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CAC1 negatively regulates RARα activity through cooperation with HDAC

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ABSTRACT

Retinoic acid (RA) plays pleiotropic roles in cellular differentiation and animal development. RA responses are mediated by transcriptional activation by the retinoic acid receptor (RAR) and retinoid X receptor (RXR) in cooperation with various types of coregulators at RA-responsive gene promoters. Here, we identified CDK2-associated cullin (CAC1) as a novel type of RARα coregulator that interacts with RARα and inhibits its transcriptional activity. The CoRNR box of CAC1 is required for the binding to and inactivation of RARα. In addition, CAC1 cooperates with histone deacetylases (HDACs) to suppress RARα, probably by associating with HDAC. Finally, depletion of CAC1 increases RA-induced neuronal differentiation of P19 cells, a response accompanied by significant upregulation of the neuronal marker *nestin*. From these results, we suggest that CAC1 is a novel corepressor of RARα that cooperates with HDACs and is involved in the regulation of RA-induced cellular differentiation.

1. Introduction

The retinoic acid receptor (RAR) is a member of the nuclear hormone receptor (NR) superfamily that regulates various physiological processes, including differentiation, proliferation, and development [1–3]. NRs including RAR are ligand-inducible transcription factors that specifically regulate the expression of target genes. All NRs share a common modular structure consisting of an N-terminal transcriptional activation domain (AF-1), a conserved DNA-binding domain (DBD), a hinge region (D), and a C-terminal ligand-binding domain (LBD) that overlaps with the second transcriptional activation domain (AF-2) [1,2,4]. Retinoid signaling is generated by two families of nuclear receptors, the RAR and retinoid X receptor (RXR), which bind as RAR/RXR heterodimers to *cis*-acting RA response elements (RAREs) located in the regulatory sequences of target genes [5]. Upon binding to RAREs, RAR/RXR heterodimers activate target gene transcription through complex interactions with coactivators, chromatin remodeling factors, and components of the basal transcription machinery [6].

Transcriptional regulation mediated by RAR/RXR involves the binding and recruitment of various coactivators and corepressors to target gene promoters [7]. The first identified coactivators of retinoid receptors were members of the SRC/P160 family, which includes SRC-1/NCoA-1, TIF-2/GRIP-1/NCoA-2/SRC-2, and pCIP/ACTR/AIB1/TRAM1/RAC3/SRC-3 [8,9]. Other coactivators are histone acetyltransferases (HATs) including CBP/p300 and pCAF, which acetylate lysine residues in the N-terminal tails of histones, and histone methyltransferases (HMTs) including CARM1 and PRMT1, which methylate arginine residues in histone H3 and H4 [10–14]. These coactivators modify the chromatin environment and thereby allow the recruitment of ATP-dependent chromatin remodeling factors to facilitate the initiation of transcription. In contrast, NR corepressors such as nuclear receptor corepressor (NCoR) and silencing mediator of retinoid and thyroid hormone receptors (SMRT) bind to NRs in the absence of ligand and actively repress transcription of target genes by recruiting large repressor complexes including histone deacetylases (HDACs) and the nucleosome remodeling complex NURD [15,16].

Here, we identified CAC1 as a novel corepressor of RARα. In this study, we demonstrated that CAC1 binds directly to RARα through the conserved CoRNR motif and suppresses RARα-mediated transcriptional activity. CAC1 also interacts with HDAC and cooperates with it in the suppression of RAR activity. The suppressive function of CAC1 is associated with its inhibitory role in RA-dependent neuronal differentiation. These results suggest that CAC1 is a novel RARα corepressor that cooperates with HDAC and negatively modulates RA-induced cellular differentiation.

2. Materials and methods

2.1. Plasmids and cloning

All cDNAs were constructed according to standard methods and verified by sequencing. Full-length CAC1 cDNA and CAC1 cDNA

Abbreviations: CAC1, CDK2-associated cullin; RARα, retinoic acid receptor alpha; HDAC, histone deacetylase; AF-1, transcriptional activation function 1; LBD, ligand binding domain; DBD, DNA-binding domain; GST, glutathione *S*-transferase; β-gal, β-galactosidase; GFP, green fluorescent protein.

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deletion mutants were amplified by PCR and subcloned into suitable vectors [Myc-tagged pcDNA3, pEGFP-C3 (BD Biosciences), and pGEX4T-1 (GE Life Sciences)].

