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Distinct leukemia phenotypes in transgenic mice and different corepressor interactions generated by promyelocytic leukemia variant fusion genes *PLZF–RARα* and *NPM–RARα*

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ABSTRACT **Acute promyelocytic leukemia (APL) is characterized by a specific chromosome translocation involving *RARα* and one of four fusion partners: *PML*, *PLZF*, *NPM*, and *NuMA* genes. To study the leukemogenic potential of the fusion genes *in vivo*, we generated transgenic mice with *PLZF–RARα* and *NPM–RARα*. *PLZF–RARα* transgenic animals developed chronic myeloid leukemia-like phenotypes at an early stage of life (within 3 months in five of six mice), whereas three *NPM–RARα* transgenic mice showed a spectrum of phenotypes from typical APL to chronic myeloid leukemia relatively late in life (from 12 to 15 months). In contrast to bone marrow cells from *PLZF–RARα* transgenic mice, those from *NPM–RARα* transgenic mice could be induced to differentiate by all-*trans*-retinoic acid (ATRA). We also studied RARE binding properties and interactions between nuclear corepressor SMRT and various fusion proteins in response to ATRA. Dissociation of SMRT from different receptors was observed at ATRA concentrations of 0.01 μM, 0.1 μM, and 1.0 μM for RARα–RXRα, NPM–RARα, and PML–RARα, respectively, but not observed for PLZF–RARα even in the presence of 10 μM ATRA. We also determined the expression of the tissue factor gene in transgenic mice, which was detected only in bone marrow cells of mice expressing the fusion genes. These data clearly establish the leukemogenic role of PLZF–RARα and NPM–RARα and the importance of fusion receptor/corepressor interactions in the pathogenesis as well as in determining different clinical phenotypes of APL.**

Acute promyelocytic leukemia (APL) is characterized in most patients by accumulation of promyelocytes containing a specific chromosomal translocation t(15;17) and a unique sensitivity to all-*trans*-retinoic acid (ATRA) treatment (1, 2). There have been three variant translocations reported, including t(11;17)(q23;q21), t(5;17)(q32;q21), and t(11;17)(q13;q21). These chromosomal translocations invariably involve the *RARα* (retinoic acid receptor α) gene on chromosome 17, and the *PML* (promyelocytic leukemia), *PLZF* (promyelocytic leukemia zinc finger), *NPM* (nucleophosmin), and *NuMA* (nuclear mitotic apparatus protein) genes on chromosomes 15q22, 11q23, 5q32, and 11q13, respectively (3–10). The breakpoints in the *RARα* gene are always fixed, regardless of its fusion partner; therefore, *RARα* retains the same functional domains (B–F), including the DNA, corepressor/coactivator, and ligand binding regions in all fusion proteins (11, 12). RARs are transcription factors that bind to RAREs (retinoic acid response elements) in a heterodimer formed with RXRs (retinoic X receptors; refs. 13, 14). In the absence of ATRA, RARα/RXRα is associated with a nuclear corepressor composed of N-CoR (nuclear receptor corepressor) or SMRT (silencing mediator for retinoid and thyroid-hormone receptors), Sin3A or Sin3B, and histone deacetylase HDAC1 or HDAC2, thus inhibiting transcription of ATRA-responsive genes. ATRA dissociates corepressors from and recruits coactivators to RARα/RXRα, resulting in transcription of RARE-responsive genes and subsequent induction of cell differentiation and growth arrest (15, 16).

PML–RARα, PLZF–RARα, and NPM–RARα (hereafter, together these are referred to as X–RARα) fusion proteins have the same RARα part but differ in the X moiety. PML is a member of the RING finger family, which may have growth suppressor activity (5, 12). PLZF is a phosphoprotein with an N-terminal BTB/POZ (Pox-virus and zinc finger) domain and a C-terminal *Kruppel* DNA binding domain (6, 7). PML and PLZF show similar nuclear distribution in structures known as nuclear bodies, whereas expression of PML–RARα fusion protein disrupts the normal subcellular localization of PML, which could be restored by an effect of ATRA (17–19). NPM is a major nonribosomal nucleolar phosphoprotein that is also implicated in a number of other malignancies (8, 12). Patients with APL with different fusions are clinically indistinguishable, except that patients with PLZF–RARα fusion are resistant to ATRA treatment (20–22). A number of studies have confirmed the leukemogenic role of PML–RARα (23–28). In an attempt to define the leukemogenic potential of variant fusion genes, we generated transgenic mice with *PLZF–RARα* and *NPM–RARα*. Consistent with a recent report (29), *PLZF–RARα* transgenic mice had leukemia phenotypes different from those of *PML–RARα*. Transforming activity of *NPM–RARα* fusion gene, on the other hand, was identified in transgenic animals. As part of the phenotype analysis, we also determined the expression of the tissue factor (TF) gene, which has been considered to be involved in the pathogenesis of coagulopathies typical of APL (30, 31).

