Journal of Cellular Biochemistry 79:103–112 (2000)

Leukemia-Associated AML1/ETO (8;21) Chromosomal Translocation Protein Increases the Cellular Representation of PML Bodies

Sandra McNeil, Amjad Javed, Kimberly S. Harrington, Jane B. Lian, Janet L. Stein, Andre J. van Wijnen, and Gary S. Stein*

Department of Cell Biology and Cancer Center, University of Massachusetts Medical School, Worcester, Massachusetts 01655

Abstract Promyelocytic leukemia (PML) nuclear bodies are important components of nuclear architecture that are functionally linked to aberrant gene expression and disease. To understand the mechanisms that modify subnuclear distribution and regulatory activities of PML domains in leukemia, we performed immunofluorescence microscopy with a panel of normal diploid cells and established cell lines. We analyzed the representation and intranuclear distribution of PML domains. We find that multiple biological parameters contribute to heterogeneity in the subnuclear organization of PML domains in a broad spectrum of cell types. The subnuclear organization of PML domains was also evaluated following transient transfection with a series of vectors expressing normal hematopoietic and leukemia-related transcription factors. Our results show that expression of a chimeric transcription factor encoded by the tumor related chromosomal translocation (8;21) involving the AML1 and ETO loci is sufficient to cause reorganization of PML domains. This finding increases our understanding of the mechanisms by which the AML1/ETO protein may contribute to modified gene expression linked to the onset and progression of t(8;21) related acute myelogenous leukemia. J. Cell. Biochem. 79:103–112, 2000.

Key words: transcription factor; acute myelogenous leukemia (AML); nuclear matrix; promyelocytic leukemia (PML); cancer

The interrelationships between nuclear structure and function are important to the regulation of gene expression. Evidence is accumulating that intranuclear distribution of genes and regulatory factors is critical to transcriptional and post-transcriptional control [Stein et al., 1998; Berezney and Wei, 1998]. When regulatory factors or genes are targeted inappropriately, altered gene expression may result in development of disease. Changes in nuclear structure are hallmarks of cancer cells [Nickerson, 1998].

As a nuclear matrix-associated structure, promyelocytic leukemia (PML) nuclear bodies may be prognostic indicators of alterations in nuclear architecture that are related to aberrant gene expression and disease. PML nuclear bodies are discrete subnuclear foci that range in size from 0.1 to 1 μm in size. These domains have been referred to as nuclear bodies (NB), Kr-bodies, ND10, and PML oncogenic domains (PODs) [Ascoli and Maul, 1991; Lamond and Carmo-Fonseca, 1993; Dyck et al., 1994]. PML bodies are disrupted in acute promyelocytic leukemia (APL) [Dyck et al., 1994; Weis et al., 1994; Koken et al., 1994] in which a t(15;17) translocation is present that fuses PML to the retinoic acid receptor alpha (RARα) gene [de The et al., 1991; Goddard et al., 1991]. The PML nuclear bodies are restored by treatment with retinoic acid which also leads to clinical remission through restored promyelocyte differentiation [Dyck et al., 1994; Weis et al., 1994; Koken et al., 1994]. However, APL may also result from the expression of other RARα fusion proteins, including PLZF-RARα, NPM-RARα, and NuMA-RARα, that do not exhibit altered PML nuclear bodies [Hodges et al.,

Grant sponsor: National Institutes of Health; Grant number: AR45688.

*Correspondence to: Gary S. Stein, Department of Cell Biology and Cancer Center, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655.

Received 18 February 2000; Accepted 4 April 2000

1998]. Aberrations in nuclear structure/function are not limited to cancer, but are also observed in neurodegenerative disorders. For example, spinocerebellar ataxia type 1 (SCA-1) results from the expression of the mutant ataxin-1 protein containing an expanded polyglutamine repeat. The expression of this mutant ataxin-1 protein leads to the formation of large aggregates that result in abnormal localization of the PML protein [Skinner et al., 1997]. Aggregation of mutant ataxin-1, however, is not required for disease development [Klement et al., 1998]. The observed changes in PML bodies are important indicators of altered gene expression accompanying progression of disease [Seeler and Dejean, 1999].

Numerous proteins are associated with PML nuclear bodies. It was initially proposed that PML nuclear bodies may function as dynamic storage or waste accumulation sites for nuclear proteins (nuclear depots or nuclear dumps). These storage sites may represent concentrations of proteins in an inactive state that are dispersed to other sites in response to an appropriate signal [reviewed in Maul, 1998]. Recent studies support an active role for PML nuclear bodies in numerous nuclear functions including gene regulation, cell growth, differentiation, apoptosis, and viral infection [reviewed in Seeler and Dejean, 1999]. The promyelocytic leukemia protein (PML) [de The et al., 1991; Goddard et al., 1991; Kakizuka et al., 1991] is a tumor suppressor protein [Mu et al., 1994; Liu et al., 1995; Mu et al., 1996], a regulator of MHC expression [Zheng et al., 1998], and is involved in apoptosis [Quignon et al., 1998; Wang et al., 1998]. The Sp100 protein, which was first identified using auto-immune antibodies present in primary biliary cirrhosis, is also consistently present in PML nuclear bodies [Szostecki et al., 1990]. Other proteins found associated with PML nuclear bodies include Daxx [Yang et al., 1997; Li et al., 2000], the recQ helicase (BLM) [Neff et al., 1999], and NDP55 [Ascoli and Maul, 1991]. In addition, two proteins that are components of an ubiquitin-related pathway reside in PML nuclear bodies. One of these, designated PML interacting clone (PIC1)/small ubiquitin-related modifier (SUMO-1)/ubiquitin-like protein-1 (UBL-1)/Sentrin [Boddy et al., 1996], plays a role in modifying PML and targeting to the PML nuclear bodies [Muller et al., 1998]. The second ubiquitin pathway-related factor present in PML nuclear bodies is the herpes simplex-associated ubiquitin-specific protease (HAUSP), a member of a family of proteins that removes ubiquitin adducts from proteins and protects them from degradation by the ubiquitin-proteasome pathway [Everett et al., 1997].

