Trans-Repressive Effect of NUP98-PMX1 on PMX1-Regulated c-FOS Gene through Recruitment of Histone Deacetylase 1 by FG Repeats

Xue-Tao Bai, Bai-Wei Gu, Tong Yin, Chao Niu, Xiao-Dong Xi, Ji Zhang, Zhu Chen, and Sai-Juan Chen

State Key Laboratory of Medical Genomics and Shanghai Institute of Hematology, Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, P.R. China

Abstract

The formation of fusion genes between NUP98 and members of the HOX family represents a critical factor for the genesis of acute leukemia or acute transformation of chronic myeloid leukemia (CML). To gain insights into the molecular mechanisms underlying the leukemogenesis of NUP98-HOX fusion products, we cloned NUP98-PMX1 from a CML-blast crisis patient with t(1;11) as a secondary chromosomal translocation, and functionally studied the fusion products in detail through various molecular and protein biochemical assays. In addition to many interesting features, we have found that the NUP98-PMX1 fusion protein exerts a repressive effect on PMX1 or serum response factor–mediated c-FOS activation, probably through the recruitment of a common corepressor histone deacetylase 1 by FG domains of the NUP98-PMX1 fusion protein. Moreover, we have provided evidence that the FG domains of NUP98-PMX1 and two other NUP98-containing fusion proteins, i.e., NUP98-HOXA9 and NUP98-HOXC11, all exhibit dual binding ability to both CREB binding protein, a coactivator, and histone deacetylase 1, a corepressor. Accordingly, we have hypothesized that this dual binding activity is shared by most, if not all, NUP98-HOX-involved fusion proteins, enabling these fusion proteins to act as both trans-activators and trans-repressors, and contributing to the genesis of acute leukemia or acute transformation of CML.

Introduction

Leukemia is frequently associated with recurrent chromosome translocations. Genes involved in the translocations often encode transcription factors or protein kinases that may play important roles in regulating cell proliferation, differentiation, or apoptosis in the hematopoietic system (1), and thus, possess leukemogenic features, as shown by numerous in vitro transformation assays or in vivo transgenic studies (2).

Translocations involving members of the nucleoporin (NUP) family have drawn attention over the past decade (3). These translocations were detected in de novo as well as therapy-related acute myeloid leukemia, myelodysplastic syndromes, acute lymphoid leukemia, and chronic myeloid leukemia (CML)-blast crisis (BC). To date, NUP98 gene has been found to fuse with 19 different fusion partners (4–6). These partner genes are diverse in terms of physiologic functions, belonging to two distinct categories, homeobox genes, such as HOXA9, HOXC11, HOXD11, and PMX1, and non–homeobox genes, such as DDX10, TOP1, and RAP1GDS. Interestingly, the NH2 terminus of NUP98, which remains in the fusion proteins, seems to be highly similar to the COOH terminus of NUP214, the remaining fragment of another nucleoporin member that is frequently involved in translocation (7, 8), containing a region of multiple Phe-Gly (FG) repeats and thus implicating the functional significance of FG repeats to leukemogenesis.

We and others have previously shown that the NUP98-PMX1 and NUP98-HOXA9 fusion genes could effectively transform NIH-3T3 cells, and induce a leukemia-like syndrome in transplantsions as well as transgenic models (9–11). The leukemogenic features of some other NUP98-containing fusion genes such as NUP98-TOP1 and NUP98-HOXD13 have been also shown previously (12, 13). Moreover, these NUP98-containing fusion genes seem to be able to induce acute myeloid leukemia cooperatively with MEIS1 or BCR-ABL (14, 15). Detailed biochemistry studies have provided further evidence that the FG domain is necessary for the NUP98-HOXA9 fusion protein to transform NIH-3T3, which is probably mediated by transcriptional activation through the recruitment of coactivator CREB binding proteins (CBP; ref. 10). The DNA binding activity of the NUP98-HOXA9 fusion protein seems to be dependent on the HOXA9 fragment. However, the binding affinity and spectrum seem to be both higher and wider than those of the wild-type HOXA9 (16). Accordingly, it has been proposed that fusion products between NUP98 and homeobox genes may encode aberrant transcription factors with aberrant trans-activation activities (17, 18). However, data from some of above studies (16, 19) also implies that fusion proteins between NUP98 and members of the homeobox family may exert some repressor effects on gene transcription, although these effects seem to be minor under the reported experimental conditions.

