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PDF issue: 2025-01-23

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(Degree)
博士（保健学）

(Date of Degree)
2021-03-25

(Date of Publication)
2022-03-01

(Resource Type)
doctoral thesis

(Report Number)
甲第8049号

(URL)
https://hdl.handle.net/20.500.14094/D1008049

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博　士　論　文

PML–RARα induces all-trans retinoic acid-dependent transcriptional activation through interaction with MED1

(PML-RARα は MED1 との結合を介して全トランス型レチノイン酸依存性の転写活性化を誘導する)

令和 3 年 1 月 14 日

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PML–RARα induces all-trans retinoic acid-dependent transcriptional activation through interaction with MED1

Abstract

Transcriptional activation by PML–RARα, an acute promyelocytic leukemia-related oncofusion protein, requires pharmacological concentrations of all-trans retinoic acid (ATRA). However, the mechanism by which the liganded PML–RARα complex leads to the formation of the preinitiation complex has been unidentified. Here we demonstrate that the Mediator subunit MED1 plays an important role in the ATRA-dependent activation of the PML–RARα-bound promoter. Luciferase reporter assays showed that PML–RARα induced significant transcription at pharmacological doses (1 μM) of ATRA; however, this was submaximal and equivalent to the level of transcription driven by intact RARα at physiological doses (1 nM) of ATRA. Transcription depended upon the interaction of PML–RARα with the two LxxLL nuclear receptor recognition motifs of MED1, and LxxLL→LxxAA mutations led to minimal transcription. Mechanistically, MED1 interacted ATRA-dependently with the RARα portion of PML–RARα through the two LxxLL motifs of MED1. These results suggest that PML–RARα initiates ATRA-induced transcription through its interaction with MED1.

Keywords: PML–RARα; transcriptional activation; Mediator; MED1; LxxLL nuclear receptor recognition motifs

Introduction

Acute promyelocytic leukemia (APL), a highly aggressive subtype of acute myeloid leukemia (AML), is characterized by an arrest in myeloid maturation due to chimeric oncofusion proteins containing retinoic acid receptor α (RARα). Almost 98% of cases with APL carry promyelocytic leukemia (PML)-fused RARα due to chromosomal translocation t(15;17)(q22;q12) [1-2; reviewed in 3]. In these cases, the breakpoint clustering within the *PML* gene (intron 3, exon 6 and intron 6) causes the formation of three different isoforms of *PML–RARA*, termed the short (S or bcr2), long (L or bcr1), and variant (V or bcr3) forms, with frequencies of 40%, 55% and 5%, respectively [4; reviewed in 5]. Although the affinity of all-trans retinoic acid (ATRA) for these forms of PML–RARα is similar to that for RARα [6], at physiological levels of ATRA, PML–RARα does not transactivate target genes and behaves as a constitutive repressor [reviewed in 7-9]. Under these conditions, PML–RARα interacts with epigenome-modifying corepressors, such as NCoR- and SMRT-containing histone deacetylase complexes, as well as enzymes modifying DNA and histone methylations, causing the hypoacetylation and silencing of chromatin structures [10-11; reviewed in 8-9]. Based on ChIP-seq analyses, the recruitment of PML–RARα to promoter sequences appears to be less stringently regulated than that of intact RARα [12]. Hence, PML–RARα may suppress a broader range of genes than previously anticipated on the basis of promoters bearing canonical retinoic acid response elements (*RARE*), and thus may contribute to both the inhibition of ATRA-induced differentiation and leukemogenesis.

Although PML–RARα functions as a constitutive repressor under physiological concentrations of ATRA, in the presence of pharmacological concentrations of ATRA, PML–RARα liberates corepressor complexes, and functions as an activator to induce

the differentiation of APL cells [2,13; reviewed in 7-8]. Both RARα and PML–RARα are subject to degradation upon exposure to ATRA, likely through a ligand-induced desensitizing mechanism [reviewed in 8]. Upon degradation of PML–RARα in the presence of pharmacological concentration of ATRA, previously disrupted PML nuclear bodies are restored, leading to the eradication of leukemic stem cells *in vivo* and curing the disease [reviewed in 7-8]. Thus, the therapeutic effects of pharmacological ATRA are derived from targeting the oncoprotein PML–RARα in two ways: PML–RARα-mediated transactivation of target genes in an ATRA-dependent manner followed by the rapid differentiation of APL cells, and ATRA-dependent degradation of the oncoprotein resulting in the restoration of PML nuclear bodies. Regarding the former mechanism, it is still unclear how PML–RARα-mediated transcription is initiated after the release of chromatin from silencing.

The multisubunit complex Mediator is a master transcriptional coregulatory complex, and an end-point hub of intracellular signals. As a constituent of the RNA polymerase II holoenzyme, Mediator facilitates the formation of a functional preinitiation complex (PIC) [reviewed in 14-16]. The Mediator subunit MED1 serves as an interface for ligand-dependent interaction with nuclear receptors through its two nuclear receptor recognition (NR) motifs and functions as a nuclear receptor specific coactivator [reviewed in 15,17]. The function of MED1/Mediator, which is related to PIC formation and distinct from histone modifying activities, suggests that the MED1/Mediator acts subsequent to the action of chromatin-modifying coactivators [reviewed in 17-18]. MED1 is known to be required for optimal RARα function and RARα-mediated neutrophilic differentiation [19], but it is unknown how PML–RARα leads to the

formation of functional PICs and subsequent activation of target genes and whether MED1 is involved in this process.

