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Down Regulation of DHFR Promoter Activity by PML Transcription Repressor

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Abstract

The *PML* gene encodes a nuclear protein with transcriptional regulatory activity. It directly interacts with the Sp1 transcription factor and abrogates its activation of the epidermal growth factor receptor (EGFR) gene promoter. Here, the effects of PML on the activity of dihydrofolate reductase (DHFR) promoter, mainly regulated by Sp1, were investigated. On functional analysis, transient transfection of PML into mammalian cells, U2Os and HeLa, resulted in a significant repression of the DHFR promoter. The DHFR promoter also contains binding sites for the E2F transcription factor. When the Sp1 DNA-binding site, but not the E2F-binding site of the promoter, was replaced with an unrelated binding site (GAL4), the repressive effects of PML were lost. Moreover, electrophoretic mobility shift assay (EMSA) using Sp1-containing oligonucleotide probes showed significant reduction in Sp1 binding in the presence of PML, confirming the necessity of the Sp1 DNA-binding sites for PML's repressive effects. Analysis of DNA synthesis using [3H]thymidine incorporation assay showed a significant reduction in DNA synthesis in HeLa cells overexpressing PML. Together, the data demonstrated that PML could function as a negative regulator of the DHFR promoter, which may represent a novel mechanism for the known repressive effects of PML on cellular growth.

Keywords: Promyelocytic leukemia; Transcription repression; Dihydrofolate reductase; Cell cycle

Introduction

The *PML* gene was originally identified by cloning the translocation breakpoint of t(15q;17q), a consistent feature of acute promyelocytic leukemia (APL) [1-2]. *PML* encodes a nuclear protein with several domains resembling transcription factors. These domains include a RING-finger motif and two other cys/his domains designated the B boxes [3]. PML also contains an α-helical domain at its C-terminus, which is involved in PML/PML and PML/PMLRAR α dimerization [3-4].

The PML protein is localized in the nucleus in form of nuclear bodies designated PML oncogenic domains (PODs) or PML nuclear bodies (PML NBs)[5]. Many

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proteins with transcription regulatory function have been identified to localize in the PODs. These include SP100, the ubiquitin-like protein modifier SUMU-1/PIC-1/sentrin, the interferon induced protein ISG20, immediate early viral proteins IE1 and IE4 and the transcription co-regulator CBP (CREB binding protein) [5-9,25]. Moreover, direct interaction of PML with several transcription factors including Sp1, pRB, PLZF and p53 have been reported [12,29,22,34,35]. These findings supported the notion that PML might be involved in transcription regulation.

In fact, direct role of PML in transcription regulation both as an inhibitor or activator, depending on the promoter tested, has been reported. It has been shown that PML could function as an activator of transcription of steroid hormone receptors and transcription mediated by Fos/AP-1 [9,10,13]. When fused to the GAL4 DNA-binding domain, PML acted as an inhibitor of transcription from the GAL4-responsive promoter [10]. Specific domains of the PML protein were involved in the transcription repressing events [10-12]. PML suppresses the promoter of EGFR by inhibition of EGFR's Sp1-dependent activity [12].

PML also functions as a growth and transformation suppressor [14-16]. The *PML* gene knock-out study reported by Wang et al. [26] strongly supported a crucial role for PML in the control of cell growth. Recent studies demonstrated that PML is essential for multiple pathways of programmed cell death by using $PML^{-/-}$ mice and in the cells overexpressing PML [27].

In this study the effects of PML on dihydrofolate reductase (DHFR) promoter was investigated. The results showed that the promoter activity is significantly repressed in the presence of the PML protein through the Sp1 site, but not E2F site presented in the promoter region. Since DHFR promoter functions as a key regulator in DNA synthesis and cellular growth, its repression by PML represents a novel mechanism for the well documented growth inhibitory effects of PML [14-17]. Also, these data provide a link between growth suppression and transcription regulatory function of the PML protein.

Materials and Methods

Plasmids and Cell Lines

The pSG5/PML plasmid was constructed by sub-cloning of cDNA coding for the long isoform of the PML protein in the *Eco*RI site of the pSG5 vector [11]. The DHFR-Leu contained the DHFR promoter linked to the firefly Luciferase gene [12]. The plasmids GAL4-Sp1-Leu and E2F-GAL4-Luc contained the GAL-4-binding sites in place of the E2F- and Sp1-binding sites of the DHFR, respectively [12].

