**t(9;11)(p22;p15) in Acute Myeloid Leukemia Results in a Fusion between NUP98 and the Gene Encoding Transcriptional Coactivators p52 and p75-Lens Epithelium-derived Growth Factor (LEDGF)**

Harish G. Ahuja, Jiaming Hong, Peter D. Aplan, Lucene Tcheurekdjian, Stephen J. Forman, and Marilyn L. Slovak

Department of Medicine, Roswell Park Cancer Institute, Buffalo, New York 14263 [H. G. A., J. J.]; Division of Clinical Sciences, National Cancer Institute, Gaithersburg, Maryland 20877 [P. D. A.]; and Departments of Hematology and Bone Marrow Transplantation [S. J. F.] and Cytogenetics [L. T., M. L. S.], City of Hope, National Medical Center, Duarte, California 91010

**Abstract**

A t(9;11)(p22;p15) chromosomal translocation was identified in an adult patient with de novo acute myelogenous leukemia. Fluorescence in situ hybridization and Southern blot analysis mapped the 11p15 breakpoint to the NUP98 gene. Using ³²P rapid amplification of cDNA ends, we have identified a chimeric mRNA that fused the NUP98 3′Frag repeats in frame to the COOH-terminal portion of the gene encoding the transcriptional coactivators p52 and p75, as well as known as lens epithelium-derived growth factor (LEDGF). As expected, both NUP98-p52 and NUP98-p75 (LEDGF) chimeric mRNAs were detected by reverse transcription-PCR; however, the reciprocal p52/p75 (LEDGF)-NUP98 fusion mRNA was not detected. Our results demonstrate that this is the most frequent NUP98 fusion reported and reveal a previously unrecognized genetic target, the gene encoding p52/p75 (LEDGF).

**Materials and Methods**

**Case Report.** Patient 96-1322 was a 52-year-old Caucasian male who was diagnosed with AML (French-American-British classification M1). His presentation karyotype was characterized by a 46,XY,t(9;11)(p22;p15). Induction therapy consisted of cytarabine and idarubicin followed by an autologous stem cell transplant using cyclophosphamide, etoposide, and total body irradiation as the conditioning regimen. Interleukin 2 was administered after the transplant. The patient achieved a complete remission; however, he relapsed after 9 months. His karyotype at relapse was essentially unchanged, with the t(9;11)(p22;p15) detected in all 20 metaphases analyzed. Molecular characterization studies were performed on the sample obtained at relapse.

**Cytogenetics.** A bone marrow sample was cultured and harvested for classical cytogenetic analysis using standard methods. A 20-cell analysis was done on GTG-banded metaphases. The PACs 1173K (NUP98) and 47G3 (which maps telomeric to 1173K) were labeled with Spectrum Green and Spectrum Red, respectively, by nick translation (Vysis, Downers Grove, IL). Because 47G3 maps telomeric to NUP98 on chromosome 11, translocations involving NUP98 will exhibit a split NUP98 signal with a translocation of the complete 47G3 signal to the derivative chromosome 9. Residual cell pellets were used for FISH analysis following standard procedures. A total of 200 interphase cells were scored.

**Nucleic Acid Isolation.** Genomic DNA and RNA were obtained from frozen cell pellets of leukemic cells (4). Control DNA and RNA were obtained from peripheral blood cells from a normal volunteer. The studies were approved by the Institutional Review Board, and informed consent was obtained for use of patient materials in research studies.

**Southern Blots and Probes.** Southern blots were performed as described previously (7). Probe A was a 1.24-kb EcoRI-HindIII NUP98 cDNA fragment encompassing nucleotides 1–1248 (exons 1–9 and a portion of exon 10). Probe B was a 1.4-kb HindIII–EcoRV fragment encompassing nucleotides 1249–2628 (a portion of exon 10 and exons 11–19) of the NUP98 cDNA fragment (GenBank accession number U41815) and was a kind gift from Dr. Julian Burrow (Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA; Fig. 1B).

**3′ RACE.** The 3′ RACE was performed as described previously (4). The PCR was performed using a NUP98-specific forward primer (NUP1100; nucleotides 608–630; GenBank accession number U41815) and an abridged universal adapter primer (AUAP; Life Technologies, Inc., Gaithersburg, MD) as the reverse primer. A nested end-labeled NUP98 oligonucleotide (NUP1100; nucleotides 1102–1123) was used to screen for positive clones.

**Reverse Transcription-PCR Analysis.** A NUP98 sense primer (NUP1100) and antisense primers from the unique 3′ untranslated regions of gene encoding p52 (911–94; nucleotides 1165–1141; GenBank accession number AF098482) and p75 (LEDGF) (911–3; nucleotides 2039–2015; GenBank accession number AF063020) were used to amplify potential NUP98-p52 and NUP98-p75 (LEDGF) chimeric cDNAs. Similarly, a p52/p75 (LEDGF) sense primer (911–03; nucleotides 40–61; GenBank accession number AF098482) and a NUP98 antisense primer (NUP8006; nucleotides 1981–1957) were used to amplify potential p52/p75 (LEDGF)-NUP98 fusion mRNAs as described previously (7).

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2 To whom requests for reprints should be addressed. Present address: University of Wisconsin Cancer Center, Waukesha Hospital, 215 North 28th Street, Waukesha, WI 53186. Phone: (715) 847-2866; Fax: (715) 847-2869; E-mail: harisha@waukshop.org.

3 The abbreviations used are: AML, acute myelogenous leukemia; FISH, fluorescence in situ hybridization; RACE, rapid amplification of cDNA ends; HDGF, hepatoma-derived growth factor; PAC, P1 artificial chromosome.

