NEOPLASIA

NUP98 Is Fused to *PMX1* Homeobox Gene in Human Acute Myelogenous Leukemia With Chromosome Translocation t(1;11)(q23;p15)

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The nucleoporin gene *NUP98* was found fused to the *HOXA9*, *HOXD13*, or *DDX10* genes in human acute myelogenous leukemia (AML) with chromosome translocations t(7;11)(p15; p15), t(2;11)(q35;p15), or inv(11)(p15;q22), respectively. We report here the fusion between the *NUP98* gene and another homeobox gene *PMX1* in a case of human AML with a t(1;11)(q23;p15) translocation. The chimeric *NUP98-PMX1* transcript was detected; however, there was no reciprocal *PMX1-NUP98* fusion transcript. Like the *NUP98-HOXA9* fusion, *NUP98* and *PMX1* were fused in frame and the N-terminal GLFG-rich docking region of the NUP98 and the PMX1 homeodomain were conserved in the NUP98-PMX1 fusion, suggesting that PMX1 homeodomain expression is upregulated and that the fusion protein may act as an oncogenic transcription factor. The fusion to NUP98 results in the addition of the strong transcriptional activation domain located in the N-terminal region of NUP98 to PMX1. These findings suggest that constitutive expression and alteration of the transcriptional activity of the PMX1 homeodomain protein may be critical for myeloid leukemogenesis.

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HOMEOBOX GENES represent a class of transcription factors that share a conserved DNA binding motif called the homeodomain. This domain defines the DNA binding specificity of the protein and plays an important role in transcriptional regulation of downstream target genes.[1] Homeobox proteins control embryonic development in organisms and function in adult tissue, including the hematopoietic system,[2,3] in which expression of class I *HOX* genes has been well characterized. The *HOXA* and *HOXB* genes in the 3′ region of the clusters are expressed in most primitive bone marrow cells and are downregulated as CD34$^+$ hematopoietic progenitors differentiate toward erythroid and myeloid phenotypes. Genes in the 5′ regions are expressed at nearly equal levels in CD34$^+$ subpopulations.[4] There is also good evidence that several non-class I homeobox genes, such as *Hox11*,[5] *Hex*,[6] and *Oct-2*,[7] are important in maintaining normal myeloid, erythroid, or lymphoid populations. These findings indicate that a broad range of homeobox genes are required for proper differentiation and function of hematopoietic lineages.

The important role of homeobox genes in the hematopoietic system is also indicated by the fact that several homeobox genes are involved in leukemogenesis.[2,3,8] In the mouse, a retrovirus-like intracisternal A particle is integrated in the 5′ end of the *Hoxb8* gene in the myelo-monocytic cell line WEHI-3B.[9,10] Recently, cooperative activation of *Hoxa7/a9* and another homeobox gene *Meis1* has been identified in retrovirus-induced BXH-2 mouse myeloid leukemia.[11] Cooperative leukemogenic action of *Hoxa9* and *Meis1* was further demonstrated by the induction of leukemia using retroviral transfer of the genes to mouse primary bone marrow cells.[12] In humans, overexpression of the *HOX11* gene occurs in rare cases of human T-cell acute lymphoblastic leukemia (ALL) that have the chromosomal translocations t(10;14)(q24;q11) or t(7;10)(q35;q24) in which the *HOX11* gene is activated due to juxtaposition of promoter elements from the T-cell receptors.[13-16] In these cases, upregulation of the homeobox genes is thought to cause inhibition of hematopoietic differentiation and/or abnormal proliferation of primitive bone marrow cells by deregulating expression of downstream target genes.

Another type of homeobox gene involvement in human leukemias is the synthesis of a fusion protein owing to specific chromosomal translocation. Gene fusion between *E2A* and *PBX1* was identified in pre-B ALL with t(1;19)(q23;p13.3).[17,18]

Expression of *E2A-PBX1* induces both myeloid and T-cell leukemia in a transgenic mouse model,[19] and expression of *E2A-PBX1* in cultured bone marrow cells blocks differentiation of myeloid progenitors.[20,21] We and another group have recently found that the nucleoporin gene *NUP98* is fused to *HOXA9* in human acute myelogenous leukemia (AML) with the chromosomal translocation t(7;11)(p15;p15).[22,23] As a result of the gene fusion, the major part of the *HOXA9* including the DNA binding homeodomain was fused in frame to a *NUP98* domain encoding the N-terminal GLFG repeat. Both mouse and human data indicated that abnormal expression of the *HOXA9* homeodomain might play an important role in leukemogenesis.

NUP98 has been found fused to the putative RNA helicase gene *DDX10* in a few cases of human AML with inv(11)(p15; q22),[24] suggesting that the N-terminal domain of NUP98 might also have a leukemogenic potential. In this study, we have shown that the *NUP98* is involved in the t(1;11)(q23;p15) translocation in a case of the secondary AML and that the gene is fused to another homeobox gene *PMX1* located at chromosome 1q23. *NUP98-PMX1* fusion transcripts were detected, and our data suggested that the chimeric protein consisting of the NUP98 N-terminal docking site and the PMX1 homeodomain might function as an oncogenic transcriptional activator.

MATERIALS AND METHODS

Patient material. Leukemia cells were obtained from a 55-year-old man with AML of an M2 subtype associated with a t(1;11)(q23;p15)

translocation. Details of clinical data and karyotypic analysis will be reported elsewhere (Hatano et al, manuscript submitted). The patient had a history of treatment for non-Hodgkin's lymphoma as the primary malignancy and had received chemotherapy, including the use of topo II inhibitors. He developed AML 3 years after the initial chemotherapy against the lymphoma.

