

LETTER TO THE EDITOR

Identification of *GSX2* and *AF10* as *NUP98* partner genes in myeloid malignancies

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The *Nucleoporin 98* gene (*NUP98*) is a promiscuous gene implicated in chromosomal aberrations in hematopoietic disorders. *NUP98* encodes a 98-kDa protein of the nuclear pore complex that regulates nucleocytoplasmic transport of protein and RNA. Twenty-eight different *NUP98* partner genes have been identified across various human hematological malignancies,^{1,2} many of which encode for homeodomain (HD) transcription factors and chromatin-modifying factors. We report here two new oncogenic fusions for *NUP98*, involving the homeobox gene *genetic screened homeobox 2* (*GSX2*, formerly *Gsh2*) and the putative zinc-finger transcription factor gene *ALL-1 fused gene from chromosome 10* (*AF10*)/*MLLT10*.

Patient 1 is a 57-year-old woman with a post-myeloproliferative neoplasm M4 acute myeloid leukemia (AML) associated with a t(4;11)(q12;p15) translocation and trisomy 8. Patient 2 is an 83-year-old man who developed an atypical myelodysplastic syndrome resembling chronic myelomonocytic leukemia associated with a t(10;11)(p12;p15) translocation. Molecular cytogenetic techniques demonstrated the *NUP98-GSX2* and *NUP98-AF10* fusions for the t(4;11) and the t(10;11) respectively (not shown). Reverse transcriptase (RT)-PCR using primers located within *NUP98*, *GSX2* and *AF10* exons validated the presence of the *NUP98-GSX2* and *NUP98-AF10* fusion transcripts in patient samples (Supplementary Figure 1). Nucleotide sequencing showed an in-frame fusion of the *NUP98* exon 12 with *GSX2* exon 2, predicting a putative chimeric protein of 59 kDa that joins the Gly-Leu-Phe-Gly (GLFG) repeats of the amino-terminal part of *NUP98* at the carboxy (C)-terminal part of *GSX2* containing a HD (Figure 1). Sequence analysis of the *NUP98-AF10* fusion transcript showed an in-frame fusion of *NUP98* exon 11 with *AF10* exon 15, predicting a 1027-amino-acid protein with the *NUP98* GLFG repeats fused to the C-terminal part of the *AF10* protein including the octapeptide motif-leucine-zipper (OM-LZ) domain and the glutamine-rich (Q-rich) sequence of *AF10*, but without the nuclear localization signal (NLS) region.

Both *AF10* and *GSX2* genes have been involved in human hematological malignancies. *GSX2* is a brain-specific class II homeobox gene of the Antennapedia family that regulates the development of mouse embryonic telencephalon.³ It has been associated with acute leukemia through its involvement in the recurrent t(4;12)(q11q12;p13) translocation.⁴ *AF10* belongs to a

family of proteins that includes *AF17* and *BR140* characterized by the presence of a C-terminal OM-LZ domain. *AF10* is considered a putative transcription factor, binding DNA through an AT hook motif and interacting with the SWI/SNF chromatin remodeling complex.⁵ Through its OM-LZ domain, *AF10* interacts with the histone methyltransferase *hDOT1L* that methylates the lysine 79 residues of histone H3 (H3K79),^{6,7} a mark associated with an open-chromatin configuration. In hematological malignancies, *AF10* is fused to the *mixed lineage leukemia* (*MLL*) gene by the t(10;11)(p12;q23) translocation⁸ and to the *clathrin assembly lymphoid myeloid leukemia* (*CALM*) gene by the t(10;11)(p12;q14) translocation.⁹

The transformation potential of the two *NUP98* fusions was investigated by transducing murine primary bone marrow (BM) hematopoietic progenitors, defined as lineage-negative cells, by murine stem cell virus (MSCV) retroviral vectors containing different versions of Flag-tagged *NUP98-GSX2* and *NUP98-AF10* sequences as reported.¹⁰ Cells were transduced with MSCV expressing the native *NUP98-GSX2* and *NUP98-AF10* fusions, fusions deleted for the conserved functional domains (*NUP98-GSX2-ΔHD* and *NUP98-AF10-ΔOM-LZ*) or with the empty MSCV. Transduced cells were seeded in methylcellulose medium for serial plating assays. We observed that *NUP98-GSX2*-transduced progenitors formed numerous colonies after the fourth round of replating, whereas control cells were not replated after the third round (Figure 2a). Cytological analysis of the colonies showed that cells expressing *NUP98-GSX2* exhibit a blast morphology, whereas empty MSCV-transduced progenitors formed monocytic and mast cell colonies (not shown). In contrast, *NUP98-GSX2-ΔHD*-transduced progenitors showed no proliferative advantage and were not able to form colonies after the second replating, thus illustrating that the transforming effect of the *NUP98-GSX2* fusion requires the *GSX2* HD. Immunofluorescence analysis showed a marked nuclear presence for the *NUP98-GSX2* protein, whereas *NUP98-GSX2-ΔHD* was located both in the nucleus and in the cytosol with a diffuse staining pattern (Supplementary Figure 2). These data demonstrated that the *NUP98-GSX2* fusion encodes a nuclear protein with a *GSX2* HD-dependent oncogenic capacity. Progenitors transduced with *NUP98-AF10* were able to produce a significantly increased number of colonies up to the third round of plating, contrary to the *NUP98-AF10-ΔOM-LZ*-transduced cells, which did not generate a significant number after the second round of plating (Figure 2a). Thus, expression of *NUP98-AF10* is sufficient to induce a proliferative advantage in progenitor BM cells and this proliferative effect depends on the integrity of the OM-LZ region. *CALM-AF10* (as well as some *MLL* fusions) participates in gene deregulation by virtue of *hDOT1L* recruitment at target gene loci, in particular for certain *HOXA* genes. As its transforming potential is linked to the integrity of the OM-LZ domain, we hypothesized that the oncogenic capacity of *NUP98-AF10* is linked to *HOXA* gene deregulation by a process similar to that involved in *MLL-AF10* and *CALM-AF10* fusions. We performed quantitative RT-PCR to measure the levels of the *HOXA5*, *HOXA7*, *HOXA9*, *HOXA10* transcripts and that of the *HOX* cofactor *MEIS1* transcript in patient 2's (*NUP98-AF10* fusion) BM cells (Figure 2b) (no material was available for patient 1). Expression analyses were performed

