Cryptic chromosome 9q34 deletion generates *TAF-Iα/CAN* and *TAF-Iβ/CAN* fusion transcripts in acute myeloid leukemia

Roberto Rosati,* Roberta La Starza,* Gianluca Barba, Paolo Gorello, Valentina Pierini, Caterina Matteucci, Giovanni Roti, Barbara Crescenzi, Silvia Romoli, Teresa Aloisi, Franco Aversa, Massimo Fabrizio Martelli, Cristina Mecucci

From the Hematology and Bone Marrow Transplantation Unit, University of Perugia, 06122 Perugia, Italy.

Funding: this work was supported by grants from MIUR (Ministero per l'Istruzione, l'Università e la Ricerca Scientifica), FIRC (Fondazione Italiana per la Ricerca sul Cancro, to B. C.) AIRC (Associazione Italiana Ricerca sul Cancro), FIRB (Fondo per gli Investimenti per la Ricerca di Base), Fondazione Cassa di Risparmio di Perugia, AULL (Association for the Study and Therapy of Leukemias and Lymphomas in Umbria), and Associazione "Sergio Luciani", Fabriano, Italy.

Acknowledgments: DNA clones were kindly provided by Dr. M. Rocchi (DAPEG, University of Bari, Italy). The authors wish to thank Dr. Geraldine Boyd for assistance in preparing the manuscript.

**These two authors contributed equally to this work.*

Manuscript received July 13, 2006. Accepted November 20, 2006.

Correspondence: Cristina Mecucci, Hematology and Bone Marrow Transplantation Unit, University of Perugia, Policlinico Monteluce, via Brunamonti 51, 06122 Perugia, Italy. E-mail: crimecux@unipg.it

ABSTRACT

In hematologic malignancies chromosome aberrations generating fusion genes include cryptic deletions. In a patient with acute myeloid leukemia and normal karyotype we discovered a new cryptic 9q34 deletion and here report the cytogenetic and molecular findings. The 9q34 deletion extends 2.5 megabases and juxtaposes the 5' *TAF-I* to the 3' CAN producing a *TAF-I/CAN* fusion gene. *TAF-I/CAN* transcribes into two fusion proteins bearing either TAF-Iα or TAF-Iβ moieties. We set up molecular assays to monitor the chimeric *TAF-Iα/CAN* and *TAF-Iβ/CAN* transcripts which, after hematopoietic stem cell transplantation from an HLA-identical sibling, were no longer detected.

Key words: AML, 9q34 cryptic deletion, *TAF-I/CAN* fusion.

Haematologica 2007; 92:232-235

Chromosome rearrangements are diagnostic and prognostic markers which serve to classify acute myeloid leukemia (AML) into categories with specific clinical and hematologic features and to orienteer therapeutic choices. In 40-50% of AML with normal karyotype, *FLT3* and *NPM1* gene mutations are frequent and molecular cytogenetics have occasionally detected cryptic chromosome changes and genomic rearrangements. In a patient with AML-M4 with normal karyotype, and no *FLT3* or *NPM1* gene mutations we identified a cryptic interstitial del(9)(q34) during interphase fluorescence *in situ* hybridization (FISH) screening for the *BCR/ABL1* fusion. del(9)(q) has been reported as an isolated cytogenetic abnormality or in association with t(8;21)(qq2;q22) in AML;[1] cryptic del(9)(q34) has been found at the t(9;22)(q34;q11) translocation breakpoint in chronic myeloid leukemia (CML).[2] Here we demonstrate for the first time that a cryptic del(9)(q34) produces a *TAF-I/CAN* fusion gene.

The *CAN* gene (also known as NUP214, nuclear pore complex protein 214kDa), mapping at chromosome 9q34, encodes a nucleoporin that participates in nucleocytoplasmic transport through interactions with import/export proteins such as hCRM1 and TAP/NFX1.[3] TAF-1 (template activating factor-I) is a multi-tasking phosphoprotein involved in chromatin modeling, transcription, cell cycle control and protein phosphatase 2A inhibition.[4-7] *TAF-I* transcribes two isoforms, TAF-Iα and TAF-Iβ, which bear different N-terminal sequences since they are transcribed from alternative first exons. As they homo- and hetero-dimerize, TAF-Iα and TAF-Iβ might modulate overall TAF-Iβ activity.[8] Different levels of TAF-Iα and TAF-Iβ expression have been observed in various tissues and hematopoietic cell lines suggesting that their expression is tissue and cell-type specific.[9] Moreover, functional assays on native and recombinant proteins indicate that TAF-Iα mediates chromatin remodeling more weakly than does TAF-Iβ.[4] As part of the endoplasmic reticulum-associated SET complex, TAF-Iβ is cleaved by granzyme A during cytotoxic T-lymphocyte-mediated apoptosis.[10]