Herein we present evidence that the initiation of transcription by PML–RARα depends upon both of the MED1 NR motifs as well as pharmacological concentrations of ATRA. We propose that MED1 is a specific coactivator for PML–RARα-mediated transcription and is dependent upon pharmacological doses of ATRA, indicating a role for MED1 in differentiation therapy using ATRA in APL.

Results

MED1 is necessary for optimal PML–RARα-initiated transcription - To understand whether MED1 is responsible for PML–RARα-initiated transcription in an ATRA dose-dependent manner, we first performed luciferase reporter assays using the Gal4–luciferase reporter and constructs expressing Gal4 DNA-binding domain (DBD) fusion proteins in *Med1*$^{+/+}$ and *Med1*$^{-/-}$ mouse embryonic fibroblasts (MEFs) [20]. When Gal4 DBD-fused truncated human (h) PML (Gal4-PML; encoded by *PML* exons 1–6) (Fig 1A) was introduced to MEFs, transcription levels indicated by luciferase activity remained at a background level regardless of the presence or absence of ATRA or MED1 (Fig 1B). As expected, Gal4–hRARα induced high transcriptional activation in a ligand-dependent manner in *Med1*$^{+/+}$ MEFs, reaching a 12-fold increase at the physiological concentration of ATRA (10^{-9} M) and a 35-fold increase at the pharmacological concentration of ATRA (10^{-6} M) (Fig 1B). Both the S and L forms of hPML–RARα fused to Gal4 DBD induced significant and similar levels of transcription, reaching a maximum of 5- to 7-fold at the pharmacological ATRA concentration (Fig 1B); however, these levels were much weaker than those induced by Gal4–hRARα. The transcriptional activation in *Med1*$^{+/+}$ MEFs also depended on the amounts of these fused oncoproteins (Fig 1C). In contrast, in *Med1*$^{-/-}$ MEFs, the transcription levels induced by Gal4–hRARα and Gal4–hPML–RARα(S and L) were greatly attenuated to about one-half of the levels observed in *Med1*$^{+/+}$ MEFs (Fig 1B).

Next, similar experiments were performed using a luciferase reporter bearing natural *RARE* sequences derived from the *RARβ* promoter [21]. Truncated hPML (derived from exons 1–6) did not activate transcription regardless of the presence of MED1 or ATRA. When hRARα and hRXRα were introduced in *Med1*$^{+/+}$ MEFs, transcription was

activated over 60-fold in an ATRA dose-dependent manner. However, the levels of activation by the S and L forms of hPML–RARα, while similar, were less than half of that by hRARα. In contrast, the transcriptional activities of these activators were severely attenuated (reduced over three-fold) when analyzed in *Med1*$^{-/-}$ MEFs (Fig 1D). These results suggest (i) that the S and L forms of PML–RARα similarly activate ATRA-dependent transcription and (ii) that transcriptional activation by PML–RARα is much less efficient compared to that induced by intact RARα and, further, is initiated only at pharmacological doses of ATRA. We also found that MED1 is necessary for optimal transcription induced by PML–RARα and by RARα.

PML–RARα interacts with MED1 through LxxLL NR motifs of MED1 – MED1 has two LxxLL NR motifs that serve as ligand-dependent interfaces with nuclear receptors (Fig 2A) [reviewed in 17-18]. PML–RARα was previously reported to interact with GST-fused MED1 using a GST-pulldown assay [22]. To identify whether PML–RARα(S and L) binds intracellularly with MED1 through the NR motifs, we performed mammalian two-hybrid assays using mutants of the first NR motif (mutant I), the second NR motif (mutant II), and both NR motifs (mutant I+II) of hMED1 (Fig 2A).

Gal4–hMED1(wild-type) interacted with VP16–hPML–RARα(S and L forms) in an ATRA dose-dependent manner, with a seemingly stronger affinity for VP16–hPML–RARα(S) than for VP16–hPML–RARα(L) (Fig 2B). Gal4–hMED1(mutant I) and Gal4–hMED1(mutant II) interacted likewise with VP16–hPML–RARα(S and L), but less efficiently (especially at lower levels of expression) than with Gal4–hMED1(wild-type). Notably, however, an interaction was barely observed between Gal4–hMED1(mutant

I+II) and VP16–hPML–RARα(L and S) even at pharmacological ATRA concentrations (10^{-6} M) (Fig 2B).

Next, assays were performed with different doses of VP16–hPML–RARα. At a pharmacological ATRA concentration (10^{-6} M), Gal4–hMED1(wild-type) interacted with VP16–hPML–RARα(S and L) in a VP16–hPML–RARα dose-dependent manner, with a seemingly stronger affinity for VP16–hPML–RARα(S) than for VP16–hPML–RARα(L) (Fig 2C). Both Gal4–hMED1(mutant I) and Gal4-hMED1(mutant II) likewise interacted with VP16–hPML–RARα(S and L) but less strongly than Gal4–hMED1(wild-type). In contrast, Gal4–hMED1(mutant I+II) barely interacted with VP16–hPML–RARα(S and L) (Fig 2C). These results suggest that PML–RARα(S and L) binds to MED1 in an ATRA-dependent manner through both NR motifs of MED1, and that the affinity for PML–RARα(S) may be higher than that for PML–RARα(L).

