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PTOV1 antagonizes MED25 in RAR transcriptional activation

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ABSTRACT

Retinoic acid (RA) plays a role in cancer therapy. However, its long-term treatment is hindered by the acquired resistance which is not fully understood. Our previous study indicated that the transcriptional activity of RA receptor (RAR) is enhanced by association of MED25 with CREB-binding protein (CBP) through the PTOV domain, which is also present in prostate tumor over-expressed protein 1 (PTOV1). Here, we show that MED25 and PTOV1 reciprocally regulate RAR transcriptional activity through competitive bindings to CBP and opposite regulation of CBP recruitment to the RA-responsive gene promoter. Finally, we demonstrate that MED25 and PTOV1 differentially modulate RA sensitivity in cancer cells depending on their expression levels, suggesting a potential molecular mechanism underlying RA resistance which frequently emerges during cancer treatments.

1. Introduction

Vitamin A and its active derivatives, referred to as retinoids, regulate several important cellular processes, such as cellular differentiation and organ development [1,2]. In addition, retinoids have been implicated in cancer prevention and therapy owing to their functions in cell growth, proliferation, and apoptosis [3,4]. Although various retinoid derivatives have been developed, intrinsic or acquired resistance to these agents during cancer therapy limits their broad clinical application [5,6]. Overcoming RA resistance requires the synthesis of novel retinoids, the identification of resistance and signaling mechanisms, and the use of combination therapies with other drugs. We have focused on identifying a new resistance mechanism that is based on differential regulation of the RA receptor (RAR).

In the presence of RA, RAR binds to its response element (RARE) and acts as a transcription factor to control gene expression through associations with various coregulators [7,8]. Among the coregulators, MED25 was known to stimulate RAR activity by forming the MED25–CBP–RAR complex through its distinct domains [9]. MED25 was originally identified by differential display screening as one of two prostate tumor over-expressed genes (PTOV) [10]. Structurally, PTOV1 harbors two tandemly repeated PTOV homology blocks, whereas PTOV2, which corresponds to MED25, possesses a single PTOV domain required for CBP binding [9]. PTOV1 is over-expressed during the early and late stages of prostate cancer and is scarcely detectable in normal prostate tissues. In contrast, MED25 is expressed at higher levels in primary prostate cells than in metastatic prostate cancer cell lines [11]. Recently, PTOV1 has been implicated in tumor growth by increasing cell proliferation [12–14].

Based on the observations of different expression levels of MED25 and PTOV1 in prostate cancer and their structural similarities, we investigated the roles of these proteins in RAR regulation. In the present study, we demonstrate that MED25 and PTOV1 compete for CBP binding through the conserved PTOV domain. In addition, these proteins regulate, in opposing fashions, the transcriptional activity of RAR by modulating CBP occupancy of chromatin. These two regulatory paths exert different effects on RA cytotoxicity in RA-resistant cell lines. Overall, our findings suggest novel approaches to overcoming RA resistance in cancer therapy.

2. Materials and methods

2.1. Cell lines and cell culture

H1299 cells were grown in RPMI 1640 medium that was supplemented with 5% heat-inactivated fetal bovine serum (FBS) and an antibiotic–antimycotic mixture (all from Invitrogen, Carlsbad, CA) in a 5% CO_2 atmosphere at 37 °C. Before use with cells that were to be treated with RA, the FBS was pretreated with charcoal.

Abbreviations: NR, nuclear receptor; RAR, retinoic acid receptor; AtRA, all-*trans* retinoic acid; RXR, retinoid X receptor; CBP, CREB-binding protein; MED25, mediator protein 25; PTOV, prostate tumor over-expressed; DBD, DNA-binding domain; LBD, ligand-binding domain; GST, glutathione S-transferase; WB, Western blotting; ChIP, chromatin immunoprecipitation; GFP, green fluorescent protein; LUC, luciferase; shRNA, small hairpin RNA; β-gal, β-galactosidase.

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2.2. Plasmids and cloning

All the cDNAs were constructed according to standard methods and verified by sequencing. Most of the constructs have been described previously [13]. The desired genes were amplified by PCR and subcloned into suitable vectors: the pBTM116 and pASV3 vectors for yeast two-hybrid assay; Flag (2X)-tagged pcDNA3 for over-expression in mammalian cells; the pG4MpolyII vector for Gal4 fusion; and pSilencer 2.1-U6 Hygro (Applied Biosystems/Ambion, Austin, TX) for small hairpin (sh) RNA expression. For the *in vitro* binding assay, the indicated genes were subcloned into pGEX4T-1 (GE Healthcare, Piscataway, NJ) for the GST-fused proteins, and into pET15b vectors (Novagen, Madison, WI) to generate the His-tagged proteins.

