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Fusion of the *NUP98* gene with the *LEDGF/p52* gene defines a recurrent acute myeloid leukemia translocation

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Abstract

Background: The *NUP98* gene is involved in multiple rearrangements in haematological malignancy. The leukemic cells in an acute myeloid leukemia (AML) patient with a t(9;11)(p22;p15) were recently shown to have a fusion between the *NUP98* gene and the *LEDGF* gene but it was not demonstrated that this fusion was recurrent in other leukaemia patients with the same translocation.

Results: We used RT-PCR to analyse the leukemic cells from an AML patient who presented with a cytogenetically identical translocation as the sole chromosomal abnormality. A *NUP98-LEDGF* fusion transcript was observed and confirmed by sequencing. The reciprocal transcript was also observed. The fusion transcript was not detectable during remission and recurred at relapse. The breakpoints in the *NUP98* and *LEDGF* genes were different to those previously reported. The *NUP98* breakpoint occurs in the intron between exons 8 and 9. It is the most 5' breakpoint reported in a translocation involving the *NUP98* gene. All of the *LEDGF* gene is included in the fusion except for exon 1 which codes for the first 24 amino terminal amino acids.

Conclusions: Our results show that fusion of the *NUP98* and *LEDGF* genes is a new recurrent translocation in AML.

Background

The translocation, t(9;11)(p22;p15), was first reported in a patient with AML M1 [1]. Recently, a second AML M1 patient with a cytogenetically identical translocation was shown to have a fusion transcript between the 5' end of the *NUP98* gene on 11p15 and the 3' end of the *LEDGF* gene on 9p22 [2].

We have identified a third AML patient with a cytogenetically identical translocation. The patient, a 60 year old Caucasian woman presented with a white cell count of 1.5 x 10^9/L due to neutropenia. The bone marrow showed 50% blasts and 30% promyelocytes. She was diagnosed as AML M2. Cytogenetics showed 46,XX,t(9;11)(p22;p15) [13 cells]/46,XX [2 cells]. Induc-

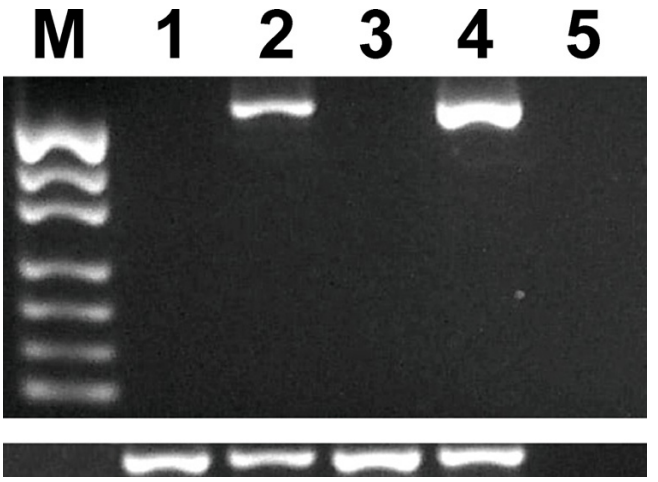

Figure 1
RT-PCR analysis of *NUP98-LEDGF* expression. RT-PCR for the *NUP98-LEDGF* fusion was performed as outlined in the materials and methods. RNA samples are from peripheral blood mononuclear cells taken from a normal donor (lane 1), bone marrow mononuclear cells taken from t(9;11) patient presentation (lane 2), and peripheral blood mononuclear cells taken from t(9;11) patient remission (lane 3) and relapse (lane 4). Lane 5 is a relapse specimen negative control RT-PCR without reverse transcriptase. M is pUC19/HpaII molecular weight marker. The lower panel shows the corresponding *PBGD* PCR control reactions.

tion chemotherapy with ARA-C, idarubicin and etoposide was abandoned after the patient developed a severe neutropenic reaction at the end of the first course. Nevertheless, complete haematological and cytogenetic remission was obtained. After 54 months, the patient relapsed with frank leukemia and a white cell count of 50 x 10^9/L. Due to the patient's wishes, only supportive therapy was given, and she died of her disease a few days later with a rapidly escalating blast cell burden. Cytogenetics of the relapse peripheral blood showed the same karyotype as at presentation 46,XX,t(9;11)(p22;p15) [19 cells]/46,XX [3 cells].

We analysed the leukemic cells from this patient in order to determine whether the fusion of *NUP98* and *LEDGF* as a result of the t(9;11)(p22;p15) is a recurrent event in AML.

