Regulation of AML2/CBFA3 in Hematopoietic Cells through the Retinoic Acid Receptor α-Dependent Signaling Pathway*

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AML2 is a member of the acute myelogenous leukemia, AML family of transcription factors. The biologic functions of AML1 and AML3 have been well characterized; however, the functional role of AML2 remains unknown. In this study, we found that AML2 protein expressed predominantly in cells of hematopoietic origin is a nuclear serine phosphoprotein associated with the nuclear matrix, and its expression is not cell cycle-related. In HL-60 cells AML2 expression can be induced by all three natural retinoids, all-trans-retinoic acid (RA), 13-cis-RA, and 9-cis-RA in a dose-dependent manner. A synthetic retinoid acid derivative, 4HPR, which neither activates RA receptor (RAR) α nor retinoic X receptor α was unable to induce the expression of AML2. A RAR-selective activator, TTPB, induced AML2 expression similar to RA. Our study further showed that AGN193109, a potent RARα antagonist, suppressed AML2 expression induced by RA and that a retinoic X receptor pan agonist AGN194204 had no effect on its expression. Taken together, these studies conclusively demonstrated that the expression of AML2 in HL-60 cells is regulated through the RARα-specific signaling pathway. Our study further showed that all-trans-retinoic acid priming, AML2 expression could be augmented by vitamin D₃. Based on these studies we hypothesize that AML2 expression is normally regulated by retinoid/vitamin D nuclear receptors mainly through the RARα-dependent signaling pathway and that it may play a role in hematopoietic cell differentiation.

The AML transcription factor family of proteins consists of three key members: AML1 (or CBFA2) (1, 2), AML2 (or CBFA3), (3, 4), and AML3 (or CBFA1) (3, 5). The AML1 gene was identified initially by cloning the t(8;21) chromosomal translocation associated with acute myelogenous leukemia (1, 6). AML1 is a sequence-specific DNA binding protein and a transcription factor, and it has been shown to be the human counterpart of the mouse polyomavirus enhancer binding protein (7, 8). Sequence analysis revealed a region of 128 amino acids that is highly homologous to the product of the Drosophila segmentation gene, runt (3–5, 9–11). Runt plays an essential role in segmentation, sex determination, neurogenesis, and regulation of differentiation (9, 12). The runt domain of AML1 is involved in both DNA binding and protein-protein interaction (9, 13).

AML1 expresses in most tissues (14–16) and at a high level in hematopoietic cell (15). The biologic function of AML1 is regulated by extracellular signal-regulated kinase (17), and its expression can be induced by all-trans-retinoic acid (ATRA) (18). AML1 function is absolutely necessary for liver definitive hematopoiesis as demonstrated by gene knock-out study (19, 20). Therefore, it is not surprising that AML1 gene is the most common target of chromosomal abnormalities in leukemia (21). The leukemia-associated chimeric oncoproteins involving AML1 such as AML1/ETO created by t(8;21), AML1/Evi-1, AML1/MDS1, and AML1/EAP created by t(3;21), and TEL/AML1 created by t(12;21) are dominant negative inhibitors of AML1 function, and they believed to contribute to the development of leukemia.

The AML1 gene promoter is TATA-less and consists of the binding sites for Sp1, PU.1, Oct, CRE, Myb, and Ets; its expression is controlled by two different promoters in an orientation-dependent manner (22). Both promoters are active in hematopoietic and nonhematopoietic cells, suggesting that additional factors are necessary for regulation of its expression in a tissue-specific manner (22). A recent study demonstrated that AML1 interacts with corepressor TLE1 and suppressed transcription activation of the T cell receptor enhancer, indicating that AML1 acts as a transcription activator as well as a transcription repressor (23).

