OPEN

Citation: Blood Cancer Journal (2013) **3**, e124; doi:10.1038/bcj.2013.20

www.nature.com/bcj

LETTER TO THE EDITOR

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

Blood Cancer Journal (2013) **3,** e124; doi:10.1038/bcj.2013.20; published online 12 July 2013

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. Molécular 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) translocation[8] and to the *clathrin assembly lymphoid myeloid leukemia* (*CALM*) gene by the t(10;11)(p12;q14) translocation.[9]

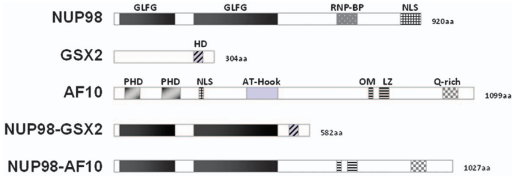

Figure 1. Schematic representation of the native or chimeric AF10 and GSX2 and NUP98 proteins. RNP-BD, ribonucleoparticle binding domain; PHD, plant homeodomain; Q-rich, glutamine-rich region.

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-⊗HD* 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-⊗HD*-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 G*SX*2 HD. Immunofluorescence analysis showed a marked nuclear presence for the NUP98–GSX2 protein, whereas NUP98–GSX2-⊗HD 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

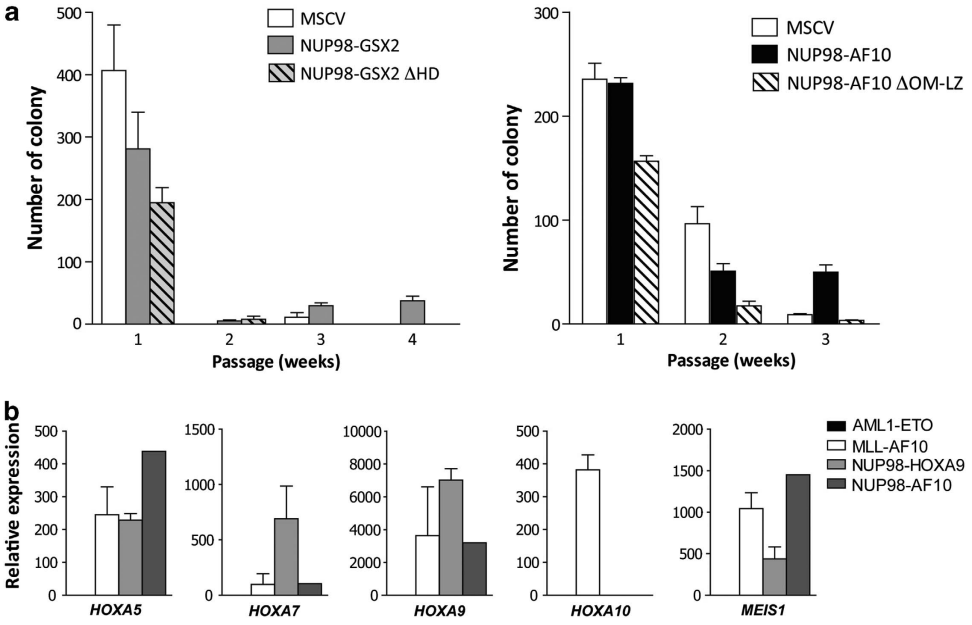

Figure 2. (**a**) Serial colony-plating assay of bone marrow progenitors transduced by wild-type, NUP98, NUP98–GSX2, NUP98–AF10 or empty vector. The average colony numbers ± s.d. values are shown for three independent experiments. (**b**) Quantitative RT-PCR analyses of the *HOXA5*, *HOXA7*, *HOXA9*, *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.

ACKNOWLEDGEMENTS

This works was supported by grants from The Ligue Nationale Contre le Cancer (LNCC). GS was a recipient from the Fondation pour la Recherche Médicale (FRM).