Both PML–RARα and PLZF–RARα bind to RAREs as homodimers or in a complex with RXRα (32). Previous studies have shown that these two fusion proteins have ligand-binding affinities similar to the wild-type molecule RARα/RXRα and

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Abbreviations: APL, acute promyelocytic leukemia; ATRA, all-*trans*-retinoic acid; TF, tissue factor; BM, bone marrow; RT-PCR, reverse transcription–PCR; GST, glutathione *S*-transferase; CMV, cytomegalovirus; SV40, simian virus 40; CML, chronic myeloid leukemia.

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can form a complex with nuclear corepressors. In interacting with nuclear corepressors, however, these fusion proteins have different ligand sensitivities, which may be the underlying molecular mechanism for differential responses to ATRA (15, 16, 33, 34). To understand better the molecular mechanisms underlying APL pathogenesis and response to ATRA, we addressed the interactions between NPM–RARα and nuclear corepressor in response to ATRA in comparison with RARα/RXRα, PML–RARα, and PLZF–RARα.

MATERIALS AND METHODS

Construction of Transgenes. Both *PLZF–RARα* and *NPM–RARα* fusion gene cDNAs were PCR amplified and cloned into the pUC9-hCG vector (kindly provided by Timothy J. Ley, Washington University Medical School, St. Louis) by using restriction sites *Cla*I and *Not*I. Transgenic mice (C57) were generated by using the purified transgene fragments as described (26). Constructs also were made in which the expression of *PLZF–RARα* was driven by simian virus 40 (SV40) or cytomegalovirus (CMV).

Detection of Transgene Integration/Expression and TF Gene Expression. The murine genomic DNA was isolated from tail tissue. Integration of the transgenes was detected by PCR and Southern blot analysis. Primers were designed to amplify fragments spanning the breakpoints of *PLZF–RARα* and *NPM–RARα* cDNAs, respectively. Total RNA was extracted from bone marrow (BM) cells of transgenic mice. Expression of the transgenes was detected by a nested reverse transcription–PCR (RT-PCR) approach, by using two pairs of primers spanning the first intron of *hCG* (26, 29). RT-PCR primers were designed for the detection of mouse TF gene expression according to its mRNA sequence (35).

Morphological and Histochemical Analyses. Peripheral blood smears or BM cells prepared by using conventional methods were stained with Wright's Giemsa. Sections (4-μm thick) of a frozen spleen or liver from a transgenic or nontransgenic mouse were stained histochemically with the Wright's Giemsa or the myeloperoxidase. Femurs, spleens, or livers from either transgenic or nontransgenic mice were isolated, fixed in 10% formalin, and subsequently embedded in paraffin. Paraffin was then removed from the 4-μm-thick sections, and they were stained with hematoxylin and eosin.

Immunofluorescence. BM and spleen cells were fixed with 4% paraformaldehyde for 10 min and made permeable with methanol at −20°C for 7 min. The cells were incubated subsequently with a primary PLZF antibody (29).

ATRA Treatment of BM Cells. BM cells were isolated from transgenic leukemic mice before death. Cultures were performed at a cell concentration of 10^6 cells per ml in RPMI medium 1640 with or without ATRA at a final concentration of 1 μM. Cells were harvested at 0, 24, 48 and 72 h for quantitative evaluation of differentiation induction and morphological studies.

Flow Cytometry. After blocking the murine FcII/III receptors with an unlabeled FcR (CD16/32) antibody, a single-cell suspension was incubated with Mac-1 (CD11b) antibody and Gr-1 (myeloid differentiation antigen) or c-Kit (CD117) antibodies. All antibodies were obtained from PharMingen. At least 1×10^4 cells were analyzed from each sample on a FACScan flow cytometer (Becton Dickinson).

Expression Plasmids, *in Vitro* Translation and Gel-Shift Assay. The *RARα*, *RXRα*, *PML–RARα*, *PLZF–RARα*, and *NPM–RARα* expression vectors were constructed as described (32). The RARα-specific binding sequence DR5G was end-labeled with [γ-^{32}P]ATP. The interactions between receptor proteins and DR5G as well as the specificity of the interactions were examined as described (32, 34).