AML3 has been identified to be essential for normal osteoblasts differentiation and skeletal morphogenesis by gene targeting (24–27). Deletion, insertion, or mutation of AML3 gene could result in skeletal disorders such as cleidocranial dysplasia (26–28). Suppression of AML3 gene expression in primary rat osteoblasts by antisense oligonucleotides significantly inhibited osteoblasts differentiation (29). AML3 expression has been shown to be strictly restricted to cells of the osteoblast lineage (24), regulated by BMP4/7 heterodimer and vitamin D₃ (26, 30).

All members of the AML family of proteins are capable of binding the consensus enhancer core motif, PyGPyGTT (31), and form heterodimers with the partner subunit, CBFβ (13–34). This core motif has been found in the promoter of

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1 The abbreviations used are: ATRA, all-trans-retinoic acid; RA, retinoic acid; RXR, retinoic X receptor; RAR, retinoic acid receptor; TPA, 12-O-tetradecanoylphorbo 12-acetate; VDR, vitamin D receptor; 4HPR, all-trans-4-hydroxyphenylretinamide; TTPB, 9-[2-(5,6,7,8-tetrahydro-5,8,8-tetramethyl-2-naphthyl)-1-propenyl]-benzoic acid.
The AML2 gene has been mapped to human chromosome 1p35–36 (3, 4, 37) and mouse chromosome 4 (38). The biologic function of AML2 is relatively unknown. It has been shown that AML2 activates transcription of the TCRβ gene promoter and that AML1/ETo and TEL/AML1 inhibited this transactivation event (39). It is therefore believed that AML2, in addition to AML1, could also be a target of these oncogenic fusion proteins. Recent study demonstrated that similar to AML1, AML2 is also capable of interacting with TLE1 and acting as a transcription repressor for T cell receptor enhancers (23).

To further understand the biologic function of AML2, our study presented here demonstrated that AML2 is a serine phosphoprotein associated with the nuclear matrix. We found that AML2 is expressed predominantly in cells of hematopoietic origin. In the human myeloid leukemia cell line HL-60, AML2 expression can be induced specifically by the natural and synthetic retinoids through the RARo signaling pathway. The results presented here suggest that AML2 may play a role in hematopoietic cell differentiation.

Materials and Methods

Cell Lines and Culture Conditions—NIH/3T3 cells were maintained in Dulbecco’s modified Eagle’s medium containing 10% bovine calf serum (Atlanta Biologicals, Norcross, GA), 100 units/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Inc.). Raji, U937, HL-60, and HL-60R (obtained from Dr. S. J. Collins, Fred Hutchinson Cancer Center, Seattle, WA) cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (Atlanta Biologicals), 100 units/ml penicillin, and 100 μg/ml streptomycin. The NB4 and Kasumi-1 cell lines were obtained from Dr. D. M. Lanotte (St. Louis, MO), Dr. D. H. Hsu (Paris, France) and Dr. N. Kamada (Hiroshima University, Hiroshima, Japan), respectively. All other cell lines were obtained from the American Tissue Culture Collection (Rockville, MD). Cell viability was determined by trypan blue exclusion assay.

Clinical Samples—Bone marrow or peripheral blood samples were obtained from normal donors at our institution with informed consent. Blood samples were collected with 7.5% buffered 3% saline containing 0.5 mM KHCO3 and 10 mM EDTA to lyse the red blood cells. Total protein from each sample was isolated from the nucleated cells.

Antibodies—Polyclonal anti-AML2 antibody was raised in rabbits against a 270-amino acid fragment at the C terminus of AML2, which was expressed in prokaryotic expression vector pRSETB-AML2. Working dilution at 1:350 to 1:1000 of different batches of AML2 antibodies were used to perform Western blotting and immunofluorescence staining. Monoclonal antibodies to actin and lamin B were purchased from Amersham Life Science Inc. and Oncogene Research Products (Cambridge, MA), respectively.