G Soler[1,2,3,8], S Kaltenbach[1,2,3], S Dobbelstein[4], C Broccardo[4,5], I Radford[1,2], M-J Mozziconacci[6], OA Bernard[3,7], V Penard-Lacronique[3,7], E Delabesse[4,5] and SP Romana[1,2]
[1]*Service de Cytogénétique, Hôpital Necker, AP-HP, Paris, France;*
[2]*Université Paris Descartes, Paris, France;*
[3]*INSERM U985, Institut Gustave Roussy, Villejuif, France;*
[4]*Laboratoire d'Hématologie, CHU Toulouse, Hôpital Purpan, Toulouse, France;*
[5]*INSERM U563, Toulouse, France;*
[6]*Département de Biopathologie-Institut Paoli Calmettes, Marseille, France and*
[7]*Université Paris Sud 11, Orsay, France*
E-mail: serge.romana@nck.aphp.fr
[8]*Present address: Laboratoire de Cytogénétique Médicale CHU Estaing, Clermont Ferrand, France.*

REFERENCES

1 Romana SP, Radford-Weiss I, Ben Abdelali R, Schluth C, Petit A, Dastugue N *et al.* NUP98 rearrangements in hematopoietic malignancies: a study of the Groupe Francophone de Cytogenetique Hematologique. *Leukemia* 2006; **20**: 696–706.
2 Gough SM, Slape CI, Aplan PD. NUP98 gene fusions and hematopoietic malignancies: common themes and new biologic insights. *Blood* 2011; **118**: 6247–6257.
3 Pei Z, Wang B, Chen G, Nagao M, Nakafuku M, Campbell K. Homeobox genes Gsx1 and Gsx2 differentially regulate telencephalic progenitor maturation. *Proc Natl Acad Sci USA* 2011; **108**: 1675–1680.
4 Cools J, Mentens N, Odero MD, Peeters P, Wlodarska I, Delforge M *et al.* Evidence for position effects as a variant ETV6-mediated leukemogenic mechanism in myeloid leukemias with a t(4;12)(q11-q12;p13) or t(5;12)(q31;p13). *Blood* 2002; **99**: 1776–1784.
5 Debernardi S, Bassini A, Jones LK, Chaplin T, Linder B, de Bruijn DR *et al.* The MLL fusion partner AF10 binds GAS41, a protein that interacts with the human SWI/SNF complex. *Blood* 2002; **99**: 275–281.
6 Mohan M, Herz HM, Takahashi YH, Lin C, Lai KC, Zhang Y *et al.* Linking H3K79 trimethylation to Wnt signaling through a novel Dot1-containing complex (DotCom). *Genes Dev* 2010; **24**: 574–589.
7 Okada Y, Feng Q, Lin Y, Jiang Q, Li Y, Coffield VM *et al.* hDOT1L links histone methylation to leukemogenesis. *Cell* 2005; **121**: 167–178.

8 Chaplin T, Ayton P, Bernard OA, Saha V, Della Valle V, Hillion J *et al.* A novel class of zinc finger/leucine zipper genes identified from the molecular cloning of the t(10;11) translocation in acute leukemia. *Blood* 1995; **85**: 1435–1441.
9 Dreyling MH, Martinez-Climent JA, Zheng M, Mao J, Rowley JD, Bohlander SK. The t(10;11)(p13;q14) in the U937 cell line results in the fusion of the AF10 gene and CALM, encoding a new member of the AP-3 clathrin assembly protein family. *Proc Natl Acad Sci USA* 1996; **93**: 4804–4809.
10 Petit A, Ragu C, Della-Valle V, Mozziconacci MJ, Lafage-Pochitaloff M, Soler G *et al.* NUP98-HMGB3: a novel oncogenic fusion. *Leukemia* 2010; **24**: 654–658.
11 Wang GG, Cai L, Pasillas MP, Kamps MP. NUP98-NSD1 links H3K36 methylation to Hox-A gene activation and leukaemogenesis. *Nat Cell Biol* 2007; **9**: 804–812.
12 Palmqvist L, Pineault N, Wasslavik C, Humphries RK. Candidate genes for expansion and transformation of hematopoietic stem cells by NUP98-HOX fusion genes. *PLoS One* 2007; **2**: e768.
13 Hirose K, Abramovich C, Argiropoulos B, Humphries RK. Leukemogenic properties of NUP98-PMX1 are linked to NUP98 and homeodomain sequence functions but not to binding properties of PMX1 to serum response factor. *Oncogene* 2008; **27**: 6056–6067.
14 DiMartino JF, Ayton PM, Chen EH, Naftzger CC, Young BD, Cleary ML. The AF10 leucine zipper is required for leukemic transformation of myeloid progenitors by MLL-AF10. *Blood* 2002; **99**: 3780–3785.
15 Okada Y, Jiang Q, Lemieux M, Jeannotte L, Su L, Zhang Y. Leukaemic transformation by CALM-AF10 involves upregulation of Hoxa5 by hDOT1L. *Nat Cell Biol* 2006; **8**: 1017–1024.

Supplementary Information accompanies this paper on Blood Cancer Journal website (http://www.nature.com/bcj)