Because altered subnuclear organization of PML domains has been functionally linked to deregulation of gene expression during tumorigenesis, it is important to understand the mechanisms that modify intranuclear distribution and regulatory activities of PML domains. In this study, we show that expression of a chimeric transcription factor encoded by a single tumor related chromosomal translocation, (8;21), involving the AML1 (RUNX1)[1] and ETO loci, is sufficient to cause a reorganization of the PML domains. Our data provide novel insight into the mechanisms by which the AML1/ETO protein may contribute to the onset and progression of leukemia.

MATERIALS AND METHODS

Cell Culture, Transient Transfections, and Plasmids

SaOS-2, HeLa, C2C12, and IMR90 cells were acquired from the American Tissue Culture Collection (ATCC). ROB d7 cells were derived from rat calvarial osteoblasts. Human osteoblasts (i.e., HOB-04T8, HOB-03-CE10, HOB-03-CE6) were kindly provided by Barry Komm and Peter Bodine (Wyeth-Ayerst Research, Radnor, PA) [Prince et al., submitted]. Marrow cells transformed with v-myc (MLB13-myc clone 14 and MLB13-myc clone 17) were kindly provided by Vicky Rosen (Genetics Institute, Cambridge, MA) [Rosen et al., 1994]. C2C12, HeLa, and MLB13-MYC (clone 14 and clone 17) cells were maintained at 37°C in DMEM medium supplemented with 10% (v/v) fetal bovine serum (FBS). SaOS-2 cells were maintained at 37°C in McCoy's 5A medium supplemented with 15% FBS. IMR90 cells were maintained at 37°C in BME medium supplemented with 10% FBS. HOB 03-CE6, HOB 03-CE10, and HOB-04T8 cells were maintained at 34°C in phenol red-free DMEM/F-12 medium containing 10% heat-inactivated FBS. ROB cells were grown at 37°C in MEM medium sup-

[1]The nomenclature committee of the Human Genome Organization has recently designated the AML1 locus as RUNX1.

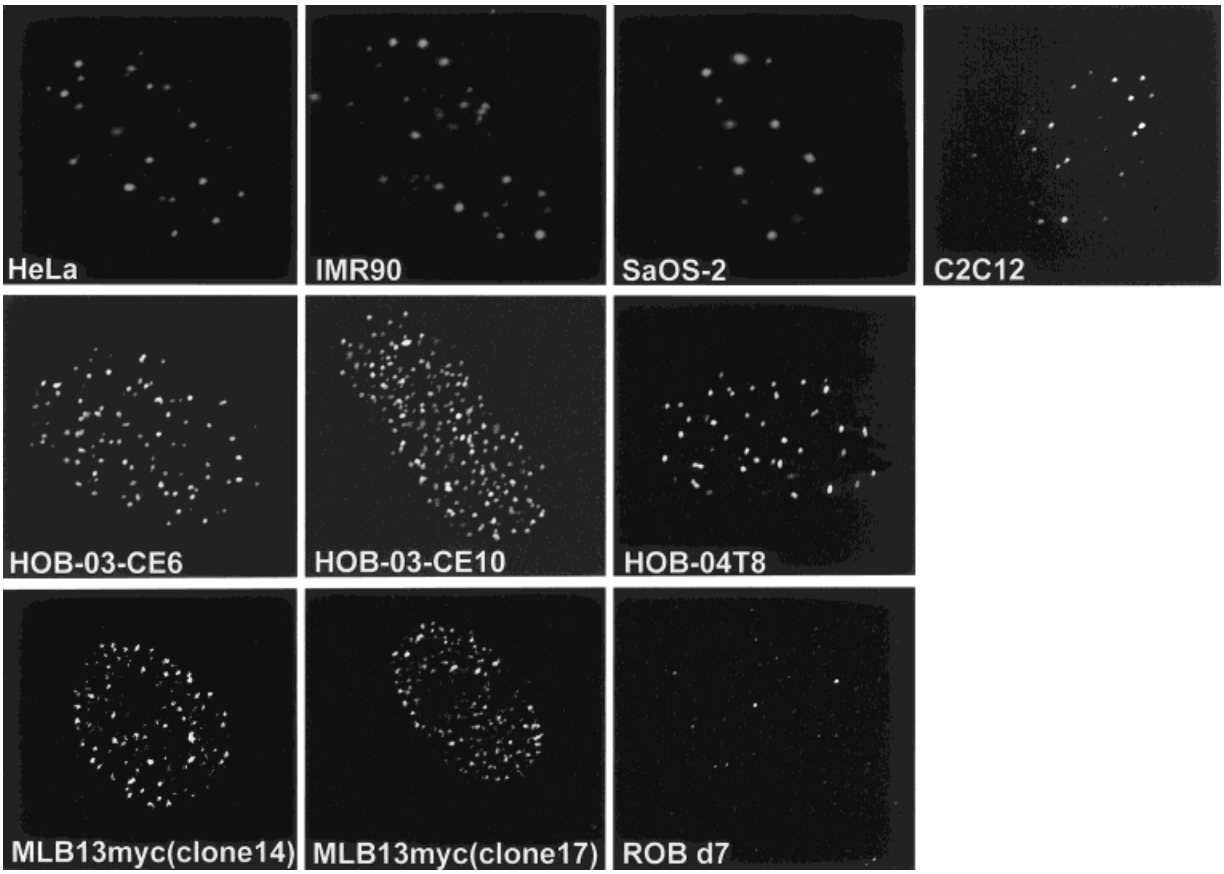

Fig. 1. Immunofluorescence staining of PML protein in various cell types reflects the heterogeneity of PML domains. Various cell types were analyzed by immunostaining of the nuclear matrix intermediate filament (NMIF) preparations and PML domains were detected using a mouse anti-PML primary antibody and a Texas red conjugated secondary antibody (shown in black and white).

plemented with 10% fetal calf serum (FCS). All cells were maintained in the presence of 1% (v/v) Penicillin-Streptomycin and 2 mM L-glutamine. Cells were plated on 0.5% gelatin-coated coverslips (Fisherbrand, #12-545-101, 22cir-1; Fisher, Pittsburgh, PA) in six-well tissue culture plates at a density of 0.2×10^6 cells/well. SaOS-2 cells were grown approximately 18 h after plating on coverslips in the McCoy's 5A supplemented with 15% FBS. Cell density was approximately 50% at the time of transfection. SaOS-2 cells were transfected using Superfect as described by the manufacturer (Qiagen, Valencia, CA). Transfection conditions were optimized to achieve low levels of expression by limiting the amount of expression vector and Superfect reagent. Optimization of the Superfect procedure included using 250 ng of expression vector and 5 μl of Superfect reagent per well. Cells were processed for immunofluorescence 48 h following transfection as described below. Epitope-tagged DNA constructs used were HA-AML1, HA-AML1/ETO, and Flag-ETO [Zeng et al., 1997; Meyers et al., 1995; Meyers and Hiebert, 1995; Lutterbach et al., 1998].