Plasmids—The expression plasmid pGK-AML2 contains the full-length AML2 cDNA driven by the mouse phosphoglycerate kinase promoter followed by the phosphoglycerate kinase polyadenylation signal (3). The pCEV15-AML3 expression plasmid contains the full-length AML3 coding region and some 3’ noncoding sequences that were originally derived from the pCEV15 cDNA library as described previously (3). The plasmid pCMVAML1B containing the full-length cDNA of AML1b was kindly provided by Dr. S. W. Hiebert (St. Jude Children’s Hospital, Memphis, TN).

Chemicals—ATRA, 13-cis-RA, 9-cis-RA, 4HPR, and TTNPB were obtained from Sigma. A stock solution at a concentration of 10⁻³ M was prepared in 95% ethanol, protected from lights with foil, and stored at −80°C. The RXR agonist AGN194204 and the RARα-specific antagonist AGN193109 were provided by Dr. R. Chanderwarad of Allergan Inc, Irvine, CA (54). Both the reagents were dissolved at 10⁻³ M stock concentration in Me₂SO and stored at −80°C away from light prior to use. Vitamin D₃ was dissolved in 100% ethanol at a stock concentration of 10⁻³ M and stored at −20°C. 12-O-Tetradecanoylphorbo 12-acetate (TPA) was dissolved in 100% acetone at a stock concentration of 10⁻⁴ M and stored at −20°C.

Cell Culture and Differentiation Induction—HL-60 or U937 cells were cultured in RPMI 1640 in the presence of 10% fetal bovine serum in a humidified CO₂ incubator at 37°C. Cultured cells were treated with the following differentiation-inducing agents at a cell density of 4 × 10⁵ cells/ml: ATRA (10⁻⁶ to 10⁻¹² M), 9-cis-retinoic acid ATRA (10⁻⁶ to 10⁻¹² M), 13-cis-retinoic acid ATRA (10⁻⁷ to 10⁻¹² M), 4HPR (10⁻⁷ M), sodium butyrate (5.0 mM), 1, 25 (OH)₂D₃ (10⁻³ M), Ara-C (3.6 × 10⁻⁷ M), TPA (12 M), 13-cis retinoic acid (10⁻⁷ M), MeSO (2.5% v/v), and granulocyte-macrophage colony-stimulating factor (100 ng/mL) ATRA priming was performed by incubating the HL-60 cells with 10⁻⁷ M of ATRA for 30 min. Cells were then washed twice with phosphate-buffered saline and reincubated with various differentiation inducing agents for 2 days. Differentiation of cells was assessed by their ability to produce superoxide as measured by reduction of nitro blue tetrazolium and by Wright-Giemsa staining (60).

Gene Transfection, Preparation of Nuclear Protein and Total Protein, Isolation of Nuclear Matrix, Western Immunoblot Analysis, and Immunofluorescence Staining—Procedures for gene transfection, preparation of nuclear protein and total protein, isolation of the nuclear matrix, Western immunoblot analysis, and immunofluorescence staining were performed as described in our previous reports (43, 64). Quantification of AML2 expression in Western blot analysis was determined by a Microtek Scan Maker, model MRS-1200TP (Microtek International Corp., Taiwan, R.O.C.). Results presented in Figs. 5–7 were repeated at least once to confirm our observation.

Protein Phosphorylation and Phosphoaminoacid Analysis—Phosphorylation of the AML2 protein and identification of the phosphoamino acids were determined as described in our previous report (65).

Centrifugal Elutriation—U937 cells at various phases of the cell cycle were isolated by centrifugal elutriation as described previously (66). Cells growing in logarithmic phase were fractionated using a Beckman JE-6B elutriator rotor mounted in a J-6ME centrifuge at 19°C. Forty fractions (50 ml/fraction) were collected for further analysis. Cell cycle distribution was determined by a FACSCAN flow cytometry (Becton Dickinson).