DNA extraction and Southern blot analysis. High molecular weight genomic DNA was extracted from frozen leukemia cell suspensions. Samples of 5 μg of genomic DNA were subjected to restriction endonuclease digestion, agarose gel electrophoresis, Southern blot transfer, and hybridization according to the methods previously described.[25] A 350-bp *Hae*III fragment[22] was used as a probe to detect *NUP98* rearrangements.

cDNA cloning of the NUP98 fusion partner. Poly(A)$^+$ RNA was isolated from frozen leukemia cell suspensions using the Fast-Track 2.0 kit (Invitrogen, Carlsbad, CA). To isolate the unknown gene at chromosome 1q23, 3′-rapid amplification of cDNA ends (3′-RACE)[26] was performed using the Marathon cDNA Amplification kit (Clontech, Palo Alto, CA) with *NUP98* gene-specific primers 3R-1 (5′-TCTTGGTACAGGAGCCTTTGGG-3′) and 3R-2 (5′-CTC-TTGGTGCTGGACAGGCATC-3′) and adaptor primers AP-1 (5′-CCA-TCCTAATACGACTCACTATAGGGC-3′) and AP-2 (5′-ACTCAC-TATAGGGCTCGAGCGGC-3′). The 3′-RACE products were subcloned into the pCR2.1 vector (Invitrogen), and plasmid clones were sequenced using a Thermo Sequenase dye terminator cycle sequencing kit (Amersham, Cleveland, OH).

Reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted from leukemic cell suspensions by using the RNAzol method (TelTest, Friendswood, TX). First-strand cDNA was synthesized from 1 μg total RNA using random hexamers and Superscript II reverse transcriptase (Life Technologies, Rockville, MD) in a total volume of 20 μL. The mixture was diluted to 50 μL, and 2 μL was used for PCR. PCR was performed using 2.5 U of Taq DNA polymerase (Boehringer Mannheim, Mannheim, Germany) and a Perkin Elmer Thermal Cycler (Perkin Elmer, Norwalk, CT) using the following temperature cycling protocol: 94°C for 30 seconds, 60°C for 1 minute, and 72°C for 2 minutes for 35 cycles. PCR products were separated

by electrophoresis through a 2.5% GTG gel (FMC Bioproducts, Rockland, ME), subcloned into the pCR2.1 plasmid, and sequenced. The PCR primers for *NUP98* and *PMX1* used were as follows: n1, 5′-CTCTTGGTGCTGGACAGGCATC-3′; n2, 5′-CTAGGGATGGTT-CATCGTC-3′; p1, 5′-AGCTACGGGCACGTTCTGG-3′; P2, 5′-AGG-GGTCTGCGATGGTGGTGTGG-3′; and P3, 5′-GCACAAAAGCAT-CAGGATAGTG-3′.

Northern blot analysis. The full-length human *PMX1* cDNA was used to probe Human Multiple Tissue Northern blots (Clontech). Membranes were hybridized and washed according to the method of Church and Gilbert.[27]

Construction of expression plasmids. cDNAs containing full-length *NUP98-PMX1* and wild-type *PMX1* coding sequences were obtained by RT-PCR on RNA samples extracted from leukemia cells and by PCR of the human heart cDNA (Clontech), respectively. The 5′ and 3′ oligonucleotides used for PCR contained unique *Eco*RI and *Bam*HI restriction sites to facilitate cloning into the pM eukaryotic expression plasmid in frame with sequences encoding the GAL4 DNA binding domain.[28] These full-length cDNAs were also subcloned into pCDNA3.1 expression vector (Invitrogen). Truncated *NUP98* and *PMX1* sequences were obtained by PCR amplification of full-length clones. To generate the pMCK1-1 reporter plasmid that contains the 246-bp murine muscle creatine kinase (MCK) basal enhancer,[29] the region from bp −1250 to −1048 relative to the MCK transcription start site was amplified by PCR and subcloned into the pGL3 promoter vector (Promega, Madison, WI). All constructs were verified by sequencing.

Transient transfection assays. HeLa cells were seeded 18 to 24 hours before transfection at 5×10^5 cells per 60-mm dish in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. One microgram of each pM fusion construct, 1 μg of 5 × GAL4-pGL2 plasmid,[30] and 0.1 μg of pRL-SV40 plasmid (Promega) were cotransfected by using Lipofectamin and Opti-MEM (Life Technologies). The pCDNA vectors containing full-length cDNAs of *PMX1* or *NUP98-PMX1*, and pMCK1-1 were cotransfected into Jurkat cells (2×10^6 cells) using DIMRIE-C reagent (Life Technologies) and Opti-MEM. Cells were harvested 24 hours posttransfection in 1× passive lysis buffer (Promega). Luciferase activity was measured in a luminometer
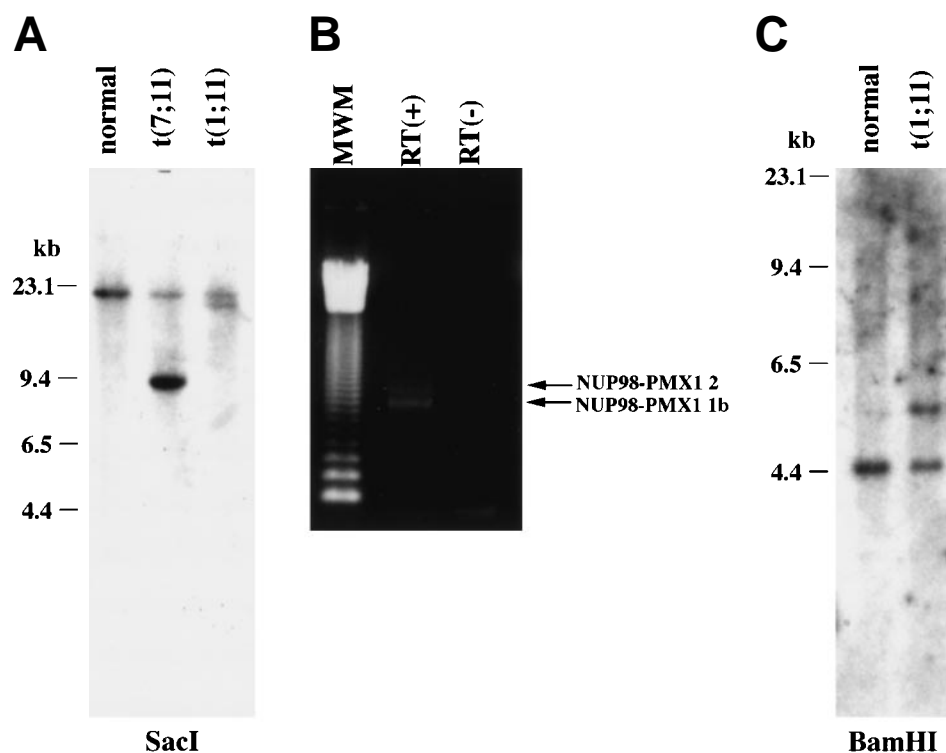