Cell Extraction, Fixation, and Immunofluorescence Microscopy

Cells were processed for nuclear matrix intermediate filament (NMIF) as described previously [McNeil et al., 1998]. PBS-A (PBS containing 0.5% bovine serum albumin) was used to block non-specific antibody binding, as the wash solution, and for antibody dilution, unless otherwise indicated. Antisera were as follows: a rabbit polyclonal antiserum to the HA epitope was diluted 1/1,000 (Santa Cruz Biotechnology, Santa Cruz, CA #SC805); a mouse monoclonal antibody to the FLAG-epitope was

TABLE IA. Distribution of PML Nuclear Bodies

Cell type	Species	Average number (SD)	Range	Comments	Reference for PML index
HEp2	Human	12.2 (3.2)	7–21	Epidermoid carcinoma, larynx cell line	Ascoli and Maul, 1991
WI38	Human	10.1 (4.2)	4–24	Normal embryonic lung fibroblast cell line	Ascoli and Maul, 1991
Fibroblast	—	26.4 (4.1)	6–40	—	Ascoli and Maul, 1991
Keratinocyte	—	8.5 (2.9)	4–17	—	Ascoli and Maul, 1991
Melanocyte	—	14.9 (4.4)	6–23	—	Ascoli and Maul, 1991
MCF-7	Human	14.6 (4.4)	4–40	Breast adenocarcinoma cell line	Ascoli and Maul, 1991
HeLa	Human	10	—	Epitheloid cervical carcinoma cell line	Cho et al., 1998
MCF-7	Human	10	—	Breast adenocarcinoma cell line	Cho et al., 1998
ML-2	Human	16	—	Myeloid leukemia cell line	Gordon et al., 2000
AML-Dx	Human	8.4	1–20	Peripheral blood monocytes from AML patient[a]	Gordon et al., 2000
ALL-Dx	Human	5.2	1–6	Peripheral blood monocytes from ALL patient[b]	Gordon et al., 2000
ALL-CR	Human	2.2	1–3	Peripheral blood monocytes from ALL patient in remission[c]	Gordon et al., 2000
Normal	Human	2.6	1–3	Normal peripheral blood monocytes[d]	Gordon et al., 2000

[a]Peripheral blood monocytes collected from a 30-year-old male AML patient, monosomy 10 and 21, at time of diagnosis.
[b]Peripheral blood monocytes collected from a 20-year-old female ALL patient, del(9;p21), at time of diagnosis.
[c]Same as in "b" with patient at time of remission.
[d]Peripheral blood moncoytes collected from a control individual without hematologic/oncologic disorders.

diluted 1/1,000 (Kodak, #IB13010 or Sigma #F3165); a mouse monoclonal antibody to human PML was diluted 1/1000 (Santa Cruz Biotechnology, #SC966); and a rabbit polyclonal to the ETO protein was diluted 1/500 (kindly provided by Scott Hiebert, Vanderbilt University, Nashville, TN). Diluted antibody was added as a 50 μl drop to coverslips in wells, covered lightly with Parafilm, and incubated for 1 h at 37°C or overnight at 4°C. Coverslips were rinsed four times with PBS-A and secondary antibody was added. Secondary antibody was goat anti-rabbit IgG conjugated to fluorescein or Texas Red or donkey anti-mouse IgG conjugated to fluorescein or Texas Red (Jackson Immunoresearch Laboratories, West Grove, PA) diluted 1/500, added to coverslips, and incubated 1 h at 37°C. Following incubation coverslips were rinsed four times with PBS-A; once with PBS-A containing 0.1% Triton X-100 and 0.05 μg per ml of the DNA counterstain DAPI; 1 time with PBS-A containing 0.1% Triton X-100; and twice in PBS. Coverslips were mounted in Vectashield (Vector Laboratories, Burlingame, CA) as an antifade mounting media. A Zeiss Axioplan 2 microscope equipped with epifluorescence filters and a CCD camera interfaced with the MetaMorph Imaging System (Universal Imaging Corporation, West Chester, PA) was used for analysis.

RESULTS AND DISCUSSION

Multiple Biological Parameters Contribute to Heterogeneity in the Subnuclear Organization of PML Domains

The number of PML nuclear bodies reflects structural and functional properties of nuclear

TABLE IB. Distribution of PML Nuclear Bodies

Cell type	Species	Average number	Range	Number	Comments
HOB-04T8	Human	—	40–49[a]	5	ts, SV40 T-antigen tranformed osteoblast
HOB-03-CE10	Human	—	180–195[a]	5	ts, SV40 T-antigen tranformed osteoblast
HOB-03-CE6	Human	—	150–175[a]	5	ts, SV40 T-antigen tranformed osteoblast
MLB13-MYC(Clone 14)	Mouse	—	110–125[a]	5	ts, v-myc transformed marrow cells
MLB13-MYC(Clone 17)	Mouse	—	90–110[a]	5	ts, v-myc transformed marrow cells
C2C12	Mouse	—	10–12[a]	5	Normal diploid myoblast
ROB d7	Rat	—	6–8[a]	5	Primary osteoblast
SAOS-2	Human	5.8	1–18[b]	575	Osteogenic sarcoma
HeLa	Human	19	5–34[b]	75	Epitheloid cervical carcinoma
IMR90	Human	22	5–39[b]	75	Normal diploid fetal lung