To better understand the oncogenic roles potentially played by these fusion products in leukemia, and to test the hypothesis that the fusion proteins may act as both trans-activators and trans-repressors, we cloned a NUP98-PMX1 fusion gene from a CML-BC patient with t(1;11) as a secondary chromosomal translocation and conducted a series of functional studies. In addition to many interesting features, we have found that the FG domain of NUP98-PMX1 and some other NUP98-containing fusion proteins is capable of recruiting CBP as well as histone deacetylase 1 (HDAC1), a common corepressor of transcriptional regulation, implicating dual effects of aberrant transcription factors encoded by these fusion proteins. Moreover, we have found that NUP98-PMX1 may exert dominat-negative effects on PMX1-regulated c-FOS gene expression.
Materials and Methods

Case report. The patient was a 51-year-old man suffering from acute myeloid leukemia transformation after 2.5 years of chronic phase CML. He received hydroxyurea during the chronic phase and chemotherapy including arabinosylcytosine, aclamycin, and daunorubicin during BC.

Fluorescence in situ hybridization. Fluorescence in situ hybridization (FISH) studies were done as described elsewhere (18). BAC RP11-120E20 spans the breakpoint of NUP98 and the BAC RP11-79C4 contains the entire PMX1.

Reverse transcription-PCR and real-time RT-PCR. DNA isolation and reverse transcription were done as previously described (20). The primers for amplification of NUP98-PMX1 were designed to span the breakpoint between the fusion partners: forward primer, 5'-CTCTTGTGGTCTGAAGGACATCT-3' and reverse primer, 5'-TTAGAATCGGTATGAAGCC-3'. For some experiments, real-time reverse transcription-PCR was done with an ABI PRISM 7000 sequence detection system using a SYBR Green PCR Master Mix (PE Biosystems, Foster City, CA) according to the manufacturer's instructions. The primer pairs were as follows: c-FOS, 5'-AGAA-TCCGAAGGGAAGGAAATA-3' and 5'-CTCTGAAAGCAGACTTCTCT-3'; GAPDH, 5'-GAAGGTGAAGGCTGACGTG-3'; and 5'-GAGAATGGTTAGG-GAATTC-3'. The threshold amount of c-FOS expression in each sample was normalized by GAPDH expression.

Cell culture and transfection. HeLa, COS-7, and 293T cells were grown in DMEM supplemented with antibiotics and 10% FCS. All transient transfections were done using SuperFect (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The primers used were as follows: c-FOS, 5'-AGAA-TCCGAAGGGAAGGAAATA-3' and 5'-CTCTGAAAGCAGACTTCTCT-3'; GAPDH, 5'-GAAGGTGAAGGCTGACGTG-3'; and 5'-GAGAATGGTTAGG-GAATTC-3'. The threshold amount of c-FOS expression in each sample was normalized by GAPDH expression.

Expression plasmids constructs. Expression plasmids were constructed using standard molecular biology techniques and verified through DNA sequencing. SRE, PMX1, NUP98-PMX1, NUP98-HOXA9, NUP98-HOXC11, the truncated form of NUP98 (designated ΔNUP98), the truncated form of PMX1 (ΔPMXI), the truncated form of HOXA9 (ΔHOX9), the truncated form of HOX11 (ΔHOX11), fragments of NUP98 and NUP54 were subcloned into pSG5M or pCI-neo vectors for mammalian cell expression and in vitro translation. NUP98, NUP98-PMX1, NUP98-HOXA9, NUP98-HOXC11, ΔNUP98, ΔPMXI, and NUP54 were also subcloned in pEGFP-C1 vector to study cellular localizations, establish cell lines, and perform communoprecipitation assays. PMX1 was fused in frame to RED for cotransfection with pEGFP-C1-NUP98-PMX1. Cloning details are available on request. SRE-Luc (21) was obtained from J.E. Pessin (Department of Biochemistry and Molecular Biology, Mayo Clinic and Mayo Graduate School, Rochester, MN; ref. 22).