Design and Methods

Case report. A 35-year old man was admitted to our Department because of asthenia, arthralgia, and persistent fever. Clinical examination revealed an enlarged liver. Peripheral blood counts were: hemoglobin 7.9 g/dL, platelets 61×10^9/L, white blood cells 40×10^9/L

with 90% blasts. Acute myelomonocytic leukemia (AML M4 according to the French-American-British classification) was diagnosed from a bone marrow aspirate. Immunophenotyping was positive for myeloperoxidase, CD34, CD33, CD13, CD45, CD66b, CD15 and CD11b antigens. The karyotype was normal. Sequencing of the *FLT3* gene did not show any internal tandem duplications or activating loop mutations. *NPM1* exon 12 was normal as assessed by denaturing high-Performance liquid chromatography.[11]

The patient received daunorubicin and cytosine arabinoside as induction therapy and achieved remission according to hematologic parameters (<5% blasts on bone marrow smears) and FISH analysis (1.4% of nuclei with one fluorescence signal for *ABL1* which is within the cut-off established in our laboratory for *ABL1* monosomy/deletion in ten healthy controls). Reverse transcriptase polymerase chain reaction (RT-PCR) detected residual *TAF-I/CAN* positive clones (*data not shown*). Four months after diagnosis, the patient underwent hematopoietic stem cell transplantation from his HLA-identical brother.

Fluorescence in situ hybridization

FISH was performed according to previously published protocols.[12] The *BCR/ABL* rearrangement was studied with the LSI BCR/ABL Dual Color, Single Fusion probe (Vysis). A panel of 22 DNA clones was applied to delineate the 9q34 deletion (Figure 1).

Array comparative genomic hybridization (CGH)

The patient's sample was hybridized to the SpectralChip 2600 BAC Array (SpectralGenomics) following the manufacturer's instructions. Fluorescent signals were acquired with a GenePix 4000B scanner (Axon Instruments, Union City, CA, USA) running a GenePix Pro 6.0 software (Axon Instruments). Data were analyzed using SpectralWare™ BAC Array Analysis Software (Spectral Genomics).

Polymerase chain reaction

Bone marrow samples were obtained at diagnosis, after induction therapy, and at 14, 19, and 24 months after the HLA identical transplant. RNA and DNA extraction and cloning were done as already described.[13] The *TAF-I(SET)/CAN* fusion transcripts were amplified using the following primer pairs: SET_540F (GAAGAGGCAGCATGAGGAAC) + CAN_2916R (TACTTTGGGCAAGGATTTGG), 643 bp; SET_540F + CAN_2705R (CGATTGTTGGCTAGGGTGTT) 432 bp; SET_87F (GCAAGAAGCGATTGAACACA) + CAN_2916R, 1096 bp. Isoform-specific PCR were performed with the common reverse primer CAN_2601R (ATCATTCACATCTTGGACAGCA) and either TAFIa_42F (GAAACCAAGACCACCTCCTG) for the TAF-Iα isoform, or TAFIb_38F (AGCTCAACTCCAACCACGAC) for TAF-Iβ. Del(9)(9) genomic breakpoints were amplified from 100 ng of the patient's DNA with primers SET_747F (TGACGAAGAAGGGGATGAGGAT) and CAN_IN17_2R (CTGAGGCATTCAATTAAGTATGTC).

For nested PCR, the first amplification round was performed using primers SET_540F and CAN_2916R. The second round of amplification was performed with primers SET_747F and CAN_2601R. *GAPDH* (positive control) was amplified with primers GAPD247F (AATCCCATCACCATCTTCCA) and GAPD1147R (AGGGGAGATTCAGTGTGGTG).