2.3. Yeast two-hybrid (Y2H) assay, Immunoprecipitation (IP) and Western blotting (WB)

Y2H assay, IP and WB were performed as previously reported [9]. The amounts of whole cell lysates used for IP and WB were 500 μg and 20–50 μg, respectively. For IP, the lysates were incubated overnight at 4 °C with the indicated antibodies (1:200 dilution). After 2 h of incubation at 4 °C with A/G-agarose beads (Santa Cruz Biotechnology, Santa Cruz, CA), the beads were washed three times with RIPA buffer. The immune complexes were released from the beads by boiling, and analyzed by WB using the indicated antibodies. For WB, lysates or proteins were separated by electrophoresis on 8–12% SDS–polyacrylamide gels, transferred to nitrocellulose, and incubated with the following primary antibodies: anti-MED25 [13]; anti-PTOV1 (Ab81173; Abcam, Cambridge, MA); anti-His (ab1187; Abcam); anti-Flag M2 (F-3165; Sigma Chemical Co., St. Louis, MO); anti-CBP (sc-1211; Santa Cruz Biotechnology); anti-RARα (sc-551; Santa Cruz Biotechnology); and anti-GFP (sc-8334; Santa Cruz Biotechnology). The blots were then incubated with peroxidase-conjugated mouse or rabbit IgG secondary antibodies (GE Healthcare).

2.4. Glutathione S-transferase (GST) pull-down assays

A GST-fusion of CBP (aa 1-460) and His-tagged MED25 or PTOV1 were expressed in *Escherichia coli* and purified on glutathione–sepharose beads (GE Healthcare) and a HiTrap™ chelating HP column (GE healthcare), respectively. An approximately equal amount of GST or GST-CBP was mixed with His-tagged MED25 or PTOV1. For the competition assays, the level of either MED25 or PTOV1 was increased gradually. Bound proteins were detected using WB with an anti-His-tag antibody (Abcam).

2.5. Transient transfection and luciferase assay

H1299 cells were seeded in 12-well culture plates at a density of 1.5×10^5 cells per well. After overnight incubation, the cells were transiently transfected with the Gal4- or RARE-tk-luciferase reporter and an SV40-driven β-gal expression vector (internal control). Depending on the experimental conditions, the Gal4-MED25-, MED25-, PTOV1- or CBP-expressing vector was co-transfected using the Lipofectamine Plus reagent (Invitrogen). The luciferase activity was measured and normalized to the β-gal activity.

2.6. Reverse transcriptase-PCR (RT-PCR) and Real-Time RT-PCR

Total RNA was extracted using the TRIzol Reagent (Invitrogen) from H1299 cells that stably expressed Flag-tagged vectors (for over-expression) or sh-vectors (for knockdown), and 5 μg of RNA were reverse-transcribed using Superscript II reverse transcriptase (Invitrogen) and random oligo(dT) primers (New England Biolabs, Beverly, MA). The reverse transcripts were amplified by PCR using the following primer pairs (forward and reverse, respectively): for *RARβ2* (113-bp fragment), 5′-TTGTGTTCACCTTTGCCAAC-3′ and 5′-CGGTTCCTCAAGGTCCTGG-3′; for *CYP26* (111-bp), 5′-CTTCAGCC GCGAGGCACTC-3′ and 5′-TCGGGGTAGACCAGGAGGC-3′; for *p21W AF1* (100-bp), 5′-TGCGCTAATGGCGGGCTG-3′ and 5′-CACACGCTCC CAGGCGAAG-3′; and for *GAPDH* (120-bp), 5′-CTGCACCACCAAC TGCTTAGC-3′ and 5′-GGGCCATCCACAGTCTTCTGG-3′. For Real-Time PCR, the cDNA species were amplified using the same primer pairs, and analyzed using the iQ™ SYBR Green Supermix and Icycler CFX96 Real-Time PCR detection system (Bio-Rad, Hercules, CA). All the gene expression levels were normalized using *GAPDH* as an internal standard in each well. Fold-expression was defined as the fold-increase relative to controls.

2.7. Chromatin immunoprecipitation (ChIP)

H1299 cells were transfected with the Flag-MED25 or Flag-PTOV1 expression vector overnight. Subsequent ChIP assay was performed as described previously [9].

2.8. Construction of stable cell lines

For the over-expression of MED25 or PTOV1, the cDNA species were amplified by PCR and subcloned into the G418 resistance-bearing pcDNA3 vector. To knock down the expression of MED25 and PTOV1, small hairpin (sh) sequences (5′-TCTTGTGCAGCAGA TTGGG-3′ and 5′-CTTTGTCAACGGCATCCGG-3′, respectively) were used. A synthetic duplex that harbored additional restriction enzyme sites (*Hin*dIII and *Bam*HI) was subcloned into the hygromycin-resistance-bearing pSilencer 2.1-U6 Hygro vector. H1299 or PC3 cells were transfected for 48 h, and then treated with 600 μg/ml G418 or 400 μg/ml hygromycin. After 3–5 days, the cells were incubated with fresh culture medium that contained G418 or hygromycin, and resistant colonies were selected after 2 weeks of incubation. For the controls, the pcDNA3 vector or sh-luciferase (sh-Luc) vector was used.

