NUP98-HOXD13 Gene Fusion in Therapy-related Acute Myelogenous Leukemia

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Abstract

A novel chromosomal translocation, t(2;11)(q31;p15), was identified in a patient with therapy-related acute myelogenous leukemia (t-AML). Fluorescence in situ hybridization experiments mapped the breakpoint near NUP98; Southern blot analysis demonstrated that the nucleoporin gene NUP98 was disrupted by this translocation. We used rapid amplification of cDNA ends to identify a chimeric mRNA. An in-frame, chimeric mRNA that fused NUP98 sequences to the homeobox gene HOXD13 was cloned; the predicted fusion protein contains both the GLFG repeats from NUP98 as well as the homeomain from HOXD13. The NUP98-HOXD13 fusion is structurally similar to the NUP98-HOXA9 fusion previously identified in patients with AML, leading to the speculation that NUP98-homeobox gene fusions may be oncogenic. Moreover, this report, along with a recent study that demonstrated NUP98-DDX10 fusions in patients with t-AML, raises the possibility that NUP98 may be a previously unsuspected target for chromosomal translocations in patients with t-AML.

Introduction

Nonrandom, recurrent, chromosomal aberrations such as translocations and inversions are frequently associated with a wide spectrum of hematological malignancies and are generally thought to be causal events in the process of leukemic transformation (1). In general, these translocations or inversions are thought to be oncogenic either through the activation of a latent proto-oncogene [such as activation of c-myc by the t(8;14) translocation] or through the generation of a chimeric oncoprotein, such as the bcr-abl protein produced by the t(9;22) translocation (1). The identification of the genes involved in these chromosomal aberrations, as well as characterization of the fusion proteins produced, has proven to be a valuable starting point for understanding the process of leukemic transformation. NUP98 is a Mr 98,000 component of the NPC. NUP98 is localized at the nucleoplasmic side of the NPC and is involved in the transport of RNA and protein between the cytoplasm and nucleus (2). Recently, NUP98 has been reported to be involved in a rare but recurrent chromosomal translocation, t(7;11)(p15.5;q31)(3), as well as a chromosomal inversion, inv(11)(p15.5;q31) (5). The t(7;11) fuses NUP98 in-frame to a homeobox gene (HOXA9), whereas the inv(11)(p15.5;q31) fuses NUP98 to DDX10, a putative RNA helicase gene. Both of these chromosomal aberrations involving NUP98 were recognized in patients with AML. Moreover, the inv(11)(p15.5;q31) has been associated with t-AML and t-MDS (5). t-AML and t-MDS are malignancies that occur following treatment of a primary malignancy with cytotoxic chemotherapeutic agents or ionizing radiation. As a first approximation, there seem to be two forms of t-AML, which can be distinguished by clinical and cytot genetic features. The first form is typified by the therapeutic use of alkylating agents; a long (5–10-year) latency period, often associated with a preceding myelodysplastic phase; and the loss of chromosome 5 or 7 material. The second form is associated with the use of topoisomerase II inhibitor, a short (<24-month) latency period, and balanced translocations involving either the MLL or AML1 gene (6). The speculation that these t-AMLs are caused by chromosomal aberrations induced by cytotoxic, DNA-damaging chemotherapy is strengthened by the observation that both Etoposide (a topoisomerase II inhibitor) and Melphalan (an alkylating agent) can induce frequent gross chromosomal abnormalities, such as translocations, inversions, and deletions, in peripheral blood leukocytes (7, 8).

Here, we describe the characterization of a novel chromosomal translocation, a t(2;11)(q31;p15.5), which occurred in a patient with t-MDS that progressed to t-AML.

Materials and Methods

FISH. Cytogenetic analysis of bone marrow cells was performed in accordance with standard techniques, as described previously (9). Fixed cells stored at –20°C were used for FISH studies with previously mapped PAC clones from 1p15.5 (Fig. 1A), as described previously (9, 10).

Nucleic Acid Isolation. In accordance with institute guidelines, informed consent to participate in research studies was obtained from the patient’s parents. Leukemic blasts were isolated from the bone marrow of patient 229 by Ficoll-Hypaque (Sigma Chemical Co.) density centrifugation. Genomic DNA was isolated using a salting-out technique, as described previously (11). Total RNA was isolated using Trizol reagent (Life Technologies, Inc.) and the manufacturer’s recommended protocol. Plasmid DNA was isolated using Qia gen reagents and protocols.