Results

Expression of AML2 Protein in Various Cell Lines and Hematopoietic Cells—To understand the possible role of AML2, we examined AML2 expression in various cell lines, normal human peripheral blood, and bone marrow. AML2 protein was found to express predominantly in cell lines of hematopoietic origin (Fig. 1A and Table I). B-lymphocyte lymphoma cell line Raji, myelomonoblastic leukemia cell line U937, and early myeloblast cell line KG-1 expressed high basal level of AML2. All three erythroblast cell lines HEL, K562, and EM2 expressed very low level of AML2 protein (Table I). The t(8;21)-positive Kasumi-1 cell line also expressed low level of AML2 protein (Table I). Our study showed that nonhematopoietic cell lines either do not express or express a very low level of AML2 (Fig. 1B and Table I). All three normal bone marrow and four peripheral blood samples were found to express high level of AML2 (Fig. 1C).

Cellular Localization of the AML2 Protein in Hematopoietic Cells—Cellular localization of the AML2 protein was first determined by immunofluorescence staining of the NIH/3T3 cells transiently transfected with the AML2 expression plasmid pGK-AML2. The results shown in Fig. 2 (A and B) demonstrated that the AML2 antibody did not detect any signal in the NIH/3T3 cells, but a nuclear diffused staining pattern was detected consistently in cell transfected with pGK-AML2. We next performed immunofluorescence staining of AML2 in various cell lines highly expressing this protein. This study is consistent with the result obtained from the transient transfection experiment as shown in Fig. 2 (A and B). Therefore, AML2 is normally localized to the nucleus in a nuclear diffused pattern (Fig. 2, D–F) similar to those of the AML1 protein reported previously (39–43).
The AML2 Protein Is a Serine Phosphoprotein Associated with the Nuclear Matrix—Polyonal anti-AML2 antisera generated in rabbit against an AML2 fusion protein detected two bands migrated closely together at about 48 kDa by Western blotting in NIH/3T3 cells transiently transfected with the expression plasmids pCMV-AML1B, pGK-AML2, and pCEV15-AML2, respectively. Lanes 4–9 show the expression of AML2 in 32D.3, K562, RS1, HEI, ML-3, and KG1 cells, respectively. B, lanes 1–8 show the expression of AML2 protein in SK-BR-3, MDA-MB-435, Rat-1, Cos-1, CCD-37, GM637D, and HSF-23, respectively. C shows the AML2 protein expression in three normal bone marrow samples (lanes 1–3) and four normal peripheral bloods sample (lanes 4–7). Lane C in panels B and C represents the protein sample isolated from NIH/3T3 cells transiently transfected with the expression plasmid pGK-AML2.

Expression of AML2 Protein during the Progression of Cell Cycle—AML1 has been shown to regulate expression of genes associated with cell proliferation and differentiation (31, 35). Because AML2 is also a transcription factor recognizing the same AML1 target site, TGTGGT (39), it is therefore of interest to examine whether the expression of AML2 protein varies at different phases of the cell cycle. To study this, U937 cells at various phases of the cell cycle were fractionated by centrifugal elutriation. The DNA content of each fraction was analyzed by "Materials and Methods." We found that only the serine residues of AML2 protein were phosphorylated (Fig. 3B, panel a). We next determine the phosphoamino acids of the AML2 protein by thin layer chromatography as described under "Materials and Methods." We found that the serine residues of AML2 protein were phosphorylated (Fig. 3B, panel b).

Expression of AML2 Protein in hematopoietic and nonhematopoietic cells

<table>
<thead>
<tr>
<th>Cell types</th>
<th>Cell lines</th>
<th>AML2</th>
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<tr>
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<tr>
<td>Early myeloblast</td>
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<td>Late myeloblast (RA resistance)</td>
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<td>Burkitt's lymphoma</td>
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<td>Murine hematopoietic progenitor</td>
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<td>Adrenal adenocarcinoma</td>
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AML2 expression in HL-60 cells at different time points by Western blot analysis. As shown in Fig. 5, ATRA-treated HL-60 cells induced AML2 protein expression by 9.6-fold after 24 h and continue to maintain a high level of expression 96 h post-treatment (Fig. 5A). At this time point most of the HL-60 cells have become differentiated into mature granulocytes as indicated by Wright-Giemsa staining (Fig. 2, G and H). Two other retinoic acids analogs, 13-cis-RA and 9-cis-RA, were also capable of inducing AML2 protein (Fig. 5B) in a dose-dependent manner. As shown in Fig. 5, as low as 10⁻⁹ mol/liter of 13-cis-RA induced AML2 protein expression by 9.3-fold in 96 h. Induction of AML2 by 9-cis-RA was less sensitive, requiring a concentration of 10⁻⁶ mol/liter to induce a maximum level of its expression.