[a]The number of PML nuclear bodies was determined by counting and comparing several planes of a z-series.
[b]The number of PML nuclear bodies was determined by counting all of the PML nuclear bodies in nuclei.

architecture and varies significantly under different biological conditions (Table I, Fig. 1). PML domains were visualized in various cell types by immunofluorescence staining of nuclear matrix intermediate filament (NMIF) preparations with monoclonal antibodies against PML protein. Primary rat osteoblasts (ROB, d7), which are normal diploid cells derived from ex vivo cultures of rat calvarial tissue, exhibited a limited number of PML bodies. Similar results were obtained by Cho et al. [1998], who observed modest numbers of PML domains in many normal fetal and adult tissues. Consistent with previous results (Table IA), many established cell lines (C2C12, IMR90, HeLa, and SaOS-2), including both normal and tumor derived cells, were observed to have between five and 25 PML nuclear bodies (Table IB). Interestingly, several SV40 T-antigen transformed (HOB-04T8, HOB-03-CE10, and HOB-03-CE6) and v-MYC transformed cell lines (MLB-13-MYC-clone 14, and MLB-13-MYC-clone 17) contain a much greater number of PML nuclear bodies (Table IB). In general, we observed higher numbers of PML nuclear bodies in transformed cell lines than in other cell lines. One conclusion that emerges from our findings and those of others is that there is no strict correlation between PML organization and a single biological parameter (Table I). Thus, it appears that multiple biological factors contribute to the intranuclear distribution of PML domains.

PML nuclear bodies may be increased by many different biological events and by physiological mediators of gene expression. For example, Cho et al. [1998] have reported that the number and size of PML bodies is increased in malignant neoplasms relative to benign tumors and normal tissues. These authors also observed increased PML body staining in sex hormone related normal tissues (endometrium and myometrium). Hormonal changes in the uterine endometrium and mammary epithelial cells were observed to be associated with size variation in nuclear bodies [Padykula et al., 1981; Fitzgerald and Padykula, 1983]. Cho et al. [1998] observed that the number of PML bodies was increased in MCF7 cells treated with 17-hydroxy-β-estradiol, but not in hormone insensitive cells (MDA-MB-231). PML nuclear bodies are dispersed following heat shock or heavy metal exposure and redistributed to a great number of sites throughout the nucleus [Maul et al., 1995]. Viral infection with Herpes simplex virus-1 [Maul et al., 1993], human cytomegalovirus [Kelly et al., 1995; Korioth et al., 1996], or Adenovirus 5 [Carvalho et al., 1995; Doucas et al., 1996; Ishov and Maul, 1996] also results in dispersal of PML nuclear bodies. Interferon induced upregulation of PML and Sp100 result in increases in both size and number of PML nuclear bodies [Lavau et al., 1995; Grotzinger et al., 1996]. These studies indicate that tumorigenesis, hormone-signaling pathways, stress factors, viral infection, and