MED1 LxxLL NR motifs are necessary for optimal PML–RARα-initiated transcription – Since we established that MED1 NR motifs are required for PML–RARα(S and L)-MED1 interactions, we next analyzed the function of MED1 NR motifs using luciferase reporter assays in MEFs bearing LxxLL to LxxAA mutations in MED1 NR motifs (*Med1* LxxAA or *Med1(lx)*$^{KI/KI}$ MEFs) [23]. First, Gal4 DBD-fused proteins were tested using a 5×Gal4–luciferase reporter. We found that Gal4 DBD-fused truncated hPML was completely inactive in *Med1* LxxAA MEFs (Fig 3A). When compared to its transcription activity in wild-type (*Med1(lx)*$^{+/+}$) MEFs (*Med1* WT MEFs), the activity induced by Gal4–hRARα in *Med1* LxxAA MEFs was reduced by more than two-fold (Fig 3A) and to levels similar to those observed in *Med1*$^{-/-}$ MEFs (Fig 1B). Transcriptional activation by Gal4–hPML–RARα(S and L) was also strongly

attenuated (reduced over three-fold) in *Med1* LxxAA MEFs compared to wild-type MEFs (Fig 3A), similar to above-described observations in *Med1*$^{-/-}$ MEFs (Fig 1B).

When the luciferase reporter bearing *RARβ* promoter-derived natural *RARE* sequences was used, truncated hPML did not activate transcription in *Med1* LxxAA MEFs (Fig 3B), consistent with findings described above. RARα-mediated transcription levels were severely attenuated (less than one-fifth) in *Med1* LxxAA MEFs compared to those in *Med1* WT MEFs (Fig 3B), as was observed in *Med1*$^{-/-}$ MEFs (Fig 1C). However, there was a residual (6-fold) activation by ATRA at the pharmacological concentrations (10^{-6} and 10^{-5} M) (Fig 3B). Notably, however, hPML–RARα(S and L)–mediated transcription was barely detected (1.5- to 3-fold) in *Med1* LxxAA MEFs at the pharmacological ATRA concentration (Fig 3B), as seen earlier in *Med1*$^{-/-}$ MEFs (Fig 1C). These results provide compelling evidence that both RARα and PML–RARα(S and L) require MED1 NR motifs for optimal transcription.

Discussion

PML–RARα-targeted differentiation therapy by ATRA in APL has become the standard of care in clinics and has been so successful that APL is now a curable disease. This study demonstrates, for the first time, that transactivation by PML–RARα is executed through its ATRA-dependent interaction with the Mediator subunit MED1. Our model provides a mechanism of direct transcriptional activation by PML–RARα, and signifies the importance of MED1 NR motifs in ATRA therapy in APL.

Interaction between PML–RARα and MED1 – PML–RARα, through its RARα portion, appears to bind equally well to both NR motifs of MED1 (this study). However, this contrasts with the model proposed for the interaction of the receptors TRα, VDR, and PPARα with MED1. These receptors interact strongly with the second NR motif of MED1, while their heterodimerization partner RXR interacts with the first NR motif. The RXR receptor heterodimer has been proposed to simultaneously interact with MED1 through both NR motifs in a unidirectional manner (Fig 4A) [reviewed in 18]. PML–RARα dimerizes (or oligomerizes) through PML coiled-coil domains, and RXRs associate with the homo-oligomerized PML–RARα, forming a heterotetramer (or heterooligomer) (Fig 4B,C) [reviewed in 8-9]. Unifying the above ideas, the PML–RARα/RXR heterooligomer may bind MED1 *in vivo* in two ways: either through the PML–RARα homodimer (Fig 4B) or through the PML–RARα/RXR heterodimer (Fig 4C).

The affinity of ATRA has been reported to be slightly higher for PML–RARα(L) than for PML–RARα(S) [6], and liganded PML–RARα(S) appears to interact with MED1 more efficiently than liganded PML–RARα(L) (this study). The net binding efficiencies

of these interfaces may explain the similar levels of transactivation achieved by liganded PML–RARα(S) and PML–RARα(L) (this study), which may lead to similar and extremely high rates of complete remission achieved by ATRA therapy for APL cases with both PML–RARα(S) and PML–RARα(L) [reviewed in 3,8].

Interestingly, PML–RARα(S and L) showed a residual ATRA-dependent interaction with mutant MED1 having no intact NR motifs in our sensitive mammalian two-hybrid assays (Fig 2). One explanation is the existence of a hitherto unknown ATRA-dependent interface within MED1. A putative intermediating factor that binds to MED1 at a domain other than NR motifs and, simultaneously, to the RARα portion of PML–RARα in an ATRA-dependent fashion may be proposed. One candidate for this factor might be CCAR1, which was originally discovered as a bridging coactivator bypassing estrogen receptors to MED1 [24] and was subsequently implicated in bridging other nuclear receptors and other activators to MED1 [reviewed in 25].

MED1 as coactivator for PML–RARα: implications in APL cell differentiation therapy – This study establishes MED1 as a coactivator for PML–RARα. However, the coactivation function of MED1 is weak and requires a thousand-fold molar excess of ATRA to induce transcriptional levels comparable to those observed using intact RARα at physiological ATRA doses. Therefore, PML–RARα-initiated transactivation requires pharmacological ATRA concentrations. The fact that the affinity of ATRA for PML–RARα is close to that of intact RARα [6] suggests that all PML–RARα molecules within an oligoheteromer complex must be liganded for transcription initiation.