Fig 1. (A) Southern blot analysis of the leukemia cell sample derived from t(1;11)(q23;p15) AML. Genomic DNA samples from the t(7;11)(p15;p15) AML patient as well as healthy control were also shown. Rearranged bands were detected both in t(1;11) patient as well as t(7;11) by *Sac* I digestion using a *NUP98* probe. (B) 3′-RACE products of *NUP98* fusion cDNA. MWM, 123-bp ladder molecular weight marker; RT(+), reverse transcribed and PCR-amplified patient poly(A)$^+$ RNA sample; RT(−), patient RNA without reverse transcription. (C) Southern blot analysis of the patient genomic DNA with the 3′-fragment of shorter RACE product as a probe. Rearranged band was detected in patient sample digested with *Bam*HI.

using a Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's protocol. The firefly luciferase activity derived from the reporter constructs was normalized to levels of renilla luciferase activity as an internal control for transfection efficiency. Experiments were repeated three times. The expression of GAL4 fusion proteins was analyzed by Western blotting as described below. The $5 \times$ GAL4-pGL2 expression plasmid was a generous gift of Cory Abate-Shen (UMDNJ, Piscataway, NJ).

Western blot analysis. Each transfectant (5×10^5 cells) was lysed in 100 μL of sodium dodecyl sulfate (SDS) sample buffer containing 50 mmol/L Tris (pH 6.8), 2% SDS, 10% glycerol, 100 mmol/L dithiothreitol, and 0.1% bromophenol blue, and 10-μL aliquots were subjected to SDS-polyacrylamide gel electrophoresis. The size-fractionated proteins were transferred by electrical field (Trans-Blot cell; Bio-Rad, Hercules, CA) onto a nitrocellulose membranes. Membranes were blocked in Tris-buffered saline (pH 8.0) containing 5% nonfat dry milk. The monoclonal anti-GAL4 DNA binding domain antibody (Santa Cruz Biotechnology, Santa Cruz, CA) was used as a primary antibody. The signals were detected using ECL Western blotting detection reagents (Amersham).

RESULTS

Cloning the breakpoint gene at 1q23. Chromosomal analysis of the patient bone marrow cells at the onset of AML showed a t(1;11)(q23;p15) (Hatano et al, manuscript submitted). This finding suggested the involvement of *NUP98* in chromosome 11 break. Southern blot analysis using a *NUP98* intron probe

showed the DNA rearrangement in the t(1;11) patient sample (Fig 1A). The result indicated that the chromosome 11 break was within the same intron as was seen in t(7;11) AML, resulting in a *NUP98-HOXA9* fusion.[22] Because the vast majority of human AML with reciprocal chromosomal translocations have shown that chimeric transcripts of the fused genes are synthesized,[8,31] we attempted to isolate the affected gene on chromosome 1q23 by using the 3′-RACE method.[25] Two RACE products of different sizes were identified (Fig 1B), and the t(1;11) DNA rearrangement was observed using the 3′-end of the shorter product as a probe (Fig 1C), indicating that the product contained a cDNA fragment derived from chromosome 1q23.