2.9. MTT and FACS analysis

To determine the effect of RA on cell viability, the IC_{50} values were measured using MTT (Sigma) as described previously [15]. Stable H1299 or PC3 cells were seeded in 96-well plates at a density of $3–5 \times 10^5$ cells per well. After 24 h, the cells were incubated with serially diluted AtRA (0–100 μM) for 72 h, and then incubated with MTT reagent (2 mg/ml, 50 μl) for 4 h. The fraction of living cells was measured as the optical density (OD) at 540 nm in an ELISA plate reader. IC_{50} values were calculated using the Sigma-plot software.

FACS analysis was used to determine the fraction of apoptotic cells. Stable cells were treated with 30 μM AtRA for 72 h. Each cell cycle fraction was measured by fixing 1×10^6 cells with 80% ethanol, staining with propodium iodide, and analysis in the FACSCalibur™ Flow cytometer according to the manufacturer's recommendations (BD Biosciences). Data analysis was performed using the WinMDI software (version 2.8).

3. Results and discussion

3.1. Interactions of CBP with MED25 and PTOV1

Sequence analysis of MED25 revealed a single PTOV (or ACID) domain between the SD1 and SD2 domains, in contrast to the PTOV1 protein, which contains two tandemly arranged PTOV

domains (Fig. 1A). MED25 also contains the VWA domain, which is required for mediator binding, and the NR box, which is required for RAR binding. In line with our previous study [13], yeast two-hybrid assays showed that the PTOV domain (amino acid [aa] residues 395–545) of MED25 interacted with the N-terminal region (aa 1–460) of CBP, which is a co-activator with histone acetyltransferase (HAT) activity (Fig. 1B). In similar fashion, the region of PTOV1 that contains the second PTOV domain (aa 253–416) was found to be responsible for CBP binding. To confirm the CBP interactions with MED25 and PTOV1 *in vivo* and *in vitro*, we performed immunoprecipitation (IP) and GST pull-down assays, respectively. For the IP assay, H1299 cells were co-transfected with HA-tagged CBP and Flag-tagged MED25 or Flag-tagged PTOV1. IP with an anti-HA antibody and subsequent Western blotting (WB) with an anti-Flag antibody demonstrated that both MED25 and PTOV1 interacted with CBP (Fig. 1C). The *in vivo* interaction was further verified by IP using an anti-CBP antibody and WB with an anti-PTOV1 or anti-MED25 antibody (Fig. 1D). GST pull-down assays were performed using purified GST-fused CBP (aa 1–460), His-tagged MED25, and His-tagged PTOV1. Binding reactions were monitored by WB with an anti-His-tag antibody. The direct interaction between CBP and PTOV1 decreased gradually as the level of MED25 was increased (Fig. 1E, *left*). The reciprocal experiment confirmed a direct interaction between CBP and MED25, and competition for CBP binding by PTOV1 and MED25 (Fig. 1E, *right*). Overall, our data suggest that both MED25 and PTOV1 interact with CBP through the common PTOV domain, and therefore compete for CBP binding.

3.2. Reciprocal effects of MED25 and PTOV1 on the transcriptional activity of RAR

To examine the interplay between CBP, MED25, and PTOV1 with respect to transcription, we performed luciferase reporter assays. Following transfection of the Gal4 DBD-responsive reporter into H1299 cells, Gal4 DBD-fused MED25 showed autonomous transcriptional activity, which was enhanced by CBP (Fig. 2A). The CBP-mediated increase in MED25 activity was significantly impaired by PTOV1 (Fig. 2B). Either CPB or PTOV1 alone had no effect on the transcriptional activity of Gal4 DBD (data not shown). In transfection experiments using the RARE-luciferase reporter, MED25 increased RA-induced RAR activity, and this was diminished by PTOV1 (Fig. 2C). Taken together, these results suggest that PTOV1 suppresses MED25-enhanced RAR activity, probably by

