

ORIGINAL ARTICLE

Molecular cloning of t(2;7)(p24.3;p14.2), a novel chromosomal translocation in myelodysplastic syndrome-derived acute myeloid leukemia

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In this study, we report the molecular structure of the breakpoint region in a new chromosomal translocation, t(2;7)(p24.3;p14.2), in a case of acute myeloid leukemia transformed from myelodysplastic syndrome (MDS). An extensive fluorescence *in situ* hybridization (FISH) analysis showed that *NAG* (2p24.3) and *ELMO1* (7p14.2) were involved at the breakpoints of t(2;7)(p24.3;p14.2). Furthermore, we detected a novel chimeric transcript consisting of *NAG* and *ELMO1*. Interestingly, this transcript encoded a truncated molecular form of 3' *ELMO1* as the result of a frameshift caused by the chromosomal translocation. Although this study does not provide direct evidence that a defect in *NAG-ELMO1* plays a role in the pathogenesis or the leukemic change in MDS, it does suggest that defects in *NAG-ELMO1* potentially contributed to the leukemic progression in this case.

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INTRODUCTION

Myelodysplastic syndrome (MDS) is a clonal hematopoietic stem-cell disorder characterized by ineffective hematopoiesis, trilineage dysplasia, marrow dysfunction and susceptibility to acute myeloid leukemia (AML).^{1–6} Although the molecular mechanisms of MDS and its progression to AML have not been clarified, frequent chromosomal abnormalities, such as an entire or a partial deletion of the long arm of chromosome 5 or 7 (–5/5q– or –7/7q–), trisomy 8, der(1;7), deletion 20 and Y-missing, have been reported, suggesting an unidentified oncogene and/or tumor-suppressor gene to be involved in the pathogenesis and/or progression of this disease.^{5,7} Balanced-type chromosomal translocations are infrequently observed in MDS, for example, t(5;12)(q33;p13), t(11;16)(q23;p13) and t(3;21)(q26;q21),^{6,8} generating *TEL/PDGFR-β*,⁹ *MLL/CBP*¹⁰ and *EVI-1/AML1*¹¹ chimeric genes, respectively, which are involved in this disorder. Specific chromosomal abnormalities, especially translocations, found in hematological and nonhematological malignancies have provided important insights into the pathogenesis of malignant transformation, prompting us to explore and characterize novel chromosomal translocations in MDS or MDS-derived AML to understand the pathogenesis as well as to establish novel therapeutic strategies of this malignancy.

Here, we report a new chromosomal translocation, t(2;7)(p24.3;p14.2), observed in leukemic cells in a patient with AML transformed from MDS. Through a detailed characterization of the molecular structure of the break points involved in this translocation, we have identified a novel chimeric transcript consisting of the neuroblastoma-amplified gene (*NAG*)^{12,13} at 2p24.3 and the engulfment cell motility gene 1 (*ELMO1*)¹⁴ at 7p14.2. The expression of *NAG-ELMO1*, in which the 3' terminal of *ELMO1* was truncated as a result of a frameshift caused by the chromosomal translocation, was detected.

MATERIALS AND METHODS

Case report and chromosomal analysis

A 62-year-old man was admitted to hospital in May 1997 with fever and pneumonia. At diagnosis, his white blood cell count was 1800 μl⁻¹, hemoglobin level was 4.6 g dl⁻¹ and platelet count was 30 000 μl⁻¹, indicating pancytopenia. Bone marrow aspirated with informed consent was hypocellular with 17.6% blasts, expressing CD13, CD33 and CD34. On the basis of these findings and a normal cytogenetic pattern, he was diagnosed as having myelodysplastic syndrome (RAEB) with a normal karyotype, 46,XY and treated with granulocyte colony-stimulating factor. After 5 months, he developed AML-M4 with t(2;7)(p24.3;p14.2) in all 20 bone marrow metaphase cells that were analyzed

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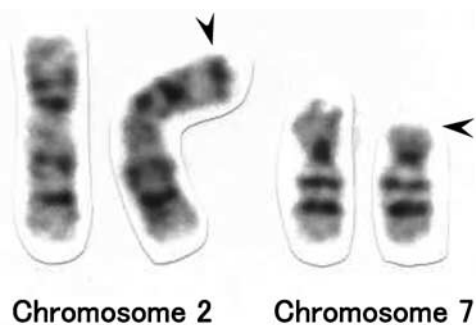


Figure 1 G-banded partial karyogram from a bone marrow cell. Arrowheads indicate each of the break points of the reciprocal translocation, t(2;7)(p24.3;p14.2).