HeLa and U2OS cells were maintained in Dulbeccos modified Eagles medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 100 µg/ml streptomycin (GIBCO/BRL, Gaithersburg, MD) in 5% CO_2 at 37°C in a humidified incubator.

Transfection and Luciferase Assays

Cells were cultured to semiconfluence and were transfected with the plasmids using the Superfect reagent (Qiagen, Germany). For a 5-cm tissue culture dish, 5 µg of total DNA was used. This contained 0.5 µg of the reporter and 1-3 µg of the expression plasmids (see the legends to the figures), and 0.5 µg of pCMV-β-GAL plasmid as an internal control. The latter expresses the bacterial β-galactosidase gene constitutively. The luciferase assay was performed with the Promega (Madison, Wis.) luciferase assay system according to the supplier's instructions. The luciferase activity was measured with a Luminometer (Turner Design, Sunnyvale, Calif.) and normalized against β-galactosidase activity. The activity of each sample was measured and calculated relative to the activity exhibited by cells transfected with empty expression vector (pSG5), and is shown as the fold increase. The β-galactosidase activity was measured as described before [11].

Electrophoretic Mobility Shift Assay (EMSA)

DNA-protein interactions were analyzed using EMSA essentially as described previously [12]. Briefly, 5 µg nuclear proteins from HeLa cells first preincubated in a binding buffer (4% Ficoll 400, 20 mM HEPES [pH 7.9], 2 mM MgCl2, 1 µg of salmon sperm DNA with final concentration of KCl in the reaction mixture adjusted to 100 mM) for 10 min at room temperature in a total volume of 19 µl. In each reaction mixture, 10 fmol (1 µl) of the ^{32}P-labeled (Amersham Bioscience, USA) probe was then added, and the reaction mixture was incubated at room temperature for an additional 30 min. Double stranded Sp1 or E2F binding sites oligonucleotide probes were made by annealing the complementary strands of the following oligonucleotides:

Sp1:
5'-CATTCGATCGGGGCGGGGCGAG-3';
E2F:
5'-TCCGTAGTTTTCGCGCTTAAATTTGAGAAAG
GGCGCGAAACTAGGTC-3';

In competition assays, 100-fold molar excess of Sp1

or E2F double stranded oligonucleotides were preincubated with the extracts for 10 min before addition of the labeled probes. Similarly in supershift assays, 1 µl monoclonal anti Sp1 antibody (Santa Cruz Biotechnology, Inc., USA) was pre-incubated with the extracts. The PML protein was a gift from Dr. ZM Mu (MD Anderson Cancer Center, Texas). The protein-DNA complexes were resolved on a 4% native polyacrylamide gel in 0.25 TBE (44.5 mM Tris-HCl, 44.5 mM boric acid, 1 mM EDTA), and visualized by autoradiography.

[^{3}H]thymidine Incorporation Assay

The DNA synthesis rate of HeLa cells which reflects cell proliferation and growth was determined using the [^{3}H]thymidine incorporation assay. Cells were plated at 1×10^4 cells/plate in 24-well plates and cultured in complete medium. Cells were then transfected with 5 µg of pSG5/PML vector. [^{3}H]thymidine (2 mCi) (Amersham Bioscience, USA) was added to each well at 24, 48, and 72 h intervals, and the cells were incubated for additional 2 h at 37°C. Cells were then washed in PBS and harvested as described previously [16]. Radioactivity of the incorporated [^{3}H]thymidine was determined in a scintillation counter.

Results

DHFR Promoter Activity Is Inhibited by PML

The ability of PML as a growth suppressor has been documented in several studies *in vivo* and *in vitro* [14-17]. However, the mechanism by which PML exerts its growth suppressive effects is not understood very well. In this study, the effects of PML on dihydrofolate reductase (DHFR) promoter were investigated. The cDNA encoding the PML protein in pSG5 vector (pSG5/PML) was cotransfected with a reporter promoter containing the DHFR promoter in front of the firefly Luciferase (the reporter) gene, into mammalian HeLa and U2OS cells. Analysis of the effects of PML on DHFR promoter activity in HeLa cells showed a sharp repression of the promoter activity in the presence of PML (Fig. 1A). As shown in Figure 1B, similar results were found in U2OS cells. However, the background level of the promoter activity in U2OS cells was lower compared to the activity in HeLa cells.