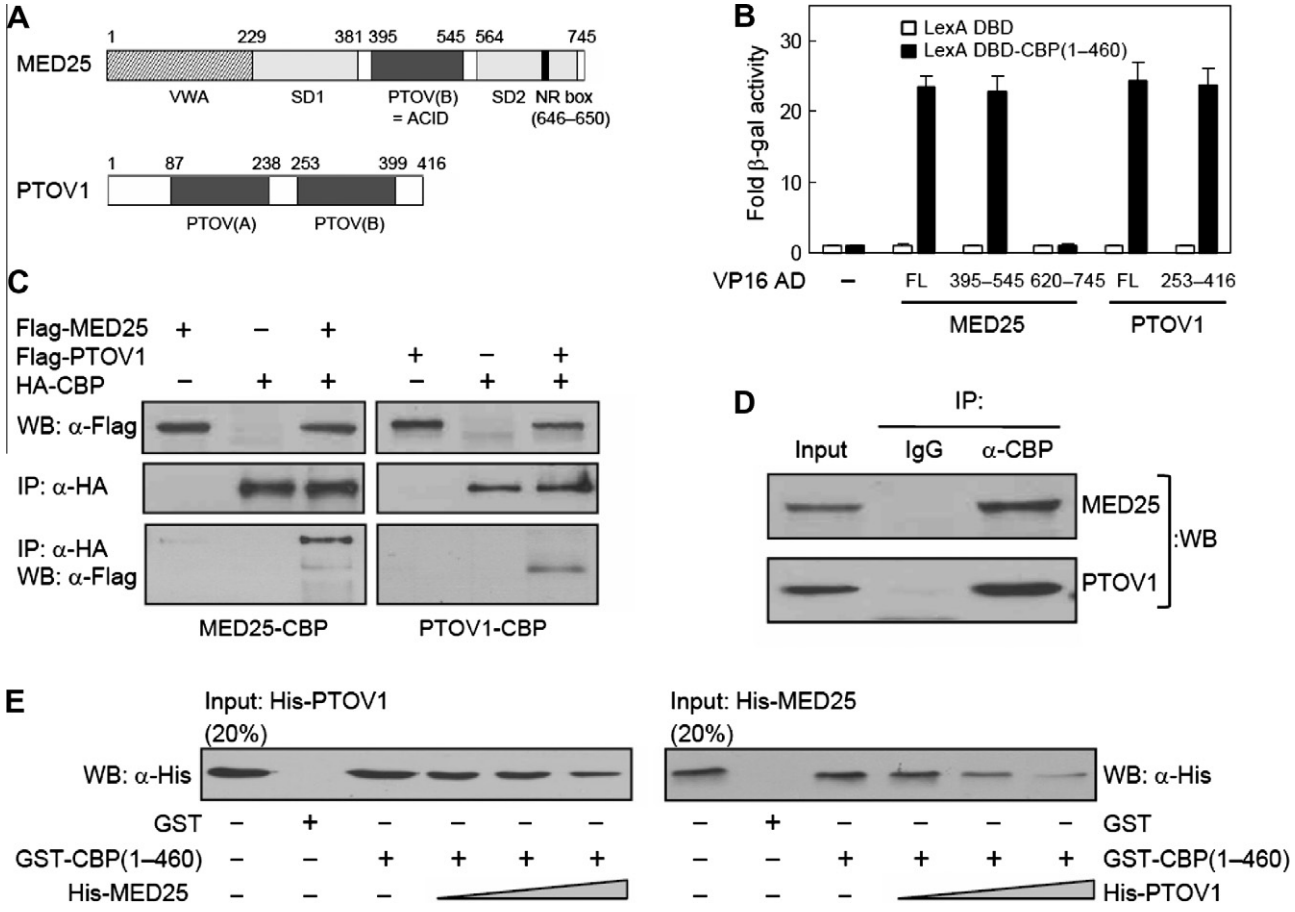


Fig. 1. CBP interacts with MED25 and PTOV1. (A) Structural features of MED25 and PTOV1. VWA, von Willebrand factor type A domain; SD, synapsin 1 domain; PTOV, a conserved domain found in prostate tumor over-expressed protein 1; ACID, VP16 activator-interacting domain; NR box, nuclear receptor binding motif. (B) Identification of the PTOV domain responsible for the CBP interaction in yeast. Yeast two-hybrid assays were performed using LexA-fused CBP (aa 1–460) and VP16 AD-fused MED25 or PTOV1. Interactions were evaluated using β-gal assays. The fold-activity is relative to the value for the VP16 AD empty control, and the results are shown as means ± S.D. of three independent experiments. (C) CBP interactions with MED25 and PTOV1 *in vivo*. H1299 cells were transfected with HA-CBP and the Flag-MED25 (*left*) or Flag-PTOV1 (*right*) expression vector, and cell lysates were prepared. The cell lysates were subjected to immunoprecipitation (IP) using an anti-HA antibody, and the precipitated proteins were analyzed by Western blotting (WB) using an anti-Flag antibody. (D) Endogenous interaction between CBP and MED25 or PTOV1. H1299 cell lysates were immunoprecipitated with pre-immune serum (IgG) or anti-CBP antibody. The bound proteins were identified by WB with an anti-MED25 or anti-PTOV1 antibody. (E) Competition between MED25 and PTOV1 for CBP binding. Purified His-PTOV1 (*left*, 0.2 μg) or His-MED25 (*right*, 0.2 μg) was incubated with 0.2 μg GST or GST-CBP (aa 1–460) and then reacted with increasing amounts of purified His-MED25 or His-PTOV1 (0.2, 0.5, and 1.0 μg), respectively. The bound proteins were visualized by SDS–PAGE and subsequent WB with the anti-His-tag antibody.