Results and Discussion

A DNA probe used to study the *BCR/ABL1* rearrangement in our patient with AML M4 did not detect a *BCR/ABL1* fusion but gave an atypical hybridization pattern of one red and two green signals in 85% of nuclei, indicating an *ABL1* monoallelic loss. The other 15% of nuclei had a normal hybridization pattern. Array CGH confirmed loss of genomic material from chromosome 9q34.1 (clones RP11-98H23 and RP11-17O4, Figure 1A). No other genomic imbalances were identified. Metaphase FISH with a panel of 22 DNA clones for the 9q arm showed the deleted region was flanked by clone RP11-216B9, containing the 5'-end of *TAF-I*, and clone RP11-544A12, containing the 3'-end of *CAN* (Figure 1B). The intervening sequence, which included the 3' region of *TAF-1* (RP11-550J21) and the 5' region of *CAN* (RP11-143H20), was lost (Figure 1C-D), suggesting that a 2.5 Mb cryptic deletion at 9q34 juxtaposed *TAF-1* and *CAN*. RT-PCR on a bone marrow sample obtained at diagnosis amplified a *TAF-I/CAN* fusion transcript joining *TAF-I* exon 7 with *CAN* exon 18 (Figure 2A, B). Isoform-specific RT-PCR detected *TAF-Iα/CAN* and *TAF-Iβ/CAN* chimeric transcripts (*data not shown*). Long-range PCR on the patient's genomic DNA identified breakpoints at *TAF-1* intron 7 and *CAN* intron 17 (Figure 3). After induction therapy nested RT-PCR detected residual *TAF-I/CAN*-expressing clones which were no longer detected after hematopoietic stem cell transplantation from the patient's HLA-identical brother (*data not shown*). At a follow-up of 24 months, the patient is still in hematologic, FISH and molecular remission.

We demonstrate for the first time that a cryptic 9q34 interstitial deletion, which is easily detected by FISH using a commercially available probe for studying the t(9;22)(q34;q11)-BCR/ABL1, produces a *TAF-I/CAN* fusion gene. FISH, RT-PCR and genomic PCR were used to monitor our *TAF-I/CAN* positive AML, which responded partially to chemotherapy and was fully eradicated after hematopoietic stem cell transplantation from an HLA-identical sibling. Finding a cryptic del(9)(q34) as the genomic mechanism underlying a *TAF-I/CAN* fusion suggests that as long as only cytogenetics and PCR for known fusion proteins are used for genomic studies on AML the incidence of this rearrangement will remain unknown. FISH screening with the LSI BCR/ABL Dual Color, Single Fusion probe in 18 cases of AML with normal karyotype and no *NPM1* exon

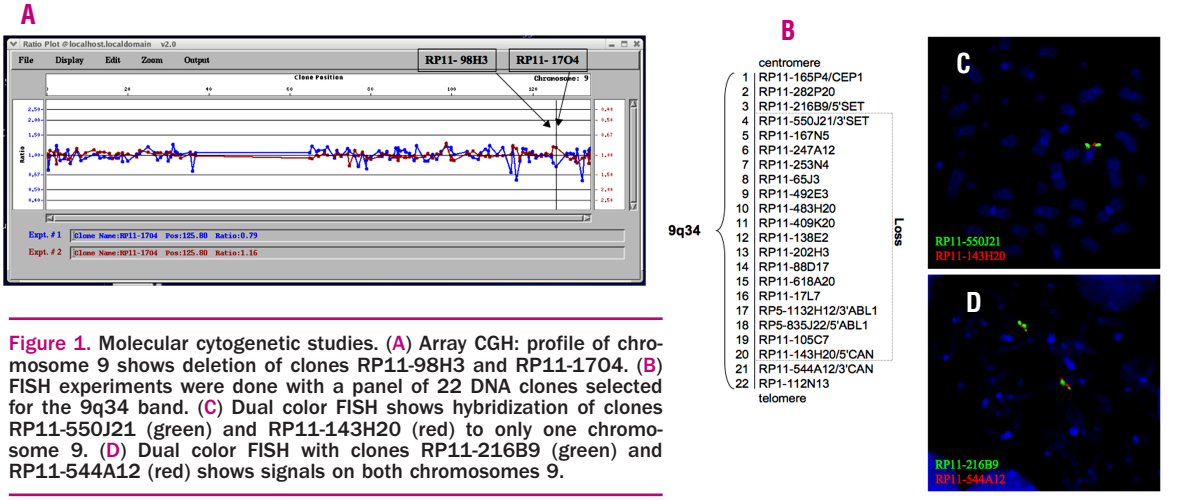

Figure 1. Molecular cytogenetic studies. (A) Array CGH: profile of chromosome 9 shows deletion of clones RP11-98H3 and RP11-17O4. (B) FISH experiments were done with a panel of 22 DNA clones selected for the 9q34 band. (C) Dual color FISH shows hybridization of clones RP11-550J21 (green) and RP11-143H20 (red) to only one chromosome 9. (D) Dual color FISH with clones RP11-216B9 (green) and RP11-544A12 (red) shows signals on both chromosomes 9.