Southern Blots and Probes. Ten μg of genomic DNA were digested with the indicated restriction enzyme (Life Technologies, Inc.), size-fractionated on 0.8% agarose gels containing 1 μg/ml ethidium bromide, photographed, de natured, neutralized, and transferred to nitrocellulose membranes (Schleicher & Schuell) by the Southern technique (12). Southern blots were hybridized to 32P-labeled probes labeled by the random priming technique using Prime-it II (Stratagene) reagents and protocols. Oligonucleotides were labeled using a terminal deoxynucleotidyl transferase end-labeling technique, and hybridization was performed as described previously (13). The probes used were a 1.4-kb HindIII-EcoRV NUP98 cDNA fragment (nucleotides 1249–2628 of GenBank accession no. U41815; a kind gift of Dr. Julian Borrow, Center for Cancer Research, MIT, Cambridge, MA), a 0.3-kb MluI-BamHI ETv6 cDNA probe (nucleotides 813–1142, which progressed to t-AML.

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3 The abbreviations used are: NPC, nuclear pore complex; AML, acute myelogenous leukemia; t-AML, therapy-related AML; t-MDS, therapy-related myelodysplastic syndrome; FISH, fluorescence in situ hybridization; RACE, rapid amplification of cDNA ends; AP, adapter primer.
Results

Case Report. The patient developed B-cell precursor acute lymphoblastic leukemia at 4 years of age. Cytogenetic analysis at that time revealed a normal 46, XY karyotype. He did not have central nervous system involvement at initial diagnosis, and he received standard induction therapy consisting of vincristine, prednisone, L-asparaginase, and “triple” intrathecal therapy (hydrocortisone, methotrexate, and 1-ß-D-arabinofuranosylcytosine). Consolidation and maintenance therapies lasted 2.5 years and consisted of intermediate-dose methotrexate and methotrexate with 6-mercaptopurine, respectively. The patient was in continuous complete remission for 3 years,
NUP98 Rearrangement. To investigate whether NUP98 was disrupted by this translocation, we searched for genomic DNA rearrangements within the NUP98 locus by Southern blot hybridization to the 1.4-kb HindIII-EcoRV NUP98 cDNA probe. As seen in Fig. 2A, non-germ-line-sized fragments are present in genomic DNA digested with multiple different restriction enzymes, suggesting a genomic DNA rearrangement had occurred within the NUP98 gene, most likely due to a chromosomal translocation.

To rule out the possibility that the non-germ-line-sized bands were due to a polymorphism, as well as to investigate whether the NUP98 rearrangement was present in the initial leukemia, we searched for NUP98 rearrangements in genomic DNA from the original B-cell precursor leukemia, genomic DNA from the t-AML, and genomic DNA from a remission sample obtained after treatment of the t-AML. In addition, because the initial leukemia had an ETV6-AML1 translocation, we were able to determine whether the initial leukemia had evolved into an AML, through a lineage shift of the initial leukemic clone. Fig. 2B shows that the ETV6 rearrangement was present in the initial leukemia but not in the t-AML or the remission sample. Similarly, the NUP98 rearrangement was present in the t-AML but not the initial leukemia nor the remission sample, demonstrating that the NUP98 rearrangement was not due to a polymorphism or to a lineage shift of the initial leukemia.

Detection of a Fusion Transcript in the t-AML Sample. We used 3′ RACE to search for a NUP98 fusion transcript in RNA from the t-AML sample. Because the previously reported NUP98 fusion transcripts (3–5) join NUP98 exons to HOXA9 or DDX10 exons at either nucleotide 1552 or 1864 of the NUP98 cDNA (GenBank accession no. U41815) in a head-to-tail fashion, we reasoned that the t(2;11) may produce a NUP98 fusion transcript with a similar fusion point. We used two complementary 3′ RACE approaches to clone the NUP98 fusion transcript. For one set of experiments, we used an oligo(dT) tailed AP and a JVWPS-specific primer. For another set of experiments, we used an oligo(dT) tailed AP and a JVWPS-specific primer.