2.2. Cell culture and differentiation

H1299 cells were grown and maintained in RPMI-1640 medium, and HeLa cells were grown and maintained in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and an antibiotic–antimycotic mix (all from Gibco-BRL). All cells were grown and maintained in a 5% CO_2 atmosphere at 37 °C. For treatment with all-*trans* retinoid acid (AtRA) (Sigma), FBS was pretreated with charcoal. P19 cell differentiation was induced as described previously [17]. P19 cells were aggregated in bacterial Petri plates at a density of 10^5 cells/ml and treated with 1 mM RA for 96 h, with subculturing in fresh medium after treatment for 48 h. On day 4, the aggregates were transferred to cell culture plates and RA was eliminated from the medium. Cells were then seeded on 10 cm-diameter plates at a density of 3×10^5 cells/plate. After 12 h, the medium was replaced with medium containing 0.5% FBS, and the cells were allowed to differentiate for an additional 6 days.

2.3. Glutathione S-transferase pull-down assays

The experimental procedures were as described previously [18]. A GST fusion of CAC1 was expressed in *Escherichia coli* and purified on glutathione-Sepharose beads (GE Life Sciences) by standard methods. RARα protein was translated in vitro using the TNT rabbit reticulocyte system (Promega). Then 2 μg of GST or GST-CAC1 was mixed with 10 μl of in vitro-translated RARα protein.

2.4. Immunofluorescence microscopy

Twenty-four hours after GFP-RARα was co-transfected with Myc-tagged CAC1 on coverslips, H1299 cells were washed with phosphate-buffered saline (PBS), fixed through incubation with 4% paraformaldehyde in PBS for 1 min, and permeabilized via incubation in PBS containing 0.5% Triton X-100 for 4 min at 4 °C. After washing, the cells were incubated with anti-Myc antibody (Millipore) in blocking buffer (PBS containing 2% bovine serum albumin) for 1 h and then incubated with rhodamine-conjugated anti-mouse antibody (Millipore). After washing and mounting with 50 μl of Vecta Shield (Vector Laboratories), cells were visualized using an immunofluorescence microscope (Carl Zeiss).

2.5. Transient transfection and luciferase reporter assay

HeLa cells were seeded in a 6-well culture plate and transiently transfected with a RARE-luciferase reporter gene, and as an internal control, an SV40-driven-galactosidase (β-gal) expression vector. Depending on the experimental conditions, the RARα or CAC1 expression vector was co-transfected using Lipofectamine Plus reagent (Invitrogen). Luciferase activity was measured using an analytical luminescence luminometer according to the manufacturer's instructions (Promega), after the addition of 20 μl of luciferin to 20 μl of cell lysate. β-Gal activity was determined in 96-well plates by measuring absorbances at 405 nm using a microplate reader. Luciferase activity was normalized to β-gal activity.

2.6. Western Blotting (WB) and immunoprecipitation (IP)

IP and WB were performed as previously reported [19]. For WB, cells were lysed in lysis buffer supplemented with a protease