Gel shift and gel supershift assays. Nuclear extracts were prepared from cells with NE-PER (Pierce, Rockford, IL) and quantified using bichinonic acid protein assay reagent kit (Pierce) with bovine serum albumin as a control. The c-FOS SRE probes are as follows: forward, 5'-AGAC-TTACAGGGATGTCAATATTAGCACATCTG-3' and reverse, 5'-CAGATGTCGCTATARATGGACATCCTGTAAGC-3'. The probe was generated by annealing the oligonucleotides after end-labeling with polynucleotide kinase (Promega) and was purified with MicroSpin G-25 column (Amersham Pharmacia Biotechnology, Piscataway, NJ). Binding reactions were carried out by mixing 10 μg of nuclear proteins and 0.2 pmol (in 1 μL) of 32P-labeled probes in a total volume of 20 μL of binding buffers (Promega). The binding reactions were allowed to proceed at room temperature for 20 minutes. Thereafter, the reaction mixture was subjected to electrophoresis on nondenaturing 4% polyacrylamide gels followed by visualization using autoradiography. For gel supershift assays, 1 μg (in 1 μL) of specific supershift antibodies against SRE (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was added to the reaction mixture containing the nuclear protein extract and incubated for an additional 30 minutes at room temperature before gel shift assays were done.

GST pull-down assay. The GST fusion protein series were expressed in the host strain BL-21 and purified with glutathione Sepharose 4B (Amersham). In vitro expression of radiolabeled proteins was done using the Quick Coupled Transcription/Translation system (Promega) in the presence of [35S]methionine. Beads coated with GST fusion proteins were incubated in binding buffer [20 mmol/L Tris (pH 8.0), 150 mmol/L KCl, 1 mmol/L EDTA, 4 mmol/L MgCl2, 0.2% NP40, 10% glycerol] with in vitro-translated proteins at 4°C for 4 hours. The beads were washed thrice with binding buffer, eluted by boiling in sample buffer, and resolved by 10% SDS-PAGE. Bound proteins were visualized using PhosphorImager.

Coimmunoprecipitation. For communoprecipitation assays, transfected 293T cells were harvested and resuspended in radiocommunoprecipitation assay buffer [50 mmol/L Tris·HCl (pH 8.0), 150 mmol/L NaCl, 0.5% deoxycholic acid, 0.1% SDS, 1% NP40] supplemented with protease inhibitor set 1 (Calbiochem Corp., La Jolla, San Diego, CA). The extracts were first precleared using 40 μL of protein A beads (Santa Cruz Biotechnology). Following centrifugation to remove the protein A-Sepharose, 2 μL of HDAC1 antibody (Abcam, Cambridge, United Kingdom) or 10 μL of irrelevant antifibronectin antibody (H-300, Santa Cruz Biotechnology) was added to the reaction and incubated for 4 hours at 4°C. The immune complexes were recovered by the addition of 50 μL of protein A-Sepharose and washed four times with PBS. After the final wash, 2× SDS sample was added, and the beads were heated to 90°C for 5 minutes, analyzed by SDS-PAGE, and immunoblotted with GFP antibodies (Santa Cruz Biotechnology) that recognize the GFP epitope fused to the NH2 terminus of each exogenously produced protein.

Western blotting assay. Cells were lysed and Western blotting was done with anti-GFP antibody (Santa Cruz Biotechnology), anti-Fos, and anti-GAPDH antibody (Abcam) as described previously (20).

Indirect immunofluorescence. HeLa cells were seeded in 12-well plates and subsequently transfected with the pEGFP-NUP98-PMX1, NUP98-HOXA9, and NUP98-HOXC11. After 24 hours, cells were washed with PBS, fixed with 4% paraformaldehyde, and permeabilized with methanol for 10 minutes at −20°C. Fixed cells were blocked with 3% bovine serum albumin/PBS at room temperature for 1 hour, then incubated with anti-HDAC1 antibodies (1:100) overnight at 4°C, followed by a 50-minute incubation with rhodamine-conjugated anti-rabbit IgG (1:200; Calbiochem). Coverslips were then mounted with DAKO fluorescence mounting medium, and images were captured using a laser scanning confocal microscope and LSM Pascal software (LSM5; Pascal; Zeiss, Jena, Germany).