In addition, because the initial leukemia had an ETV6-AML1 translocation, we were able to determine whether the initial leukemia had an ETV6-AML1 translocation. A bone marrow aspiration and biopsy at that time was consistent with a diagnosis of refractory anemia with excess blasts. Cytogenetic analysis at that time revealed a balanced t(2;11)(q31;p15) translocation. The myelodysplastic syndrome progressed to AML (M6 variant) within 3 months, and a successful remission was induced with 1-β-D-arabinofuranosylcytosine, etoposide, and mitoxantrone, followed by consolidation with an allogeneic bone marrow transplant.

FISH Analysis. We used a set of previously characterized chromosome 11p15.5 probes (9, 10) to localize the t(2;11) breakpoint. Fig. 1 demonstrates that the 1173K1 probe hybridized to the derivative 11 chromosome and the normal 11, whereas the 47G3 probe hybridized to the derivative 2 and the normal 11 (Fig. 1). Additionally, two more telomeric 11p15.5 probes (74K15 and 915F1; Ref. 10) also mapped to the derivative 11 (data not shown). These findings placed the breakpoint between 1173K1 and 47G3. Because the 1173K1 mapped quite close to NUP98 and NUP98 was known to be translocated in other AML cases, NUP98 was an obvious candidate gene for this translocation.

Fig. 2. A, Southern blot hybridized to the 1.4-kb NUP98 HindIII-EcoRV cDNA probe. The restriction enzymes used are indicated above the lanes. 229, patient DNA; C, control DNA from a lymphoblastoid cell line. Size standards are in kb. Rearranged fragments are seen in all four lanes containing patient DNA. B, comparison of primary acute lymphoblastic leukemia and t-AML. Lanes 1–4, Southern blot of BamHI-digested DNA from indicated patient samples or control lymphoblastoid DNA (C) hybridized to the ETV6 Mulu-BamHI cDNA probe. Lanes 5–8, the identical Southern blot was stripped and rehybridized to the NUP98 HindIII-EcoRV cDNA probe. Size standards are in kb. Non-germ-line sized fragments are evident in Lanes 2 and 7.
NUP98-HOXD13 IN t-AML

primer (5'-TGGAGGGCCTCTTGTTACAGG-3') complementary to NUP98 nucleotides 1461–1481. However, if the fusion transcript encoded a very large RNA, we realized that this approach may not be successful if the reverse transcriptase had to reverse transcribe a large segment prior to reaching the NUP98 fusion point. Therefore, a second set of 3' RACE experiments used a random hexamer AP and the same NUP98-specific primer. Both approaches were performed with templates from patient RNA, as well as three control cell lines (HEL, K562, and Molt4) that did not have a t(2;11) translocation; both approaches yielded PCR products that hybridized to NUP98 probes.

PCR products from the oligo(dT) primed experiment were subcloned into the pGEMT Easy vector. Twenty-five clones that hybridized to a nested, internal oligonucleotide 5' of the predicted breakpoint (5'-GCCACTTTGGGCTTTGGAGC-3'; complementary to NUP98 nucleotides 1516–1525) were evaluated. None of these clones hybridized to a NUP98 probe 3' of the predicted breakpoint (5'-AGAAGTTGGTTTrGAAGAACC-3'), suggesting that none of these clones represented germ-line NUP98 clones. We analyzed six independent subclones; these fell into two classes, with either a 0.5- or 1.6-kb insert. We sequenced one clone from each class; both clones diverged from NUP98 germ-line sequences at nucleotide 1552 of GenBank accession no. U41815, precisely where the NUP98-HOXA9 fusion occurs (Fig. 3A). The sequence 3' of the fusion in each case was a perfect match for the human HOXD13, which maps to human chromosome 2q31; we did not detect a NUP98-HOXD13 fusion among the PCR products from three control cell lines (HEL, K562, and Molt4). Therefore, the t(2;11)(q31;p15) generated a specific fusion mRNA between NUP98 and HOXD13. The 1.6-kb clones had a consensus polyadenylation signal (AATAAA) located 23 nucleotides upstream of the poly(A) tail; the 0.5-kb clones did not have a polyadenylation signal and were generated by fortuitous annealing of the AP to a complementary sequence within the HOXD13 3'-untranslated region. We were unable to amplify a potential reciprocal HOXD13-NUP98 fusion transcript; this result is not surprising in light of the observation that the HOXD13 promoter is not normally active in hematopoietic cells (14).