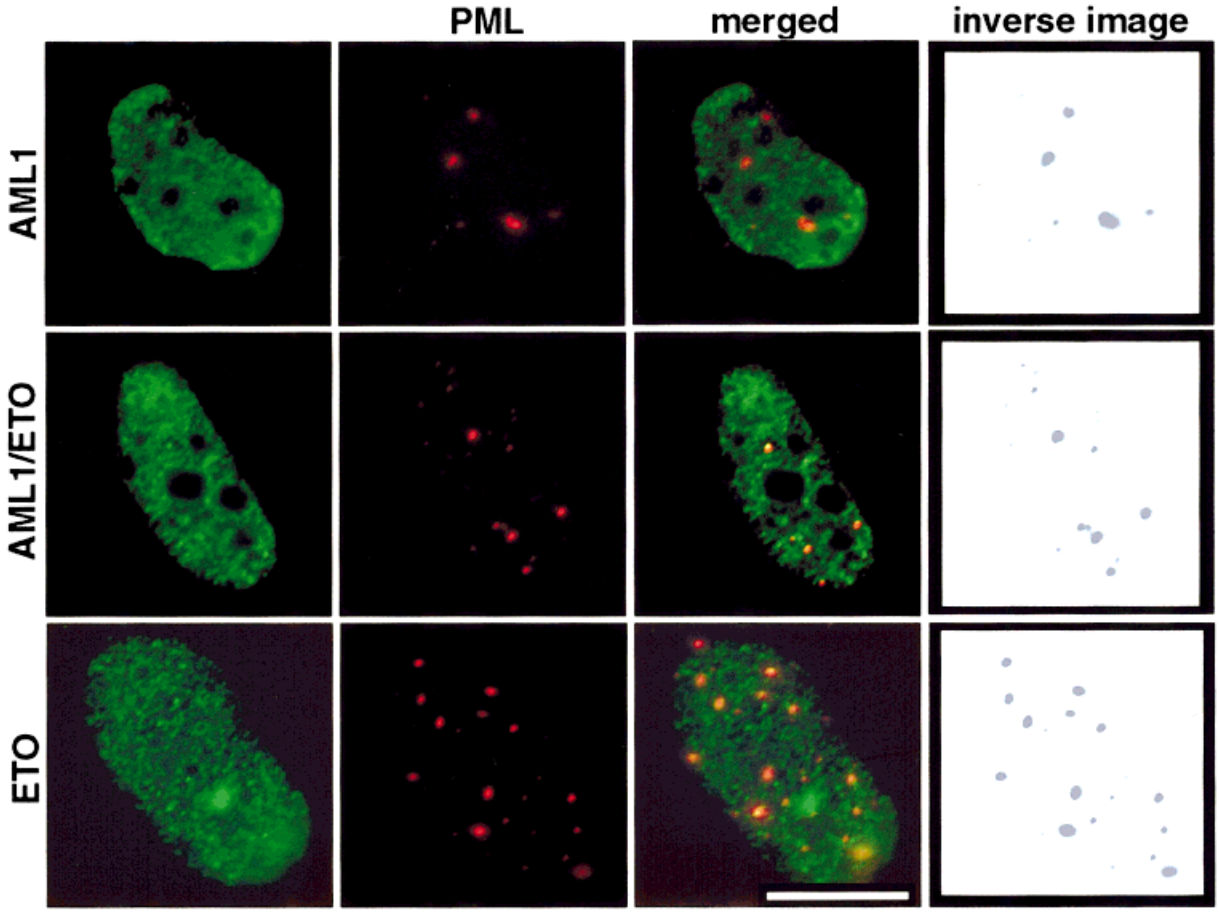

Fig. 2. Expression of the AML1/ETO fusion protein modifies NM-associated subnuclear organization of PML domains. The subnuclear distribution of PML bodies was analyzed in SaOS-2 cells transfected with either epitope tagged AML1 protein isoform AML1B, AML1/ETO, or ETO expression constructs. We determined the number of PML bodies present in expressing cells as compared to non-expressing cells. **Left**: AML1 (top), AML1/ETO (middle), and ETO (bottom) were detected using antibodies specific for epitope tags or the ETO protein and visualized using a fluorescein isothiocyanate conjugated secondary antibody detected as a green fluorescent signal. **Center**: PML domains were visualized using a mouse anti-PML primary antibody and a Texas red conjugated secondary antibody that is detected as a red fluorescent signal. **Right**: Merged images are shown. **Far right**: The PML images, shown in the center panel, were inverted in Adobe Photoshop and shown in black and white to improve visualization of PML bodies outside the focal plane. We observed that the average number of PML bodies is similar in both non-expressing and AML1 expressing cells. In contrast, this number increases in both AML1/ETO and ETO expressing cells. These results suggest that PML bodies are reorganized in response to AML1/ETO or ETO expression. Scale bar = 10 μm).

anti-viral agents all contribute to altered subnuclear organization of PML domains.

Genetic Abnormalities Present in Some Leukemias May Alter the Subnuclear Distribution of PML Domains

Apart from pleiotropic mediators of biological activity, reorganization of PML subnuclear domains has been observed in leukemic cells as a direct consequence of chromosomal translocations of the PML locus. For example, the PML-RARα fusion generated by the t(15;17) translocation in acute promyelocytic leukemia (APL) alters PML domains in a retinoic acid reversible manner [Dyck et al., 1994; Weis et al., 1994; Koken et al., 1994]. More recently, Gordon et al. [2000] observed that the number of PML nuclear bodies in hematopoietic cells are increased in both acute lymphocytic leukemia (ALL) and acute myelogenous leukemia (AML) patients at the time of diagnosis (Table IA). During remission of the disease the ALL patient had a normal number of PML nuclear bodies in peripheral blood monocytes. Neither

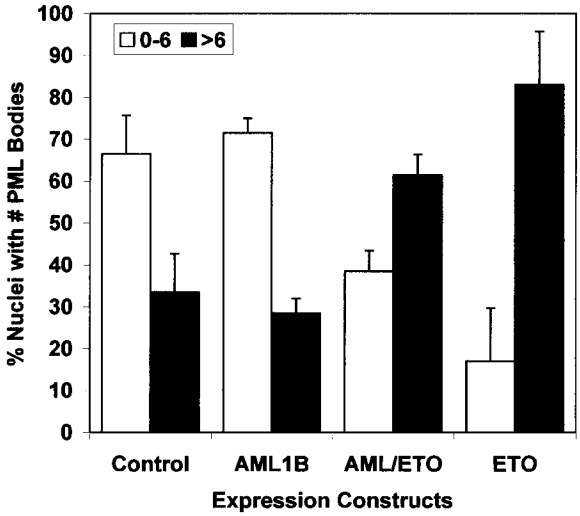

Fig. 3. The proportion of cells containing more than six PML bodies per nucleus is changed in response to expression of AML1/ETO or ETO, but not AML1. The bar graph illustrates the increased frequency of a higher than average number of PML bodies per nucleus in cells expressing AML1/ETO or ETO relative to control cells or cells expressing AML-1. Open bars represent the percentage of cells with zero to six PML bodies per nucleus and solid bars represent the percentage of cells with greater than six PML bodies per nucleus. The average number of PML bodies in the control cell population was approximately six.

ALL nor AML patients had a translocation of the PML locus, suggesting that rearrangement of the PML locus is not the only factor affecting PML domain distribution. Thus, genetic lesions present in leukemias other than APL result in altered subnuclear distribution of PML domains.

The acute myelogenous leukemia transcription factor AML1 is a key regulator of hematopoiesis [Okuda et al., 1996; Wang et al., 1996]. Numerous cytogenetic abnormalities that involve the genes encoding AML1 or its partner CBFβ have been identified in acute myelogenous leukemia and acute lymphocytic leukemia [Speck et al., 1999; Lutterbach et al., 2000; Speck and Stacy, 1995; Meyers and Hiebert, 1995; Meyers et al., 1995; Sawyers, 1997; Rowley, 1984]. The most frequently occurring translocation [Koeffler, 1987; Schiffer et al., 1989; Tashiro et al., 1992], t(8;21), produces a chimeric protein (AML1/ETO) in which the C-terminus of AML1 is replaced by the unrelated ETO (MTG8) protein [Erickson et al., 1992; Miyoshi et al., 1991, 1993; Kozu et al., 1997; Le et al., 1998]. The AML1/ETO fusion protein encoded by the (8;21) chromosomal translocation lacks a nuclear matrix targeting signal that directs the AML1 protein to distinct gene regulatory sites within the nucleus [Zeng et al., 1997]. We previously reported that substitution of the multifunctional C-terminus of AML1 with the chromosome 8-derived ETO protein precludes targeting of the factor to AML1 subnuclear domains. Instead, the AML1/ETO fusion protein is redirected by the ETO component to alternate nuclear matrix-associated foci [McNeil et al., 1999].