12 mutations did not detect any other cryptic del(9)(q) (*data not shown*). Moreover, array-CGH did not detect cryptic del(9)(q34) in AML/myelodysplastic syndrome with isolated trisomy 8 at a karyotypic level, or in CML.[14,15]

In AML, del(9)(q) are recurrent cytogenetic aberrations which, despite different boundaries, share a 2.4 Mb common deleted sequence at band 9q21.3. Molecular studies of the 11 putative suppressor genes which have been mapped within this region suggest a haploinsufficiency mechanism of one or more critical genes (1). In CML, cryptic del(9)(q34) at the translocation breakpoint of t(9;22) (q34;q11) includes the 5'*ABL1* and has a variable extension towards the centromere. Interestingly, the largest deletions seem to have a poor prognostic significance, suggesting that loss of one or more tumor suppressor genes influences disease progression.[2] Cryptic deletions generating fusion genes have occasionally been described in hematologic malignancies. In a subgroup of T-cell acute lymphocytic leukemias the del(1)(p32), a 90 Kb sub-microscopic deletion, places the *TAL1* gene under control of the *SIL* promoter.[16] In 30-40% of chronic hypereosinophilic leukemias a del(4)(q12) of roughly 800 Kb generates the FIP1L1A/ PDGFRA fusion protein.[17] In two cases of AML, cryptic 11q23 deletions juxtapose *LARG* or *CBL* genes with *MLL*.[18,19]

Although the chromosomal rearrangements underlying the *TAF-I/CAN* chimeric fusion are reported here for the first time, involvement of *CAN* and *TAF* genes has already been observed in hematologic malignancies. In young adults with AML, *CAN* was first identified as a partner of *DEK* in the reciprocal t(6;9)(p23;q34) which accounts for 2-3% of chromosomal rearrangements in AML.[20] In one case of acute undifferentiated leukemia with normal karyotype, a variant of the *DEK/CAN* translocation i.e. the *TAF-I/CAN* fusion was reported with breakpoints in intron 17 of *CAN* and 800 bp 3' of the *TAF-I* gene.[21] Although genomic breakpoints were different in our patient, the same *TAF-I/CAN* fusion was formed as exon 7 of *TAF-I* was fused to exon 18 of *CAN*.

One major difference was TAF-I isoform involvement.

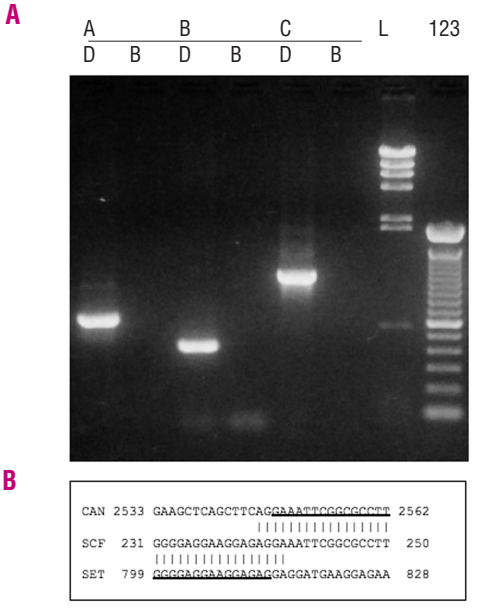

Figure 2. Amplification of *TAF-I(SET)/CAN* fusion cDNA. (A) Agarose gel electrophoresis of *TAF-I/CAN* fusion transcripts amplified by RT-PCR with the following primer pairs: *SC1*, SET_540F + CAN_2916R, 643 bp; "SC2", SET_540F + CAN_2705R, 432 bp; "SC3", SET_87F + CAN_2916R, 1096 bp. Lanes "D": amplification from patient cDNA; lanes "B": blanks (no cDNA). "L", marker λ DNA/HinDIII digest; "123", marker 123bp ladder (Invitrogen). (B) Partial sequence of the "SC2" PCR product showing in-frame fusion between *TAF-I(SET)* exon 7 (nt. 813 in GenBank accession NM_003011) and *CAN* exon 18 (nt. 2548 in GenBank accession NM_005085). Sequences corresponding to *TAF-I(SET)* exon 7 and to CAN exon 18 are underlined.