Sequence analysis confirmed that both the two 3′-RACE products contained unique sequences. Genetic database searching showed that the 3′-region of the longer product was identical to human *PMX1* (*PHOX1*).[32] The shorter product did not show significant homology to *PMX1*; however, further analysis showed that both products were located on the same genomic DNA fragment derived from chromosome 1, which was produced by Sanger Centre Human Chromosome 1 Genome Project (EMBL/GenBank Accession No. Z97200). The chromosome 1 derived parts of the RACE products were situated at 50 kb (Fig 2A). The shorter fragment was thought to
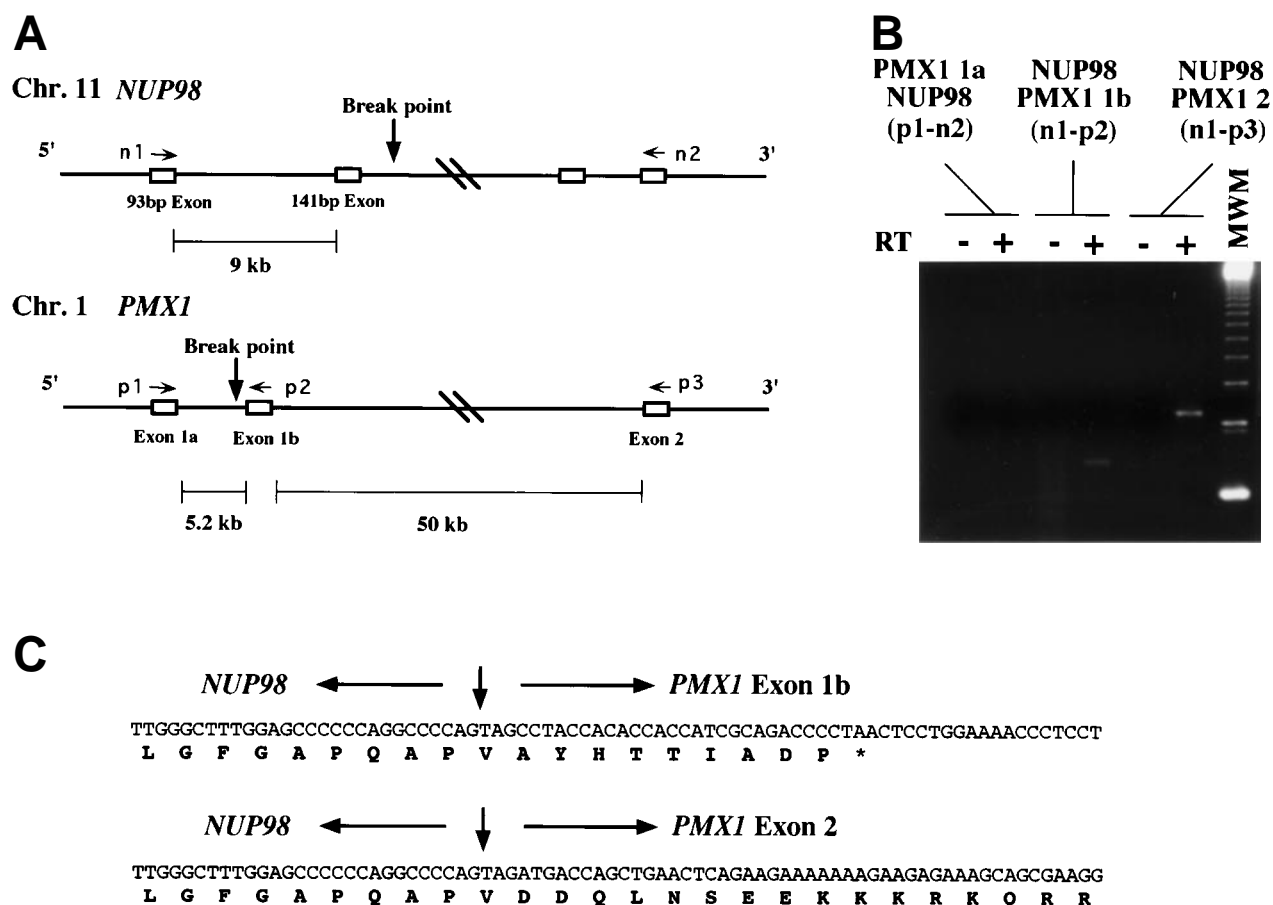

Fig 2. (A) Breakpoint at chromosomes 1 and 11. The 5′ exons of *NUP98* were previously described.[22] The location of the primers used for RT-PCR is indicated. (B) Detection of fusion transcripts between *NUP98* and *PMX1* exon 2 and between *NUP98* and *PMX1* exon 1b. A 270-bp product of *NUP98-PMX1* exon 2 fusion and a 168-bp product of *NUP98-PMX1* exon 1b fusion were observed, whereas no *PMX1* exon 1a-*NUP98* fusion was seen. MWM, 123-bp ladder molecular weight marker. + and − indicate the presence and absence of RT reaction, respectively. (C) Nucleotide and deduced amino acid sequences of *NUP98-PMX1* fusions.
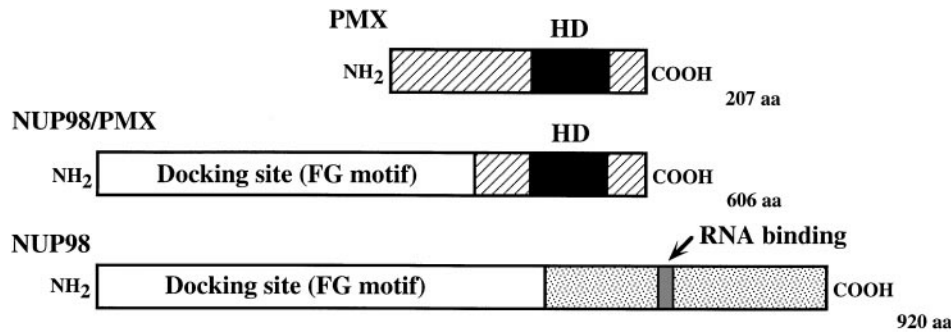
PMX

NUP98/PMX

NUP98

contain the noncoding exon 1b of the *PMX1* gene that is located 5.2 kb downstream of exon 1a (Fig 2A). The longer product contains exons 2 and 3 that encode the DNA binding homeodomain of *PMX1*.

Gene fusion between NUP98 *and* PMX1. RT-PCR analysis showed two *NUP98-PMX1*–derived chimeric transcripts, suggesting that the NUP98-PMX1 protein may have a biological function; however, no reciprocal *PMX1-NUP98* fusion transcript was detected (Fig 2B). Sequence analysis of 3′-RACE and RT-PCR products showed that *NUP98-PMX1* exon 1b encoded a protein that lacked *PMX1* functional domain (Fig 2C). *NUP98* and *PMX1* exon 2 were fused in frame, resulting in production of a chimeric protein containing the N-terminal half of NUP98[33] and most of the coding region of PMX1, including the entire homeodomain (Figs 2C and 3). HOX proteins, which show cooperative DNA binding with PBX, contain short conserved peptide motifs, including a key tryptophan required for the interaction with PBX.[34,35] The PBX-interaction domain is conserved in NUP98-HOXA9, and we observed cooperative DNA binding with PBX (Fujino and Nakamura, unpublished data). However, neither wild-type PMX1 nor NUP98-PMX1 contained such a motif.