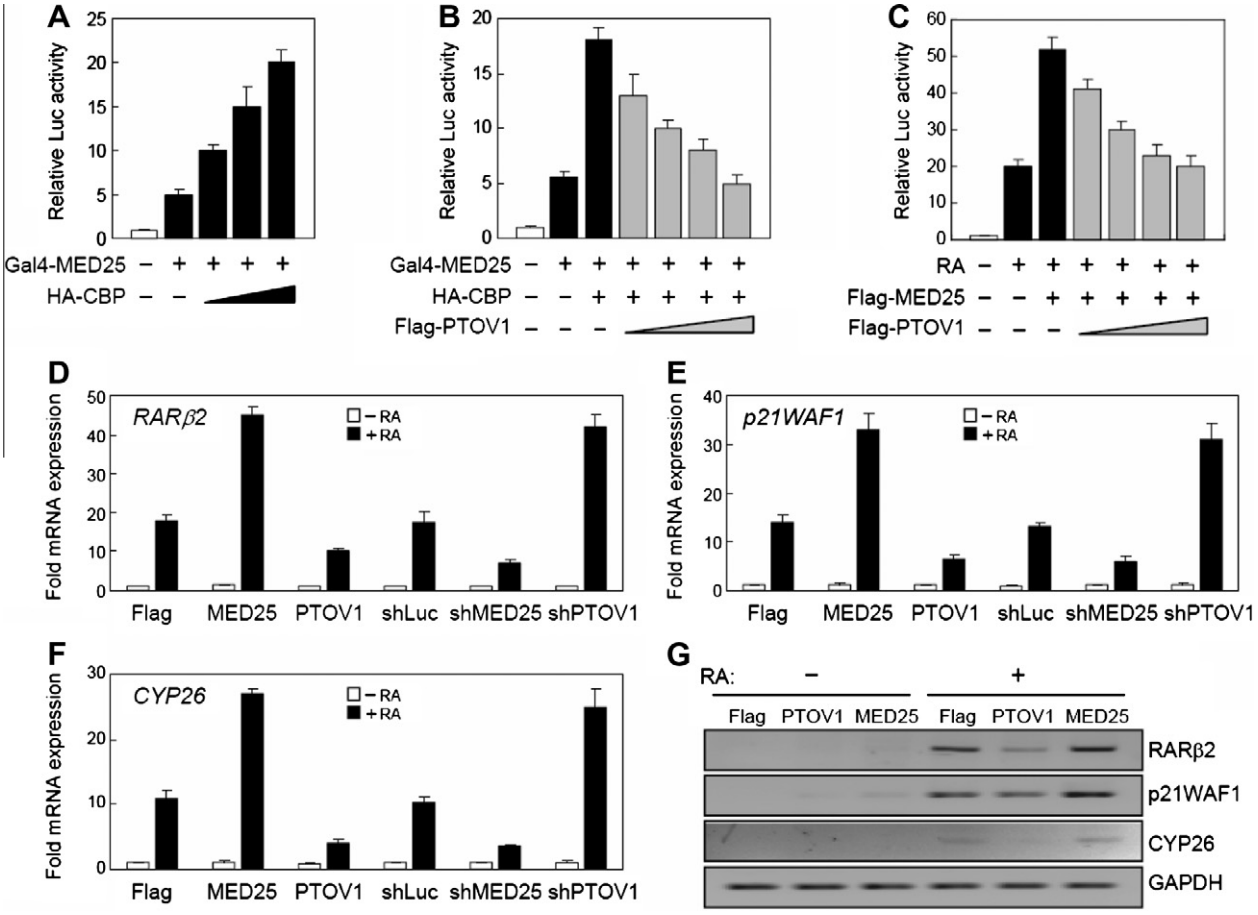


Fig. 2. MED25 and PTOV1 regulate in opposite manners the transcriptional activation of RAR. (A) Effect of CBP on the autonomous transcriptional activity of MED25. H1299 cells were transfected with the Gal4 DBD-responsive luciferase reporter, 0.4 μg of Gal4 DBD-fused MED25, and increasing amounts (0, 0.1, 0.2, 0.3, and 0.4 μg) of the CBP-expressing vector. (B) Effect of PTOV1 on CBP-enhanced MED25 activity. In the presence of 0.4 μg of Gal4-MED25 and 0.4 μg CBP, the level of PTOV1 was increased (0, 0.1, 0.2, 0.3, and 0.4 μg). (C) Effect of PTOV1 on MED25-promoted RAR activity. The RARE-tk-luciferase reporter was used with a level (0.4 μg) of MED25 and increasing levels (0, 0.1, 0.2, 0.3, and 0.4 μg) of PTOV1 in the presence of 1 μM AtRA. Extracts of the transfected cells were subjected to luciferase (Luc) activity assays (A–C). Relative luciferase activity is shown as mean ± S.D. of three independent experiments. (D–F) Effects of MED25 and PTOV1 on the expression of the RAR target genes *RARβ2* (D), *p21WAF1* (E), and *CYP26* (F). Stable H1299 cells were generated so that they over-expressed or under-expressed (knocked down) MED25 or PTOV1, as described in Section 2. Total RNA samples were extracted from stable H1299 cells that were cultured in the absence or presence of 1 μM AtRA, and subjected to Real-Time RT-PCR. The fold-increases in mRNA expression are normalized to the level of *GAPDH* mRNA. Data shown represent the means ± S.D. of three independent experiments. $P < 0.01$. (G) Normal RT-PCR. Total RNA samples were extracted and subjected to RT-PCR using primer pairs specific for the three genes. *GAPDH* was used as an internal control.

inhibiting MED25 binding to CBP, which is a co-activator required for RAR activation.