To determine whether AML1/ETO may affect other aspects of nuclear organization, we analyzed the subnuclear distribution of PML bodies in human osteosarcoma cells (SaOS-2) transfected with epitope-tagged AML1, AML1/ETO, or ETO expression constructs (Fig. 2). The transfected cells were allowed to express the fusion protein and recover from the transfection for 48 h. We processed the transfected cells to retain the nuclear matrix intermediate filament (NMIF) fraction. PML domains and epitope tags were visualized by immunofluorescence staining with monoclonal antibodies against PML protein or polyclonal antibodies against the epitope tags. The number of PML bodies present in transfected cells was determined and compared to untransfected cells. At least 100 transfected and untransfected cells were counted for each expression construct. We noted that the transfection procedure appeared to result in a modest and transient increase in the number of PML nuclear bodies within the first 18 h after transfection (data not shown). However, this procedural effect subsided by the time of our analysis (48 h).

SaOS-2 cells expressing AML1 consistently exhibited the same number of PML bodies as non-expressing cells from the same experiments (Fig. 3; Table II). In contrast to AML1 expressing cells, we observed a significant increase in the average number of PML domains and the proportion of cells with an increased number of PML bodies (greater than six) in cells expressing AML1/ETO proteins (Fig. 3; Table II). Similar to AML1/ETO expressing cells, analysis of ETO expressing cells also reveals an increase in the average number of PML bodies, as well as the fraction of cells with many PML bodies. Not all expressing cells display an altered number of PML bodies. Values in Table II show an increase in the average number of PML bodies but the range of values did not change. No obvious or

TABLE II. Distribution of PML Nuclear Bodies

	Average number	SD	Range	Number	Comments
Non-expressing	5.7	2.7	1–14	175	Experiment 1
Non-expressing	6.1	2.5	3–12	300	Experiment 2
AML1B	5.6	2.6	4–18	50	Experiment 1
AML1B	5.6	2.2	2–12	100	Experiment 2
AML/ETO	7.8	3.5	1–20	50	Experiment 1
AML/ETO	7.5	3.6	1–18	100	Experiment 2
ETO	10.2	6.2	3–26	50	Experiment 1
ETO	10.1	3.2	7–17	50	Experiment 2

consistent effect on size of PML bodies was observed for any of the expression constructs. Thus, in SaOS-2 cells, over-expression of AML1/ETO and ETO, but not AML1 results in subtle modifications of subnuclear organization. Hence, aberrant expression of the ETO moiety present in the AML1/ETO fusion protein appears to be responsible for alterations in the subnuclear organization of PML domains. Although many factors may alter PML nuclear body distribution (see Table I), we demonstrate that expression of a single fusion protein may result in altered nuclear organization as reflected by the increased number of PML nuclear bodies.

Concluding Remarks

The t(8;21) chromosomal lesion is the most frequently observed genetic abnormality in acute myelogenous leukemia [Speck et al., 1999]. The t(8;21) encoded AML1/ETO fusion protein interferes with normal hematopoietic differentiation by interfering with AML1 dependent gene regulation [Lutterbach et al., 2000]. Recent data from our laboratory indicate that the fusion of the ETO protein with AML1 causes misdirection of the AML1 transcription factor to subnuclear foci distinct from those containing the wild-type protein [McNeil et al., 1999]. Thus, apart from abrogating fidelity in the normal molecular mechanisms that regulate AML1 responsive genes, the t(8;21) lesion perturbs intranuclear trafficking of the AML1 transcription factor. The main finding of this study is that expression of the t(8;21) related AML1/ETO protein also perturbs the subnuclear organization of PML domains. We conclude that alterations in molecular mechanisms and subnuclear organization of regulatory factors may contribute to the onset and progression of acute myelogenous leukemia.

ACKNOWLEDGMENTS

We thank Scott Hiebert for providing reagents and informative comments. We thank Congmei Zeng, Shirwin Pockwinse, and Karina Barseguian for helpful discussions, and Elizabeth Buffone for expert assistance with cell culture.

REFERENCES

Ascoli CA, Maul GG. 1991. Identification of a novel nuclear domain. J Cell Biol 112:785–795.

Berezney R, Wei X. 1998. The new paradigm: integrating genomic function and nuclear architecture. J Cell Biochem Suppl 30–31:238–242.

Boddy MN, Howe K, Etkin LD, Solomon E, Freemont PS. 1996. PIC 1, a novel ubiquitin-like protein which interacts with the PML component of a multiprotein complex that is disrupted in acute promyelocytic leukaemia. Oncogene 13:971–982.

Carvalho T, Seeler J-S, Ohman K, Jordan P, Pettersson U, Akusjärvi G, Carmo-Fonseca M, Dejean A. 1995. Targeting of adenovirus E1A and E4-ORF3 proteins to nuclear matrix-associated PML bodies. J Cell Biol 131:45–56.

Cho Y, Lee I, Maul GG, Yu E. 1998. A novel nuclear substructure, ND10: Distribution in normal and neoplastic human tissues. Int J Mol Med 1:717–724.

de The H, Lavau C, Marchio A, Chomienne C, Degos L, Dejean A. 1991. The PML-RAR alpha fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. Cell 66:675– 684.

Doucas V, Ishov AM, Romo A, Juguilon H, Weitzman MD, Evans RM, Maul GG. 1996. Adenovirus replication is coupled with the dynamic properties of the PML nuclear structure. Genes Dev 10:196–207.

Dyck JA, Maul GG, Miller WH, Chen JD, Kakizuka A, Evans RM. 1994. A novel macromolecular structure is a target of the promyelocyte-retinoic acid receptor oncoprotein. Cell 76:333–343.

Erickson P, Gao J, Chang KS, Look T, Whisenant E, Raimondi S, Lasher R, Trujillo J, Rowley J, Drabkin H. 1992. Identification of breakpoints in t(8;21) acute myelogenous leukemia and isolation of a fusion transcript, AML1/ETO, with similarity to Drosophila segmentation gene, runt. Blood 80:1825–1831.

Everett RD, Meredith M, Orr A, Cross A, Kathoria M, Parkinson J. 1997. A novel ubiquitin-specific protease is dynamically associated with the PML nuclear domain and binds to a herpesvirus regulatory protein [corrected and republished in EMBO J 1997 Apr 1;16(7):1519–30]. EMBO J 16:566–577.

Fitzgerald M, Padykula HA. 1983. Differing functional responses of simple and complex nuclear bodies in uterine luminal epithelial cells following estrogenic stimuli. Anat Rec 205:131–141.