Glutathione *S*-Transferase (GST) Pull Down. To determine the interaction between various fusion proteins and nuclear receptor corepressor SMRT in response to ATRA, SMRT cDNA (kindly provided by Ronald Evans and J. Don Chen, University of Massachusetts, Worcester, MA) was inserted into a GST expression vector. GST–SMRT fusion protein was preincubated with glutathione Sepharose beads. After centrifugation and washing with cold PBS, [^{35}S]methionine-labeled receptor proteins were added and incubated with or without ATRA. Beads were then washed with buffer H (20 mM Hepes, pH 7.7/50 mM KCl 20% (vol/vol) glycerol /0.1% NP-40/0.0007% β-mercaptoethanol). Bound proteins were eluted by boiling in sample buffer containing 10% SDS and resolved by SDS/8% PAGE (15, 32).

RESULTS

Generation of Transgenic Mice and Expression of Transgenes. We generated 51 stable founder lines of *hCG–PLZF–RARα* mice and 7 founder lines of *hCG–NPM–RARα* mice (Table 1). Littermates that developed leukemic phenotypes all showed positive expression of the transgene in BM cells, whereas, in a total of 12 *PLZF–RARα* lines randomly selected, 8 showed positive expression of the transgene in F_1 mice, and the other 4 were negative. Silencing or diminished expression of the integrated transgene may be due to nearby chromatin structure or other epigenetic factors caused by the integration. Most of the transgenic mice had normal development and fertility except those with an early onset of leukemia in the *PLZF–RARα* group. Immunofluorescence analysis demonstrated that littermates expressing *PLZF–RARα* fusion transcripts also had PLZF positive reactions in the cytoplasm of BM cells in a microgranular distribution. Of note, neither fusion gene expression nor leukemia phenotype was found in 9 lines of SV40–*PLZF–RARα* and 16 lines of CMV–*PLZF–RARα* over an observation period of 2 years.

Leukemia in *PLZF–RARα* Transgenic Mice. Of the 51 founder line *hCG–PLZF–RARα* mice, 6 developed leukemia phenotype; 5 of the 6 had short latent periods (31–85 days after birth), and 1 had a long latent period of 11 months. The five leukemic mice with early onset were characterized by low body weight (Fig. 1*A*), marked splenomegaly, leukocytosis, increased percentage of mature myeloid cells, and myeloid/lymphoid ratio in the peripheral blood and BM, resembling features of human chronic myeloid leukemia (CML), though promyelocytes also were present in significantly elevated percentages (≈20%) in the BM (Table 2; Fig. 1*C*). Histopathological analysis identified extreme expansion of myeloid cells at varying stages of maturation and disrupted BM trabeculae. Damage of splenic follicular architecture was observed, and the red pulp was infiltrated by myeloid cells, mainly myelocytes, metamyelocytes, and segmented forms (Fig. 1*D*). Focal infiltration of liver with myeloid precursors also was observed, but the gross structure was unaffected (Fig. 1*E*). The leukemia mouse with late onset presented with similar features,

Table 1. Generation of transgenic mice

	No.				
Fusion gene construct	Eggs injected	Eggs implanted	Recipient mice	Offspring produced	Transgene integrated
hCG–PLZF–RARα	2,902	1,517	70	135	51
SV40–*PLZF–RARα*	270	165	8	42	9
CMV–*PLZF–RARα*	927	426	20	28	16
hCG–NPM–RARα	923	400	19	38	7
Total	5,022	2,508	117	243	83