Because murine *Pmx1* is expressed mainly in embryonic mesoderm, adult heart, and adult muscle tissue,[36] we analyzed the expression pattern of *PMX1* in human normal tissue. Northern blot analysis showed that *PMX1* was expressed in heart, muscle, thymus, prostate, testis, and ovary tissues, whereas no expression was detected in peripheral blood leukocytes (Fig 4). This suggested that *PMX1* may be downregulated in mature myeloid cells. Previous reports have shown ubiquitous *NUP98* expression,[23,24] and the expression is observed throughout the myeloid differentiation of murine 32D cells (Nakamura et al, unpublished data). Thus, expression of the C-terminal region of PMX1 as part of the fusion gene, which includes the entire homeodomain, may be driven by the *NUP98* promoter, leading to constitutive upregulation.

The N-terminal domain of NUP98 *modulates the transcriptional activity of* PMX1. *PMX1* encodes a homeodomain protein that acts as a sequence-specific transcription factor.[32] To assess the transcriptional activities of both wild-type and fusion PMX1 proteins, transient-transfection assays were performed. Wild-type *PMX1*, chimeric *NUP98-PMX1*, and deletion constructs of both *NUP98* and *PMX1* were subcloned into the pM eukaryotic expression vector in frame with sequences encoding the GAL4 DNA binding domain.[28] Constructs and the 5 × GAL4 pGL2 reporter plasmid, which contains GAL4 binding sequences, were cotransfected to HeLa cells. After production of GAL4 fusion proteins was confirmed by Western blotting (Fig 5A), luciferase activity was determined using a luminometer. As shown in Fig 5B, the wild-type PMX1 construct showed weak repression of the luciferase activity. The N-terminal part of PMX1, removed by the translocation, did not significantly
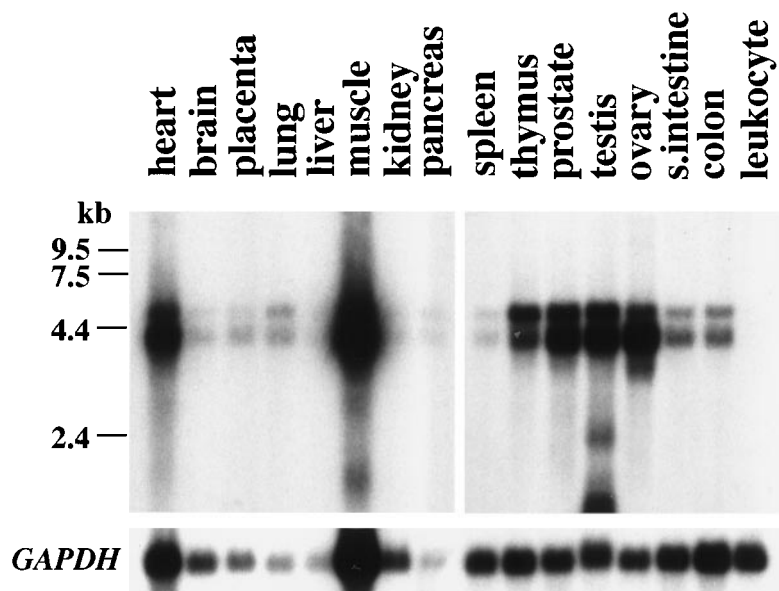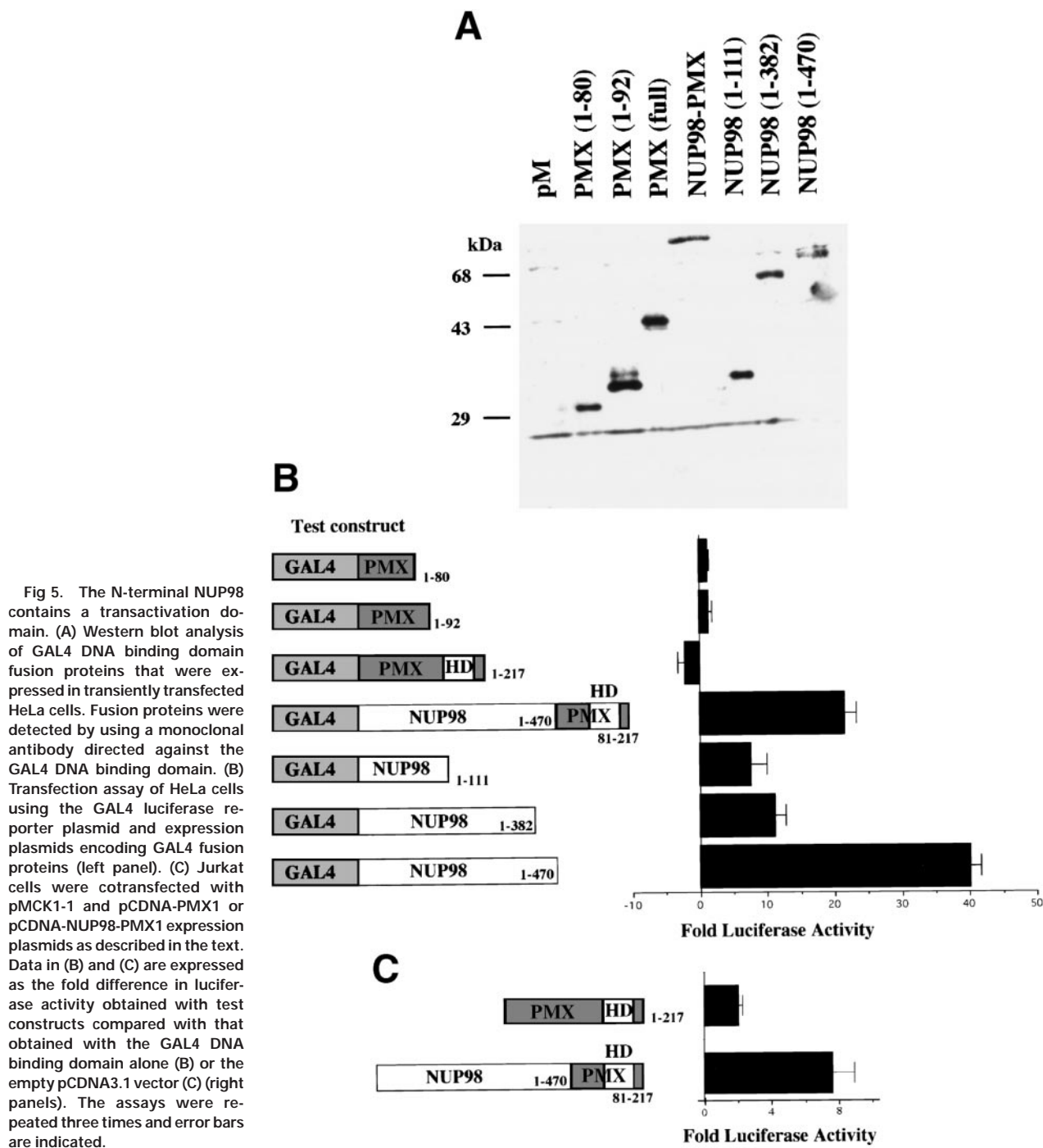
A