Next, we evaluated the effects of MED25 and PTOV1 on the endogenous expression of RAR target genes in H1299 cells when MED25 and PTOV1 were over-expressed and knocked down, monitored by WB as shown in Fig. 4. The cells were treated with vehicle or all-*trans* RA, and the gene expression levels were measured by Real-Time RT-PCR. The expression levels of *RARβ2* (Fig. 2D), *p21WAF1* (Fig. 2E), and *CYP26* (Fig. 2F) were significantly increased by MED25 over-expression, whereas they were decreased by PTOV1 over-expression. These changes in mRNA expression were reversed when MED25 or PTOV1 was knocked down using shRNA. In both cases, transfection with Flag or shLuc had no effect on gene expression. With conventional RT-PCR, similar results were obtained, *i.e.*, MED25 enhanced and PTOV1 suppressed RA-induced target gene expression (Fig. 2G). These results reveal that MED25 and PTOV1 reciprocally control the RA-induced transcriptional activity of RAR.

3.3. Effects of MED25 and PTOV1 on CBP recruitment to RA-responsive chromatin

To investigate further the molecular mechanism underlying the competitive CBP binding and reciprocal roles of MED25 and PTOV1 in RAR activation at the chromatin level, we performed chromatin immunoprecipitation (ChIP) assays using the RA-responsive *RARβ2* promoter in H1299 cells that over-expressed either Flag-empty (F-E), Flag-MED25 or Flag-PTOV1 in the absence and presence of RA (Fig. 3A). No chromatin binding was observed with the IgG control. In all cases, RAR binding to the promoter was RA-dependent. Interestingly, PTOV1 did not occupy the promoter, whereas MED25 efficiently occupied the promoter in the presence of RA. The RA-dependent CBP binding to the promoter was decreased when PTOV1 was over-expressed, whereas it was slightly increased when MED25 was over-expressed. These findings were confirmed by Real-Time PCR followed by ChIP using appropriate antibodies (Fig. 3B–E). The ChIP data suggest that PTOV1, which itself is not recruited to the promoter, inhibits CBP binding to the promoter, probably *via* a chromatin-free PTOV1–CBP interaction. In contrast, MED25, which is recruited to the promoter, potentiates CBP binding through a MED25–CBP interaction on the chromatin.

3.4. Opposite roles of MED25 and PTOV1 in RA cytotoxicity

To address the biological significance of the reciprocal function of MED25 and PTOV1 in RAR regulation, we assayed RA-induced cytotoxicity under various expression conditions of MED25 or

