t(3;11)(q12;p15)/NUP98-LOC348801 fusion transcript in acute myeloid leukemia

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ABSTRACT

In a case of acute myeloid leukemia we report molecular cytogenetic findings of a t(3;11)(q12;p15), characterized as a new NUP98 translocation rearranging with *LOC348801* at chromosome 3. *NUP98* involvement was detected by fluorescence *in situ* hybridization. 3'-RACE-PCR showed nucleotide 1718 (exon 13) of NUP98 was fused in-frame with nucleotide 1248 (exon 2) of *LOC348801*. RT-PCR and cloning experiments detected two in-frame spliced *NUP98-LOC348801* transcripts and the reciprocal *LOC348801-NUP98*. A highly specific double-color double-fusion FISH assay reliably detects *NUP98-LOC348801*.

Key words: acute myeloid leukemia, NUP98, translocation partners.

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Introduction

Chromosome translocations are recurrent features in hematologic malignancies. In primary and therapy-related acute myeloid leukemia (AML) and in T-cell acute lymphoblastic leukemia (T-ALL), the *NUP98* gene, a member of the nucleoporin gene family which maps to chromosome 11p15.5, is a frequent target in chromosomal translocations. To date, 22 diverse *NUP98* partners with different characteristics have been described and grouped into homeobox and non-homeobox genes.[1-5] Here we characterize for the first time fusion of *NUP98* and *LOC348801* in a case of primary acute myeloid leukemia (AML) with t(3;11)(q12;p15).

Design and Methods

Case report

A 28 year-old man was referred because of fatigue, fever and bleeding. Clinical examination revealed petechia, enlarged liver, spleen and lymph nodes. The peripheral blood count was: Hb 9.1 gr/dL, PLT 30×10^9/L, WBC 86.4×10^9/L with 41% blasts. Acute myeloid leukemia M2, according to the FAB classification, was diagnosed on bone marrow. The karyotype was: 46,XY,t(3;11)(q12;p15). The patient achieved hematologic remission after chemotherapy with the HOVON protocol.[6] He relapsed seven months later. Second-line therapy with cytosine arabinoside and mitoxantrone failed. He died 23 months after diagnosis.

Fluorescence *in situ* hybridization (FISH)

Interphase break-apart FISH assay was performed with clone RP11-348A20 spanning the 5' region and exons 1-27 of NUP98/11p15, and clone CTD-3234F16 spanning the rest of *NUP98* and flanking its 3' region.[7] Once the new NUP98 partner was identified at 3q12, we designed a specific double-color, double-fusion FISH assay by combining RP11-348A20/CTD-3234F16 for *NUP98* (in green) with RP11-683B14, encompassing *LOC348801* (in red).

3'-RACE- and RT-PCR

Total RNA was extracted by Trizol (Invitrogen) from the patient's cryopreserved bone marrow cells and 1µg was reverse transcribed using 3'-RACE kit (Invitrogen). cDNA was amplified in semi-nested PCR (Expand extra long PCR system; Roche Applied Science, Penzberg, Germany) using NUP_1083_1106F (exon 8) as the first gene specific primer, NUP_1400_1419F (exon 11) as the second and AUAP

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(Abridged Universal Amplification Primer, Invitrogen) as reverse primer in both steps. The PCR product was sub-cloned into the pGEM-T easy vector (Promega), sequenced and analyzed using the BLAST program (NCBI, *http://www.ncbi.nim.nih.gov/*) and BLAT Genome Search (*http://genome.ucsc.edu/cgi-bin/hgBlat*) programs.

To confirm the *NUP98-LOC348801* fusion transcript we performed RT-PCR experiments using primers NUP_1284_1303F (exon 10) and LOC_1843_1824R (exon 4) for the first amplification round and primers NUP_1400_1419F (exon 11) and LOC_1787_1768_R (exon 4) for the second.

Primers LOC_1171_1190F (exon 1) and NUP1861_1843R (exon 14) for the first round and primers LOC_1219_1238F (exon 1) and NUP1861_1843R (exon 14) for the second were used to search for the reciprocal fusion transcript.

Results and Discussion

In this first case of primary AML with t(3;11)(q12;p15) characterized by *NUP98-LOC348801* fusion, the 5'-region of *NUP98* gene encoding GLFG repeats motifs and the GLEBS-like motif was fused in-frame with the 3'-region of *LOC348801* gene (Figure 1). The reciprocal *LOC348801-NUP98* fusion transcript was also present. *LOC348801* is the 23rd gene to be described as a *NUP98* fusion partner. It maps to chromosome 3q12.2 and contains four exons encoding for a protein with 178 aminoacids still lacking functional characterization.