Expression of AML2 in HL-60 cells was not inducible by treatment with phorbol ester (TPA), sodium butyrate, granulocyte-macrophage colony-stimulating factor, actinomycin D, 1,25-dihydroxyvitamin D₃ (vitamin D₃), interferon-γ, and cytosine arabinoside (Ara-C) (Fig. 6, A and B, and data not shown). HL-60 cells pre-exposed to 1 × 10⁻⁷ mol/liter ATRA for 30 min (ATRA priming) as described under “Materials and Methods” is sufficient to induce a low expression of AML2 (a 3.6-fold induction) (Fig. 6A, lane 2). Interestingly, after ATRA priming, AML2 expression can be augmented by vitamin D₃ stimulation (an 11.4-fold induction) (Fig. 6A, lanes 5–8), which alone was unable to induce AML2 expression (Fig. 6A, lane 3). The result shown in Fig. 6A (lane 5) demonstrated that a significant increase in AML2 protein expression (2.3-fold) was detected as early as 4 h after vitamin D₃ treatment in RA primed cells, suggesting a cooperative effect of ATRA and vitamin D₃ in inducing AML2. This effect cannot be found with inducers other than vitamin D₃ such as ATP, TPA, and sodium butyrate (data not shown). These results further support that the regulation of AML2 expression is related to a retinoic acid-responsive pathway. The enhancement effect of RA and vitamin D₃ reflects a possible interaction of the nuclear signaling pathways between retinoic acid and vitamin D₃.

We also observed a significant increase in expression of the AML2 protein (4.2-fold induction) in U937 cells 48 h after ATRA treatment, although this cell line already expresses a high level of AML2 (Fig. 6C). ATRA-induced differentiation of U937 cells toward the monocytic lineage has been reported previously (45). To examine whether expression of the AML2 gene can be induced by RA in other leukemia cell lines, Raji, HEL, and K562 cells were treated with ATRA. Results shown in Fig. 6 (D, F, and G) demonstrated that RA did not induce AML2 expression in these cell lines. Interestingly, no significant change in expression of the AML2 protein was detected after RA treatment (Fig. 6E) in HL-60R cell line, which was originally derived from HL-60 and is resistant to RA-induced differentiation. HL-60R cells harbor a point mutation within the RARα gene that enables the mutated protein to act as a dominant negative inhibitor against normal RARα (46). Our findings thus suggest that RARα may be responsible for RA-induced expression of AML2 in HL-60 cells.

Expression of the AML2 Gene Is Regulated by Retinoic Acid through a RARA-dependent Signaling Pathway—Results from the above study demonstrated that AML2 expression in HL-60 was dramatically induced by retinoidic acids but not by other nonretinoid differentiation inducers. In particular, Me₃SO, which also induces HL-60 differentiation toward the granulocyte lineage similar to RA, was unable to induce AML2 expression. These results suggest that induction of AML2 by RA is not a result of granulocyte differentiation but rather an effect of RA-mediated expression of the AML2 gene. Furthermore, RA was unable to induce AML2 expression in the HL-60R cells with a defective RARA function (Fig. 6E). Together, the above study suggests that expression of AML2 in these cells was mediated through RARα-specific signaling events.