The Sp1 Mediated Activity of the DHFR Promoter Was Abrogated by PML

The DHFR promoter is regulated by Sp1 and E2F transcription factors [30-32]. In order to investigate the possible effects of PML through these sites, the E2F site of the DHFR promoter in one construct was replaced with an unrelated site (GAL4 site), and was represented as Sp1-GAL4-Leu (Fig. 2A). The effects of PML on the promoter activity were repeated as shown in Figure 1, with the new Sp1-E2F-Leu reporter construct. The results showed similar effects as with the intact promoter, although the basal promoter activity was reduced to approximately one third, apparently due to the elimination of the E2F site (Fig. 2B). These data suggested that the inhibitory effects of PML on the DHFR promoter were likely not through the E2F sites, but might be through the Sp1 site of the promoter.

In order to analyze whether the inhibitory effects of PML were imposed through the Sp1 site of the DHFR promoter, a new construct was used in which the Sp1 site was replaced with the GAL4 site (GAL4-E2F-Leu construct) (Fig. 3A). The GAL4-E2F-Leu reporter construct was cotransfected with the pSG5/PML into HeLa and U2OS cells, and the total Luciferase activity in the cell lysate was assayed 48 hrs post transfection. As illustrated in Figure 3B, a significant reduction in the promoter activity was observed in the presence of the PML protein. It is of note that the replacement of the Sp1 site of the promoter with the GAL4 site resulted in the reduction of the basal promoter activity slightly more that the condition in which the E2F site was replaced. However, the background activity of the promoter was high enough to analyze the PML effects.

Sp1 DNA Binding Is Disrupted by PML

Given the inhibitory effects of the PML protein on the DHFR promoter through the Sp1 site of the promoter, we were prompted to analyze these effects by electrophoretic mobility shift assay (EMSA) using Sp1 and E2F oligonucleotide probes corresponding to the related region of the DHFR promoter. Nuclear extracts from HeLa cells were prepared and mixed with labeled Sp1 and E2F oligonucleotides. Analysis of the DNA-protein interaction through EMSA showed a clear change in the mobility pattern in the assays containing the Sp1 probe in the presence of the PML protein (Fig. 3, left panel, compare lanes 3-5). As shown in the Figure 3, the specificity of Sp1 DNA binding was verified using the anti Sp1 antibody (left panel, lane 1). The antibody specifically detected and bound to the Sp1 protein in the complex, which resulted in a supershift due to the formation of a large Sp1/antibody complex. The addition of unlabelled Sp1 oligonucleotide at 100-fold molar excess (lane 2) competed out the Sp1 binding which further confirmed the specificity of Sp1 DNA