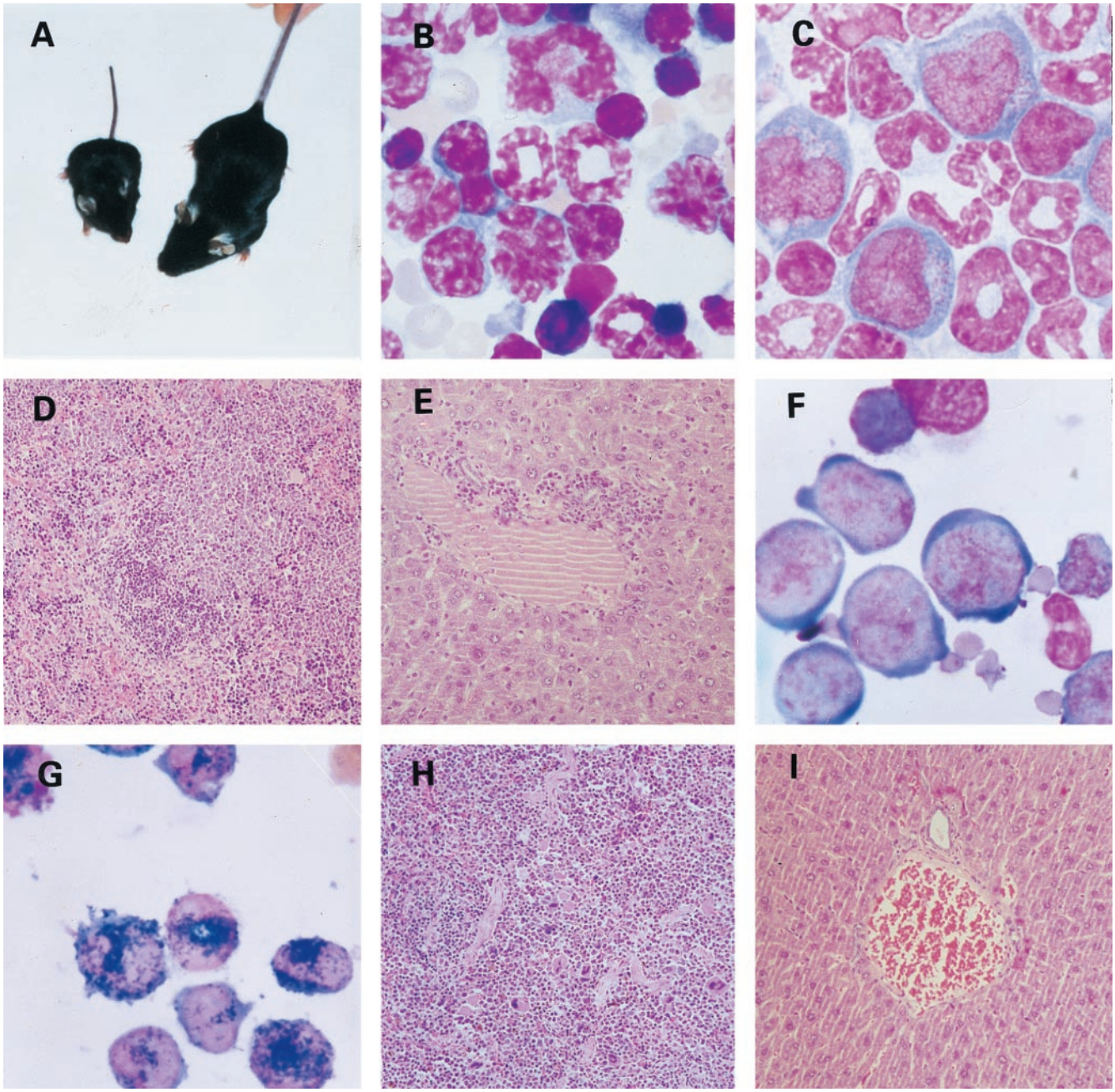

FIG. 1. Features of *PLZF–RARα* and *NPM–RARα* mice. (*A*) Growth retardation in a *PLZF–RARα* leukemia mouse of the early onset type (left, no. 153). On the right is a normal mouse of the same age. (*B*) BM picture of a wild-type mouse. (*C*) BM-cell morphology of a representative *PLZF–RARα* leukemia mouse (no. 153), showing presence of promyelocytes and predominance of mature myeloid cells. Erythroid and other lineages are severely suppressed. (*D*) Spleen section of the same mouse. Expanded red pulp with infiltration of large numbers of myeloid cells and damaged follicular structure are noted. (*E*) Liver section shows focal infiltration of myeloid cells around the sinus. (*F*) BM-cell morphology of *NPM–RARα* leukemic mouse of the typical APL type (no. 20). The BM is filled with immature myeloid cells (mainly promyelocytes) containing large amount of azurophilic granules that stain strongly with myeloperoxidase (*G*), resembling typical human APL. (*H*) Spleen section from the same mouse. Note the effacement of follicular architecture by infiltration of myeloid cells. (*I*) However, hepatic structure is not involved in this mouse (no. 20).

except that it developed normally until the onset of leukemia (Table 2, mouse no. 23).