(Figure 1), and received reinduction therapy with daunorubicin, cytarabine, 6-mercaptopurine and prednisolone. Although he temporarily achieved complete remission, he died 52 months after diagnosis from hypocellular marrow with atypical myeloid cells and serious pancytopenia. The translocation t(2;7)(p24.3;p14.2) was found as the sole anomaly, which disappeared during remission and reappeared at a relapse of the disease.

Fluorescence *in situ* hybridization (FISH)

FISH was carried out as described elsewhere.¹⁵ According to information from human genome databases (<http://www.ncbi.nlm.nih.gov/> and <http://genome.ucsc.edu/>), we selected and used a total of 12 and 17 bacterial artificial chromosome clones (BACs) and P1-derived artificial chromosome clones (PACs) as probes, respectively, spanning the breakpoints of t(2;7)(p24.3;p14.2), and made contig maps around the break points (Figure 2).

Reverse-transcription (RT)-PCR and direct sequence analysis

Mononuclear cells were separated from BM samples with Lymphoprep (Nycomed pharma, Oslo, Norway). Total RNA was extracted with TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) and single-stranded cDNA was synthesized using SuperScript First Strand Synthesis (Invitrogen). RT-PCR was carried out using FastStart Taq (Roche Diagnostics, Tokyo, Japan) according to the manufacturer's instructions, using a series of primer sets designed on the basis of the sequences of BAC/PACs (<http://www.ncbi.nlm.nih.gov/>). All primer sequences are available on request. Each PCR product was separated by gel electrophoresis. Direct sequencing of the RT-PCR product was carried out with a Cyclist DNA sequencing kit (Stratagene, La Jolla, CA, USA). The results of the sequencing analysis were compared using the Basic Local Alignment Search Tool (BLAST) of the NCBI (<http://www.ncbi.nlm.nih.gov/>).

RESULTS AND DISCUSSION

The karyotype of mononuclear cells from the bone marrow aspirate of this patient at AML transformation showed a reciprocal translocation, possessing a break point on the short arm of chromosome 2 and the short arm of chromosome 7. As we first presumed his t(2;7) from a variant of t(7;11)(p15;p15), which is known to generate the *HOXA9/NAP98* fusion gene in myeloid leukemia,^{16,17} we conducted a FISH analysis using the PAC clone, RP1-170O19, containing the *HOXA9* gene. However, clear signals of this clone were located on normal chromosome 7 and der(2)t(2;7), indicating that the break point of der(7) lies proximal to *HOXA9*. Thus, we carried out a series of FISH analyses using additional 9 BAC clones. Signals from BAC RP11-7P19 to BAC RP11-340F1 were translocated to der(2) (Figure 2a and data not shown), whereas the signal from BAC RP11-164E6 was detected on normal chromosome 7 but not on der(2) or der(7) t(2;7) (Figure 2b). Interestingly, the signal from BAC RP11-59F6 that was observed on der(7) was weaker than that on normal chromosome 7 (Figure 2c), suggesting a cryptic deletion to be involved. According to

the human genome database (<http://www.ncbi.nlm.nih.gov/> and <http://genome.ucsc.edu/>), only *ELMO1*, a human ortholog of the *Caenorhabditis elegans* gene, *Ced12*,¹⁴ is located around the break point of der(7) (Figure 2a), suggesting *ELMO1* to be involved in the rearrangement and/or deletion at break point.