Goddard AD, Borrow J, Freemont PS, Solomon E. 1991. Characterization of a zinc finger gene disrupted by the t(15;17) in acute promyelocytic leukemia. Science 254: 1371–1374.

Gordon JA, Pockwinse SM, Stewart FM, Quesenberry PJ, Nakamura T, Croce CM, Lian JB, Stein JL, van Wijnen AJ, Stein GS. 2000. Modified intranuclear organization of regulatory factors in human acute leukemias: Reversal following treatment. J Cell Biochem 77:30–43.

Grotzinger T, Sternsdorf T, Jensen K, Will H. 1996. Interferon-modulated expression of genes encoding the nuclear-dot-associated proteins Sp100 and promyelocytic leukemia protein (PML). Eur J Biochem 238:554–560.

Hodges M, Tissot C, Howe K, Grimwade D, Freemont PS. 1998. Structure, organization, and dynamics of promyelocytic leukemia protein nuclear bodies. Am J Hum Genet 63:297–304.

Ishov AM, Maul GG. 1996. The periphery of nuclear domain 10 (ND10) as site of DNA virus deposition. J Cell Biol 134:815–826.

Kakizuka A, Miller WH, Jr., Umesono K, Warrell RP, Jr., Frankel SR, Murty VV, Dmitrovsky E, Evans RM. 1991. Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RAR alpha with a novel putative transcription factor, PML. Cell 66:663–674.

Kelly C, van Driel R, Wilkinson GW. 1995. Disruption of PML-associated nuclear bodies during human cytomegalovirus infection. J Gen Virol 76 (Pt 11):2887–2893.

Klement IA, Skinner PJ, Kaytor MD, Yi H, Hersch SM, Clark HB, Zoghbi HY, Orr HT. 1998. Ataxin-1 nuclear localization and aggregation: role in polyglutamine-induced disease in SCA1 transgenic mice. Cell 95:41–53.

Koeffler HP. 1987. Syndromes of acute nonlymphocytic leukemia. Ann Intern Med 107:748–758.

Koken MH, Puvion-Dutilleul F, Guillemin MC, Viron A, Linares-Cruz G, Stuurman N, de Jong L, Szostecki C, Calvo F, Chomienne C. 1994. The t(15;17) translocation alters a nuclear body in a retinoic acid- reversible fashion. EMBO J 13:1073–1083.

Korioth F, Maul GG, Plachter B, Stamminger T, Frey J. 1996. The nuclear domain 10 (ND10) is disrupted by the human cytomegalovirus gene product IE1. Exp Cell Res 229:155–158.

Kozu T, Komori A, Sueoka E, Fujiki H, Kaneko Y, Matsui T, Uehara T, Seino Y, Ishii M. 1997. Significance of MTG8 in leukemogenesis. Leukemia 11:297–298.

Lamond AI, Carmo-Fonseca M. 1993. The coiled body. Trends Cell Biol 3:198–204.

Lavau C, Marchio A, Fagioli M, Jansen J, Falini B, Lebon P, Grosveld F, Pandolfi PP, Pelicci PG, Dejean A. 1995. The acute promyelocytic leukaemia-associated PML gene is induced by interferon. Oncogene 11:871–876.

Le XF, Claxton D, Kornblau S, Fan YH, Mu ZM, Chang KS. 1998. Characterization of the ETO and AML1-ETO proteins involved in 8;21 translocation in acute myelogenous leukemia. Eur J Haematol 60:217–225.

Li H, Leo C, Zhu J, Wu X, O'Neil J, Park EJ, Chen JD. 2000. Sequestration and inhibition of Daxx-mediated transcriptional repression by PML. Mol Cell Biol 20: 1784–1796.

Liu JH, Mu ZM, Chang KS. 1995. PML suppresses oncogenic transformation of NIH/3T3 cells by activated neu. J Exp Med 181:1965–1973.

Lutterbach B, Westendorf JJ, Linggi B, Isaac S, Seto E, Hiebert SW. 2000. A mechanism of repression by acute myeloid leukemia-1, the target of multiple chromosomal translocations in acute leukemia. J Biol Chem 275:651–656.

Lutterbach B, Westendorf JJ, Linggi B, Patten A, Moniwa M, Davie JR, Huynh KD, Bardwell VJ, Lavinsky RM, Rosenfeld MG, Glass C, Seto E, Hiebert SW. 1998. ETO, a target of t(8;21) in acute leukemia, interacts with the N- CoR and mSin3 corepressors. Mol Cell Biol 18:7176–7184.

Maul GG. 1998. Nuclear domain 10, the site of DNA virus transcription and replication. BioEssays 20:660–667.

Maul GG, Guldner HH, Spivack JG. 1993. Modification of discrete nuclear domains induced by herpes simplex virus type 1 immediate early gene 1 product (ICP0). J Gen Virol 74 (Pt 12):2679–2690.

Maul GG, Yu E, Ishov AM, Epstein AL. 1995. Nuclear domain 10 (ND10) associated proteins are also present in nuclear bodies and redistribute to hundreds of nuclear sites after stress. J Cell Biochem 59:498–513.

McNeil S, Guo B, Stein JL, Lian JB, Bushmeyer S, Seto E, Atchison ML, Penman S, van Wijnen AJ, Stein GS. 1998. Targeting of the YY1 transcription factor to the nucleolus and the nuclear matrix in situ: The C-terminus is a principal determinant for nuclear trafficking. J Cell Biochem 68:500–510.

McNeil S, Zeng CM, Harrington KS, Hiebert S, Lian JB, Stein JL, van Wijnen AJ, Stein GS. 1999. The t(8;21) chromosomal translocation in acute myelogenous leukemia modifies intranuclear targeting of the AML1/CBFA2 transcription factor. Proc Natl Acad Sci USA 96:14882–14887.

Meyers S, Hiebert SW. 1995. Indirect and direct disruption of transcriptional regulation in cancer: E2F and AML-1. Crit Rev Eukaryot Gene Exp 5:365–383.