Discussion

This report demonstrates that NUP98 is fused to HOXD13 in a novel t(2;11)(q31;p15) seen in a patient with t-AML. Because this was the sole cytogenetic abnormality seen in this t-AML sample, we suspect that the NUP98-HOXD13 fusion was a causal event in this disease. The fusion transcript is composed of the 5' region of the NUP98 gene fused in frame to exon 2 and the 3' untranslated region of the HOXD13 gene. The NUP98-HOXD13 fusion transcript is fused at the same NUP98 exon as NUP98-HOXA9 and NUP98-DDX10 fusions, suggesting the existence of a breakpoint cluster region within the NUP98 gene (14–16).

Table 1 Translocations involving nucleoporin genes

<table>
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<th>NPC gene</th>
<th>Partner gene</th>
<th>Disease</th>
<th>Refs.</th>
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<td>t(2;11)(q31;p15.5)</td>
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<td>HOXD13</td>
<td>t-AML</td>
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<td>HOXA9</td>
<td>AML</td>
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<td>Unknown</td>
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Fig. 3. A NUP98-HOXD13 fusion cDNA. The germ-line NUP98 nucleotide sequence is shown (top) in boldface type. The germ-line HOXD13 sequence (middle) and the fusion cDNA (bottom) are also shown. The fusion between NUP98 and HOXD13 occurs between two Gs (underlined). The amino acids encoded are shown below the nucleotide sequence. A, schematic diagram of wild-type proteins and deduced chimeric protein. Arrows, fusion point of NUP98 and HOXD13. Regions that correspond to structural and functional domains of the proteins are also shown: GLFG, GLFG repeats; RNA bind, RNA-binding domain (B); HD, homeodomain (D); and HOXD13 exon 1 (E).
HOX genes encode a large family of transcription factors containing a conserved DNA-binding homeobox domain (14, 15). HOX genes are important for a wide spectrum of developmental events, including axial patterning and hematopoiesis (14, 16). Most of the mammalian HOX genes are found in four conserved clusters (HOX A, B, C, and D); a minority of mammalian HOX genes (such as HOX11) are "orphans" that are located outside of one of the principal clusters (14). Members of the HOX A, B, and C clusters are normally expressed during distinct stages of hematopoietic differentiation. In contrast, with the exception of HOXD3 expression in the erythroleukemia cell line HEL (17), HOX D cluster genes are not normally expressed during hematopoietic development (16). Several homeobox genes, including HOXA9, HOX11, and PBX1, are known to be involved in recurrent, nonrandom chromosomal translocations associated with lymphoid and myeloid malignancies (3, 4, 18, 19).

The predicted NUP98-HOXD13 fusion protein (illustrated in Fig. 3B) retains the conserved GLFG repeats of NUP98, which are thought to function as docking sites for karyopherin b at the nuclear pore (20). The HOXD13 portion of the predicted fusion protein retains the homeobox domain, which is a helix-turn-helix DNA-binding domain. Therefore, the putative fusion protein retains the ability to bind both DNA as well as any transcription factors that bind to the homeodomain.

It is not clear how a NUP98-HOXD13 fusion protein might contribute to leukemogenesis. The NUP98 portion of the fusion protein does not retain the RNA-binding domain of NUP98, which is replaced by HOXD13. Therefore, aberrant RNA transport could be responsible for oncogenesis. Alternatively, the localization of HOXD13 as part of a NUP98-HOXD13 fusion protein at the nuclear pore might act as a trap for homeobox-binding transcription factors that normally bind HOXD13, resulting in their sequestration at the nuclear membrane. Of note, the erythroleukemia cell line HEL (17) demonstrates overexpression of HOXD3, raising the possibility that dysregulation of HOXD family members may be generally associated with a subset of erythroleukemias.

A number of themes regarding NUP98 translocations are underscored by this report. First, this is now the second form of NUP98 translocation associated with t-MDS or t-AML, suggesting that the NUP98 locus, like MLL and AMLI, may be a target for nonrandom chromosomal translocations or inversions occurring as a result of cytotoxic chemotherapy. Second, there are now at least five examples (Table 1) of translocations involving NPC genes in association with acute leukemia. Last, this is the second example of a translocation fusing NUP98 to a HOX gene, suggesting that both NUP98 and the homeobox domains are important for malignant transformation.

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References


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