The interphase break-apart FISH assay indicated *NUP98* was involved in t(3;11)(q12;p15) (*data not shown*). Our 3'-RACE-PCR experiments showed that nucleotide 1718 (exon 13) of *NUP98* was fused in-frame with nucleotide 1248 (exon 2) of *LOC348801* (Figure 1). The genomic breakpoints appeared to fall within intron 13 of *NUP98* and intron 1 of *LOC348801*. Double-color double-fusion FISH which gave one green signal, one red signal, and two fusion signals (Figure 2), further confirmed the reciprocal translocation t(3;11)(q12;p15) produced the *NUP98-LOC348801* fusion gene.

Interestingly, in *LOC348801* nucleotide 1248 is located 33 nucleotides upstream to the ATG start codon (1281-1283) (Figure 1). Thus, the predicted protein fuses the *NUP98* FG repeat motifs and GLEBS-like motif to the entire *LOC348801* through an 11 bridging peptide translated from non-coding sequence at the start of *LOC348801* exon 2. RT-PCR and cloning experiments detected two in-frame alternatively spliced transcripts. Isoform 1 had *NUP98* exon 12, upstream to the breakpoint region while in isoform 2 it was eliminated by alternative splicing (Figure 1). Alternative splicing mechanisms were reported in other *NUP98* fusions.[8-12] In mammalians splicing physiologically produces *NUP98* or the *NUP98-NUP96* mRNA which encodes a precursor protein of 186 kDa. The precursor is then proteolytically cleaved to produce NUP98 and NUP96 proteins.[8,13] Interestingly, in several oncogenes aberrant and alternative splicing defects may underlie susceptibility to tumor development and progression.[14,15] Our case emphasizes that alternative splicing is a frequent event in *NUP98* leukemic recombinations.

In this patient, a reciprocal *LOC348801-NUP98* fusion transcript was also found as nucleotide 1247 (exon 1) of *LOC348801* was fused with nucleotide 1719 (exon 14) of *NUP98* (*data not shown*). Reciprocal fusion transcripts were reported in *NUP98* translocations with different partners. However, since fusion with a partner may or may not produce the reciprocal transcripts generation does not seem to depend upon the characteristics of the partner gene.[16-18] Whatever the mechanism, any possible biological and clinical significance of different *NUP98* fusion isoforms and/or reciprocal transcripts is still not understood.

To date, t(3;11)(q12;p15) has been reported in two

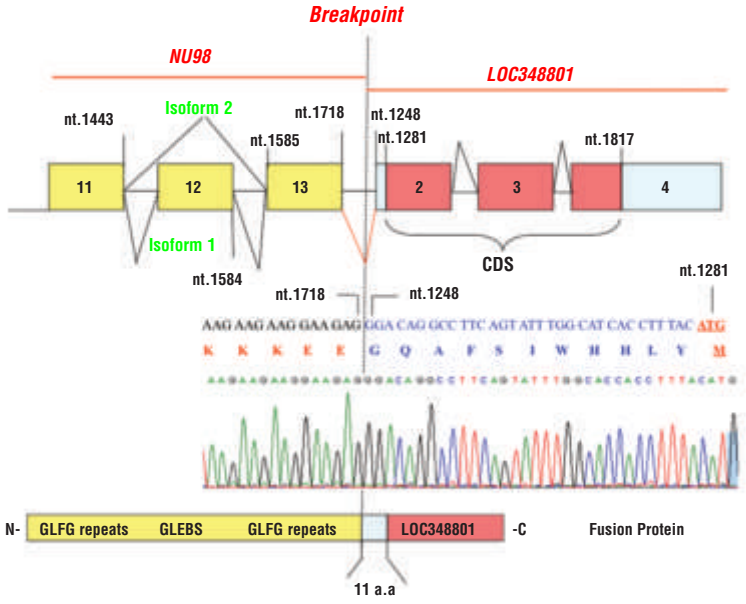

Figure 1. ***NUP98/LOC348801*** **fusion transcript and sequencing. In-frame fusion of *NUP98* and *LOC348801*, joining nucleotide 1718 (exon 13) of *NUP98* to nucleotide 1248 (exon 2) of *LOC348801*. The in-frame fusion inserts 11 aminoacids (lower panel) derived from the *LOC348801* exon 2 non-coding sequence (nt.1248 -1280). Two in-frame splicing *NUP98* isoforms (upper left) maintained the same fusion sequence. The *NUP98* exon 12 was present in Isoform 1 and absent in isoform 2. (Sequence numbers refer to GenBank accessions NM_139131.1 for *NUP98* and NM_001085451.1 for *LOC348801*) CDS: coding sequence.**