The TAF-Iβ/CAN fusion protein, which was expressed in the case of acute undifferentiated leukemia binds hCRM1, disorganizes nuclear export, causes cell cycle arrest at S phase, partially blocks vitamin D_3-induced differentiation and, like DEK/CAN, induces apoptosis in the U937 cell line.[22-24] As our patient with AML-M4 expressed both *TAF-Iα/CAN* and *TAF-Iβ/CAN* transcripts, this is the first observation of *TAF-Iα/CAN*. Although the biological impact of *TAF-Iα/CAN* in the leukemogenic process remains to be established, concomitant occurrence of transcripts with

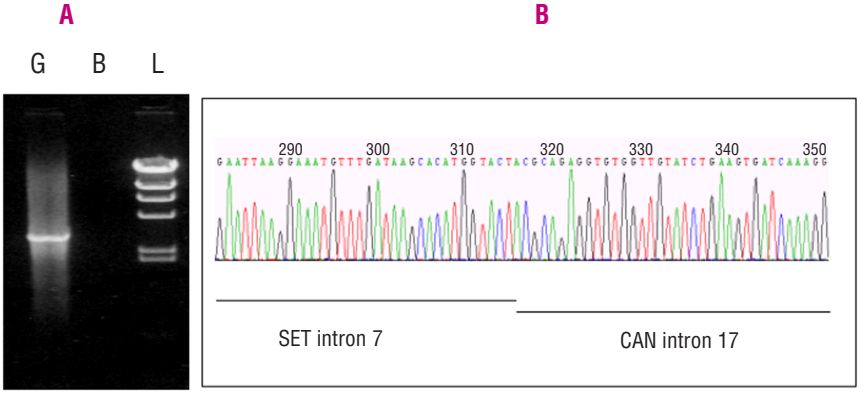

Figure 3. PCR amplification of the *TAF-I(SET)/CAN* genomic breakpoint. (A) Agarose gel electrophoresis. Patient DNA (lane "G", 100 ng) was amplified using primers SET_747F and CAN_IN17_2R. Lane "B", blank (no cDNA); lane "L", λ DNA/HinDIII digest. (B) Partial sequence of the PCR product showing fusion between intron 7 of *SET* and intron 17 of *CAN*.

both isoforms signifies molecular assays should assess each isoform transcript when monitoring the response to therapy of *TAF1/CAN* positive AML.

Authors' Contributions

RR designed the molecular studies and wrote the paper. RLS designed FISH experiments and wrote the paper. GB and CM performed and interpreted array-CGH studies. PG perfomed molecular studies. VP, selected and validated DNA clones and performed FISH experiments. BC supervised FISH analyses. SR prepared cell cultures and performed cytogenetic analysis. GR perfomed mutational analysis. TA provided clinical data. FA, and MFM were involved in the management of the patient. CM was responsible for the conception and supervision of the study.

Conflicts of Interest

The authors reported no potential conflicts of interest.