Next, we carried out FISH to determine the break point involved in der(2)t(2;7)(p24.3;p14.2). As shown in Figure 2d, the clones between BAC RP11-375P12 and BAC RP11-314E10 were translocated to der(7), whereas the clones from BAC RP11-65H13 to BAC RP11-111P18 were retained on der(2). Notably, BAC RP11-516B4 showed signals split between der(2) and der(7) (Figure 2e). According to the human genome database (<http://www.ncbi.nlm.nih.gov/> and <http://genome.ucsc.edu/>), only *NAG* is located within this clone (Figure 2d), suggesting *NAG* to be involved in the break point of der(2)t(2;7)(p24.3;p14.2) in this case.

As (a) *ELMO1* or *NAG* is located around each of the break points involved in this t(2;7)(p24.3;p14.2) and (b) these two genes are arranged in the same orientation, we speculated that a rearrangement of *ELMO1* and *NAG* might generate a chimeric gene through the chromosomal translocation. To test this hypothesis, we tried to connect exons by RT-PCR using a series of primer sets designed on the basis of the 5' of *NAG* and 3' of *ELMO1* or the 5' of *ELMO1* and 3' of *NAG*. An RT-PCR product of minimal size was detected using the former set between exon 38 of *NAG* and exon 14 of *ELMO1*, but not the latter set between exon 13 of *ELMO1* and exon 39 of *NAG* (Figure 3a). Sequencing of the product showed that the 3' end of exon 38 of *NAG* was fused to the 5' end of exon 14 of *ELMO1* in the chimeric transcript, resulting in a truncated deduced amino acid sequence of the 3'-portion of *ELMO1* because of a frameshift of the coding sequence of this gene (Figure 3b). The expression of this chimeric transcript was confirmed by RT-PCR, using primer sets designed to connect the possible start codon of *NAG* and possible novel stop codon of *ELMO1* and sequencing of the RT-PCR product (Figure 3c), suggesting that a truncated fusion protein may be expressed in the leukemia cells of the patient. The deduced amino acid sequence of *NAG-ELMO1* contains most of *NAG* and a very short additional sequence at the C terminus. As no functional characterization of the *NAG* protein has ever been reported and no functional motif has been mapped in this protein sequence, the functional significance of the truncated *NAG-ELMO1* protein to leukemogenesis remains unclear. Our results of FISH analysis showed a cryptic deletion of the 5'-portion of *ELMO1* through the translocation (Figures 2a–c), suggesting that the reciprocal *ELMO1-NAG* transcript was not expressed, possibly because of deletion of the 5'-portion of *ELMO1*. The expression of intact *NAG* and *ELMO1*, which are possibly transcribed from intact chromosomes 2 and 7, respectively, was detected by RT-PCR (data not shown).

MDS is a heterogeneous disorder, with several specific chromosomal abnormalities involved in its pathogenesis. However, it is relatively rare for a chromosomal translocation to affect the pathogenesis of AML developed from MDS. Recently, a monocytic leukemia cell line, ELAM1, was established from a patient with AML that had progressed from MDS.¹⁸ Cytogenetically, both the leukemic cells of the patient and ELAM1 cells indicated t(11;21)(q13;q22). The fusion transcripts of *RUNX1/LRP16* and the reciprocal *LRP16/RUNX1* were also detected in ELAM1 cells. Although the function of *LRP16* remains unknown, the chromosomal translocation, t(11;21), is thought to contribute to the onset of the leukemic transformation from MDS, as this translocation is reported to disappear during remission and to reappear at relapse. In relation to the leukemic transformation of fusion genes, animal models have provided important insights into the