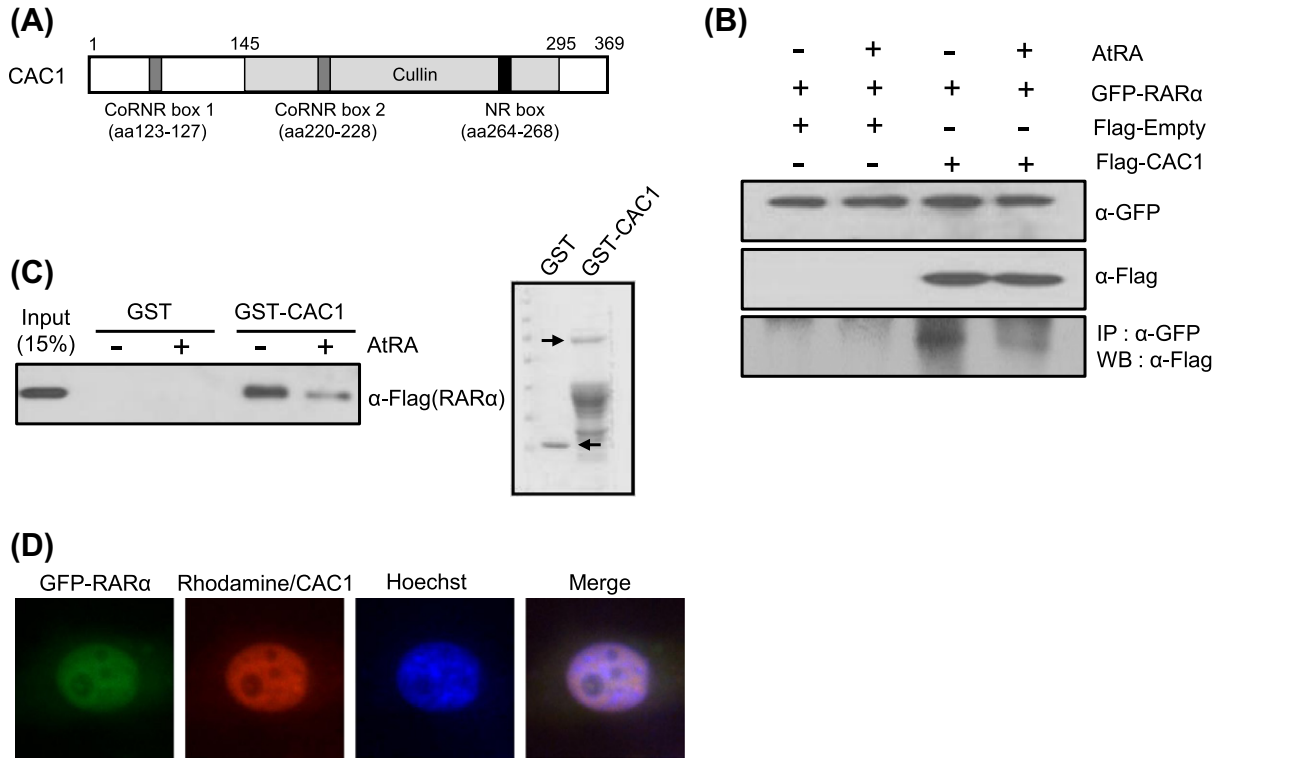

Fig. 1. CAC1 interacts with RARα. (A) Structural features of CAC1. CoRNR box, corepressor binding motif (L/IXXI/VI); NR box, coactivator binding motif (LXXLL); Cullin, cullin homology domain (amino acids 145–295). (B) IP assay. H1299 cells were co-transfected with GFP-fused RARα and Flag-empty or Flag-tagged CAC1 in the absence or presence of 2 μM AtRA. Lysates were subjected to IP using anti-GFP and bound protein was visualized by WB with anti-Flag antibody. (C) GST-pull down assay. In vitro translated Flag-tagged RARα was incubated with GST or GST-CAC1 in the absence or presence of 20 μM AtRA. The bound proteins were visualized by subsequent WB using anti-Flag antibody. (D) Immunofluorescence microscopy. H1299 cells were transfected with GFP-RARα and Myc-CAC1. The cellular location of CAC1 was visualized using rhodamine-conjugated anti-mouse IgG. The nuclei were visualized by Hoechst staining.