The effects of retinoic acid on expression of AML2 protein in HL-60 cells as shown in the above study could be mediated through RARs, RXRs, or both. ATRA, 13-cis-RA, and 9-cis-RA are the naturally occurring retinoids, each of which has a different affinity for RARs versus RXRs. ATRA binds to all the RARs and directly activates them (47). ATRA does not bind to RXRs, but it does show RXR-stimulating activity in a transactivation assay (48, 49). This activity is likely due to its conversion to 9-cis-RA under in vitro culture conditions (50). 9-cis-RA is a high affinity ligand for RXRs that also binds to and activates RARs (49–51). 13-cis-RA has fairly high affinity for RARs and very low affinity for RXRs (49). Previous studies documented that HL-60 cells express RARα, RARβ, RXRα, and RXRβ (51, 52).

To precisely understand the signaling pathway responsible for retinoid-mediated regulation of AML2, we next examined the regulation of expression of AML2 in response to various...
Cells were labeled with $^{32}$P orthophosphate and served as a positive control. Human U937 cells were transiently transfected with the expression plasmid pGK-AML2, which represents the total protein isolated from the NIH/3T3 cells transiently transfected with the expression plasmid pGK-AML2, which served as a positive control. B, AML2 is a serine phosphoprotein. U937 cells were labeled with $^{32}$P orthophosphate in vivo. Panel a, nuclear protein was isolated, immunoprecipitated with AML2-specific antibody and separated on 8% SDS-polyacrylamide gel electrophoresis. Protein size markers are indicated on the right in kDa. The lane on the right was loaded with 5-fold more protein compared with the left lane. Panel B, complete hydrolysis of the $^{32}$P-labeled AML2 protein with 6 N hydrochloric acid was performed for 1.5 h at 110 °C, and the radioactive phosphoamino acids were detected by autoradiography. Ser, Thr, and Tyr indicate the relative positions of the standard phosphoserine, phosphothreonine, and phosphotyrosine, respectively.

The pan-RAR-selective analog TTNPB, which exhibits high affinity to all three isoforms of RARs and is a potent inducer of their transactivation activity. It neither binds to RXR receptors nor transactivates their target gene expression (51). As shown in Fig. 7B, TTNPB induced AML2 expression similar to ATRA. This strongly supported our hypothesis that AML2 expression is mediated through the RARα-specific signaling pathway. To further confirm this, HL-60 cells were treated with RXRα-selective agonist, AGN19204. The results shown in Fig. 7C demonstrated no significant effect on the expression of AML2. Conversely, the HL-60 cells were treated with a RARα-specific antagonist AGN193109 (54) in the presence of ATRA. The results shown in Fig. 7D demonstrated that AGN193109 could significantly inhibit ATRA-induced expression of AML2.

Together, the above results conclusively demonstrated that AML2 is selectively regulated by retinoic acid through the RARα-dependent signaling pathway.

**DISCUSSION**

The AML transcription factor family of protein plays an important role in the regulation of mammalian cell growth and differentiation. In these studies we have determined the regulated expression of AML2 in hematopoietic cells. Our study shows that AML2 is a nuclear phosphoprotein tightly associated with the nuclear matrix, and its expression does not appear to fluctuate during the progression of the cell cycle. This protein is expressed predominantly in cell lines of hematopoietic origin and expressed at high levels in all samples of peripheral blood and bone marrow. Using the HL-60 leukemia
cell line as a model, we demonstrate that retinoids can selectively induce AML2 expression in a time- and dose-dependent manner via RARα-dependent signaling pathway.

The physiologic function of AML2 in mammalian cells has yet to be established. Based on our study presented here, we hypothesize that AML2 plays a role in regulating hematopoietic cell differentiation. This hypothesis is supported by the facts that 1) AML2 protein is predominantly expressed in cells of hematopoietic origin (Fig. 1 and Table I) and 2) expression of the AML2 protein can be up-regulated by retinoids through the
RARα signaling pathway, which is an important component in inducing hematopoietic cell differentiation.