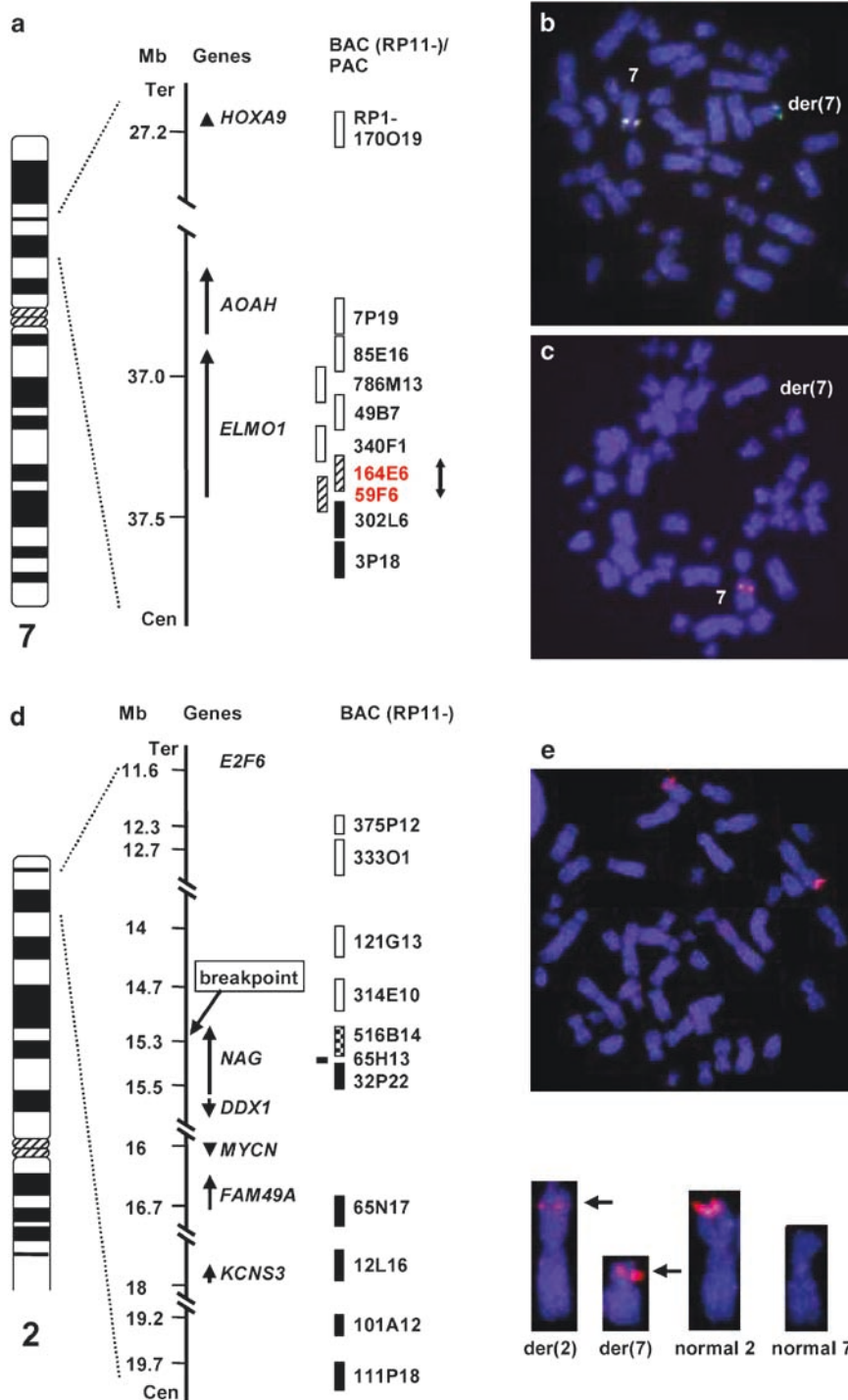


Figure 2 Maps around break points and representative results of metaphase FISH experiments. **(a and d)** The positions, according to the human genome database (<http://genome.ucsc.edu/>), of bases, genes and the BACs/PACs used as probes for FISH are represented to the right of chromosomes 7 **(a)** and 2 **(d)** ideograms. Open boxes, black boxes and oblique-lined boxes represent clones located on the partner chromosomes, clones remaining on the original chromosomes and clones with an entirely or partially deleted signal in FISH, respectively. The checked box represents the clone with a split signal pattern on FISH. A closed arrow indicates the possibly deleted region on chromosome 7. **(b)** Representative result of two-color FISH using BAC RP11-302L6 (green) and BAC RP11-164E6 (red). Green signals were observed on normal chromosome 7 and der(7), whereas red was observed only on normal chromosome 7. **(c)** Representative result of FISH using BAC RP11-59F6 (red). Red signals were weakly observed on the der(7) chromosome compared with normal chromosome 7. **(e)** Representative result of FISH using BAC RP11-516B14 (red). Red signals were observed on normal chromosome 2 as well as der(7) and der(2) (upper panel). Enlarged images of normal chromosomes 2 and 7 and those of der(2) and der(7) are shown in the lower panel.

molecular mechanisms underlying the progression from MDS to AML. Using a NUP98-HOXD13 fusion gene, Lin *et al.*¹⁹ developed a mouse model with all the key clinical features of MDS, including

peripheral blood cytopenias, bone marrow dysplasia and apoptosis and transformation to AML. In contrast, the AML1/RUNX1 gene point mutation has been shown to be one of the major causes of