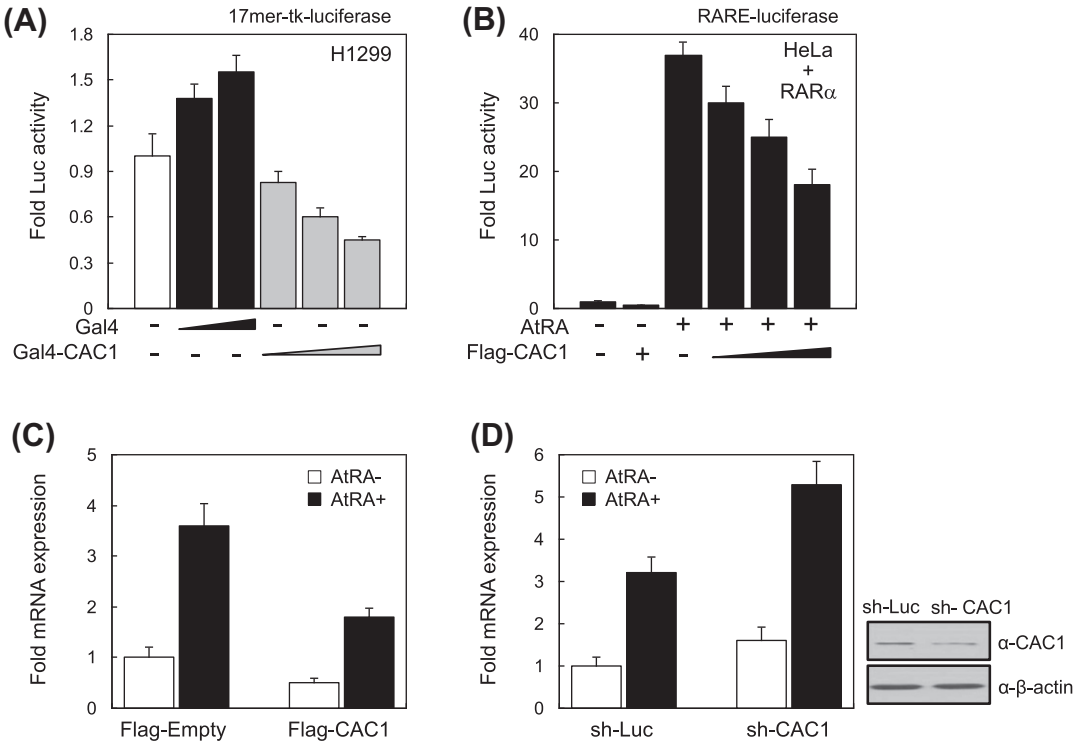

Fig. 2. CAC1 is a corepressor of RARα. (A) Autonomous transcriptional repression by CAC1. Gal4 DBD-empty (1 or 1.5 μg) or Gal4 DBD-CAC1 (0.5, 1, or 1.5 μg) was introduced into H1299 cells with a Gal4-responsive 17mer-tk-luciferase reporter (0.5 μg). (B) Effect of CAC1 overexpression on RARα-driven Luc activity. HeLa cells were transiently transfected with a RARE-Luc reporter and increasing amounts of Flag-CAC1 (0, 0.5, 1, or 1.5 μg) together with Flag-RARα in the absence or presence of 2 μM AtRA. Extracts from the transfected cells were analyzed by Luc activity assays. Relative Luc activity is shown as the mean ± SD of three independent experiments after normalization to β-gal activity. (C, D) Effect of CAC1 overexpression (C) or depletion (D) on expression of the endogenous RARα target gene *RARβ2*. HeLa cells were transfected with Flag-empty, Flag-CAC1, sh-Luc, or sh-CAC1 (2 μg) in the absence or presence of 2 μM AtRA. Total RNA was extracted, reverse-transcribed, and analyzed by real-time RT-PCR. Fold-increases in *RARβ2* mRNA expression were normalized to *GAPDH* mRNA expression levels. Data represent the mean ± SD of three independent experiments. Knockdown of CAC1 was monitored by WB and β-actin was used as an internal control.

inhibitor cocktail (Roche). Proteins were separated by electrophoresis on 8–12% SDS–polyacrylamide gels, transferred to nitrocellulose, and incubated with primary antibodies. The commercially available primary antibodies used were rabbit polyclonal antibodies specific for RARα (sc-551), GFP (sc-8334), and β-actin (sc-47778) (all Santa Cruz Biotechnology) and a mouse monoclonal anti-Flag M2 antibody (F3165; Sigma). The blots were next incubated with peroxidase-conjugated mouse or rabbit IgG secondary antibodies (Santa Cruz Biotechnology). Protein bands were detected with the ECL system (GE Life Sciences). For IP assays, cells treated under various conditions were washed with ice-cold PBS and lysed in RIPA buffer supplemented with a protease inhibitor cocktail (Roche). The lysates were incubated overnight at 4 °C with a 1:200 dilution of the indicated antibody. After incubation at 4 °C for 2 h with A/G-agarose beads (Santa Cruz Biotechnology), the beads were washed three times with RIPA buffer. The immune complexes were released from the beads by boiling and were analyzed by WB using the indicated antibodies.

2.7. Real-time RT-PCR

Conventional and real-time RT-PCR were performed as described previously [20]. Total RNA was extracted from HeLa cells using TRIzol reagent (Gibco-BRL) according to the manufacturer's instructions. RNA (5 μg) was reverse-transcribed using Superscript II reverse transcriptase (RT; Invitrogen) and random oligo (dT) primers (Invitrogen). cDNA was amplified using the following primer pairs: *RARβ2* (113-bp), forward 5′-TTGTGTTCACCTTTGCCAAC-3′ and reverse 5′-CGGTTCCTCAAGGTCCTGG-3′; *GAPDH* (120-bp), forward 5′-CTGCACCACCAACTGCTTAGC-3′ and reverse 5′-GGGCCATCCACAGTCTTCTGG-3′; and *nestin* (296-bp), forward 5′-CAGATGTGGGAGCTCAATCG-3′ and reverse 5′-GCCTCCTCGATG GTCCGCTC-3′. Real-time PCR was performed using iQ™ SYBR Green Supermix and an iCycler CFX96 Real-Time PCR detection system (Bio-Rad). Gene expression levels were normalized using *GAPDH* as an internal standard. Fold expression was defined as fold increase relative to the control.

