0%) of 5 CML cell lines, 6 (50.0%) of 12 BCR-ABL-positive B-precursor ALL cell lines, 3 (60.0%) of 5 BCR-ABL-negative B-precursor ALL cell lines, 2 (20.0%) of 10 B-ALL cell lines, 3 (30.0%) of 10 T-ALL cell lines, 2 (33.3%) of 6 AMOL cell lines, 2 (22.2%) of 9 AML cell lines, 1 (20.0%) of 5 EBV-B cell lines, and 2 (40.0%) of 5 normal MNCs (Table 1; Fig. 3B). No expression was detected in two AMKL cell lines examined.

To compare the expression of the HOXD11 gene with that of the HOXD13 gene located on 2q31, expression of the HOXD13 gene was examined by RT-PCR. The HOXD13 gene was expressed in 10 (41.6%) of 24 myeloid lineage cell lines, including 4 (44.4%) of 9 AML, 3 (50.0%) of 6 AMOL, 2 (100.0%) of 2 AMKL, and 2 (40.0%) of 5 CML cell lines by RT-PCR. No expression was found in any B-ALL cell lines, EBV-B cell lines, or normal MNC, whereas expression was found in 1 (5.9%) of 17 B-precursor ALL cell lines and 1 (10.0%) of 10 T-ALL cell lines (Table 1; Fig. 3B). Expression of the HOXD11 gene was found significantly more frequently than that of the NUP98 gene in this patient is not fused to the HOXD13 gene, but to a novel partner gene.

To isolate the novel partner gene of the NUP98, we performed a cDNA panhandle PCR for total RNA from the patient’s leukemic cells. A cDNA panhandle PCR product of 281 bp was obtained (Fig. 2B), which consisted of a 246-bp unknown sequence fused to the NUP98 gene. BLAST database searching revealed that the sequence was homologous to a part of the mouse Hoxd11. We also found that the sequence was identical to the gene that maps to 2q31 on the human chromosome (GenBank accession no. AC009336). Therefore, we considered that a 246-bp unknown sequence was a part of the human HOXD11 gene. To isolate the human HOXD11 gene, we performed RT-PCR on cDNA from AMOL cell line THP-1 using primers based on the mouse Hoxd11, and we cloned a 1017-bp product of the HOXD11 cDNA (Fig. 2E). The predicted HOXD11 protein consists of 338 amino acids with a homoeodomain (Fig. 2, D and E). The entire amino acid sequence exhibits high similarity (93.8%) to the mouse Hoxd11 protein. Southern blotting with a HOXD11 cDNA probe revealed rearranged bands in the leukemic cells (Fig. 1B).

RT-PCR analysis using NUP98–5S and HOXD11–1AS revealed two types of NUP98-HOXD11 in-frame fusion transcripts, one containing exon 12 of the NUP98 gene (up to nt 1552) fused to exon 2 of the HOXD11 gene, and the other containing exon 11 of the NUP98 gene (up to nt 1411) fused to exon 2 of the HOXD11 gene (Fig. 2, B and C). However, no reciprocal fusion transcripts (HOXD11-NUP98) were detected (Fig. 2B).

We performed RT-PCR analysis in 61 leukemic cell lines, 5 EBV-B cell lines and 5 normal MNCs. Expression of the HOXD11 gene was found in 4 (80.0%) of 5 CML cell lines, 6 (50.0%) of 12 BCR-ABL-negative B-precursor ALL cell lines, 3 (60.0%) of 5 BCR-ABL-positive B-precursor ALL cell lines, 2 (20.0%) of 10 B-ALL cell lines, 3 (30.0%) of 10 T-ALL cell lines, 2 (33.3%) of 6 AMOL cell lines, 2 (22.2%) of 9 AML cell lines, 1 (20.0%) of 5 EBV-B cell lines, and 2 (40.0%) of 5 normal MNCs (Table 1; Fig. 3B). No expression was detected in two AMKL cell lines examined.

To compare the expression of the HOXD11 gene with that of the HOXD13 gene located on 2q31, expression of the HOXD13 gene was examined by RT-PCR. The HOXD13 gene was expressed in 10 (41.6%) of 24 myeloid lineage cell lines, including 4 (44.4%) of 9 AML, 3 (50.0%) of 6 AMOL, 2 (100.0%) of 2 AMKL, and 2 (40.0%) of 5 CML cell lines by RT-PCR. No expression was found in any B-ALL cell lines, EBV-B cell lines, or normal MNC, whereas expression was found in 1 (5.9%) of 17 B-precursor ALL cell lines and 1 (10.0%) of 10 T-ALL cell lines (Table 1; Fig. 3B). Expression of the HOXD11 gene was found significantly more frequently than that of
the HOXD13 gene in ALL cell lines and BCR-ABL-positive leukemic cell lines (P = 0.0007 and P = 0.038, respectively).