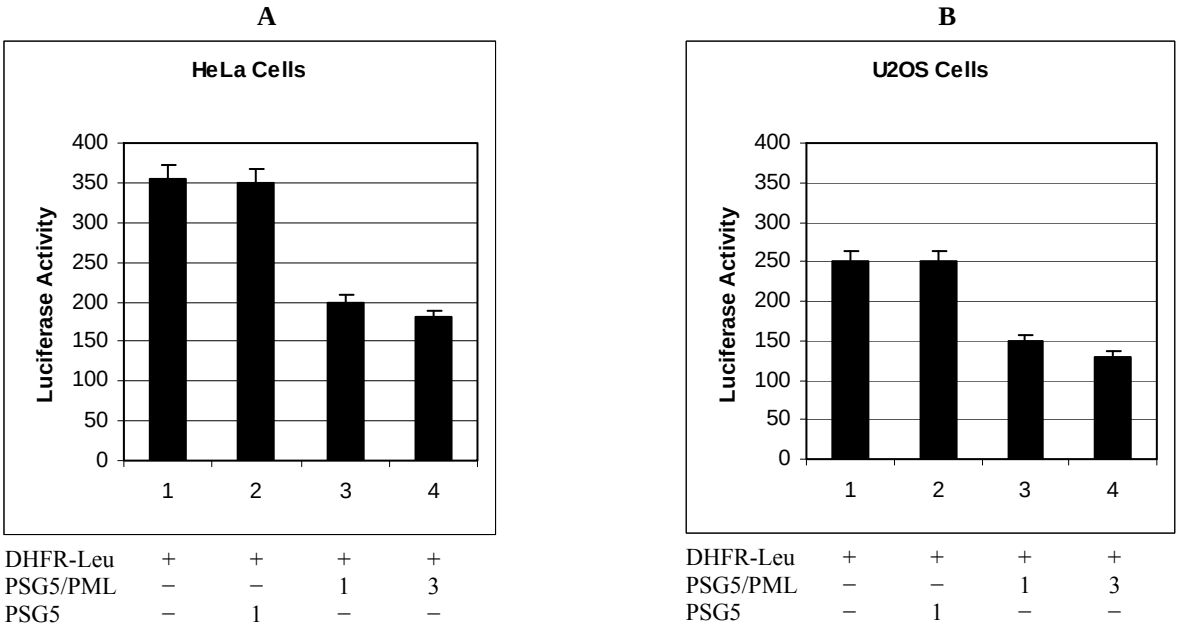

Figure 1. Inhibition of DHFR promoter activity by PML. A) HeLa cells; B) U2OS cells. Cells were cultured and transfected with control plasmid (pSG5) and plasmid containing the *PML* gene (pSG5/PML) together with the Luciferase reporter plasmid (DHFR-Leu). After transfection the Luciferase activity was measured on the total cell extract. The activity of each sample was measured and calculated relative to the activity exhibited by cells transfected with empty expression vector (pSG5), and is shown as the fold increase.

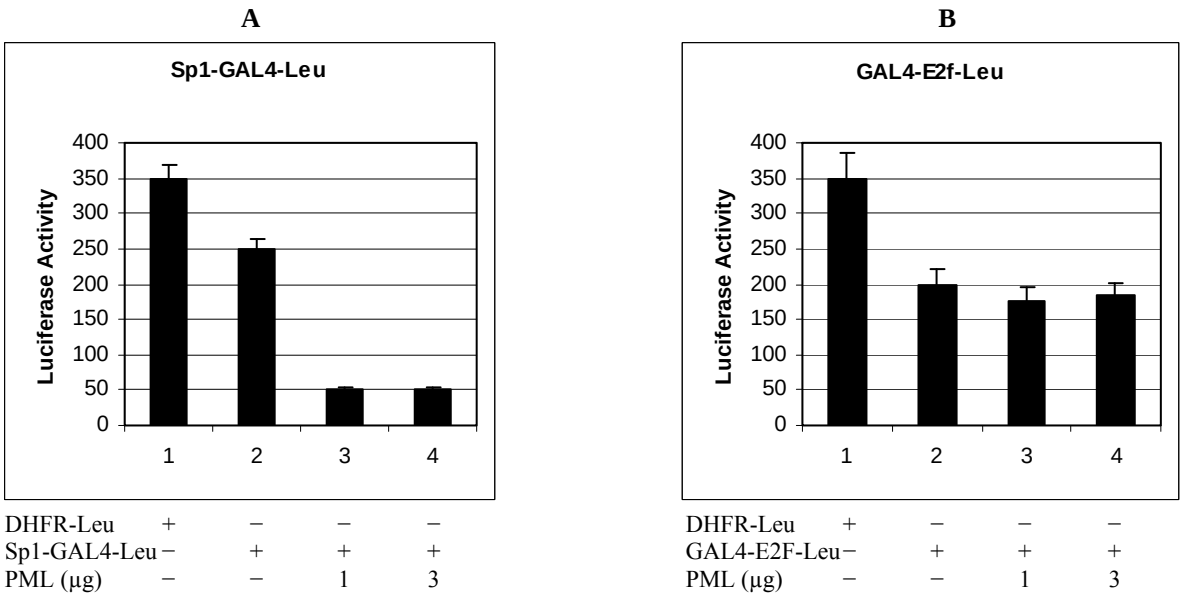

Figure 2. Abrogation of Sp1 mediated activity of DHFR promoter by PML. HeLa cells were transfected with the DHFR reporter plasmid containing the Sp1 site (Sp1-GAL4-Leuc) or the reporter plasmid containing only the E2F site (GAL4-E2F-Leuc) together with the pSG5 and pSG5/PML plasmids. After transfection the Luciferase activity was measured on the total cell extract (see the legend to Fig. 1).