Results

Cloning of NUP98-PMX1 fusion gene and localization of the fusion protein to the nucleus. As shown in Fig. 1A, a t(11;17)(q23;p15) translocation, similar to a previously reported case (17), was identified from a patient with CML-BC by conventional cytogenetic methods. Through FISH analysis, and followed by candidate gene cloning, a fusion fragment containing exons 1 to 12 of NUP98 and exons 2 to 5 of PMX1 was eventually obtained. Because the intracellular distribution of neither PMX1 protein nor NUP98-PMX1 fusion protein is reported, and thus the spatial relationship between these two potential transcription factors...
remains to be determined, a series of subcellular localization experiments have been conducted by cloning the full-length cDNA of NUP98, PMX1, and NUP98-PMX1, respectively, into expression vectors, followed by transfection assays. As shown in Fig. 1B, fluorescent signals of NUP98 are exclusively localized on the nuclear membrane, showing typical patterns of nuclear pore proteins, whereas fluorescent signals of PMX1 are mostly distributed inside the nucleus with slightly enhanced accumulation in some nuclear domains. Although the NUP98-PMX1 fusion protein is also localized in the nucleus, its distribution patterns seem to be somewhat different from those of PMX1.

Transcriptional repression of NUP98-PMX1 on PMX1-activated c-FOS gene. PMX1 is a transcription factor that activates c-FOS gene expression through complex mechanisms. In one series of reports, PMX1 is believed to enhance the binding of SRF to SRE (a cis element contained in the promoter of c-FOS gene), which results in a strong induction of c-FOS (23). In another, PMX1-mediated c-FOS induction, however, seems to be irrelevant to SRF (24). To further address the potential relationship between NUP98-PMX1 and PMX1 in terms of their roles in transcriptional regulation, we have chosen c-FOS as a model system and conducted a series of luciferase assays in two conventional cell systems, COS-7 and HeLa cell lines. As shown in Fig. 2A, in COS-7 cells, the luciferase activity is strongly increased by SRF (group IV), indicating that these cells may have the trans-activation activity of SRF on c-FOS. It also suggests that the amount of the endogenous SRF present in COS-7 cells is minor, with an inconsequential effect on c-FOS induction in comparison with the effect of the exogenous SRF. In the presence of PMX1 (group I), however, the effect of the endogenous SRF seems to be significantly enhanced, showing an even greater increase of the activity than that of the exogenous SRF and thus suggesting a powerful enhancement of the trans-activation of SRF by PMX1 in this setting. Surprisingly, cotransfection of NUP98-PMX1 with SRF (group V) or with PMX1 (group II) results in a statistically significant reduction of the trans-activation activity of SRF either exogenously (group IV versus group V; \( P = 0.047, 0.013, \) and 0.008, respectively) or endogenously (group I versus group II; \( P = 0.037, 0.007, \) and 0.001, respectively), implicating a suppression effect of NUP98-PMX1 on PMX1 or SRF-mediated c-FOS activation. In HeLa cells, however, the same reporter system only responded to PMX1 but not SRF, suggesting a distinct regulatory mechanism in this cell system. Nevertheless, cotransfection of NUP98-PMX1 into HeLa cells in the presence of PMX1 displayed statistically significant inhibition (group VII versus group VIII; \( P = 0.009, 0.010, \) and 0.026, respectively) on reporter gene expression (Fig. 2A, right). Accordingly, we have speculated that NUP98-PMX1 might be able to recruit a corepressor to the regulatory complex at the SRE site. HDAC is a common corepressor involved in the transcriptional repression of various types of genes and its repression effect seems to be releasable by TSA, known as a HDAC inhibitor. As shown in the Fig. 2A (left), the addition of TSA seems to be statistically effective in releasing the repression effect of NUP98-PMX1 (group III versus group II; \( P = 0.047, 0.027, \) and 0.005, respectively, and group VI versus group V; \( P = 0.031, 0.036, \) and 0.011, respectively). Although further investigation is needed to understand the partial releasing effect of TSA in this setting, it can be assumed that NUP98-PMX1 may exert a suppression effect on PMX1 or SRF-mediated c-FOS activation, which is probably through the recruitment of HDAC as a corepressor.