Characteristics of *NPM–RARα* Transgenic Mice. Of the *hCG–NPM–RARα* mice, three developed leukemia, and another (Table 2, mouse no. 7) was killed when signs of disease were first observed; this mouse turned out to be still at the myelodysplasia-syndrome stage. There existed heterogeneity in cytology/pathology in the three leukemic mice, with a spectrum of manifestations from typical APL to CML-like syndrome. Mouse no. 20 showed marked leukocytosis and increased percentages of both peripheral myeloid cells and myeloid/lymphoid ratio; the morphology was typical of APL, with immature myeloid cells of enlarged cell volume and increased nucleus/cytoplasm ratio (Fig. 1*F*). Azurophilic granules that showed strong positive myeloperoxidase staining were apparent in the cytoplasm (Fig. 1*G*). Mouse no. 16, in contrast, had CML features similar to *PLZF–RARα* mice. Mouse no. 6 was characterized by myeloid cells with distorted nuclei, resembling the "monocyte-like" picture in some human APL. Splenomegaly was prominent in all three leukemic mice. Pathologically, in spite of distinct morphology, the red pulps were expanded and filled with large amount of myeloid cells, including myeloblasts, promyelocytes, and mature forms. Of note, in mouse no. 20, though disrupted splenic follicular

Table 2. Features of *PLZF–RARα* and *NPM–RARα* leukemic mice

Mouse No.	Onset, days	PB M/L	BM M/L	BM pathology	Spleen pathology	Liver pathology
31*	39	2.1	ND	CML-L	ND	ND
33*	38	0.9	64	CML-L	EDI	FI
34*	85	1.6	75	CML-L	EDI	FI
74*	43	1.2	ND	CML-L	ND	FI
153*	31	3.6	90	CML-L	EDI	FI
23*	330	2.3	15	CML-L	EDI	FI
6†	368	6.7	15	APL	EDI	FI
16†	418	3.4	12.5	CML-L	EDI	FI
20†	414	1.8	ND	APL	EDI	‡
7†	441	1.4	26.3	MDS	SE‡	‡

ND, not determined; PB, peripheral blood; M/L, myeloid/lymphoid ratio; CML-L, CML-like picture, with significantly increased promyelocytes (≈20%) in BM; EDI, enlarged, damaged structure, and myeloid cell infiltration; FI, focal infiltration; SE, slightly enlarged; MDS, myelodysplasia syndrome.
**PLZF–RARα* leukemic mice.
†*NPM–RARα* mice.
‡No obvious infiltration.

architecture (Fig. 1*H*) and BM trabeculae (not shown) were observed, no obvious hepatic infiltration was visible (Fig. 1*I*).

Expression of TF Gene Was Correlated with Fusion Gene Expression. To address the possible relationship between expression of TF and that of fusion genes, RT-PCR was performed on BM cells. As shown in Fig. 2, in mice not expressing the fusion gene, TF was not detectable, whereas mice expressing either *PLZF–RARα* or *NPM–RARα* fusion gene invariably showed TF gene expression.

ATRA Sensitivity of BM Cells from Transgenic Mice. We assessed the ATRA responsiveness of BM myeloid precursors from *NPM–RARα* transgenic mice at myelodysplasia-syndrome state (no. 7) and a leukemic *PLZF–RARα* mouse (no. 23). BM cells were cultured in the absence or presence of 1 μM ATRA for 24–72 h. In cell cultures from the *NPM–RARα* mouse, mature myeloid cells tended to increase, while, at the same time, immature myeloid cells tended to decrease 24, 48 and 72 h after ATRA treatment. Significant differentiation to maturation was observed 48 h after ATRA treatment, and Gr-1 positive cells increased with time on flow cytometric analysis. On the contrary, BM cells from *PLZF–RARα* transgenic mice were not inducible for further differentiation (Fig. 3).

NPM–RARα Bound to RARE. PML–RARα and PLZF–RARα bound to RARE in forms of homodimers or heterodimers with RXRα, though less efficiently than RARα/RXRα (Fig. 4*A*). NPM–RARα also bound to RARE but seemingly not in homodimers. According to the known molecular mass, both PML–RARα and PLZF–RARα monomers and homodimers are ≈110 kDa and 220 kDa, respectively, whereas

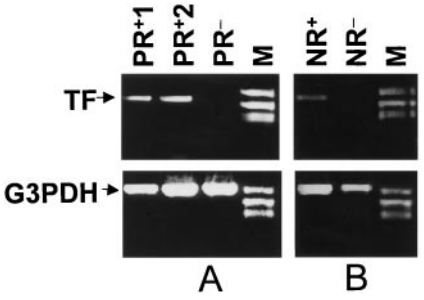

FIG. 2. RT-PCR result of TF gene expression in BM cells from *PLZF–RARα* (*A*) and *NPM–RARα* transgenic mice (*B*). Note that two mice with *PLZF–RARα* expression (PR^+1 and PR^+2) and one with *NPM–RARα* expression (NR^+) also had TF gene expression compared with mice with transgene integration but without fusion gene expression (PR^- and NR^-) where TF expression was absent. Glyceraldehyde-3-phosphate dehydrogenase control was amplified from the same RT products as TF gene but electrophoresed on separate lanes. M, marker 7zf/*Hae*III.