According to the classical model of ATRA therapy in APL, treatment with pharmacological doses of ATRA converts the constitutive repressor oncoprotein PML–RARα to an activator, dissociates corepressors, recruits histone acetyltransferases, and restores differentiation of APL cells [reviewed in 7]. APL cells indeed differentiate under this condition, as shown in *in vitro* cell cultures [13], and MED1 is involved in this process, *i.e.*, rapid APL cell clearance in remission induction therapy. In the view that the lethal bleeding tendency that characterizes APL should be alleviated as soon as possible, the ATRA-mediated rapid differentiation is indeed welcomed at the remission induction stage. However, according to the revised model, eradication of APL stem cells (leading to the subsequent cure of the disease) requires degradation of PML–RARα and restoration of PML nuclear bodies, which are achieved by ATRA and arsenic trioxide [reviewed in 7-9]. MED1, nevertheless, still plays a fundamental role in the revised model, as the restored intact RARα requires MED1 to resume transcription activation.

In conclusion, MED1 is required for optimal transactivation by PML–RARα. The coactivation function of MED1 requires pharmacological ATRA concentrations and depends upon the interaction of MED1 NR motifs with PML–RARα.

Materials and methods

Plasmids

The mammalian expression vector containing Gal4–hRARα in pCDM8 (Invitrogen) (pGal–hRARα) was described [20]. The cDNA for truncated hPML encoded by *PML* exons 1–6 was prepared by reverse transcriptase-PCR (RT-PCR) using the ReverTra Ace qPCR RT kit and KOD FX (Toyobo, Japan). *PML* cDNA was either cloned in pcDNA3.1(+) (Thermo Fisher) (pcDNA3.1–hPML), or fused to Gal4 DBD and cloned in pCDM8 (pGal–hPML). hRARα and hRXRα cDNAs were cloned in pcDNA3.1(+) (pcDNA3.1–hRARα and pcDNA3.1–hRXRα). The cDNA encoding fusion oncoprotein hPML–RARα(S or L) was prepared by 2-step PCR: the first step involved the use of the 5'-forward primer of hPML and the 3'-reverse primer encoding the chimeric sequences of each of the translocation sites of *PML* and *RARα* cDNAs, with *PML* cDNA as a template, and the use of the 5'-forward primer encoding each corresponding reverse chimeric sequence and the 3'-reverse primer of hRARα with *RARα* cDNA as a template; the second step involved mixing these PCR products and performing PCR without adding primers. The amplicons that encoded chimeric cDNAs were either cloned in pcDNA3.1(+) (pcDNA3.1–hPML–RARα(S and L)) or fused to Gal4 DBD and cloned in pCDM8 (pGal–hPML–RARα(S and L)). The Gal4-luciferase reporter consisted of an SV40 promoter-luciferase reporter pGL3 (Promega) with five Gal4-binding sites, as described [20]. For the 3×*RARE* luciferase reporter, an adaptor pair encoding the *hRARβ2* promoter (-54/-28), 5'-tcgagaagggttcaccgaaagttcactcgcataagggttcaccgaaagttcactcgcataagggttcaccgaaagttcactcgcata-3' and 5'-agcttatgcgagtgaactttcggtgaacccttatgcgagtgaactttcggtgaacccttatgcgagtgaactttcggtgaacccttc-3', was inserted in the luciferase reporter pGL4.10 (Promega).

For mammalian two-hybrid assays, Gal4–hMED1(wild-type) in pCDM8 (pGal4–hMED1) was described previously [26]. Mutant *MED1* cDNAs with the first NR LxxLL motif mutated to encode LxxAA (hMED1(mutant 1)), the second NR motif mutated to LxxAA (hMED1(mutant 2)), and both NR motifs mutated to LxxAA (hMED1(mutant 1+2)), were prepared by site-directed mutagenesis according to the manufacturer's protocol (Agilent). Mutant *MED1* cDNAs were fused to Gal4 DBD and cloned in pCDM8 to generate pGal4–hMED1(mutant 1), pGal4–hMED1(mutant 2) and pGal4–hMED1(mutant 1+2). cDNAs encoding hPML–RARα(S and L) were fused to VP16 and subcloned into pcDNA3.1(+) to generate pVP16–hPML–RARα(S and L).

Generation of MEFs

Stable lines of *Med1*$^{+/+}$ *p53*$^{-/-}$ and *Med1*$^{-/-}$ *p53*$^{-/-}$ MEFs, and primary *Med1* LxxAA MEFs, were described previously [23,26]. These MEFs were derived from embryonic day (E) 10.5 or 11.5. Animal experiments were performed according to the institutional guidelines of the Animal Research Center, Kobe University, Japan.

Cell culture

MEFs and human embryonic kidney epithelial cell line HEK 293T cells were cultured in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) under 5% CO_2 at 37°C in a humidified atmosphere.

Transient transfection and luciferase reporter assays

Cells were plated in 24-well plates at 2.0×10^4 cells/well in DMEM supplemented with 10% charcoal-stripped FBS (Biological Industries, Israel). MEFs were used for

luciferase reporter assays. For Gal4-repoter-based luciferase reporter assays, mammalian expression vectors for Gal4 DBD-fused proteins (100 ng or the indicated amounts) and Gal4-luciferase reporter (100 ng) were cotransfected with the pRL-CMV control vector (5 ng, Promega) using Lipofectamine 2000 Reagent (Thermo Fisher). For *RARE*-reporter-based assays, pcDNA3.1–hPML, pcDNA3.1–hRARα, pcDNA3.1–hPML–hRARα(S) or pcDNA3.1–hPML–hRARα(L) (50 ng), with or without pcDNA3.1–hRXRα (50 ng), and 3×*RARE* luciferase reporter were transfected together with the pRL-CMV control vector (5 ng). ATRA (0, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, or 10^{-5} M) was then added, and 24 h after transfection, firefly and Renilla luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega). Values were normalized by Renilla luciferase activities.