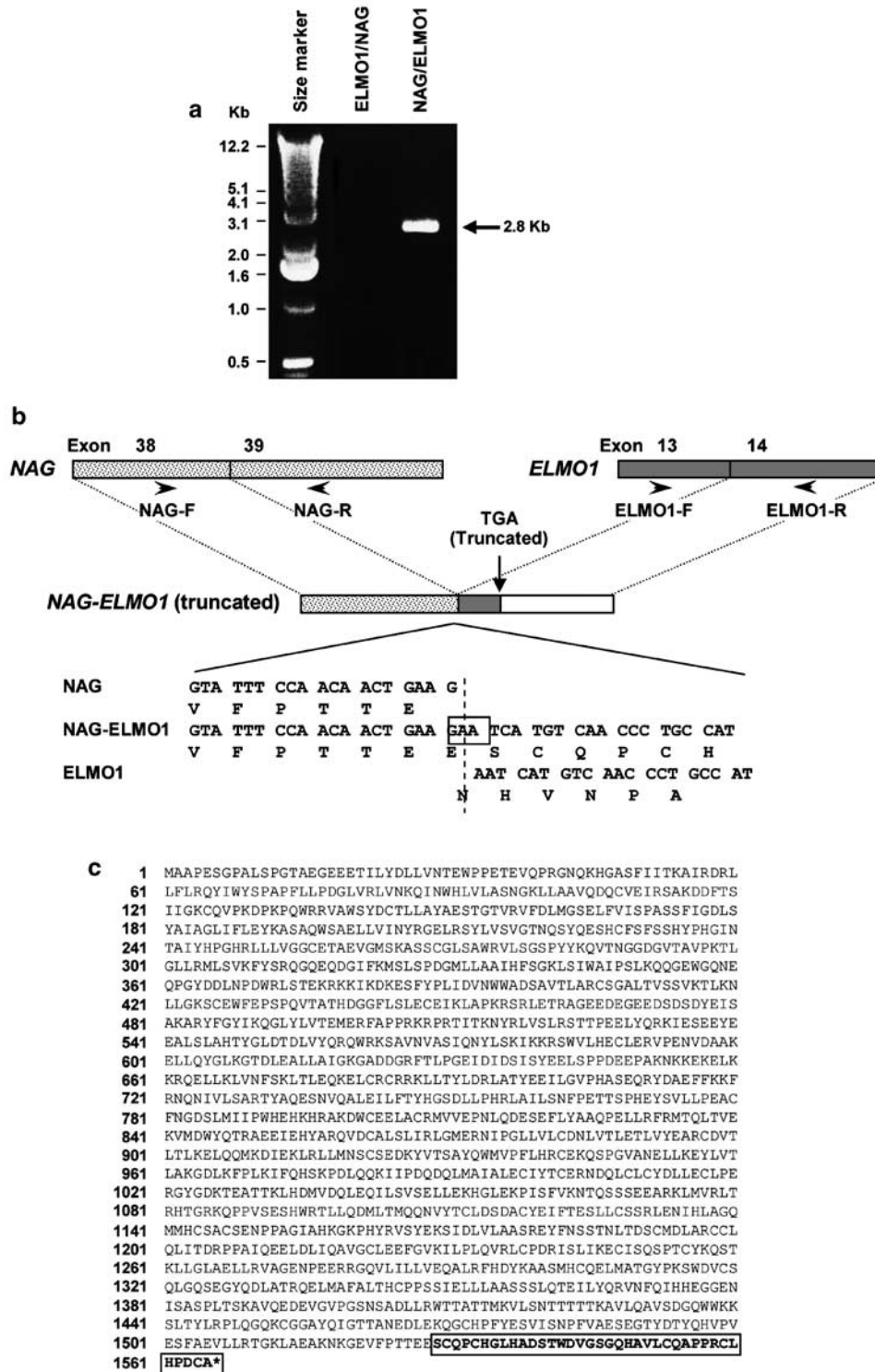


Figure 3 Structure of the fusion point of the t(2;7)(p24.3;p14.2) translocation. (a) Identification of the *NAG-ELMO1* chimeric transcripts by RT-PCR in the patient. 1 Kb DNA ladder (Invitrogen) was used as a size marker. The *NAG-ELMO1* transcript was detected by RT-PCR, but the reciprocal *ELMO1-NAG* transcript was not. (b) Schematic representation of the *NAG-ELMO1* mRNA junction and nucleotide and deduced amino acid sequences around the junction between exon 39 of *NAG* and exon 14 of *ELMO1*. Exon numbers are given above the map. Dotted boxes and gray boxes indicate exons of *NAG* and *ELMO1*, respectively. The positions and orientations of primers used in (a) are indicated by arrowheads. The *NAG-ELMO1* transcript generated by t(2;7)(p24.3;p14.2) was a truncated molecular form of 3' *ELMO1*, produced as the result of a frameshift. (c) Deduced amino acid sequence of the *NAG-ELMO1* chimeric transcript. An open box indicates the sequence from *ELMO1*. The asterisk indicates the TGA termination codon.

refractory anemia with an excess of blasts (RAEB), RAEB in transformation (RAEB-t) and AML, following the molecular alteration of MDS.²⁰ We did not investigate the AML1/RUNX1 gene point mutation in this case.

Rosenfeld and List²¹ have suggested a multi-stepped pathogenesis of MDS. In the first step, progenitor cell injury gives rise to genomic instability. This instability leads to stepwise gene mutations and/or chromosomal aberrations, the subsequent accumulation of which results in the diverse features of MDS. According to this hypothesis, the translocation t(2;7) during the second stage of pathogenesis is involved in the leukemic transformation from MDS. Interestingly, Paulsson *et al.*²² reported a 1.8-Mb hemizygous deletion at 7p14.1, which was identified in a case with MDS transforming to AML, using high resolution array-based comparative genomic hybridization, although the deleted region was more centromeric to *ELMO1* and did not include this gene. Conversely, it is possible that t(2;7) observed in leukemic cells in this case was one of the random chromosomal aberrations that were already present in a subset of RAEB cells through genomic instability. Moreover, an unknown genetic event responsible for progression to an overt leukemia occurred in one of the t(2;7) marker-positive cells, resulting in a clonal expansion.

Two molecular mechanisms resulting from chromosomal translocations are known to produce hematological malignancies.²³ The first mechanism involves the formation of a chimeric transcript that has a dominant-negative effect or strong tyrosine kinase activity. The second mechanism involves the regulation of gene functions by a promoter located upstream of target genes. Although there is no direct evidence that a defect in *NAG-ELMO1* plays a role in the pathogenesis or leukemic transformation of MDS, our observation suggests that *NAG-ELMO1* contributes to disease progression because of the consistent appearance of t(2;7) in leukemic cells. Exploration of the cytogenetic and genetic alterations in MDS may increase our understanding of the molecular mechanisms underlying this disease and aid in the development of novel therapeutic strategies.

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