Meyers S, Lenny N, Hiebert SW. 1995. The t(8;21) fusion protein interferes with AML-1B-dependent transcriptional activation. Mol Cell Biol 15:1974–1982.

Miyoshi H, Kozu T, Shimizu K, Enomoto K, Maseki N, Kaneko Y, Kamada N, Ohki M. 1993. The t(8;21) translocation in acute myeloid leukemia results in production of an AML1-MTG8 fusion transcript. EMBO J 12:2715–2721.

Miyoshi H, Shimizu K, Kozu T, Maseki N, Kaneko Y, Ohki M. 1991. t(8;21) breakpoints on chromosome 21 in acute myeloid leukemia are clustered within a limited region of a single gene, AML1. Proc Natl Acad Sci U S A 88: 10431–10434.

Mu ZM, Chin KV, Liu JH, Lozano G, Chang KS. 1994. PML, a growth suppressor disrupted in acute promyelocytic leukemia. Mol Cell Biol 14:6858–6867.

Mu ZM, Le XF, Glassman AB, Chang KS. 1996. The biologic function of PML and its role in acute promyelocytic leukemia. Leuk Lymphoma 23:277–285.

Muller S, Matunis MJ, Dejean A. 1998. Conjugation with the ubiquitin-related modifier SUMO-1 regulates the partitioning of PML within the nucleus. EMBO J 17:61–70.

Neff NF, Ellis NA, Ye TZ, Noonan J, Huang K, Sanz M, Proytcheva M 1999. The DNA helicase activity of BLM is necessary for the correction of the genomic instability of bloom syndrome cells. Mol Biol Cell 10:665–676.

Nickerson JA. 1998. Nuclear dreams: the malignant alteration of nuclear architecture. J Cell Biochem 70:172–180.

Okuda T, van Deursen J, Hiebert SW, Grosveld G, Downing JR 1996. AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. Cell 84:321–330.

Padykula HA, Fitzgerald M, Clark JH, Hardin JW. 1981. Nuclear bodies as structural indicators of estrogenic stimulation in uterine luminal epithelial cells. Anat Rec 201:679–696.

Quignon F, De Bels F, Koken M, Feunteun J, Ameisen JC, de The H. 1998. PML induces a novel caspase-independent death process. Nat Genet 20:259–265.

Rosen V, Nove J, Song JJ, Thies RS, Cox K, Wozney JM. 1994. Responsiveness of clonal limb bud cell lines to bone morphogenetic protein 2 reveals a sequential relationship between cartilage and bone cell phenotypes. J Bone Miner Res 9:1759–1768.

Rowley JD. 1984. Biological implications of consistent chromosome rearrangements in leukemia and lymphoma. Cancer Res 44:3159–3168.

Sawyers CL. 1997. Molecular genetics of acute leukaemia. Lancet 349:196–200.

Schiffer CA, Lee EJ, Tomiyasu T, Wiernik PH, Testa JR. 1989. Prognostic impact of cytogenetic abnormalities in patients with de novo acute nonlymphocytic leukemia. Blood 73:263–270.

Seeler JS, Dejean A. 1999. The PML nuclear bodies: actors or extras? Curr Opin Genet Dev 9:362–367.

Skinner PJ, Koshy BT, Cummings CJ, Klement IA, Helin K, Servadio A, Zoghbi HY, Orr HT. 1997. Ataxin-1 with an expanded glutamine tract alters nuclear matrix- associated structures. Nature 389:971–974.

Speck NA, Stacy T. 1995. A new transcription factor family associated with human leukemias. Crit Rev Eukary Gene Exp 5:337–364.

Speck NA, Stacy T, Wang Q, North T, Gu TL, Miller J, Binder M, Marin-Padilla M. 1999. Core-binding factor: A central player in hematopoiesis and leukemia. Cancer Res 59:1789s–1793s.

Stein GS, van Wijnen AJ, Stein JL, Lian JB, Pockwinse S, McNeil S. 1998. Interrelationships of nuclear structure and transcriptional control: Functional consequences of being in the right place at the right time. J Cell Biochem 70:200–212.

Szostecki C, Guldner HH, Netter HJ, Will H. 1990. Isolation and characterization of cDNA encoding a human nuclear antigen predominantly recognized by autoantibodies from patients with primary biliary cirrhosis. J Immunol 145:4338–4347.

Tashiro S, Kyo T, Tanaka K, Oguma N, Hashimoto T, Dohy H, Kamada N. 1992. The prognostic value of cytogenetic analyses in patients with acute nonlymphocytic leukemia treated with the same intensive chemotherapy. Cancer 70:2809–2815.

Wang Q, Stacy T, Binder M, Marin-Padilla M, Sharpe AH, Speck NA. 1996. Disruption of the Cbfa2 gene causes necrosis and hemorrhaging in the central nervous system and blocks definitive hematopoiesis. Proc Natl Acad Sci U S A 93:3444–3449.

Wang ZG, Ruggero D, Ronchetti S, Zhong S, Gaboli M, Rivi R, Pandolfi PP. 1998. PML is essential for multiple apoptotic pathways. Nat Genet 20:266–272.

Weis K, Rambaud S, Lavau C, Jansen J, Carvalho T, Carmo-Fonseca M, Lamond A, Dejean A. 1994. Retinoic acid regulates aberrant nuclear localization of PML-RAR alpha in acute promyelocytic leukemia cells. Cell 76: 345–356.

Yang X, Khosravi-Far R, Chang HY, Baltimore D. 1997. Daxx, a novel Fas-binding protein that activates JNK and apoptosis. Cell 89:1067–1076.

Zeng C, van Wijnen AJ, Stein JL, Meyers S, Sun W, Shopland L, Lawrence JB, Penman S, Lian JB, Stein GS, Hiebert SW. 1997. Identification of a nuclear matrix targeting signal in the leukemia and bone-related AML/CBF α transcription factors. Proc Natl Acad Sci USA 94:6746–6751.

Zheng P, Guo Y, Niu Q, Levy DE, Dyck JA, Lu S, Sheiman LA, Liu Y. 1998. Proto-oncogene PML controls genes devoted to MHC class I antigen presentation. Nature 396:373–376.