B

C

Fig 5. The N-terminal NUP98 contains a transactivation domain. (A) Western blot analysis of GAL4 DNA binding domain fusion proteins that were expressed in transiently transfected HeLa cells. Fusion proteins were detected by using a monoclonal antibody directed against the GAL4 DNA binding domain. (B) Transfection assay of HeLa cells using the GAL4 luciferase reporter plasmid and expression plasmids encoding GAL4 fusion proteins (left panel). (C) Jurkat cells were cotransfected with pMCK1-1 and pCDNA-PMX1 or pCDNA-NUP98-PMX1 expression plasmids as described in the text. Data in (B) and (C) are expressed as the fold difference in luciferase activity obtained with test constructs compared with that obtained with the GAL4 DNA binding domain alone (B) or the empty pCDNA3.1 vector (C) (right panels). The assays were repeated three times and error bars are indicated.

modulate the luciferase activity. The N-terminal NUP98 encodes a strong transcriptional activation domain that resulted in significant enhancement of transcriptional activity by the fusion protein construct. Similar results were seen when the human T-lymphocyte cell line Jurkat was transfected with the same series of constructs (data not shown).

Transcriptional properties of PMX1 and NUP98-PMX1 were also assessed using the murine MCK regulatory element, a known target of the PMX1 protein.[29,37] PMX1 showed only slight activation (1.9-fold) of luciferase activity, whereas NUP98-PMX1 showed significantly enhanced transactivation (7- to

8-fold; Fig 5C). Thus, NUP98-PMX1 fusion resulted in transactivation against the endogenous enhancer for PMX1.

DISCUSSION

We show here a novel fusion gene encoding a homeodomain protein that is synthesized due to a t(1;11) reciprocal chromosomal translocation. This is the fourth example of chimeric *NUP98* gene in leukemia. To date, this is the only case of a *NUP98-PMX1* gene fusion; however, there are similarities between the predicted NUP98-PMX1 protein structure and the NUP98-HOXA9 and NUP98-HOXD13[38] fusion. Our data

showed that the addition of the NUP98 N-terminal domain significantly altered the transcriptional activity of PMX1. In addition, upregulation of PMX1 homeodomain, under the control of the ubiquitously expressed *NUP98* promoter, may be relevant. The functional alterations of PMX1 activity might result in deregulation of target genes in bone marrow cells. Recently, Kasper et al[39] also reported potent transactivation of the NUP98-HOXA9 fusion protein.

PMX1 was first identified as a cofactor of a serum response factor.[32] PMX1 has sequence similarity to the *drosophila* paired protein; however, it contains a homeodomain as a unique DNA binding domain and lacks a paired domain.[32] The gene is mainly expressed in the embryonic mesoderm and in adult heart and skeletal muscle[36] and binds to the muscle creatine kinase gene enhancer element.[37] Mice homozygous for a mutant *PMX1* allele show defects in the formation and growth of chondrogenic and osteogenic precursors and defective skeletogenesis.[40] However, the function of PMX1 in the hematopoietic system and leukemogenesis remains unknown. Further study is required to clarify the physiological role of PMX1 in regulating myeloid differentiation and proliferation.

The present t(1;11) case of AML belongs to the M2 subtype that was also found in t(7;11) AML cases.[22,23] Although the homeodomain sequences are not highly similar between PMX1 and HOXA9, the similarities of the disease phenotype and structures of the fusion proteins suggest that PMX1 functions in a manner equivalent to that of HOXA9 in human AML. However, half of the inv(11) cases were myelodysplastic syndrome.[24] This phenotypic difference might be ascribed to functional distinctions between DDX10 RNA helicase and homeodomain proteins. NUP98-PMX1 fusion was not detected in 27 human acute leukemia samples, including AML subclasses M1 to M5, and B-cell and T-cell leukemias by RT-PCR (data not shown). The result is not very surprising, because genetic abnormalities of acute leukemias are very heterogeneous, with even common mutations seen in less than 1% of all the leukemia cases.[8] The t(1;11) case, 2 of 4 inv(11) patients,[24] and the t(2;11) case[38] were secondary leukemias and received chemotherapy, including topo II inhibitors against the primary neoplasms. There is growing evidence that cancer chemotherapies that include topo II inhibitors are one of the major causes of human AML.[41] Although the nucleotide sequences of genomic DNA at the breakpoints remain unknown, it might be speculated that topo II-sensitive sequences exist around the breakpoint of *NUP98*. The identical NUP98 N-terminal domain seen in the NUP98-HOXA9 fusion[22,23] was contained in the chimeric NUP98-PMX1 protein, suggesting that this domain may play a critical role in leukemogenesis.