2.8. RNA interference

The sequences of the shRNA duplex for CAC1 were as follows: sense 5′-GATCCATGTGTATGCCAGCAGCACTTCAAGAGAGTGCTGCTG GCATACACATTTTTTTGGAAA-3′ and antisense 5′-AGCTTTTCCA AAAAAATGTGTATGCCAGCAGCACTC TCTTGAAGTGCTGCTGGCAT-ACACATG-3′. The transfection of shRNA was performed with Lipofectamine 2000 (Gibco-BRL) according to the manufacturer's instructions. CAC1 knockdown was verified by WB using an anti-CAC1 antibody (Peptron).

3. Results and discussion

3.1. CAC1 interacts with RARα

We recently identified CAC1 through the yeast-two hybrid screening of a HeLa cDNA library using SIRT1 as bait [Kim et al., unpublished data]. CAC1 has been identified as a CDK2-associated cullin [21], but its biological function is largely uncharacterized. A comprehensive bioinformatic analysis revealed that CAC1 harbors two conserved motifs: LMNVI and LQSIVPLFI called CoRNR box (L/IXXI/VI), and LMPLL called NR box (LXXLL) (Fig. 1A). These

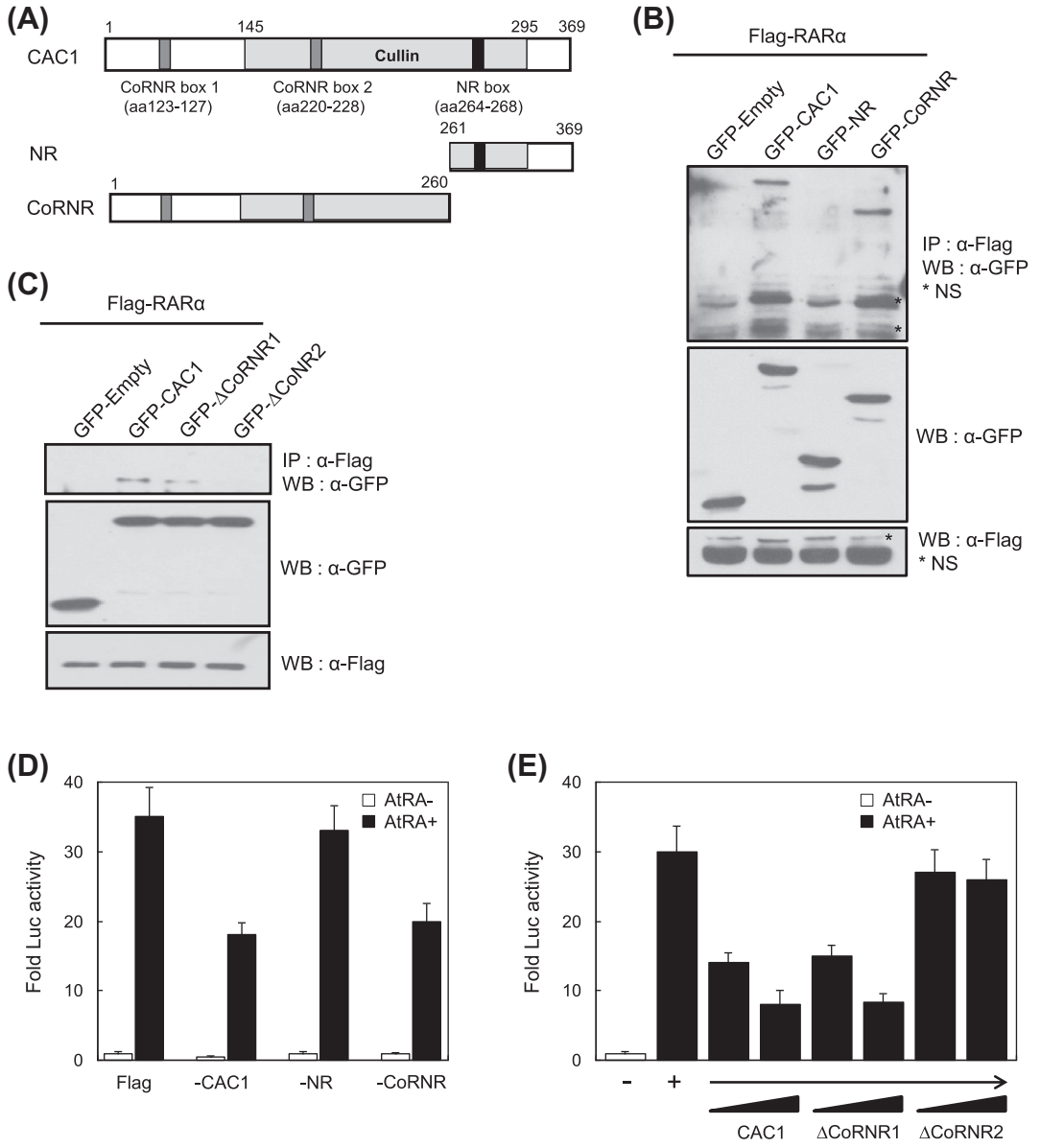

Fig. 3. CAC1 represses RARα through direct interaction. (A) Schematic representations of full-length CAC1, including conserved motifs, and two deletion mutants. (B, C) IP assays. H1299 cells were transfected with Flag-RARα and wild-type GFP-CAC1 or two GFP-CAC1 constructs carrying CoRNRs or NR box fragments (B) or two mutants with CoRNR motifs deletions (C). Interactions were monitored by IP with anti-Flag antibody and by WB using anti-GFP antibody. NS (∗) nonspecific protein band. (D, E) Effect of the CAC1 region on RARα repression. H1299 cells were transiently transfected with RARE-Luc reporter and increasing amounts of Flag-empty, Flag-CAC1 wild-type, Flag-CAC1 fragments containing the CoRNR or NR box sequences (0.5 or 1.0 μg) (D), or two CoRNR motif deletion mutants (0.5 or 1.0 μg) (E) in the presence of 2 μM AtRA. Data represent the mean ± SD of three independent luciferase experiments. $P < 0.01$.