To test the hypothesis that NUP98-PMX1 may act as a repressor to PMX1-mediated c-FOS expression, two cell lines (293T and HL-60) which endogenously expressed both PMX1 (data not shown) and c-FOS were transfected with either a control plasmid (pEGFP-C1 for 293 cells and pCI-neo for HL-60 cells) or constructs of NUP98-PMX1, and followed by G418 selection for 4 weeks before analyses were done. The expression of NUP98-PMX1 in these transfected cell systems was transcriptionally and translationally affirmed by both RT-PCR and Western blot (data not shown). When the expression of c-FOS gene was quantitatively analyzed using real-time reverse transcription-PCR (Fig. 2B and C, top), a statistically significant difference was observed between cell samples with and without expression of the fusion protein (column 5 versus columns 1 or 3) in both 293T \( (P = 0.033 \) and 0.018, respectively) and HL-60 cell lines \( (P = 0.017 \) and 0.016, respectively), indicating the suppression effect of NUP98-PMX1 on in vivo
expression of c-FOS gene. Of note, the releasing effect of TSA seems to be variable in different cell systems, which is probably due to the multifaceted deacetylation conditions near or at the SRE regulatory complex (25). There is evidence that the repression effect of NUP98-PMX1 on c-FOS gene expression seems to also be implicated at the translational level (lane 5, bottom, Fig. 2B and C). A reduced mRNA level corresponds to the reduced protein level.

To further address the potential effect of NUP98-PMX1 on the interaction between SRF and SRE, gel shift assays were done on nuclear extracts prepared from parental 293T cells, cells with GFP, and cells with GFP-NUP98-PMX1 using the c-FOS SRE sequence as the probe. As shown in Fig. 2D, two major bands corresponding to SRF/SRE complexes, i.e., complexes I and II, were detected by the SRE-specific probe in the parental cells (lane 3). In comparison with the in vitro translation product of SRF (lane 1), complex I may represent an interaction complex of SRF and SRE, whereas complex II may represent a complex of SRF with other proteins (26). The specificity of the SRE probe is validated by competition assays in the presence of excess amounts of unlabeled SRE probe, as shown in lanes 4, 5, and 6, and the specificity of SRF is further validated by anti-SRF-specific antibody, detecting a supershift band formed from complexes I and II, as shown in lane 9. By comparing band intensities between the parental cells (lane 3) and the cells with expression of the NUP98-PMX1 fusion protein (lane 8), it can be seen that the band of complex I in the cells with the fusion protein is even stronger than that in the parental cells, implicating that the DNA-binding activity of SRF to SRE is at least not reduced by NUP98-PMX1 fusion protein. Although transcriptional regulation of the c-FOS gene is rather multifaceted, probably involving different regulatory components in different cells (23, 24), we have gathered evidence from multi-experimental systems implicating that the NUP98-PMX1 fusion protein may act as a corepressor to PMX1-mediated c-FOS activation.

Recruitment of HDAC1 by NUP98-containing fusion proteins through FG domains. As described previously, TSA seems to be effective in releasing the repression effect of NUP98-PMX1, which is probably due to the multifaceted deacetylation conditions near or at the SRE regulatory complex (25). There is evidence that the repression effect of NUP98-PMX1 on SRE regulatory complex (25). There is evidence that the repression effect of NUP98-PMX1 on c-FOS gene expression seems to also be implicated at the translational level (lane 5, bottom, Fig. 2B and C). A reduced mRNA level corresponds to the reduced protein level.

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suggesting that NUP98-PMX1 may directly or indirectly interact with a corepressor complex. Accordingly, we have conducted a series of GST pull-down assays to screen potential corepressor molecules interacting with NUP98-PMX1. These screened corepressor molecules include silencing mediator for retinoid and thyroid hormone receptors, nuclear receptor corepressor 1, HDAC1 and HDAC3, of which HDAC1 seems to be the only one interacting with NUP98-PMX1 (top, Fig. 3A). Because most, if not all, NUP98-HOX fusion proteins share similar structures, it is logical to speculate that these fusion proteins may also share the feature of interacting with HDAC1. Thus, a series of pull-down experiments were done afterward using the same bait, respectively, reacting with in vitro translation products of some other members of NUP98-containing fusion proteins such as NUP98-HOXA9 and NUP98-HOXC11, as well as the partners of these fusion genes (Fig. 3A). As expected, all the fusion proteins and NUP98, the remaining fragment of NUP98 in this setting, interact with HDAC1 physically, whereas partner fragments of the HOX family seem to be incapable of interacting with HDAC1.