For mammalian two-hybrid assays, Gal 4DBD-fused proteins (50 ng) and VP-16-fused proteins (50 ng) were transfected into HEK 293T cells together with the Gal4-reporter (100 ng) and pRL-CMV control vector (5 ng), and cultured without or with various concentrations of ATRA. Luciferase activities were likewise measured 24 h after transfection.

All numerical data (N = 3) are presented as average ± S.D..

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgements

We thank members of our laboratories for helpful discussions and suggestions.

Funding

This work was supported by Grants-in-Aid for Scientific Research (26460677 and 17K09012) from the Japan Society for the Promotion of Science to M.I. and by NIH grant DK071900 to R.G.R.

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Legends to figures

Figure 1. MED1 is required for optimal transcription by PML–RARα.

(A) Schematic representation of the structures of genes encoding hPML and hRARα, and of their derived oncoprotein products hPML–RARα(S, V and L). Clustered translocation sites (S, V and L) within *PML* locus are shown. *utr*, untranslated region.

(B-C) Gal4-based luciferase reporter assays in MEFs. Gal4 DBD-fused proteins were tested with 5×Gal4 luciferase reporter in the absence or presence of indicated concentrations of ATRA (B). The same assays were performed in the presence of 10^{-6} M ATRA and different amounts of Gal4–hPML–RARα(S or L) as indicated (C). MED1 was found to be required for transactivation depending upon the pharmacological ATRA concentrations and Gal4–hPML–RARα(S or L) dose.

(D) *RARE*-based luciferase reporter assays in MEFs. hPML and intact or mutant (oncofusion) hRARα together with their heterooligomerization partner hRXRα were tested with the 3×*RARE* luciferase reporter in the absence or presence of the indicated concentrations of ATRA. MED1 was found to be required for transactivation initiated by PML–RARα(S or L)/RXRα and dependent on the pharmacological ATRA concentration-dependent transactivation.

Figure 2. MED1 interacts with PML–RARα through MED1 NR motifs.

(A) Schematic representation of hMED1 mutants used. Either one or both of the LxxLL NR motifs were mutated to LxxAA.

(B-C) Mammalian two-hybrid assays. Gal4DBD-fused wild-type or mutant hMED1 and VP16–hPML–RARα(S or L) were tested in HEK 293T cells with the Gal4-luciferase reporter in the absence or presence of the indicated concentrations of ATRA (B). The

same assays were performed in the presence of 10^{-6} M ATRA and different amounts of VP16–hPML–RARα(S or L).

Figure 3. MED1 NR motifs are required for optimal transcription by PML–RARα.
(A) Gal4-based luciferase reporter assays in MEFs. Gal4DBD-fused proteins were tested with the 5×Gal4 luciferase reporter in the absence or presence of the indicated concentrations of ATRA.
(B) *RARE*-based luciferase reporter assays in MEFs. hPML and intact or mutant (oncofusion) hRARα together with their heterooligomerization partner hRXRα were tested with 3×*RARE* luciferase reporter in the absence or presence of the indicated concentrations of ATRA. MED1 NR motifs were found to be required for transactivation initiated by PML–RARα(S or L)/RXRα and dependent upon pharmacological ATRA concentrations.

Figure 4. Model for transcription initiation by PML–RARα.
(A) Liganded RARα/RXRα heterodimer interacts with MED1 through two NR motifs, with NR1 bound to RXRα and NR2 bound to RARα. Then Mediator recruits general transcription factors to the promoter leading to the formation of the functional PIC.
(B, C) In the case of PML–RARα, the PML–RARα homodimer (or homooligomer) associates with RXRα to form a heterotetramer (or heterooligomer). In this model, pharmacological ATRA doses are assumed to be required to occupy all PML–RARα molecules within the heterooligomer, following which MED1 may interact with the oligoheteromer either through the PML–RARα homodimer (B) or through the PML–RARα/RXRα heterodimer (C). The recruited Mediator then leads to the formation of the functional PIC.