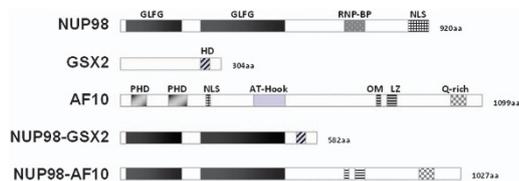


Figure 1. Schematic representation of the native or chimeric *AF10* and *GSX2* and *NUP98* proteins. RNP-BD, ribonucleoprotein binding domain; PHD, plant homeodomain; Q-rich, glutamine-rich region.

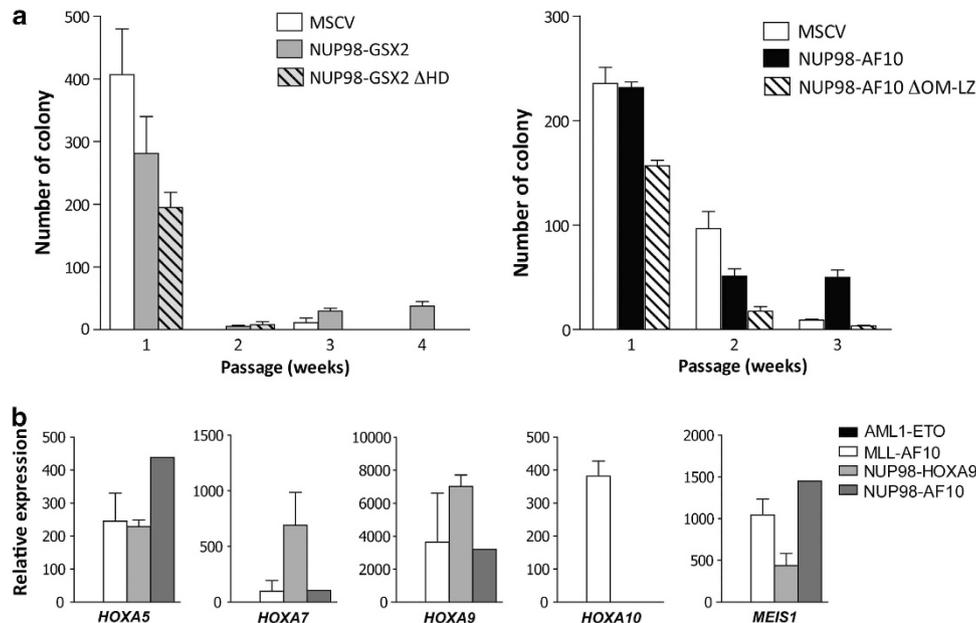


Figure 2. (a) Serial colony-plating assay of bone marrow progenitors transduced by wild-type, NUP98, NUP98-GSX2, NUP98-AF10 or empty vector. The average colony numbers \pm s.d. values are shown for three independent experiments. (b) Quantitative RT-PCR analyses of the *HOXA5*, *HOXA7*, *HOXA9*, *HOXA10* and *MEIS1* gene expression from RNA of various human AML samples carrying the following fusion genes: AML1-ETO ($n=2$), MLL-AF10 ($n=1$), NUP98-HOXA9 ($n=2$) and NUP98-AF10 ($n=1$). Expression was normalized with respect to *ABL* expression.

in parallel in samples from patients with AML1-ETO-positive leukemia ($n=2$) as a negative control for *HOXA* expression, and in samples from patients with MLL-AF10 ($n=1$)- and NUP98-HOXA9 ($n=2$)-positive leukemia as positive controls. In patient 2's BM cells, we observed an elevated expression of *HOXA5*, *HOXA7*, *HOXA9* and *MEIS1* as in leukemic cells with MLL-AF10 and NUP98-HOXA9 fusions. *HOXA10* was specifically overexpressed in the MLL-AF10 sample. No *HOXA* cluster gene expression was observed in AML1-ETO BM cells. In murine models, NUP98 fusions are often associated with upregulation of *HOXA* cluster and *MEIS1* genes in blast cells.^{11–13} Thus, NUP98-AF10-transduced BM cells were engrafted into sublethally irradiated mice. Mice transplanted with cells transduced with empty ($n=10$) or NUP98-AF10 MSCV vectors ($n=5$) remained free of hematological disease up to 12 months after transplantation (not shown), contrary to those receiving cells transduced with the NUP98-HMGB3 fusion.¹⁰ Altogether, our results indicate that contrary to the MLL-AF10 and CALM-AF10 fusions,^{14,15} NUP98-AF10 has only weak oncogenic power. This may be explained by the lack of an exclusively nuclear localization for NUP98-AF10 due to the absence of the NLS of AF10 in the chimeric protein.

In conclusion, the identification of *AF10* and *GSX2* as new NUP98 partner genes in hematological malignancies strengthens the predominance of homeobox and chromatin-modifier genes as NUP98 partners and the deregulation of *HOXA* cluster genes as an oncogenic mechanism in several NUP98-associated leukemia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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