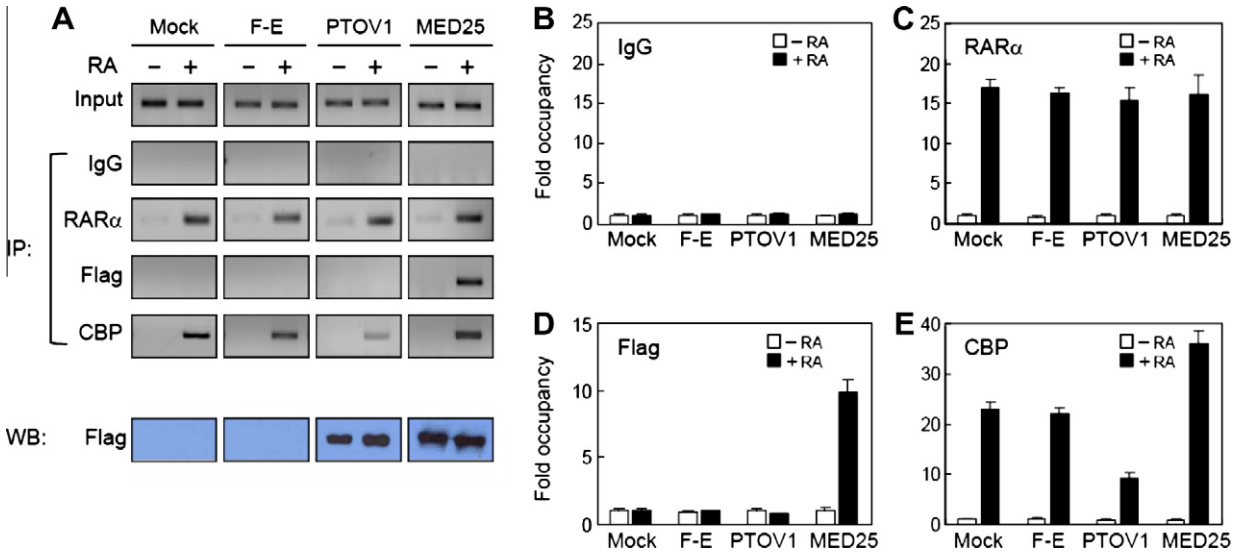
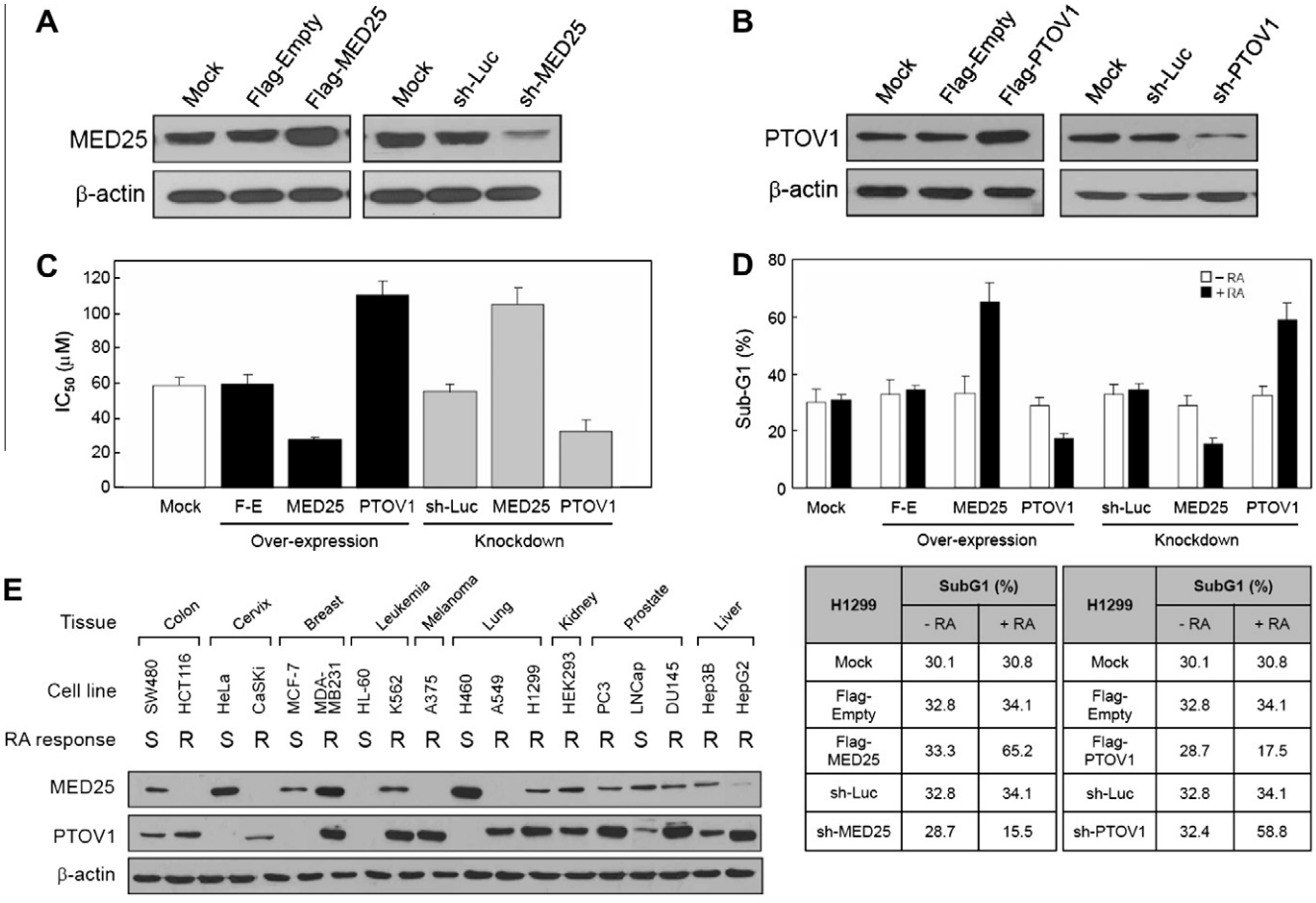


Fig. 3. PTOV1 impairs, while MED25 promotes, CBP binding to RA-responsive chromatin. (A) Effects of MED25 and PTOV1 on CBP recruitment to RA-responsive chromatin. After the H1299 cells were transfected with Flag-MED25 or Flag-PTOV1 in the absence or presence of 1 μM AtRA, ChIP and PCR were performed using a primer pair specific for the *RARβ2* promoter region, as described in Section 2. The antibodies used for IP are indicated. The levels of exogenous MED25 and PTOV1 were monitored by WB using the anti-Flag antibody. (B–E) ChIP and Real-Time PCR. ChIP assays were performed using pre-immune serum (IgG) (B), anti-RARα (C), anti-Flag for MED25 or PTOV1 (D), and anti-CBP (E) antibodies. The amount of bound DNA to each antibody was monitored by Real-Time PCR. Relative occupancy represents the fold-increase in the percentage of input over that of the mock (set as 1). Error bars indicate S.D. ($n = 3$). $P < 0.01$.