NPM–RARα monomer and homodimer should be ≈65 kDa and 130 kDa, respectively. Nevertheless, the NPM–RARα band moved slightly slower than the PML–RARα and PLZF–RARα homodimers (≈220 kDa), suggesting that NPM–RARα could form homotetramer (≈260 kDa). In addition, NPM–RARα formed heterodimer and a variety of other complexes with RXRα. Shifted bands tended to disappear with increasing concentrations of cognate DR5G, indicating specificity of RARE binding (Fig. 4*B*).

Association of NPM–RARα with SMRT in Response to ATRA. In the gel-shift assay, to analyze the interactions between GST–SMRT and wild-type as well as different fusion receptors, RARα/RXRα, PML–RARα, PLZF–RARα, and NPM–RARα showed different sensitivities to ATRA. Dissociation of receptors (Fig. 4*C*, open arrows) from high molecular mass complexes with SMRT (Fig. 4*C*, solid arrow) occurred at physiological concentrations (0.01 μM) for RARα/RXRα and at pharmacological concentrations of 0.1 μM and 1 μM, for NPM–RARα and PML–RARα, respectively. However, even in the presence of 10 μM ATRA, PLZF–RARα remained associated with SMRT. These results were confirmed by GST pull down. As shown in Fig. 4*D*, radio-labeled RARα, PML–RARα, PLZF–RARα, and NPM–RARα were retained specifically on the immobilized GST–SMRT without ATRA treatment. However, in the presence of various concentrations of ATRA, these proteins had different ligand sensitivities in binding with GST–SMRT. Notable was the diminished association of SMRT with RARα, PML–RARα, and NPM–RARα with increasing concentrations of ATRA, whereas PLZF–RARα binding to SMRT remained unchanged at 1 μM of ATRA (Fig. 4*D*).

DISCUSSION

After initial reports on leukemogenic potential of NPM–RARα in different systems (23–28), we investigated the phenotypes of *PLZF–RARα* and *NPM–RARα* transgenic mice and interactions of fusion proteins with RAREs and nuclear corepressors in response to ATRA on a comparative basis. Our results show that both PLZF–RARα and NPM–RARα play an essential role in leukemogenesis and in response to ATRA. However, these two fusion proteins are not identical in their leukemogenic potential.

Leukemia in *PLZF–RARα* and *NPM–RARα* Transgenic Mice. He *et al.* (29) reported that all *PLZF–RARα* transgenic mice developed CML 6–18 months after birth. In our series, however, five of the *hCG–PLZF–RARα* transgenic mice developed CML-like phenotype and died within 3 months after birth; one had a late onset at 11 months. Another striking feature was the growth retardation in early onset mice, which might be due to severe anemia caused by extreme expansion of myeloid cells in hemopoietic tissues. Unlike the accumulation of myeloid blasts blocked at the promyelocyte stage in BM of *PML–RARα* mice (26–28),