The HOXD13 gene has been identified as a fusion partner of the NUP98 gene in t(2;11)(q31;p15)-AML (7). In the present study, we isolated a novel chimeric transcript, NUP98-HOXD11, in a de novo pediatric patient with AML (M4) having t(2;11)(q31;p15), which was the same translocation breakpoint as NUP98-HOXD13. Recent molecular analysis has shown that different genes in the same chromosomal region were identified as fusion partners of MLL, such as MLL-ELL, MLL-ENL, and MLL-EEN in t(11;19)(q23;p13) (2). Our result suggests that it is difficult to predict the fusion partner of the NUP98 gene in t(2;11)(q31;p15) only by cytogenetics.

Two NUP98-HOXD11 fusion transcripts are predicted to encode a protein of 499 amino acids and 547 amino acids, respectively. Both proteins are caused by the alternative splicing consist of an NH2-terminal FG repeat motif and COOH-terminal homeodomain. The difference between these chimeric proteins is attributable to the number of FG peptide repeats. Both the NH2-terminal FG repeat motif and COOH-terminal homeodomain are retained in the NUP98-HOXD11, NUP98-HOXD13, and NUP98-PMX1 fusion proteins. Mice transplanted with bone marrow cells expressing NUP98-HOXA9 through retroviral transduction acquire a myeloproliferative disease and eventually succumb to AML (23). This suggests that NUP98-HOXD11 could use a similar mechanism that leads to leukemogenesis.

**HOXD11**, a human homologue of mouse Hoxd11, is a homeobox gene. The function of the HOXD11 gene in hematopoiesis remains unclear, although other HOX genes, such as the HOXC cluster and HOX9, were reported to be important in hematopoiesis (15, 24). The Hoxd11 gene contributes to the development of front legs in mice (25).

<table>
<thead>
<tr>
<th>Classifications of cell lines</th>
<th>Total no. examined</th>
<th>Expression</th>
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<tr>
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<tr>
<td>EBV-B</td>
<td>5</td>
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<tr>
<td>Normal</td>
<td>3</td>
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*Phi, B-precursor ALL with BCR-ABL transcripts; Normal, peripheral blood mononuclear cells from healthy adults.

**Table 1** Expression of the HOXD11 and HOXD13 genes in leukemia and EBV-B cell lines and normal lymphocytes by RT-PCR

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Fig. 2. Detection of the novel NUP98 partner gene, the **HOXD11** gene. A, schematic representation of primers for a cDNA panhandle PCR strategy and RT-PCR. B, The NUP98-HOXD11 chimeric transcript detected by cDNA panhandle PCR and RT-PCR. M, size marker; Lane 1, cDNA panhandle PCR products; Lanes 2, 4, and 5, NUP98-HOXD11 chimeric transcripts detected by RT-PCR; Lane 2, patient; Lane 4, leukemic cell line (NB4); Lane 5, water; Lane 3, patient (HOXD11-NUP98 chimeric transcripts). C, sequencing of two NUP98-HOXD11 chimeric transcripts. Arrows, the fusion point. D, structure of the predicted NUP98, HOXD11, and NUP98-HOXD11 fusion proteins. GLEBS, Gle2p-binding like motif; HD, homeodomain. E, cDNA sequence (1017 bp) of human HOXD11 with predicted amino acid sequence. Underlined, the homeodomain. Vertical arrow, the position of the t(2;11) breakpoint.
Expression of the HOX genes in hematopoietic cell lines results in differences in lineage commitment and maturation of these cells (15); for example, expression of the HOXA10 gene was restricted to myelomonocytic cell lines (26) and expression of the HOXC4 gene was predominant in lymphoid cell lines (27). In HOXD cluster genes, it was reported that expression of the HOXD3 gene was found in HEL and K562 but not in HL-60 (28), whereas expression of the HOXD13 gene was found in HEL, Kasumi-1, and MOLT-4 but not in U937 (18). Our study revealed that expression of the HOXD11 gene was significantly higher in BCR-ABL-positive leukemic cell lines than in BCR-ABL-negative leukemic cell lines (P = 0.028). The reason for high expression of the HOXD11 gene in BCR-ABL-positive leukemic cell lines is unclear. Expression of the HOXD11 gene was found more frequently in BCR-ABL-negative B-precursor ALL cell lines and CML cell lines than in other cell lines, but these differences were not significant statistically. Notably, expression of the HOXD13 gene was significantly higher in myeloid cell lines than in lymphoid cell lines (P < 0.0001). Overexpression of Hoxb8 with inter leukin-3, Hoxa10, Hoxb3, and Hoxa9, with Meis1 in murine hematopoietic cells was reported to lead to the development of AML (29–32). Our results, combined with previous reports, suggest that the expression of one or more HOX genes, including the HOXD11 and HOXD13, is associated with the development or proliferation of leukemia.

In conclusion, our study revealed that t(2;11)(q31;p15) was not a single chromosomal abnormality; it results from not only the NUP98-HOXD13 but also the NUP98-HOXD11 fusion gene. Although expression of the HOXD11 and HOXD13 genes in leukemic cell lines was different, the NUP98-HOXD fusion genes encode similar fusion proteins, suggesting that the NUP98-HOXD11 as well as NUP98-HOXD13 fusion protein play a role in leukemogenesis through similar mechanism. To clarify the roles of NUP98-HOXD11 and NUP98-HOXD13 fusion proteins in leukemogenesis, further functional analysis of both fusion proteins and accumulation of cases with t(2;11) is needed.

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References

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