H1299	SubG1 (%)	
	- RA	+ RA
Mock	30.1	30.8
Flag-Empty	32.8	34.1
Flag-MED25	33.3	65.2
sh-Luc	32.8	34.1
sh-MED25	28.7	15.5

H1299	SubG1 (%)	
	- RA	+ RA
Mock	30.1	30.8
Flag-Empty	32.8	34.1
Flag-PTOV1	28.7	17.5
sh-Luc	32.8	34.1
sh-PTOV1	32.4	58.8

Fig. 4. MED25 increases, while PTOV1 decreases RA toxicity for H1299 cells. (A) Expression of MED25. H1299 cells were stably transfected with Flag-MED25 (for over-expression of MED25) or sh-MED25 (to knock down the expression of MED25). Flag-empty and sh-luciferase (shLuc) vectors were used as controls. The MED25 expression levels were measured by WB using the anti-MED25 antibody. (B) Expression of PTOV1. The same conditions were applied as in Fig. 5a, except that MED25 was replaced with PTOV1. (C) Effects of MED25 and PTOV1 levels on RA-regulated cellular proliferation. To determine the IC_{50} values, stable H1299 cells were subjected to the MTT assay in the presence of various amounts of AtRA (0–100 μM). (D) Effects of MED25 and PTOV1 levels on the RA-regulated cell cycle. Stable H1299 cells incubated in the presence of 30 μM AtRA for 3 days were subjected to FACS analysis. The sub-G1 fraction (apoptotic cell fraction) was calculated using the WinMDI software. $P < 0.01$. (E) Correlation between RA sensitivity and the expression levels of MED25 and PTOV1. The RA sensitivities of various cancer cells have been previously described elsewhere [16–18]. The levels of endogenous MED25 and PTOV1 were analyzed by WB. The RA responses labeled as 'R' and 'S' are resistant ($IC_{50} > 50$ μM) and sensitive ($IC_{50} < 50$ μM) to AtRA treatment, respectively.

PTOV1. To this end, the RA-resistant H1299 cells were chosen to generate stable cell lines that over-expressed or under- expressed MED25 or PTOV1. The levels of protein expression were monitored by WB under conditions of over-expression (Fig. 4A) and knockdown (Fig. 4B). Subsequent MTT assays of stable H1299 cells treated with different concentrations of RA indicated that the IC_{50} value for MED25-over-expressing cells decreased from 58.9 μM (Flag control) to 27.5 μM, whereas that for PTOV1 cells increased to 110.5 μM (Fig. 4C). Conversely, the IC_{50} values for MED25- and PTOV1-depleted cells were 105.3 μM and 32.5 μM, respectively. This suggests that MED25 increases, whereas PTOV1 decreases, RA toxicity for H1299 cells. The effects of these proteins were further evaluated by FACS analysis (Fig. 4D). Upon the treatment with 30 μM RA, the sub-G1 fraction, comprising apoptotic cells, was increased in the MED25-over-expressing cells and decreased in the PTOV1 over-expressing cells: compare 34.1% (Flag control) to 65.2% (MED25) and 17.5% (PTOV1). These changes were reversed when MED25 or PTOV1 expression was depleted. Similar results were obtained for RA-induced toxicity in PC3 cells, as measured by MTT and FACS analysis (data not shown). These results suggest that MED25 increases RA sensitivity by increasing RAR activation, whereas PTOV1 increases RA resistance by suppressing RAR activation.

Based on our results, we hypothesized that RA signaling and cytotoxicity is regulated by balancing the levels of MED25 and PTOV1 in various cancer cells. To test this hypothesis, we examined the expression levels of MED25 and PTOV1 in various cancer cell lines. The RA responses of various cancer cells have been described in previous studies conducted by our group [17,18] and others [16]. In general, most of the RA-sensitive cells expressed higher levels of MED25 than PTOV1, while the RA-resistant cells expressed higher levels of PTOV1 than MED25. For example, the RA-sensitive HeLa and MCF-7 cells expressed only MED25, while the RA-resistant HCT116, CaSki, A375, A549, and HepG2 cells expressed only PTOV1. These data support the notion that the RA response is closely linked to the expression levels of MED25 and PTOV1 (Fig. 4E). Taken together, these findings suggest that MED25 and PTOV1 are determinants of RA sensitivity.

In the present study, we present evidence that MED25 and PTOV1 reciprocally regulate RAR transcriptional activity through differential usage of CBP and determine RA sensitivity in cancer cells. MED25 forms a complex with the Mediator protein through the VWA domain, CBP through PTOV domain, and RAR through the LxxLL motif in the presence of RA, and triggers transcriptional activation of RAR. CBP, which possesses histone acetyltransferase activity, acts as a co-activator of RAR [19]. PTOV1 is also capable of CBP binding through PTOV domain, thus competing with MED25 for CBP binding and leading to RAR repression. The limited amount of CBP in cells has been proposed as the main cause of the mutual antagonism between RAR and AP-1 [20]. Similarly, we speculate that RAR inhibition by PTOV1 is the result of PTOV1 competition with MED25 for the limited amount of CBP in cells.

Since RA activity is mainly manifested through association with the RAR, the opposite effects of MED25 and PTOV1 on RA cytotoxicity are ascribed to the reciprocal roles of these proteins in RAR activation. Given our overall findings, we propose that MED25 and/or PTOV1, owing to their differential regulation of RAR activity, are determinants of RA sensitivity, which is critical for cancer therapy with retinoids.

Acknowledgments

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