To validate that the interaction between NUP98 involved fusion proteins and HDAC1 also occurs in vivo, a series of Co-IP assays were done using anti-HDAC1 antibody to react with lysates of 293T cells, respectively, transfected with GFP tagged NUP98-PMX1, NUP98-HOXA9, NUP98-HOXC11, NUP98, NUP54, and NUP54, another FG domain containing nucleoporin. As illustrated in Fig. 3B, NUP98-related fusion proteins and NUP54 indeed interact with HDAC1 in vivo, as communoprecipitated by anti-HDAC1 antibody and followed by the detection with anti-GFP antibody, but not with an irrelevant (anti-fibrinectin) antibody. Moreover, the interaction between NUP98 and HDAC1 provides evidence that the recruitment of HDAC1 occurs in FG domain containing NUP98 fragment. To further confirm the interaction of HDAC1 with NUP98-containing fusion proteins, we carried out transfection assays in HeLa cells, respectively, with GFP-NUP98-PMX1, GFP-NUP98-HOXA9, and GFP-NUP98-HOXC11 expression vectors. As shown in Fig. 3C, partial but obvious colocalization between HDAC1 and the three NUP98 fusion proteins was visible in the cell nucleus.

Fine mapping of CBP binding region and recognition of the same FG repeats interacting with both CBP and HDAC1. Based on our observations, we have proposed that FG repeats of NUP98 may interact with both HDAC1 and CBP. Because a total of 39 FG repeats are present in the NH₂-terminal of the NUP98 protein (amino acids 1-514) and most of them are retained in NUP98-containing fusion proteins (Fig. 4A), a confined mapping of HDAC1 and CBP binding regions seems to be necessary. Furthermore, the reported CBP fragment that reacts with FG repeats of NUP98 (amino acids 1-469) also contains a large number of amino acids (amino acids 1-1,892; ref. 10). Accordingly, we first applied GST pull-down assays to narrow down the CBP region that interacts with FG repeats of NUP98. As shown in Fig. 4B, only the portion of amino acids 451 to 721 (271 amino acids) from CBP interacts with N-NUP98, permitting the determination of a much smaller than previously published region (1,892 amino acids) that binds to NUP98. Interestingly, this portion of CBP seems to retain binding activities with many other transcription factors and cofactors as well (27). Then, the full-length sequence of NUP98 was sequentially broken into FG1, FG2, FG3, and C-NUP98 fragments, and each of them was in vitro-translated, and individually reacts with GST-tagged HDAC1, the GST-tagged CBP interacting fragment (271 amino acids) and GST only (Fig. 4A). Interestingly, the translation products of all the NH₂-terminal fragments of NUP98 seem to be capable of recruiting both CBP and HDAC1, supporting the notion that NUP98-PMX1 and probably some other NUP98-containing fusion proteins as well, may function as a transcriptional coactivator and a corepressor.