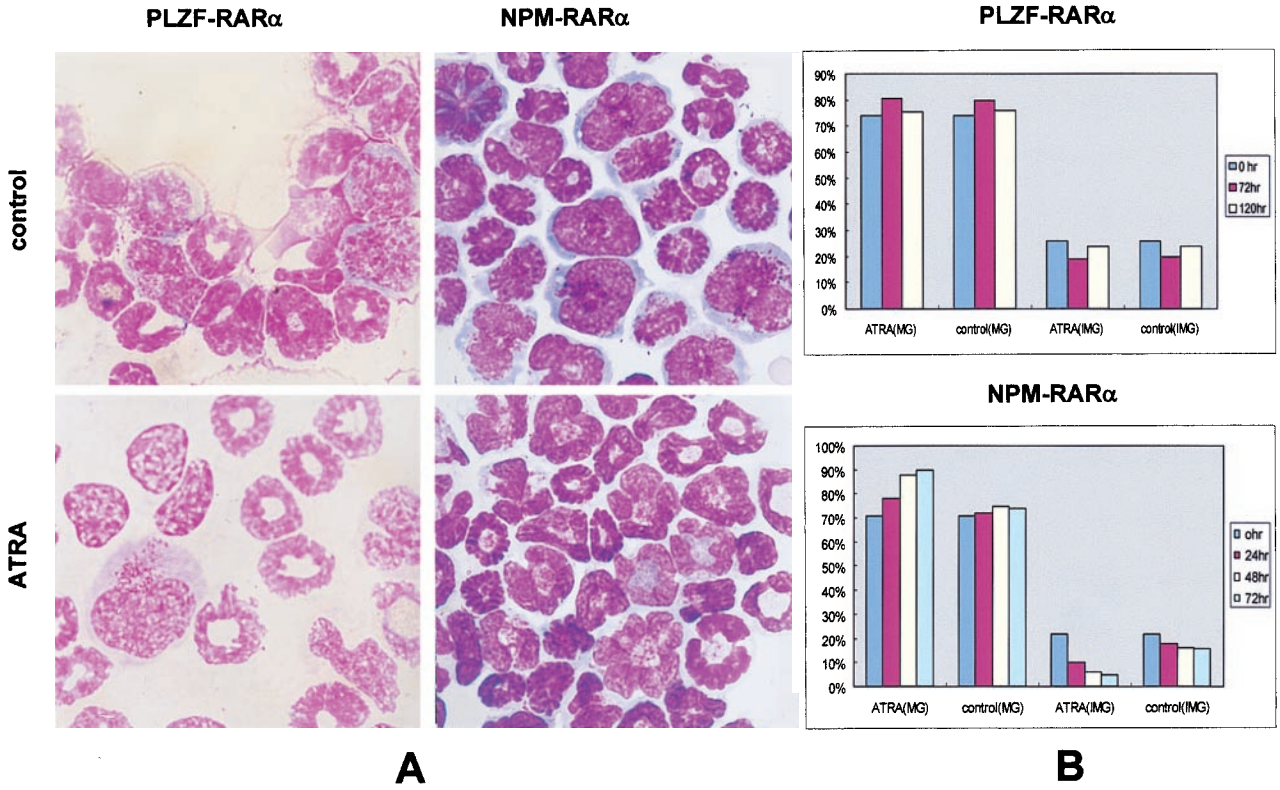

FIG. 3. ATRA response of BM cells from *PLZF–RARα* and *NPM–RARα* transgenic mice. (*A*) Morphology of the cells treated 72 h in the absence (control) or presence of 1 μM ATRA. (*B*) Percentages of mature granulocytes (MG) and immature granulocytes (IMG) are shown at different time intervals with or without ATRA treatment. (*Upper*) BM cells from *PLZF–RARα* leukemic mouse no. 23. (*Lower*) BM cells from *NPM–RARα* mouse no. 7.

the BM blasts in *PLZF–RARα* transgenic mice still retained the potential to differentiate terminally. It is possible that the *PLZF–RARα* oncogenic event occurred at a different stage of myeloid differentiation; the reciprocal transcript RARα–PLZF also may play a role in APL cell differentiation block, because it retains the DNA binding domain of PLZF and thus may disturb the normal

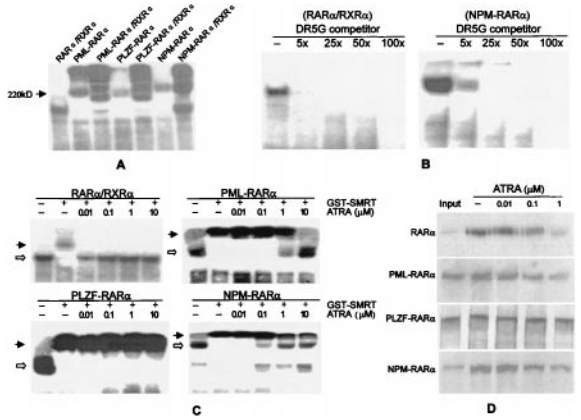

FIG. 4. (*A*) RARE binding of various fusion proteins. PML–RARα and PLZF–RARα bound to DR5G in homodimers or in complexes with RXRα. NPM–RARα also bound to DR5G possibly in the form of homotetramer or in different complexes with RXRα. (*B*) Disappearance of shifted bands with increasing concentrations of cognate DR5G indicates high specificity of RARE binding. (*C*) Gel-shift analysis showed different ligand sensitivities in interactions with SMRT. Compared with the wild-type receptor, PML–RARα and NPM–RARα had decreased ligand sensitivities, whereas PLZF–RARα was insensitive to ATRA even at pharmacological concentrations. Solid arrows indicate high molecular mass receptor/SMRT complex, whereas open arrows point to receptors dissociated from SMRT. (*D*) GST–SMRT pull down confirmed the above result.

trans-regulatory function of PLZF (12, 34). A recent report offered clues for this notion (36). In transgenic mice expressing the *RARα–PLZF* fusion gene, though no overt leukemia was observed, myelopoiesis was impaired. It is not clear whether there is any synergism between PLZF–RARα and RARα–PLZF in cell transformation. Transgenic mice expressing both transcripts of the fusion may clarify this issue.