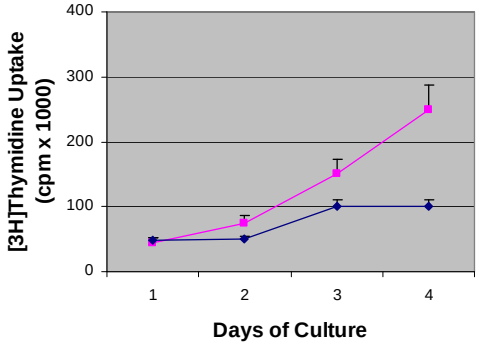

Figure 4. Effects of PML on DNA synthesis. HeLa cells were transfected with pSG5/PML or only the control vector, pSG5 as indicated. Cells were cultured and incubated in the [3H]thymidine and harvested at different times as shown. The content of cellular DNA was measured based on the amounts of labeled thymidine uptake by the cells. See materials and methods for details.

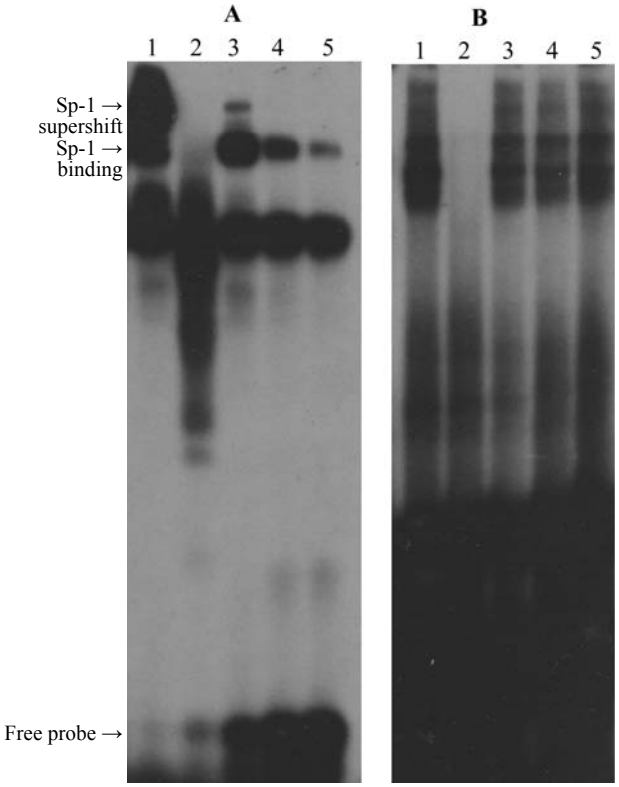

Figure 3. Effects of PML on Sp1-DNA binding. DNA-binding activity of Sp1 was analyzed using EMSA in the presence of the PML protein. HeLa nuclear extracts were incubated (A) with labeled Sp1 probe in the presence of 1 and 3 µg of the PML protein, lanes 4 and 5, respectively. In lanes 1 and 2, anti Sp1 antibody and cold Sp-1 probe were used, respectively. Lane 3 contains HeLa nuclear extracts, and (B) with labeled with E2F probe in the presence of 1 and 3 µg of PML, lanes 4-5, respectively. The supershifted Sp1 protein by anti Sp1 antibody, Sp1 binding and the free probe are shown by arrows.

binding. In parallel assays, addition of the PML protein showed no effects on the mobility pattern of the E2F binding (Fig. 3, right panel, lanes 3-5). These data clearly showed that the inhibitory effects of PML on the DHFR promoter were exerted through the Sp1 site of the promoter. Since the Sp1 site functions as a major regulator of the DHFR promoter, its inhibition by PML suggests a significant role for the PML protein in regulation of the promoter. Moreover, given the key role of the DHFR promoter in DNA synthesis and cellular growth, its inhibition by PML protein could provide a mechanism for inhibitory effects of PML on the cellular growth.

DNA Synthesis Is Significantly Reduced by PML

The DHFR promoter functions as a key regulator in DNA synthesis [30,32,33]. Given the significant repression of the DHFR promoter activity by the PML protein, and the fact that PML functions as a negative regulator of the cell growth, it was interesting to investigate the effects of PML on the rate of the DNA synthesis. Therefore, the rate of DNA synthesis in HeLa cells was examined in the presence of PML. Cells were transfected with pSG5/PML or pSG5 plasmids, incubated with [^{3}H]thymidine and analyzed at 24 h intervals. The incorporation of [^{3}H] thymidine into DNA was measured as an index for the rate of the DNA synthesis. As shown in Figure 4A, the cells expressing PML showed much lower incorporation of [^{3}H] thymidine, compared to that of the control cells (pSG5 transfected), suggesting a reduced DNA synthesis rate in the presence of PML.