motifs are responsible for interactions with NRs as a corepressor and coactivator, respectively [22,23]. Previously, we showed that SIRT1 interacts with RARα [17]. Based on this finding, we first explored whether CAC1 physically interacts with RARα. Flag-empty or Flag-tagged CAC1 was co-transfected with GFP-tagged RARα into H1299 cells treated with and without the ligand AtRA. IP with an anti-GFP antibody and subsequent WB with an anti-Flag antibody indicated that CAC1 interacts with RARα in the absence of AtRA (Fig. 1B). This ligand-independent interaction decreased in the presence of AtRA. Through GST pull-down assays, performed by GST-fused human CAC1 and in vitro-translated RARα, we confirmed the ligand-independent interaction of CAC1 with RAR in vitro (Fig. 1C). To further verify their interaction, we examined the subcellular localization of CAC1 and RARα in H1299 cells. Immunofluorescence microscopy showed that Myc-CAC1 and GFP-RARα co-localized to the nucleus (Fig. 1D). Overall, these results suggest that CAC1 physically associates with RARα in a ligand-independent manner.

3.2. CAC1 suppresses the transcriptional activity of RARα

To investigate the significance of the physical interaction between CAC1 and RARα, we examined the effect of CAC1 on the transcriptional activity of RARα. Prior to performing RARα-mediated transcription assays, we measured the autonomous transcriptional activity of CAC1 using a Gal4-driven system. H1299 cells were co-transfected with Gal4 DBD-fused CAC1 and a Gal4-responsive luciferase reporter. As shown in Fig. 2A, Gal4-CAC1 significantly reduced luciferase activity in a dose-dependent manner, suggesting that CAC1 may function as a transcriptional corepressor. Next, we