Figure 1

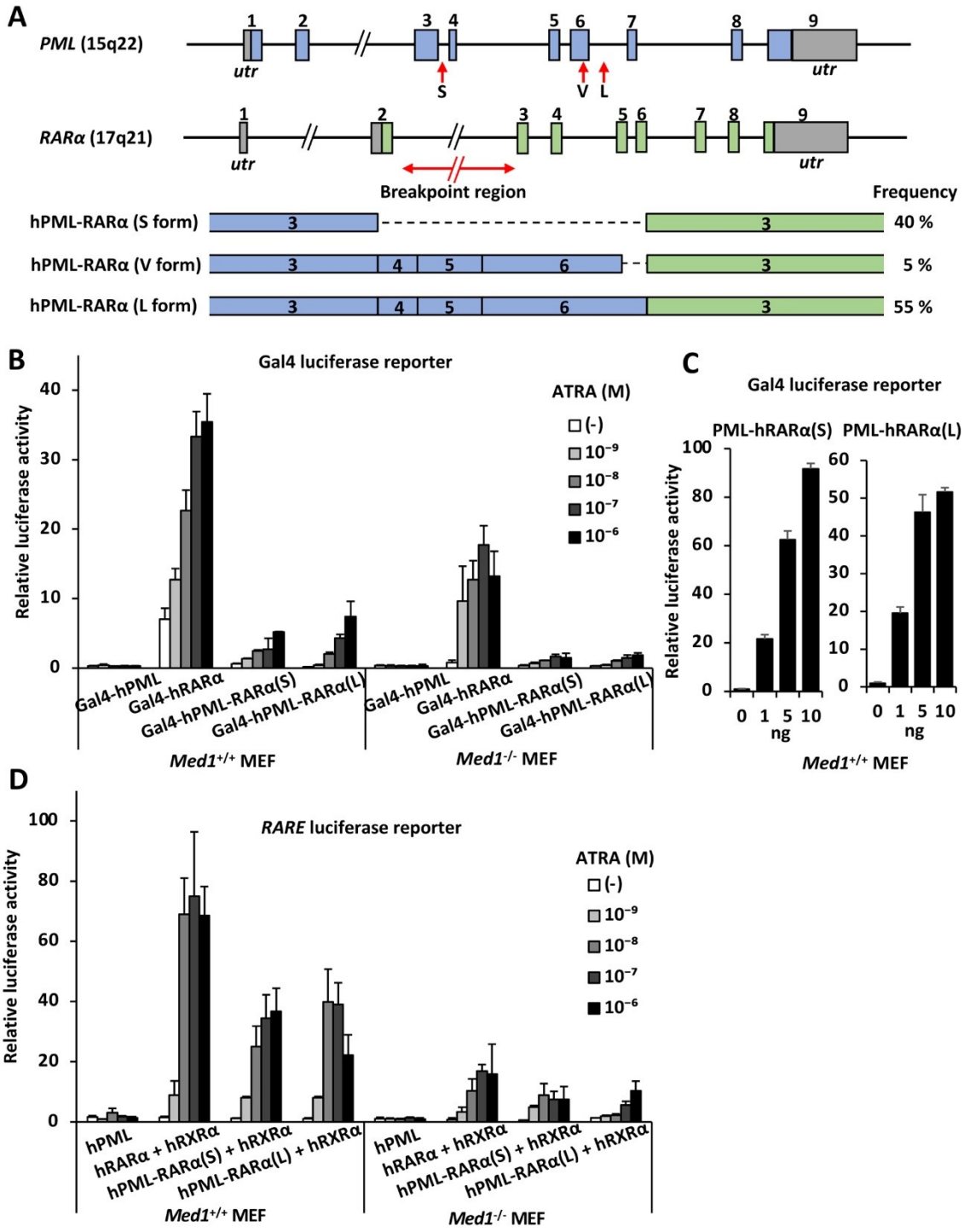

A
PML (15q22)
RARα (17q21)
utr
S
V
L
Breakpoint region
Frequency
hPML-RARα (S form)
hPML-RARα (V form)
hPML-RARα (L form)
40 %
5 %
55 %
B
Gal4 luciferase reporter
Relative luciferase activity
ATRA (M)
(-)
10⁻⁹
10⁻⁸
10⁻⁷
10⁻⁶
Gal4-hPML
Gal4-hRARα
Gal4-hPML-RARα(S)
Gal4-hPML-RARα(L)
Med1+/+ MEF
Med1-/- MEF
C
Gal4 luciferase reporter
PML-hRARα(S)
PML-hRARα(L)
Relative luciferase activity
0 1 5 10
ng
Med1+/+ MEF
D
RARE luciferase reporter
Relative luciferase activity
ATRA (M)
hPML
hRARα + hRXRα
hPML-RARα(S) + hRXRα
hPML-RARα(L) + hRXRα
Med1+/+ MEF
Med1-/- MEF