Our studies demonstrated that the induction of AML2 expression in HL-60 is mediated by the retinoid signaling pathway that involves RARα. Several lines of evidence support this conclusion: 1) The two natural retinoids 13-cis-RA and ATRA that act mainly via RARs remarkably induce AML2 protein expression; 2) ATRA had no effect on AML2 expression in the HL-60R cells, which harbor a defective nonfunctional RARα as a result of point mutation in RARα gene (46); 3) 4HPR, a potent transactivator of RARγ and RARβ but not RARs and RXRs (53), had no effect on AML2 expression; 4) TTNPB, a specific activator of RARs, is capable of inducing AML2 expression similar to ATRA and 13-cis-RA; 5) the RXR-specific agonist had no effect on the expression of AML2; and 6) the RARα-specific antagonist inhibited ATRA-induced expression of AML2. Together, these results conclusively demonstrated that AML2 expression is regulated by retinoid through the RARα-specific signaling pathway.

Retinoids are a group of vitamin A derivatives that have potential application in chemoprevention and therapy in many types of malignancies. They act via interaction with two major classes of nuclear receptors, namely, RARs and RXRs. Each class of receptor includes three subtypes, α, β, and γ (48, 50, 52, 55). These different subtypes of nuclear receptors are expressed during various developmental stages in a cell type-specific manner and regulate expression of specific set of gene (55). It is well documented that retinoid nuclear receptor ligand-mediated transcription factors play major roles in cell growth regulation, differentiation, and oncogenesis (55). In particular, ATRA and its derivatives could be used to induce cell growth arrest and differentiation of leukemic cells and have been used successfully in inducing complete remission in acute promyelocytic leukemia (56). Similar to AML2, as presented in this study, AML1 was also reported to be up-regulated by ATRA (18), but it is unknown whether such regulation is selective and which specific signaling pathway is involved. It is thus possible that AML1 and AML2 may jointly function in the hematopoietic cells to act as downstream regulators of RA-induced cell growth and differentiation.

Our finding that AML2 expression can be induced significantly by vitamin D₃ following ATRA priming in HL-60 cells is interesting. The nuclear signaling pathways for retinoids and vitamin D differ in specificity of their respective receptors and their respective cis-acting elements. Two pathways for the actions of both RARs and vitamin D receptors (VDRs) have been identified, the RAR-dependent and the RAR-independent pathways. The dihydroxylated form of vitamin D₃ mediates a biological response by binding to its receptor, VDR. VDR can form homodimers and heterodimers with RARs and RXRs (57, 58). Only in the presence of ATRA, vitamin D enhances VDR-RAR heterodimer-mediated transcription activation of target gene (58). Our study showed that AML2 expression was not induced by vitamin D₃ alone but that its expression was significantly enhanced after ATRA priming and subsequently treated with vitamin D₃. This observation supports a mechanism of AML2 gene expression via RARα- and VDR-RAR heterodimer-mediated gene regulation.

Induction of AML2 expression by ATRA was found in U937 as well as HL-60. However, in cell lines of erythroid origin (e.g. K562 or HEL), AML2 cannot be induced by ATRA. These results suggest that induction of AML2 expression is lineage-specific. Further studies will be necessary to elucidate the mechanism that regulate the expression of AML2 in these cells.

It has been reported previously that AML1 was able to transform NIH/3T3 cells (17, 59). We have tested the ability of pGK-AML2 in transforming NIH/3T3 cells in transient transfection and foci forming assay as described in our previous study.
A recent study showed that AML2 protein was undetectable in approximately 50% of the patient blasts with AML-M2 subtype based on a total of 55 samples (62). Statistical analysis of the patient data showed that the AML2-negative patients had a significantly higher incidence of relapse and a poor survival rate. This finding has the important implication that AML2 may be involved in the pathogenesis of acute leukemia. Further analysis of patient samples is currently ongoing in our laboratory to establish the possibility of dysregulation of the AML2 gene in leukemia.

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REFERENCES