Discussion

The promoter of the dihydrofolate reductase (DHFR) gene, contains Sp1 and E2F response elements in the vicinity of its transcription initiation region [31,33], and both of these elements have been shown to be involved in the promoter activity. Our previous studies showed that PML physically and functionally interacted with Sp1, disrupting the binding of the Sp1 protein to the promoter [12]. *In vivo* and *in vitro* experiments demonstrated that the association of the Sp1 and PML exerts a significant effect on the EGFR promoter activity, which is mainly regulated by Sp1 [12,18,19,23,24]. The data demonstrated that PML could function as a negative regulator of Sp1 transcriptional activity. In the present study, the association of PML and Sp1 was further investigated using the DHFR promoter. The data showed that PML could significantly affect the promoter activity of the DHFR promoter. Interestingly,

this effect was found to be mainly exerted through the Sp1 site of the promoter rather than its E2F site. The effects of PML on the DHFR promoter activity were found to be specific. This specificity was supported by three findings: i) On functional analysis, PML could reduce the DHFR promoter activity, when the Sp1 site but not the E2f site was present; ii) In EMSA assays, PML disrupted the Sp1 DNA-binding; iii) The rate of DNA synthesis was significantly reduced by PML.

One of the most characterized functions of PML is its inhibitory role in cellular growth [14-17,20,21]. However, the mechanisms underlying this function are poorly understood. The finding that PML is associated with several transcription factors involved in cellular growth including the retinoblastoma protein (pRb) and p53 [22,25,29,34,35], as well as negative and positive effects of PML on transcription regulation depending on the promoter tested, clearly suggested that the growth inhibitory and transcriptional regulatory activity of PML might be related to each other [9,19,12,17,36,37]. The findings from this study on the inhibition of DHFR promoter activity by PML and its significant role in suppression of DNA synthesis, provides a strong support for this hypothesis.

Furthermore, it has been previously reported that PML could functionally and physically interact with all three isoforms of histone deacetylase enzymes (HDAC) through specific domains [11,38]. Both the C-terminal and the N-terminal of the PML protein were found to be necessary for its efficient binding to HDAC. These data demonstrated that PML transcriptional inhibitory activity was associated with histone deacetylases that were recruited to the promoter. Therefore, the inhibition of DHFR promoter by PML might also involve HDAC. In fact, the Sp1 mediated transcriptional activation of DHFR promoter was found to be efficiently repressed by HDAC1 [32,33].

Previous studies showed that overexpression of PML induced a G1 cell cycle arrest both in cancer cell line and in normal human lung fibroblasts and stable overexpression of PML in HeLa cell led to the alteration of cell cycle progression by lengthening G1 [14-17,28]. Based on these findings it was hypothesized that PML normally plays a role in regulating cell cycle progression. The finding that PML interacted with Sp1 and inhibited its binding to the target promoters such as DHFR together with the reports that Sp1 interacts with E2F and synergistically activated transcription of G1/S checkpoint genes, suggested that PML could play a role in regulating cell cycle progression [12,14,33,39].

Our study presented in this report clearly showed that PML inhibited the DHFR promoter by disrupting the Sp1 binding to the promoter. The fact that PML interacted with HDACs to repress transcription strongly support a significant role for PML in cell cycle regulation. Therefore, it could be speculated that PML normally regulates cell cycle progression by modulating the functional activity of the Sp1/HDAC complex. In this content, possibly disruption of PML function by t(15;17) in APL may contribute to the development of acute leukemia.