NPM–RARα transgenic mice needed relatively long latent periods to develop leukemia with a spectrum of morphological features. Morphological heterogeneity in mice expressing the same transgene may be the result of an integration positional effect, or some other genetic "hits" may be required for the occurrence of full-blown APL. In *PML–RARα* transgenic mice, however, only APL phenotypes were observed unanimously by several groups (26–28); therefore, a more detailed study is required to identify the true mechanism for phenotypic heterogeneity in *NPM–RARα* transgenic mice. The different phenotypic features in mice expressing different transgenes may be attributed to the distinct biological properties of the X moiety, the possible effect of the reciprocal transcripts and the positional effect of transgene integration. Nevertheless, our data indicate that both PLZF–RARα and NPM–RARα possess leukemogenic potential.

Abnormal TF expression in APL cells is related to the coagulopathy in APL, though a number of other mechanisms also may be involved (30, 31). Our results suggest that TF expression may be correlated with expression of X–RARα fusion proteins. Cell lines harboring the translocation t(15;17) or transfected with *PML–RARα* also expressed TF (37, 38), suggesting that abnormal TF expression in promyelocytes may be due to novel transcriptional properties acquired by the X–RARα fusion products. Because a number of other tumor cell types also express TF (39), further investigation of the possible specific signaling pathways between TF expression and X–RARα is needed.

Altered Corepressor Interaction May Underlie Pathogenesis and Retinoic Acid Response of APL. Although they have different chromosome translocations and fusion gene formations, the similar phenotypes of human APL indicate a common biochemical pathway for APL pathogenesis. We showed that like PML–RARα and PLZF–RARα, NPM–RARα could also bind to RAREs, albeit possibly in homotetramers instead of homodimers. NPM–RARα was able to form a variety of complexes with RXRα. This result may explain the dominant negative effect of NPM–RARα against retinoic acid signaling mediated by wild-type RARα/RXRα (8). Recently, it was shown that, compared with wild-type RARα/RXRα, X–RARαs had reduced ATRA sensitivity with regard to their binding with nuclear corepressors (15, 16, 29, 34), which may arise from conformational changes or aberrant protein–protein interactions caused by the fusion. We confirmed that PML–RARα retained binding ability to nuclear corepressor SMRT under physiological concentration (0.01 μM) of ATRA, whereas pharmacological concentrations (0.1–1 μM) of ATRA dissociated the PML–RARα/SMRT complex. In contrast, PLZF–RARα formed a very stable complex with SMRT, most likely because of additional corepressor binding sites on the BTB/POZ domain of PLZF (15, 16, 29, 34), and this association was not responsive to ATRA. NPM–RARα also bound with SMRT, but this binding could be dissociated by ATRA as could PML–RARα. Studies with primary mononuclear cells from *NPM–RARα* patients with APL also suggested ATRA sensitivity (40), and BM cells from *NPM–RARα* transgenic mice in this study also responded to ATRA, providing direct evidence that *NPM–RARα* fusion is the underlying molecular mechanism for retinoic acid responsiveness.

Implication of Histone Deacetylase in Human Leukemia. The involvement of N-CoR/Sin3/HDAC transcriptional repressor complex in the development of APL and some other leukemia subtypes (41, 42) represents one of the highlights in recent leukemia research. Interestingly, acquired retinoic acid resistance has been reported in APL cells harboring somatic mutations in the ligand-binding domain of PML–RARα. This resistance results in decreased ligand-dependent transcriptional activity (43, 44). Histone deacetylase inhibitors were found to be able to overcome, at least partly, the ATRA resistance of cells containing the *PLZF–RARα* fusion gene (13, 29, 45, 46). Recently, complete remission was induced in an ATRA-resistant patient with APL by using sodium phenylbutyrate in combination with ATRA (47), further pointing to the functional role of transcriptional repression mediated by recruitment of histone deacetylase in myeloid differentiation block in APL. Our findings in *PLZF–RARα* and *NPM–RARα* transgenic mice and fusion receptor/SMRT interaction also provide support for the unifying model that APL fusion proteins lead to malignant transformation and distinct responses to ligand modulation through abnormal complex formation with nuclear corepressors (12, 15, 16, 34). Drug design based on the regulation of histone acetylation may open new perspectives for the treatment of a number of human malignancies, including APL.

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