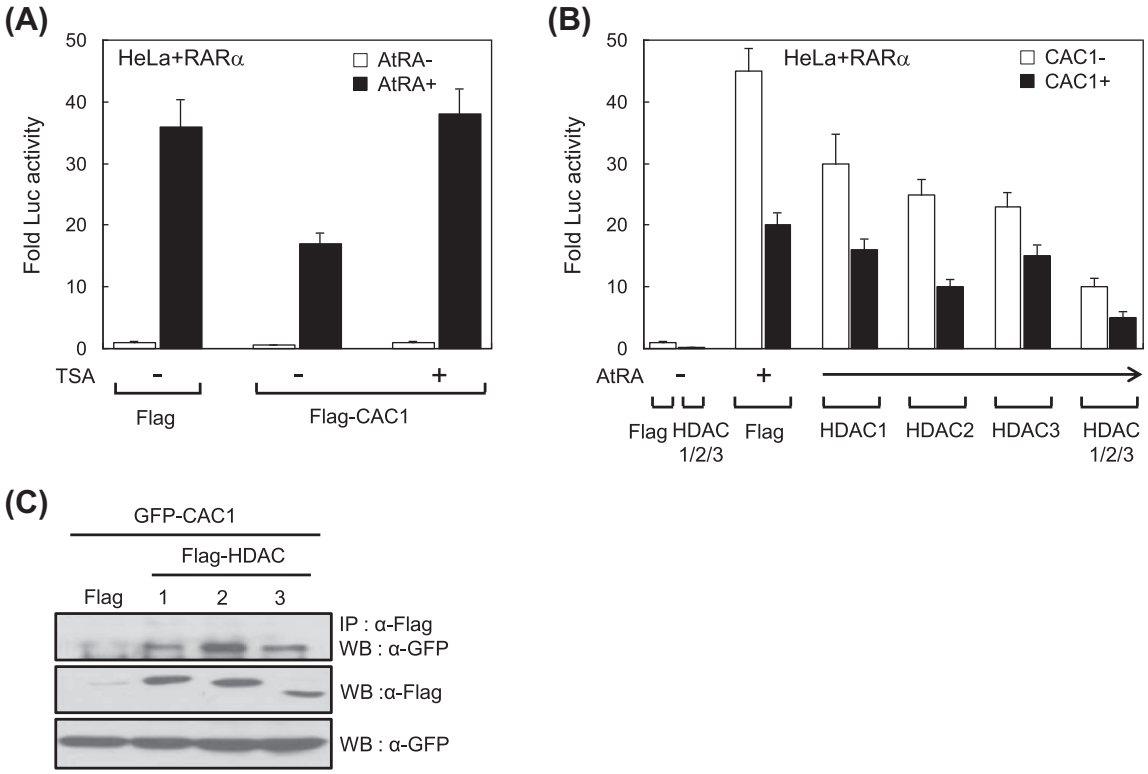

Fig. 4. CAC1 cooperates with HDAC in RARα suppression. (A) Effect of the HDAC inhibitor Trichostatin A (TSA). HeLa cells were transfected with Flag-empty or Flag-CAC1, and treated with 300 nM TSA in the presence of 2 μM AtRA. (B) Effect of HDACs on CAC1-mediated RARα inactivation. HeLa cells were co-transfected with CAC1 and HDAC1, HDAC2, or HDAC3 together with the RARE-luciferase reporter. Data represent the mean ± SD of three independent luciferase experiments. $P < 0.01$. (C) Interaction between CAC1 and HDACs. H1299 cells were co-transfected with GFP-CAC1 Flag-empty or Flag-HDACs. Interactions were detected by IP with anti-Flag antibody and subsequent WB using anti-GFP antibody.

determined whether CAC1 regulated the transcriptional activity of RARα in HeLa cells using an RA-responsive reporter. CAC1 greatly repressed RARα-mediated transcriptional activity in a dose-dependent manner (Fig. 2B). In addition, we monitored the effect of CAC1 on endogenous expression of the RARα target gene *RARβ* in HeLa cells by real-time RT-PCR. RA-induced mRNA expression of the *RARβ* gene was markedly reduced by CAC1 overexpression (Fig. 2C) and increased by depletion of CAC1 through shRNA treatment (Fig. 2D). These data, combined with our interaction data, suggest that CAC1 is a corepressor of RARα.

3.3. The CoRNR box of CAC1 is critical for RARα binding and repression

To determine whether a direct correlation exists between binding of CAC1 with RARα and CAC1-mediated RARα repression, we decided to map the CAC1 region responsible for the interaction with RARα by constructing truncated mutants of CAC1 (Fig. 3A). Flag-RARα was co-transfected with GFP or GFP-CAC1 truncated mutants into H1299 cells, and IP was performed with an anti-Flag antibody and WB using an anti-GFP antibody. As shown in Fig. 3B, the CAC1 fragment covering CoRNR1 and CoRNR2 (amino acids 1–260) was sufficient, but the fragment including the NR box (amino acids 261–369) was not required for RARα binding. Furthermore, we generated CAC1 mutants lacking CoRNR1 or CoRNR2 to determine which CoRNR box is critical for the interaction. As shown in Fig. 3C, CAC1 lacking CoRNR1 retained binding to RARα, whereas the CoRNR2 deletion mutant was defective in RARα binding, suggesting that the CoRNR2 box of CAC1 is critical for the interaction. To confirm the role of the CoRNR box in RARα repression, transcription assays were performed using an RARE-luciferase reporter and CAC1 mutants. Consistent with the above data, the CoRNR fragment of CAC1, but not the NR fragment, was sufficient for RARα repression (Fig. 3D). In addition, deletion of CoRNR2, but not CoRNR1 and the NR box, caused a defect in RARα inactivation (Fig. 3E). Overall, our data suggest that binding of CAC1 to RARα through CoRNR box 2 is required for repressing the transcriptional activity of RARα.