Results and discussion

The *NUP98* gene is known to be involved in multiple rearrangements in haematological malignancy [3–10]. The 11p15 breakpoint in our patient suggested possible disruption of the *NUP98* gene. We therefore attempted a 3'RACE approach to test for the presence of a fusion mRNA using a method similar to that used to identify RAP1GDS1 as a fusion partner of NUP98 [9]. As the most 5' break in *NUP98* known at the time occurred in the intron after exon 10 [9], we used a *NUP98* forward primer from exon 9 for 3'RACE. This approach resulted in a number of RT-PCR products from the t(9;11) patient that were different in size from the *NUP98* 3'RACE products amplified from normal individuals. However sequence analysis of these products showed that they resulted from partially spliced *NUP98* mRNAs rather than novel fusion mRNAs (results not shown).

Subsequently, an AML patient was reported in which the t(9;11)(p22;p15) resulted in an in-frame fusion of the *NUP98* and *LEDGF* genes [2]. This involved fusion of *NUP98* exon 9 to *LEDGF* exon 6. As RACE of our patient had failed to detect any fusions including exon 9 of *NUP98,* we used a *NUP98* primer from exon 8 with a *LEDGF* reverse primer from exon 6 for RT-PCR (Figure 1). An RT-PCR product was obtained in which exon 8 of *NUP98* was fused in-frame to exon 2 of *LEDGF*. The *NUP98* breakpoint thus maps to the 5.5 kb intron between exons 8 and 9 and is the most 5' *NUP98* breakpoint reported to date. The *LEDGF* breakpoint in our patient is more 5' than that found in the patient reported by Ahuja et al [2] and occurs within the 3.5 kb intron between exons 1 and 2.

We also used RT-PCR to assess expression of the *NUP98-LEDGF* fusion mRNA in remission peripheral blood taken twenty months after presentation. Using standard PCR conditions with 35 cycles of PCR (Figure 1), there was no fusion transcript visible. Even after first round PCR with 45 cycles followed by fully nested PCR with 45 cycles we did not detect *NUP98-LEDGF* fusion mRNA in the remission sample (data not shown). However, RT-PCR of the relapse specimen showed that the *NUP98-LEDGF* transcript was re-expressed consistent with the association of the translocation with the disease.

We were able to amplify the reciprocal *LEDGF-NUP98* fusion transcript at presentation and relapse. Sequencing of this product showed an in-frame fusion of exon 2 of *LEDGF* to exon 9 of *NUP98* as expected. The reciprocal transcript is unlikely to be important in the pathogenesis of the disease as the previous report did not observe a reciprocal fusion transcript [2]. However, it is possible that the reciprocal fusion transcript may modulate the course of the disease particularly in the light of the less aggressive form of the disease seen in our patient. Similar patient to patient differences in whether or not the reciprocal transcript is expressed have also been noted for other fusions including *BCR-ABL and NUP98-RAP1GDS1*[9,11].

We were also able to obtain RNA using fixed leukemic cells from the first reported t(9;11) patient [1]. This RNA

was highly degraded and had an average length of 200 bases. Despite being able to amplify a 400 bp *NUP98* RT-PCR product, we were unable to amplify a a smaller product corresponding to either of the 2 known *NUP98-LEDGF* fusions nor any of a number of other potential *NUP98-LEDGF* fusions that we tried.

The previously reported *NUP98-LEDGF* transcript [2] encodes a protein fusing the amino terminus of the *NUP98* gene containing 28 of the 38 FG repeats with exon 6 onwards of p52/75. FG repeats in NUP98 fusion proteins have been shown to act as transactivation domains which recruit CBP/p300 [8,12]. The minimum number of FG repeats which are important for the transforming properties of NUP98 fusion proteins has not yet been defined. The fusion in our patient contains 23 FG repeats from *NUP98*. Interestingly, just after the breakpoint *LEDGF* exon 2 codes for an additional FG residue. It is unknown whether this residue is important for the function of the NUP98-LEDGF fusion protein.

The *LEDGF* gene codes for 2 transcriptional co-activators, p75 (*LEDGF:* Lens Epithelium Derived Growth Factor) and p52 which have different 3' ends generated by alternative splicing [15,13]. Both *NUP98*-p52 and *NUP98*-p75 fusion mRNAs were seen in our patient at presentation and relapse (data not shown) as well as in the previously characterised t(9;11) patient [2]. It remains to be determined whether one transcript is more important in the leukemogenic process.

p52 and p75 both contain a PWWP domain at their amino terminus [14]. PWWP is the core motif of a 70 amino acid domain found in a variety of nuclear proteins [14]. The PWWP domain was lost in the *NUP98-LEDGF* fusion described by Ahuja et al [2] and disrupted in the fusion described here. Interestingly, the PWWP domain is also found in NSD1, the most recently identified *NUP98* partner gene and is absent in the *NUP98-NSD1* fusion transcript [10].