The importance of the NUP98 N-terminal domain to leukemogenesis is also suggested by other examples of nucleoporin involvement in human AML. NUP214/CAN is fused to DEK in t(6;9)(p23;q34) AML[42] and to SET in a case of acute undifferentiated leukemia with an apparently normal karyotype.[43] In both NUP214/CAN-DEK and NUP214/CAN-SET fusion, the C-terminal domain of NUP214, which contains multiple FG repeat-rich docking sites, is retained in the fusion products. Both NUP98 and NUP214 are components of the nuclear pore complex.[35,44] When proteins with nuclear localization signals (NLS) are imported to the nucleus, they bind karyopherin α and β. After the binding of the karyopherin heterodimers to the FXFG motif of nucleoporin docking sites and release caused by GTP bound form of Ran protein, the NLS-proteins are transferred into the nucleus.[45] This suggests that the docking site may facilitate the interaction of homeodomain proteins with their partners. The human homologue of yeast CRM1 protein, which interacts with NUP214, DEK-NUP214, and SET-NUP214, has been recently identified.[46] Although it is currently unknown whether hCRM1 can interact with homeodomain proteins such as PMX1 and HOXA9, specific cofactors of these homeodomain proteins may be recruited by the NUP98 docking domain.

REFERENCES

1. Manak JR, Scott MP: A class act: Conservation of homeodomain protein functions. Development Suppl 61, 1994

2. Kerl JH: Homeobox genes in hematopoiesis. Crit Rev Oncol Hematol 16:145, 1994

3. Lawrence HJ, Sauvageau G, Humphries RK, Largman C: The role of *HOX* homeobox genes in normal and leukemic hematopoiesis. Stem Cells 14:281, 1996

4. Sauvageau G, Lansdorp PM, Eaves CJ, Hogge DE, Dragowska WH, Reid DS, Largman C, Lawrence HJ, Humphries RK: Differential expression of homeobox genes in functionally distinct CD34+ subpopulations of human bone marrow cells. Proc Natl Acad Sci USA 91:12223, 1994

5. Lu M, Zhang N, Ho AD: Genomic organization of the putative human homeobox proto oncogene HOX-11 (TCL-3) and its endogenouos expression in T cells. Oncogene 7:1325, 1992

6. Bedford FK, Ashworth A, Enver T, Wiedemann LM: *HEX*: A novel homeobox gene expressed during haematopoiesis and conserved between mouse and human. Nucleic Acids Res 21:1245, 1993

7. Clerc RG, Corcoran LM, LeBowitz JH, Baltimore D, Sharp PA: The B-cell-specific Oct-2 protein contains POU box- and homeobox-type domains. Genes Dev 2:1570, 1988

8. Look AT: Oncogenic transcription factors in the human acute leukemias. Science 278:1059, 1997

9. Blatt C, Aberdam D, Schwarts R, Sachs L: DNA rearrangement of a homeobox gene in myeloid leukaemic cells. EMBO J 7:4283, 1988

10. Kongsuwan K, Allen J, Adams JM: Expression of *Hox2.4* homeobox gene directed by proviral insertion in a myeloid leukemia. Nucleic Acids Res 17:1881, 1989

11. Nakamura T, Largaespada DA, Shaughnessy JD Jr, Jenkins NA, Copeland NG: Cooperative activation of *Hoxa* and *Pbx1*-related genes in murine myeloid leukaemias. Nat Genet 12:149, 1996

12. Kroon E, Krosl J, Thorsteinsdottir U, Baban S, Buchberg AM, Sauvageau G: *Hoxa9* transforms primary bone marrow cells through specific collaboration with *Meis1a* but not *Pbx1b*. EMBO J 17:3714, 1998

13. Dube ID, Kamal-Reid S, Yuan CC, Lu M, Wu X, Corpus G, Raimondi SC, Crist WM, Carroll AJ, Minowada J, Baker JB: A novel human homeobox gene lies at the chromosome 10 break-point in lymphoid neoplasias with chromosomal translocation. Blood 78:2996, 1991

14. Hatano M, Roberts CWM, Minden M, Crist WM, Korsmeyer SJ: Deregulation of a homeobox gene, *HOX11*, by the t(10;14) in T cell leukemia. Science 253:79, 1991

15. Kennedy MA, Gonzalez SR, Kees UR, Lampert F, Dear N,

Boehm T, Rabbits TH: HOX11, a homeobox-containing T-cell onco-gene on human chromosome 10q24. Proc Natl Acad Sci USA 88:8900, 1991

16. Lu M, Gong ZY, Shen WF, Ho AD: The *TLC-3* proto-oncogene on human chromosome 10q24. EMBO J 10:2905, 1991

17. Nourse J, Mellentin JD, Galilli N, Wilkinson J, Stanbridge E, Smith SD, Cleary ML: Chromosomal translocation t(1;19) results in synthesis of a homeobox fusion mRNA that codes for a potential chimeric transcription factor. Cell 60:535, 1990

18. Kamps MP, Murre C, Sun XH, Baltimore D: A new homeobox gene contributes the DNA binding domain of the t(1;19) translocation protein in pre-B ALL. Cell 60:547, 1990

19. Dedera DA, Waller EK, LeBrun DP, Sen-Majumdar A, Stevens ME, Barsh GS, Cleary ML: Chimeric homeobox gene *E2A-PBX1* induces proliferation, apoptosis, and malignant lymphomas in trans-genic mice. Cell 74:833, 1993

20. Kamps MP, Baltimore D: E2A-Pbx1, the t(1;19) translocation protein of human pre-B-cell acute lymphocytic leukemia, causes acute myeloid leukemia in mice. Mol Cell Biol 13:351, 1993

21. Kamps MP, Wright DD: Oncoprotein E2A-Pbx1 immortalizes cultured myeloid progenitors without abrogating their factor-depen-dence. Oncogene 9:3159, 1994