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References

1. Borrow J., Goddard A.D., Sheer D., and Solomon E. Molecular analysis of acute promyelocytic leukemia breakpoint cluster region on chromosome 17. *Science*, **249**: 577-580 (1990).
2. De The H., Lavau C., Marchio A., Chomienne C., Degos L., and Dejean A. The PML-RARalpha fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. *Cell*, **66**: 675-684 (1991).
3. Freemont P.S., Hanson I.M., and Trowsdale J. A novel cystein-rich sequence motif. *Cell*, **64**: 483-484 (1991).
4. Kakizuka A., Miller W.H.J., Umesono K., Warrell R.P.J., Frankel S.R., Murty V.V.V.S., Dmitrovsky E., and Evans R.M. Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RARalpha with a novel putative transcription factor, PML. *Cell*, **66**: 663-674 (1991).
5. Doucas V. and Evans R.M. The PML nuclear component and cancer. *Biochim. Biophys. Acta*, **1288**: M25-M29 (1996).
6. Sternsdorf T., Jensen K., and Will H. Evidence for covalent modification of the nuclear dot-associated proteins PML and Sp100 by PIC1/SUMO-1. *J.Cell Biol.*, **139**: 1621-1634 (1997).
7. Ahn J.-H. and Hayward G.S. The major immediate-early proteins IE1 and IE2 of human cytomegalovirus co localize with and disrupt PML-associated nuclear bodies at very early times in infected permissive cells. *J.Virol.*, **71**: 4599-4613 (1997).
8. Muller S., Matunis M.J., and Dejean A. Conjugation with the ubiquitin-related modifier SUMO-1 regulates the partitioning of PML within the nucleolus. *EMBO J.*, **17**: 61-70 (1998).
9. Vallian S., Gaken J., Gingold E.B., Kouzarides T., Chang K.-S., Farzaneh F. Regulation of Fos-mediated AP-1 transcription by the promyelocytic leukemia protein. *Oncogene*, **16**: 2843-2853 (1998).
10. Vallian S., Gaken J.A., Trayner I.D., Gingold E.B., Kouzarides T., Chang K.-S., and Farzaneh F. Transcriptional repression by the promyelocytic leukemia protein, PML. *Exp.Cell Res.*, **237**: 371-382 (1997).