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Recruitment of HDAC1 by NUP98-containing fusion proteins. A, GST pull-down assays were done with in vitro translation products of NUP98-containing fusion genes (i.e., NUP98-PMX1, NUP98-HOXA9, and NUP98-HOXC11) and related fragments (i.e., ΔNUP98, ΔPMX1, ΔHOXA9, and ΔHOXC11) in incubating with GST-HDAC1 or GST alone. Input, 20% of in vitro-translated proteins used for the pull-down assays. B, 293T cells were respectively transfected with GST-tagged NUP98-containing fusion genes or related fragments (i.e., NUP98-PMX1, NUP98-HOXA9, NUP98-HOXC11, ΔNUP98, ΔPMX1, and NUP54) and cell lysates were immunoprecipitated with anti-HDAC1 antibody (α-HDAC1) or irrelevant antibody (α-Fibrinectin). Input, 5% of whole cell lysates used for each immunoprecipitation. C, colocalization assays were done in HeLa cells. Cells were respectively transfected with different constructs (GFP-NUP98-PMX1, GFP-NUP98-HOXA9, and GFP-NUP98-HOXC11), immunoreacted with specific anti-HDAC1 antibody and then followed by immunofluorescence detection.
that of CBP. Thus, it can be deduced that mechanisms underlying the oncogenicity of NUP98-PMX1 can be far more complex than currently recognized, probably involving both gene activation and repression, and enabling cells to gain growth advantage and differentiation/apoptosis resistance. This notion seems to be well in accordance with the observation derived from a NUP98-HOXA9-related study. Out of the 102 NUP98-HOXA9 target genes, 92 were up-regulated, and 10 were down-regulated (16). Transcriptional repression of c-FOS gene by NUP98-PMX1 can also be supportive to this notion. Fos, a protein, well known transcriptional factor, plays a crucial role in the differentiation of hematopoietic precursors into mature blood cells (28, 29). As the cofactor of PU.1, Fos is detectable in myelocytes and metamyelocytes, and increased in amount with maturity (30). Blocking c-FOS expression using antisense oligomers seems to impair terminal differentiation of normal and M1 leukemic myeloblasts (31). When human U-937 and HL-60 leukemia cells were induced to differentiate toward the lineage of the macrophage, c-FOS gene was dramatically induced (32). Accordingly, transcriptional repression of c-FOS gene might also be contributive to differentiation resistance in the cases of CML-BC in which the NUP98-PMX1 fusion gene was identified.

Nucleoporins, components of nuclear pore complexes, play pivotal roles in docking, translocation, and termination of the transport of cellular molecules across the nuclear membrane (33). The formation of NUP98-PMX1 may therefore disrupt the normal structure and function of the nuclear pore complex, which can be relevant to leukemogenesis as well. Importantly, NUP98-PMX1 shares many features with other members of NUP98-involved fusion proteins such as NUP98-HOXA9 and NUP98-HOXC11. All these fusion proteins contain FG domains, capable of interacting with HDAC1 and CBP. Although regulating different sets of genes due to different fusion partners, common features associated with ANUP98 may enable them to act as both trans-activators and trans-repressors.

In addition, a number of new notions seem to be derived from this study, which may drive us to pursue further detailed investigations. For instance, coregulation of CBP and HDAC1 by the same/similar FG repeats may imply that these two important cofactors may share the same/similar binding features. Further detailed studies may allow us to better understand the mechanisms underlying the dual effects of aberrant transcription factor encoded by NUP98-PMX1 and probably some other NUP98-related fusion genes as well.

Discussion
Through intensive studies, we have found a number of interesting features, which could be of importance for the understanding of mechanisms underlying the leukemogenicity of NUP98-PMX1 and probably some other NUP98-containing fusion proteins as well, such as NUP98-HOXA9 and NUP98-HOXC11. First, NUP98-PMX1 seems to exert a dominant-negative effect on PMX1/ SRF-mediated c-FOS activation to some degree, as implicated by both in vitro luciferase assays and in vivo expression studies of c-FOS gene. This effect seems to be largely mediated by the recruitment of a corepressor molecule. By screening of commonly recognized corepressor molecules, we have found that HDAC1 is the only corepressor that can interact with the NUP98-PMX1 fusion protein, particularly with respect to its FG repeats. Accordingly, we have hypothesized that the FG domain containing NUP98-PMX1 is capable of recruiting both CBP and HDAC1, acting as an aberrant transcription factor with the activities of both a coactivator and corepressor. This hypothesis also seems to be supported by GST pull-down and detailed mapping assays in which the binding activity of HDAC1 to FG repeats seems to always be correlated with the binding region and recognition of the same FG repeats interacting with both CBP and HDAC1. A schematic representations of the NUP98 and truncated fragments used in the study. Horizontal bars, FG repeats; RD, RNA-binding domain. Numbers correspond to the amino acids of the full-length NUP98. B, [35S]methionine-labeled in vitro translation products of N-NUP98 were incubated with GST-tagged CBP fragments or GST alone, respectively. Input, 20% of in vitro-translated NUP98 proteins used for the pull-down assays. C, [35S]-labeled FG1, FG2, FG3, and C-NUP98 were respectively incubated with GST-HDAC1, GST-CBP (amino acids 451-721), and GST alone. Input, 20% of in vitro-translated proteins.


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