References

1. Sweetser DA, Peniket AJ, Haaland C, Blomberg AA, Zhang Y, Tanweer Zaidi S, et al. Delineation of the minimal commonly deleted segment and identification of candidate tumor-suppressor genes in del(9q) acute myeloid leukemia. Genes Chromosom Cancer 2005;44:279-91.
2. Sinclair PB, Nacheva EP, Leversha M, Telford N, Chang A, Reid A, et al. Large deletions at t(9;22) breakpoint are common and may identify a poor-prognosis subgroup of patients with chronic myeloid leukemia. Blood 1999;95:738-44.
3. Fornerod M, van Deursen J, van Baal S, Reynolds A, Davis D, Murti KG, et al. The human homologue of yeast CRM1 is in a dynamic subcomplex with CAN/Nup214 and a novel nuclear pore component Nup88. EMBO J 1997; 16: 807-16.
4. Okuwaki M, Nagata K. Template activating factor-I remodels the chromatin structure and stimulates transcription from the chromatin template. J Biol Chem 1998; 273:34511-8.
5. Cervoni N, Detich N, Seo SB, Chakravarti D, Szyf M. The oncoprotein Set/TAF-1β, an inhibitor of histone acetyltransferase, inhibits active demethylation of DNA, integrating DNA methylation and transcriptional silencing. J Biol Chem 2002; 277:25026-31.
6. Canela N, Rodriguez-Vilarrupla A, Estanyol JM, Diaz C, Pujol MJ, Agell N, et al. The SET protein regulates G2/M transition by modulating cyclin B-cyclin-dependent kinase 1 activity. J Biol Chem 2003;278:1158-64.
7. Li M, Makkinje A, Damuni Z. The myeloid leukemia-associated protein SET is a potent inhibitor of protein phosphatase 2A. J Biol Chem 1996;271: 11059-62.
8. Miyaji-Yamaguchi M, Okuwaki M, Nagata K. Coiled-coil structure-mediated dimerization of template activating factor-I is critical for its chromatin remodeling activity. J Mol Biol 1999;290: 547-57.
9. Nagata K, Saito S, Okuwaki M, Kawase H, Furuya A, Kusano A, et al. Cellular localization and expression of template-activating factor I in different cell types. Exp Cell Res 1998;240:274-81.
10. Fan Z, Beresford PJ, Oh DY, Zhang D, Lieberman J. Tumor suppressor NM23-H1 is a granzyme A-activated DNase during CTL-mediated apoptosis, and the nucleosome assembly protein SET is its inhibitor. Cell 2003;112:659-72.
11. Roti G, Rosati R, Bonasso R, Gorello P, Diverio D, Martelli MF, et al. DHPLC: a valid approach for identifying NPM1 mutations in acute myeloid leukemia. J Mol Diagn 2006; 8:254-9.
12. Crescenzi B, La Starza R, Romoli S, Beacci D, Matteucci C, Barba G, et al. Submicroscopic deletions in 5q- associated malignancies. Haematologica 2004; 89:281-5.
13. Rosati R, La Starza R, Luciani L, Gorello P, Matteucci C, Pierini V, et al. TPM3/PDGFRB fusion transcript and its reciprocal in chronic eosinophilic leukemia. Leukemia 2006; 20:1623-4.
14. Paulsson K, Heidenblad M, Strömbeck B, Staaf J, Jönsson G, Borg A, et al. High-resolution menome-wide array-based comparative menome hybridization reveals cryptic chromosome changes in AML and MDS cases with trisomy 8 as the sole cytogenetic aberration. Leukemia 2006;20:840-6.
15. Hosoya N, Sanada M, Nannya Y, Nakazaki K, Wang L, Hangaishi A, et al. Genomewide screening of DNA copy number changes in chronic myelogenous leukemia with the use of high-resolution array-based comparative genomic hybridization. Genes Chromosom Cancer 2006;45:482-94.
16. Breit TM, Mol EJ, Wolvers-Tettero IL, Ludwig WD, van Wering ER, van Dongen JJ. Site-specific deletions involving the tal-1 and sil genes are restricted to cells of the T cell receptor α/β lineage: T cell receptor δ gene deletion mechanism affects multiple genes. J Exp Med 1993;177:965-77.
17. Cools J, DeAngelo DJ, Gotlib J, Stover EH, Legare RD, Cortes J, et al. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N Engl J Med 2003;348:1201-14.
18. Fu JF, Hsu JJ, Tang TC, Shih LY. Identification of CBL, a proto-oncogene at 11q23.3, as a novel MLL fusion partner in a patient with de novo acute myeloid leukemia. Genes Chromosomes Cancer 2003;37:214-9.
19. Kourlas PJ, Strout MP, Becknell B, Veronese ML, Croce CM, Theil KS, et al. Identification of a gene at 11q23 encoding a guanine nucleotide exchange factor: evidence for its fusion with MLL in acute myeloid leukemia. Proc Natl Acad Sci USA 2000;97:2145-50.
20. Soekarman D, von Lindern M, Daenen S, de Jong B, Fonatsch C, Heinze B, et al. The translocation (6;9) (p23;q34) shows consistent rearrangement of two genes and defines a myeloproliferative disorder with specific clinical features. Blood 1992;79:2990-7.
21. von Lindern M, van Baal S, Wiegant J, Raap A, Hagemeijer A, Grosveld G. CAN, a putative oncogene associated with myeloid leukemogenesis, may be activated by fusion of its 3' half to different genes: characterization of the set gene. Mol Cell Biol 1992;12: 3346-55.
22. Fornerod M, Boer J, van Baal S, Morreau H, Grosveld G. Interaction of cellular proteins with the leukemia specific fusion proteins DEK-CAN and SET-CAN and their normal counterpart, the nucleoporin CAN. Oncogene 1996;13: 1801-8.
23. Boer J, Bonten-Surtel J, Grosveld G. Overexpression of the nucleoporin CAN/NUP214 induces growth arrest, nucleocytoplasmic transport defects, and apoptosis. Mol Cell Biol 1998;18: 1236-47.
24. Kandilci A, Mientjes E, Grosveld G. Effects of SET and SET-CAN on the differentiation of the human promonocytic cell line U937. Leukemia 2004;18:337-40.