Figure 2

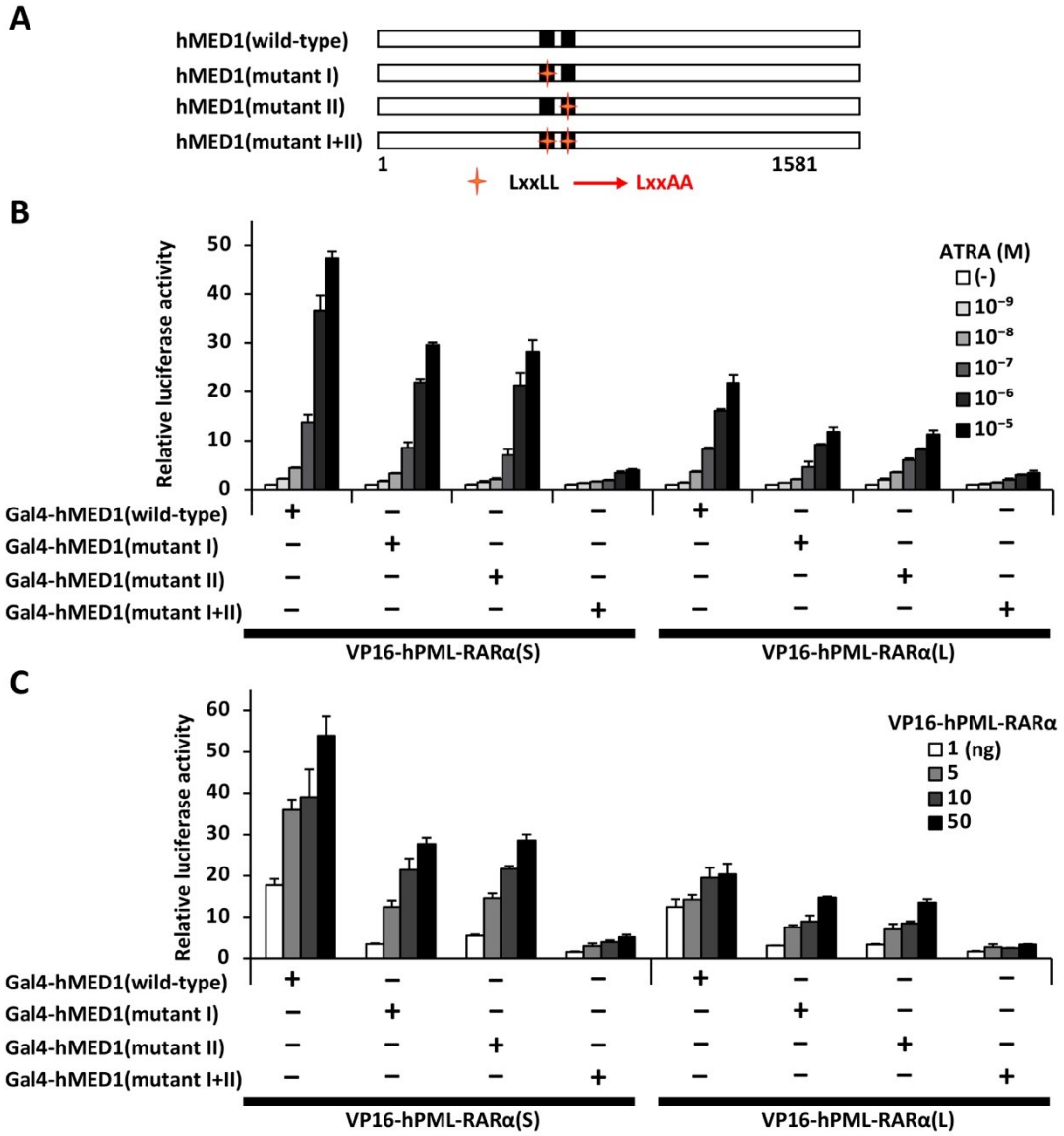

A
hMED1(wild-type)
hMED1(mutant I)
hMED1(mutant II)
hMED1(mutant I+II)
1
1581
LxxLL
LxxAA
B
Relative luciferase activity
ATRA (M)
(-)
10⁻⁹
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
Gal4-hMED1(wild-type)
Gal4-hMED1(mutant I)
Gal4-hMED1(mutant II)
Gal4-hMED1(mutant I+II)
VP16-hPML-RARα(S)
VP16-hPML-RARα(L)
C
Relative luciferase activity
VP16-hPML-RARα
1 (ng)
5
10
50
Gal4-hMED1(wild-type)
Gal4-hMED1(mutant I)
Gal4-hMED1(mutant II)
Gal4-hMED1(mutant I+II)
VP16-hPML-RARα(S)
VP16-hPML-RARα(L)