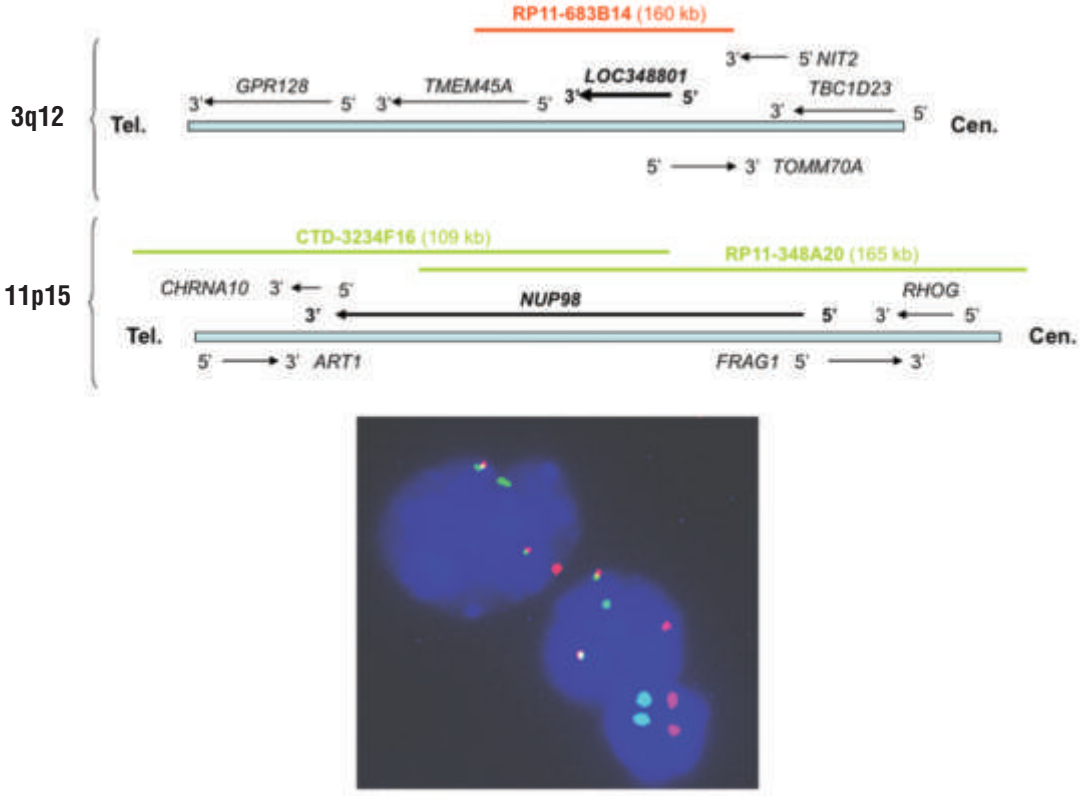

Figure 2. Double-color double-fusion FISH assay. Schematic representation of parts of chromosome 3 long arm and chromosome 11 short arm with localization/orientation of *LOC348801* and *NUP98* genes. DNA clones used for FISH studies are shown with their relative position and size (upper panel). In interphase nuclei, RP11-348A20/CTD-3234F16 for *NUP98* (green) and RP11-683B14 for *LOC348801* (red) gave one green signal, one red signal, and two fusion signals (lower panel).

females, one with myelodysplastic syndrome and the other with T-ALL. In both cases *NUP98* involvement was proven but partner(s) were not characterized.[1] In another case of primary T-ALL/AML a t(3;11) recombination, which cytogenetically resembled translocation of our patient, was really a 3q translocation/inversion in which NUP98 recombined with the IQCG gene at 3q29.[4]

In conclusion *NUP98*, like MLL, is another promiscuous genes, that rearranges with many partners. In MLL-leukemia the translocation partner may influence clinical and phenotype features.[19] Here we describe a new recombination between *NUP98* and *LOC348801* at 3q12 resulting in two in-frame spliced products and a reciprocal transcript. In our view, molecular characterization of new partners is a step towards a better understanding of the pathogenesis and mechanism of *NUP98*-positive leukemias. The highly specific double-color double-fusion FISH we set up for the *NUP98-LOC348801* fusion provides the differential diagnosis between *NUP98-IQCG* and *NUP98-LOC348801* and rapidly confirms genomic rearrangements, particularly in the event of ambiguous results with conventional cytogenetics.

Authorship and Disclosures

PG: designed molecular studies and wrote the paper; LB: performed molecular studies and sequencing analyses; RLS: designed FISH studies and wrote the paper; VP: selected DNA clones and performed FISH experiments; LB: performed molecular studies and sequencing analyses; RR: supervised molecular studies; MFM: supervised clinical and experimental findings; PV: performed cytogenetic analysis and was involved in the management of the patient; IW: performed FISH experiments and provided clinical data; CM: was responsible for the conception and supervision of the study.

The authors reported no potential conflicts of interest.

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