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Fig. 1. A. FISH analysis of a (9;11)(p22;p15) sample. The NUP98 clone was labeled with Spectrum Green, and the more telomeric 4FG3 probe was labeled with Spectrum Red. The (9;11)(p22; p15) rearrangement (inset) resulted in a split NUP98 signal on der(11) (upper left in the metaphase cell; arrow in the interphase cell). The segment distal to the NUP98 breakpoint, containing the remainder of the NUP98 signal and the distal 4FG3 signal, has been translocated to the derivative 9 chromosome at band 9p22. Inset, GTG-banded chromosome 9 and 11 pairs. The right chromosome of each pair is involved in (9;11)(p22;p15). Breaks have occurred in bands 9p22 and 11p15. The segments distal to these two breakpoints have been exchanged. B, map of the human NUP98 gene obtained by comparing the NUP98 cDNA sequence with that obtained from a human PAC clone (RP11-348A20; GenBank accession number AC018803). The size of the introns was derived from plasmid subclones of the NUP98 PAC clone (1173K) and from sequences deposited in GenBank (accession number AC018803). The region of the gene encompassed by probes A and B is shown. The location of the (9;11) breakpoint in relation to previously reported NUP98 breakpoints is indicated. G, Bgl II. C, NUP98 gene rearrangements. Southern blots of Bgl II-digested DNA from the patient sample and control hybridized to probe B (left) and probe A (right) are shown. Size standards are in kb. An identical non-germ-line restriction fragment of 9 kb is seen with both probes.

Results and Discussion

FISH analysis revealed a split signal with 1173K, indicating that NUP98 was disrupted as a result on the translocation, and the segment distal, including the more telomeric 4FG3 signal, was translocated to the derivative chromosome 9 (Fig. 1A). To map the breakpoint, we searched for genomic DNA rearrangements within the NUP98 locus by Southern blot hybridization to both probes A and B (Fig. 1B). As shown in Fig. 1C, a novel Bgl II restriction fragment of approximately 8 kb hybridized to both probes. These results suggested that the most likely site of the breakpoint was a Bgl II fragment encompassed by both probes A and B. Based on the genomic structure of the NUP98 gene (see Fig. 1B), we hypothesized that the Bgl II fragment that included exons 9, 10, and 11 was the most likely site of the breakpoint.

We used 3′ RACE to identify a potential fusion transcript involving NUP98. Sequence analysis of one of the positive clones revealed that the sequence diverged from the NUP98 cDNA sequence (GenBank accession number U41815) at nucleotide 1230. A BLAST search showed that the sequence 3′ of the divergence point was a perfect match for the gene encoding the transcriptional coactivator p52 and p75, also known as lens-epithelium derived growth factor (LEDGF), both of which are derived by alternative splicing of the same gene on chromosome band 9p22 (10). The resultant in-frame chimeric mRNA joined nucleotide 1230 of NUP98 to nucleotide 534 of the gene encoding p52 (GenBank accession number AF098482), which corresponds to nucleotide 767 of the published sequence for the cDNA encoding p75 (GenBank accession number AF063020; Fig. 2A). This is the most 5′ NUP98 fusion point reported.

To investigate whether both NUP98-p52 and NUP98-p75 chimeric mRNAs were generated as a result of the fusion and to determine whether the reciprocal p52/p75 (LEDGF)-NUP98 fusion was expressed, we designed gene-specific primer pairs (see “Materials and Methods”) to amplify the potential fusion mRNAs. As shown in Fig. 2B, we were able to amplify NUP98-p52 as well as NUP98-p75. The reciprocal fusion mRNA was undetectable. These results are consistent with previously reported observations on NUP98 fusions (2–8).

The p52/p75 proteins are homologous to the HDGF and the HDGF-related proteins 1 and 2. The highest degree of sequence identity (≥80%) is found in the NH2-terminal 98 amino acid residues, also known as the HATH region (homologous to the NH2 terminus of HDGF, Fig. 2C). The COOH-terminal region of p52 (also present in p75) shows similarity to HMG-1, a multifunctional non-histone protein involved in many steps of gene regulation. p52 is a potent general transcriptional coactivator and is thought to mediate functional interactions between upstream sequence-specific activators and the general transcription apparatus (11). It has also been shown to interact with the essential splicing factor ASF/SF2 to modulate pre-mRNA splicing (12). p75 is a less potent coactivator than p52 and does not functionally interact with ASF/SF2 (11, 12). However, it has been shown to function as a growth and survival factor for lens epithelial cells, keratinocytes, and skin fibroblasts (13).

The exact mechanism of leukemogenesis remains to be elucidated. One possible scenario is the disruption of functions normally attributable to NUP98 and/or p52/p75. Another scenario is a gain of function attributable to the fusion protein(s). The COOH-terminal region of p52/p75, which has similarity to HMG-1, is preserved in the critical fusion message. However, the NH2-terminal HATH region is lost and replaced by the NUP98 FXFG repeats (Fig. 2C), which have been shown to interact with cAMP-responsive element-binding protein and possess strong transactivating properties (14). This raises the possibility that the fusion proteins are aberrant transcription factors. Finally, the possibility that replacement of the NH2-terminal HATH sequences by the NUP98 FXFG repeats in p75 results in a fusion protein with deregulated growth-promoting and survival properties must also be considered. The reason for the heterogeneous group of fusion partners remains unknown. They are reminiscent of the various

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by the black bar 8 residues (derived from intron sequences) and has a unique region of 205 residues at its COOH terminus. The highly conserved HATH region at the NH2 terminus of p52/p75 is shown.

References

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References


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