Figure 3

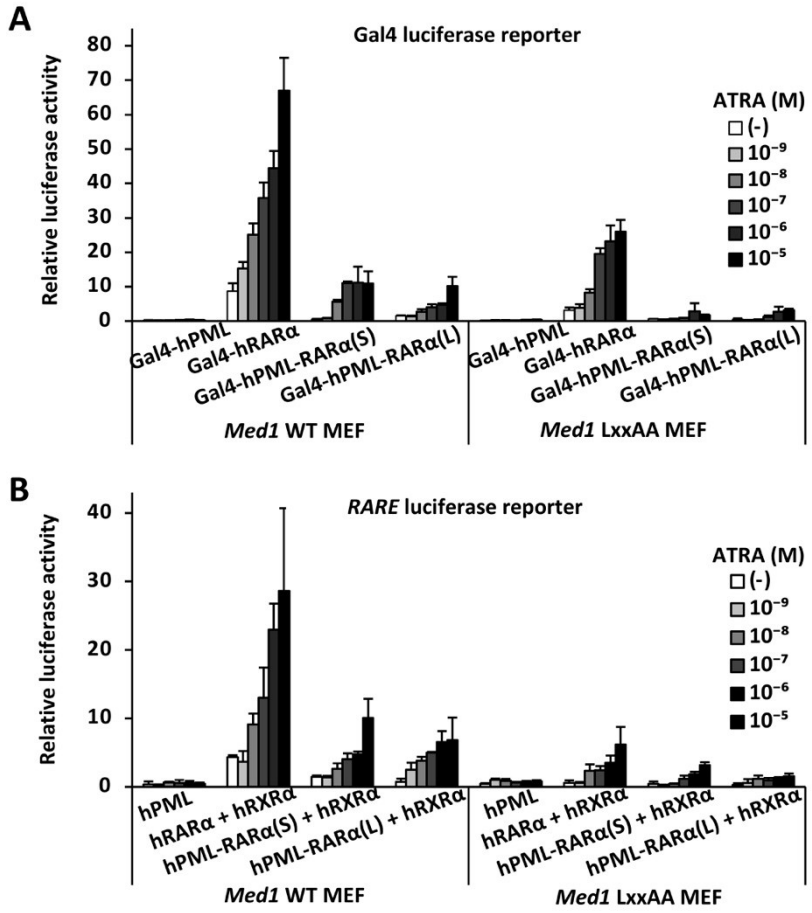
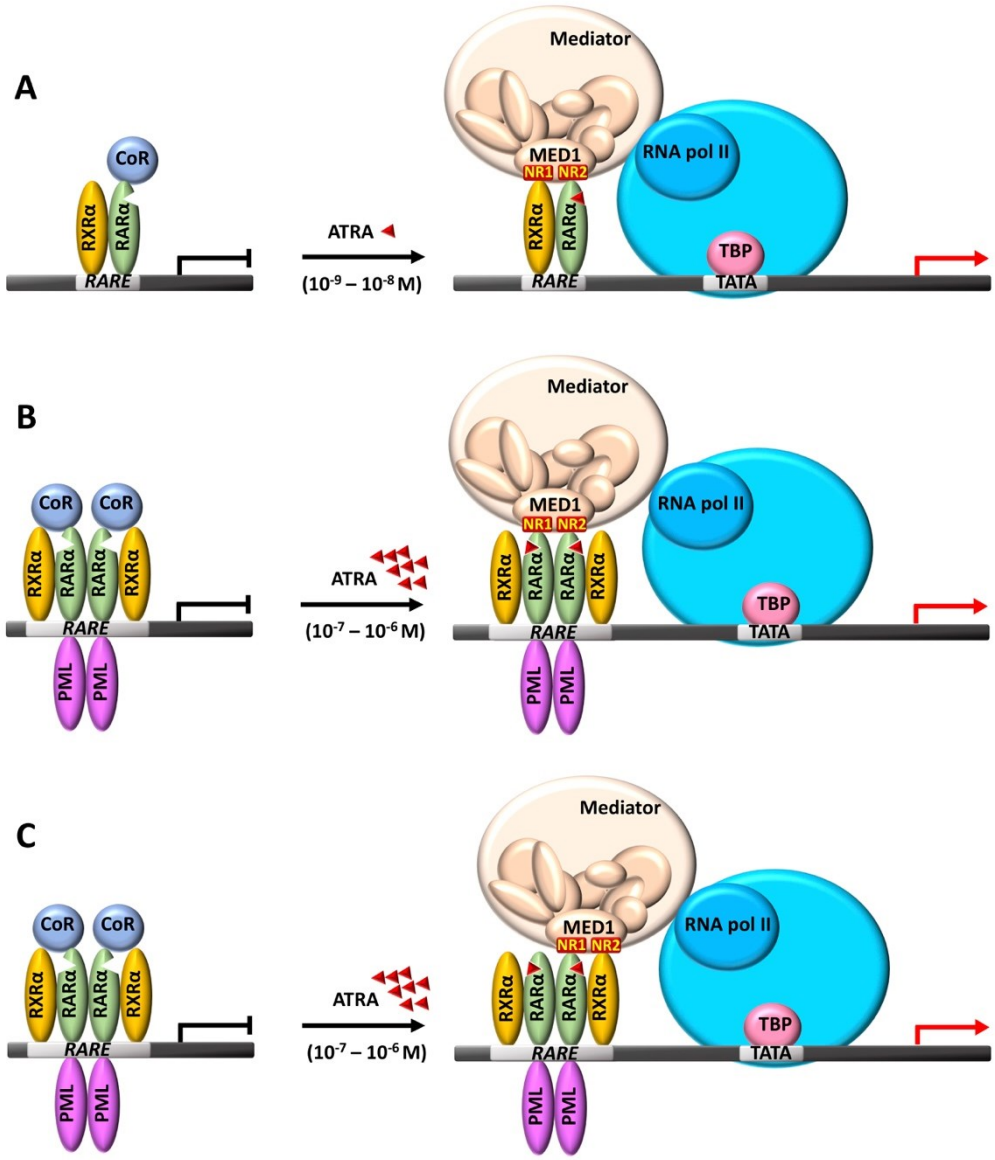

A
Gal4 luciferase reporter
Relative luciferase activity
80
70
60
50
40
30
20
10
0
ATRA (M)
(-)
10⁻⁹
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
Gal4-hPML
Gal4-hRARα
Gal4-hPML-RARα(S)
Gal4-hPML-RARα(L)
Med1 WT MEF
Med1 LxxAA MEF
B
RARE luciferase reporter
Relative luciferase activity
40
30
20
10
0
ATRA (M)
(-)
10⁻⁹
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
hPML
hRARα + hRXRα
hPML-RARα(S) + hRXRα
hPML-RARα(L) + hRXRα
Med1 WT MEF
Med1 LxxAA MEF

Figure 4


A
CoR
RXRα
RARα
RARE
ATRA
(10-9 – 10-8 M)
Mediator
MED1
NR1 NR2
RNA pol II
TBP
TATA
B
CoR
CoR
RXRα
RARα
RARα
RXRα
RARE
PML
PML
ATRA
(10-7 – 10-6 M)
Mediator
MED1
NR1 NR2
RNA pol II
TBP
TATA
C
CoR
CoR
RXRα
RARα
RARα
RXRα
RARE
PML
PML
ATRA
(10-7 – 10-6 M)
Mediator
MED1
NR1 NR2
RNA pol II
TBP
TATA