The three AML patients with the t(9;11)(p22;p15) vary in their clinical picture (Table 1). At this stage, it is too early to determine which clinicopathological features are a hallmark of this translocation, especially as it is not clear as to whether the first patient's translocation has a similar molecular basis to the other two. Patients 2 and 3 have a more mature myeloid phenotype than that seen in patient 1. All three presented with the t(9;11)(p22;p15) as their sole cytogenetic abnormality. At relapse, the leukemic cells of both patients 2 and 3 had the same karyotype as at presentation whereas the relapse karyotype of patient 1 no longer showed the translocation.

Some *NUP98* translocations are associated with secondary leukemias that occur after treatment with topoisomerase II inhibitors [3–8,15]. The patient in this report had not received chemotherapy prior to her disease and prior chemotherapy was not mentioned in either of the other two case reports [1,2]. It seems that t(9;11)(p22;p15) is preferentially associated with de-novo AML rather than therapy related AML.

Conclusions

Our results show that fusion of the *NUP98* and *LEDGF* genes is a recurrent translocation in AML. Further study is required to determine how this fusion gene promotes leukemia.

Table 1: Clinical features, immunophenotype of patients with the t(9;11)(p22p15).

Patient	Sex/Age	WCC $\times 10^9$/L	Diagnosis	Survival (months)	Immunophenotype (where less than 50% of cells are involved, the fraction is given).	Reference
1	F/20	63.8	AML M1	3	CD33 (50%), CD13 (10%) negative for CD11b, CD 14 HLA DR, CD34 CD 19, CD22, negative for CD 10 negative for CD2, CD5, CD7, CD41, CD61, CD62 Nuclear TdTnegative. Blasts were positive for PAS, Sudan black and myeloperoxidase and negative for non-specific esterase.	1
2	M/52	50.5	AML M1	9	CD33, CD13, CD11c, CD15 (15%), CD36 HLA-DR, negative for CD34 negative for CD10, CD19, CD20, CD56 CD4 (20%), negative for CD5, CD7 Blasts were positive for myeloperoxidase with a subset positive for non-specific esterase.	2
3	F/60	1.5	AML M2	54	CD33, CD13, CD11b (14%) negative for CD34, HLA-DR negative for CD10, CD19, CD20 negative for CD2, CD3, CD5	This report

Details of the three patients discussed in this study are presented. All features except for survival are at presentation. Where surface markers are positive in less than 50% of cells, the proportion of positive cells is indicated in parentheses.

Materials and methods

RT-PCR analysis

RNA was obtained from bone marrow or peripheral blood mononuclear cells using Trizol (Invitrogen). RNA was reverse transcribed using Superscript II (Invitrogen). 3' RACE was performed using the 3' RACE System for Rapid Amplification of cDNA Ends (Invitrogen). The manufacturer's protocol for "First Strand cDNA Synthesis of Transcripts with High GC Content" was used. RT-PCR to amplify the *NUP98-LEDGF* fusion was performed using a *NUP98* forward primer from exon 8 (N988F 5'ACCAGCCTCTTCAGCAAACCATTTG3') and a *LEDGF* reverse primer from exon 6 (L826R 5'AACAGATGCTGTTGCTGTTGTCAC3'). *NUP98* exons are numbered according to Genbank AB040538 and *LEDGF* exons are numbered according to reference 16. Subsequently, the N988F primer was used in combination with either a p52 reverse primer (5'CTTCATCTCTTGTTTGCTCCACTTG3') or a p75 reverse primer (5'CTCAGCATGTATCCTTTGAAGTCG3') to amplify *NUP98-p52* and *NUP98-p75* fusion transcripts respectively. The *PBGD* transcript was amplified using primers specific for the housekeeping isoform, (5'CTTTCCAAGCGGAGCCATGTCTGG3' and 5'CATGAGGGTTTTCCCGCTTGCAGA3'). PCR was performed using HotStarTaq (Qiagen) according to the manufacturer's instructions with an initial incubation at 95°C for 15 minutes followed by 35 cycles of 96°C for 30 seconds, 65°C for 45 seconds and 72°C for 1 minute.

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