11. Wu W.-S., Vallian S., Seto E., Yang W.-M., Edmondson D., Roth S., and Chang K.-S. The growth suppressor PML Represses Transcription by functionally and physically interacting with histone deacetylases. *Mol. Cel. Biol.*, **21**: 2259-2268 (2001).
12. Vallian S., Chin K.-V., and Chang K.-S. The promyelocytic leukemia protein interacts with Sp1 and inhibits Sp1-mediated transcription of the epidermal growth factor receptor (EGFR) promoter. *Mol. Cell. Biol.*, **18**: 7147-56 (1998).
13. Guiochon-Mantel A., Savouret F.J., Quignon F., Delaber K., Milgrom E., and De The H. Effect of PML and PML-RAR on the transactivation properties and subcellular distribution of steroid hormone receptors. *Mol. Endocrin.*, **9**: 1791-1803 (1995).
14. Le X.-F., Vallian S., Mu Z.-M., Hung M.-C., and Chang K.-S. Recombinant PML adenovirus suppresses growth and tumorigenicity of human breast cancer cells by inducing G1 cell cycle arrest and apoptosis. *Oncogene*, **16**: 1839-1849 (1998).
15. Liu J.H., Mu Z.M., and Chang K.-S. PML suppresses oncogenic transformation of NIH/3T3 cells by activated Neu. *J. Exp. Med.*, **6**:1965-1973 (1995).
16. Mu Z.-M., Le X.-F., Vallian S., Glassman A.B., and Chang K.S. Stable overexpression of PML alters regulation of cell cycle progression in HeLa cells. *Carcinogenesis*, **18**: 2063-2069 (1997).
17. Mu Z.M., Chin K.V., Liu J.H., Lozano G., and Chang K.S. PML,a growth suppressor disrupted in acute promyelocytic leukemia. *Mol.Cell.Biol.*, **14**: 6858-6867 (1994).
18. Hudson L.G., Santon J.B., and Gill G.N. Regulation of epidermal growth factor receptor gene expression. *Mol. Endocrin.*, 3: 400-408 (1989).
19. Johnson A.C., Ishii S., Jinno Y., Pastan I., and Merlino G.T. Epidermal growth factor receptor gene promoter. *J. Biol. Chem.*, **263**: 5693-5699 (1989).
20. He D., Mu Z.-M., Le X., Hsieh J.-T., Pong R.-C., Chung L.W.K., and Chang K.-S. Adenovirus-mediated expression of PML suppresses growth and tumorigenicity of prostate cancer cells. *Cancer Res.*, **57**: 1868-1872 (1997).
21. Koken M.H.M., LinaresCruz G., Quignon F., Viron A., ChelbiAlix M.K., SobczakThepot J., Juhlin L., Degos L., Calvo F., and De Thé H. The PML growth-suppressor has an altered expression in human oncogenesis. *Oncogene*, **10**: 1315-1324 (1995).
22. Alcalay M., Tomassoni L., Colombo E., Stoldt S., Grignani F., Fagioli M., Szekely L., Helin K., and Pelicci P.G. The promyelocytic leukemia gene product (PML) forms stable complexes with the retinoblastoma protein. *Mol. Cell. Biol.*, **18**: 1084-1093 (1998).
23. Toi M., Osaki A., Yamada H, and Tog T. Epidermal growth factor receptor expression as a prognostic indicator in breast cancer. *Eur. J. Cancer*, **27**: 977-980 (1991).
24. Morishige K.I., Kurachi H., Amemiya K., Fujita Y., Yamamato T., Miyake A., and Tanizawa O. Evidence for the involvement of TGF alpha and EGFR autocrine growth mechnism in primary human ovarian cancers in vitro. *Cancer Res.*, **51**: 5322-5328 (1991).
25. LaMorte V.J., Dyck A.J., Ochs R.I., and Evans R.M. Localization of nascent RNA and CREB binding protein with PML-containing nuclear body. *Proc. Natl. Acad. Sci. USA*, **95**: 4991-4996 (1998).
26. Wang Z.G., Ruggero D., Ronchetti S., Zhong S., Gaboli M., Rivi R., and Pandolfi P.P. PML is essentail for multiple apoptotic pathways. *Nat. Genet.*, 20: 266-272 (1998).
27. Wang Z.G., Delva L., Gaboli M., Rivi R., Giorgio M., Cordon-Cardo C., Grosvels F., and Pandolfi P.P. Role of PML in cell growth and the retinoic acid pathway. *Science*, **279**: 1547-1551 (1998).
28. Sherr C.j. G1 phase progression: cycling on cue. *Cell*, **79**: 551-553 (1994).
29. Pearson M. and Pelicci P.G. PML interaction with p53 and its role in apoptosis and replicative senescence. *Oncogene*, **20**: 7250-7256 (2001).
30. Slansky J.E. and Farnham P.J. Transcriptional regulation of the dihydrofolate reductase gene. *Bioessays*, **18**: 55-62 (1996).
31. Jensen D.E., Black A.R., Swick A.G., and Azizkhan J.C. Distinct roles for Sp1 and E2F sites in the growth/cell cycle regulation of the DHFR promoter. *J. Cell. Biochem.*, **67**: 24-31 (1997).
32. Noe V., Chen C., Alemany C., Nicolas M., Caragol I., Chasin L.A., and Ciudad C.J. Cell-growth regulation of the hamster dihydrofolate reductase gene promoter by transcription factor Sp1. *Eur. J. Biochem.*, **249**: 13-20 (1997).
33. Park K.K., Rue S.W, Lee I.S., Kim H.C., Lee I.K., Ahn J.D., Kim H.S., Yu T.S., Kwak J.Y., Heintz N.H., Magae J., and Chang Y.-C. Modulation of Sp1-dependent transcription by a *cis*-acting E2F element in *dhf* promoter. *Biochem. Biophys. Res.*, **306**(1): 239-243 (2003).
34. He L.Z., Guidez F., Tribioli C., Peruzzi D., Ruthardt M., Zelent A., and Pandolfi P.P. Distinct interactions of PML-RARalpha and PLZF-RARalpha with co-repressors determine differential responses to RA in APL. *Nat. Genet.*, **18**: 126-135 (1998).
35. Li H., Leo C., Zhu J., Wu X., O'Neil J., Park E-J., and Chen J.D. Sequestration and inhibition of Daxx-mediated transcription repression by PML. *Mol. Cell. Biol.*, **20**: 1784-1796 (2000).
36. Luo R.X., Postigo A.A., and Dean D.C. Rb interacts with histone deacetylase to repress transcription. *Cell*, **92**: 463-473 (1998).
37. Magnaghi-Jaulin L., Groisman R., Robin P., Lorain S., Le Villain J.P., Troalen F., Trouche D., and Harel B. Retinoblastoma protein represses transcription by recruiting a histone deacetylase. *Nature*, **391**: 601-605 (1998).
38. Struhl K. Histone acetylation and transcriptional regulatory mechanisms. *Genes Dev.*, **12**: 599-606 (1998).
39. Doetzlhofer, Rotheneder H., Lagger G., Koranda M., Kurtev V., Brosch G., Wintersberger E., and Seiser C. Histone deacetylase 1 can repress transcription by binding to Sp1. *Mol. Cell. Biol.*, **19**: 5504–5511 (1999).