22. Nakamura T, Largaespada DA, Lee MP, Johnson LA, Ohyashiki K, Toyama K, Chen SJ, Willman CL, Chen IM, Feinberg AP, Jenkins NA, Copeland NG, Shaughnessy JD Jr: Fusion of the nucleoporin gene *NUP98* to *HOXA9* by the chromosome translocation t(7;11)(p15;p15) in human myeloid leukaemia. Nat Genet 12:155, 1996

23. Borrow J, Shearman AM, Stanton VP Jr, Becher R, Collins T, Williams AJ, Dube I, Katz F, Kwong YL, Morris C, Ohyashiki K, Toyama K, Rowley J, Housman DE: The t(7;11)(p15;p15) translocation in acute myeloid leukaemia fuses the genes for nucleoporin *NUP98* and class I homeoprotein *HOXA9*. Nat Genet 12:159, 1996

24. Arai Y, Hosoda F, Kobayashi H, Arai K, Hayashi Y, Kamada N, Kaneko Y, Ohki M: The inv(11)(p15q22) chromosome translocation of de novo and therapy-related myeloid malignancies results in fusion of the nucleoporin gene, *NUP98*, with the putative RNA helicase gene, *DDX10*. Blood 89:3936, 1997

25. Jenkins NA, Copeland NG, Taylor BA, Bedigian HG, Lee BK: Ecotropic murine leukemia virus DNA content of normal and lympho-matous tissues of BXH-2 recombinant inbred mice. J Virol 42:379, 1982

26. Chenchik A, Moqadam F, Siebert P: Marathon cDNA amplifica-tion: A new method for cloning full-length cDNAs. CLONTECHniques 10:5, 1995

27. Church GM, Gilbert W: Genomic sequencing. Proc Natl Acad Sci USA 77:1991, 1984

28. Sadowski I, Bell B, Broad P, Hollis M: GAL4 fusion vectors for expression in yeast or mammalian cells. Gene 118:137, 1992

29. Cserjesi P, Lilly B, Hinkley C, Perry M, Olson EN: Homeodo-main protein MHox and MADS protein myocyte enhancer-binding factor-2 converge on a common element in the muscle creatine kinase enhancer. J Biol Chem 269:16740, 1994

30. Carton KM, Shang H, Marshall SC, Inostroza JA, Wilson JM, Abate C: Transcriptional repression by MSX-1 does not require homeodomain DNA-binding sites. Mol Cell Biol 15:861, 1995

31. Rabitts TH: Chromosomal translocations in human cancer. Nature 372:143, 1994

32. Grueneberg DA, Natesan S, Alexandre C, Gilman MZ: Human and *drosophila* homeodomain proteins that enhance the DNA-binding activity of serum response factor. Science 257:1089, 1992

33. Radu A, Moore MS, Blobel G: The peptide repeat domain of nucleoporin Nup98 functions as a docking site in transport across the nuclear pore complex. Cell 81:215, 1995

34. Mann RS, Chan SK: Extra specificity from *extradenticle*: The partnership between HOX and PBX/EXD homeodomain proteins. Trends Genet 12:258, 1996

35. Chang CP, Brocchieri L, Shen WF, Largman C, Cleary ML: Pbx modulation of Hox homeodomain amino-terminal arms establishes different DNA-binding specificities across the *Hox* locus. Mol Cell Biol 16:1734, 1996

36. Kern MJ, Witte DP, Valerius MT, Aronow BJ, Potter SS: A novel murine homeobox gene isolated by a tissue specific PCR cloning strategy. Nucleic Acids Res 20:5189, 1992

37. Cserjesi P, Lilly B, Bryson L, Wang Y, Sassoon DA, Olson EN: MHox: A mesodermally restricted homeodomain protein that binds an essential site in the muscle creatine kinase enhancer. Development 115:1087, 1992

38. Raza-Eglimez SZ, Jani-Sait SN, Grossi M, Higgins MJ, Shows TB, Aplan PD: *NUP98-HOXD13* gene fusion in therapy-related acute myelogenous leukemia. Cancer Res 58:4269, 1998

39. Kasper LH, Brindle PK, Schnabel CA, Pritchard CEJ, Cleary ML, van Deursen JMA: CREB binding protein interacts with nucleopo-rin-specific FG repeats that activate transcription and mediate NUP98-HOXA9 oncogenicity. Mol Cell Biol 19:764, 1999

40. Martin JF, Bradley A, Olson EN: The *paired*-like homeo box gene *MHox* is required for early events of skeletogenesis in multiple lineages. Genes Dev 9:1237, 1995

41. Felix CA: Secondary leukemias induced by topoisomerase-targeted drugs. Biochim Biophys Acta 1400:233, 1998

42. von Lindern M, Fornerod M, van Baal S, JaegleM, de Wit T, Buijs A, Grosveld G: The translocation (6;9), associated with a specific subtype of acute myeloid leukemia, results in the fusion of two genes, *dek* and *can*, and the expression of a chimeric, leukemia-specific *dek-can* mRNA. Mol Cell Biol 12:3346, 1992

43. von Lindern M, van Baal S, Wiegant J, Raap A, Hagemeijer A, Grosveld G: *can*, a putative oncogene associated with myeloid leukemo-genesis, can be activated by fusion of its 3′ half to different genes: Characterization of the *set* gene. Mol Cell Biol 12:3346, 1992

44. Kraemer D, Wozniak RW, Blobel G, Radu A: The human CAN protein, a putative oncogene product associated with myeloid leukemo-genesis, is a nuclear pore complex protein that faces the cytoplasm. Proc Natl Acad Sci USA 91:1519, 1994

45. Rexach M, Blobel G: Protein import into nuclei: Association and dissociation reactions involving transport substrate, transport factors, and nucleoporins. Cell 83:683, 1995

46. Fornerod M, van Deursen J, van Baal S, Reynolds A, Davis D, Murti KG, Fransen J, Grosveld G: The human homologue of yeast CRM1 is in a dynamic subcomplex with CAN/Nup214 and a novel nuclear pore component Nup88. EMBO J 16:807, 1997