3.4. CAC1 cooperates with HDAC for RARα inactivation

NR corepressors are widely believed to utilize histone HDAC for transcriptional repression [24–26]. To explore the molecular mechanism underlying CAC1-mediated inactivation of RARα, we first assessed whether the HDAC inhibitor Trichostatin A (TSA) influences CAC1 activity. Our luciferase assays revealed that CAC1-mediated RARα repression was released by TSA treatment (Fig. 4A). Furthermore, CAC1 cooperates with HDACs, especially HDAC2, in RARα repression (Fig. 4B). To confirm this cooperation, we examined whether CAC1 interacts with HDACs. IP assays confirmed the interaction between CAC1 and HDACs, and that CAC1 interacts with HDAC2 with particularly high affinity (Fig. 4C). Taken together, these data suggest that CAC1 interacts and cooperates with HDACs in the suppression of RARα activity.

3.5. CAC1 depletion sensitizes P19 cells to RA-induced neuronal differentiation

P19 is a murine embryonic carcinoma cell line that efficiently differentiates into neurons and glial cells upon RA treatment [27,28]. To investigate the possibility that CAC1 is involved in RA-dependent cellular differentiation in P19 cells, we generated cell lines with stable depletion of CAC1 using sh-CAC1 (Fig. S1A).

Upon RA treatment, the P19 cells acquired neurite-like structures, indicative of neuronal differentiation. Note that CAC1 knockdown significantly increased the formation of neurite-like structures (Fig. S1B). To corroborate this morphological change, we measured the mRNA expression of *nestin*, a known neuronal marker [29], by RT-PCR. As expected, RA-induced expression of *nestin* was strongly augmented in CAC1-depleted cells (Fig. S1C). These data support the conclusion that CAC1 negatively regulates RA-induced neuronal differentiation of P19 cells, likely through inactivation of RARα.

In summary, we identified CAC1 as a novel RARα corepressor. Our data demonstrate that CAC1 interacts with RARα and inhibits its transcriptional activity. The CoRNR box of CAC1 is required for RARα binding and inactivation. Note that CAC1 also binds to HDACs, binding to HDAC2 with particularly high affinity, and cooperates with them in the suppression of RARα activity. Finally, we observed that depletion of CAC1 greatly sensitizes P19 cells to RA-induced neuronal differentiation by increasing RARα activation. From our data, we speculate that CAC1, as a novel RARα corepressor, modulates RA signaling pathways, including those that regulate cellular differentiation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.08.142.

References

[1] P. Chambon, The molecular and genetic dissection of the retinoid signaling pathway, Recent Prog. Horm. Res. 5 (1995) 317–332.
[2] D.J. Mangelsdorf, C. Thummel, M. Beato, P. Herrlich, G. Schutz, K. Umesono, B. Blumberg, P. Kastner, M. Mark, P. Chambon, R.M. Evans, The nuclear receptor superfamily: the second decade, Cell 83 (1995) 835–839.
[3] M.J. Tsai, B.W. O'Malley, Molecular mechanisms of action of steroid/thyroid receptor superfamily members, Annu. Rev. Biochem. 63 (1994) 451–486.
[4] M. Resche-Rigon, H. Gronemeyer, Therapeutic potential of selective modulators of modulators of nuclear receptor action, Curr. Opin. Chem. Biol. 2 (1998) 501–507.
[5] P. Chambon, A decade of molecular biology of retinoic acid receptors, FASEB J. 10 (1996) 940–954.
[6] N.J. McKenna, B.W. O'Malley, Minireview: nuclear receptor coactivators, Endocrinology 143 (2002) 2461–2465.
[7] N.J. McKenna, B.W. O'Malley, Combinatorial control of gene expression by nuclear receptors and coregulators, Cell 108 (2002) 465–474.
[8] N.J. McKenna, R.B. Lanz, B.W. O'Malley, Nuclear receptor coregulators: cellular and molecular biology, Endocr. Rev. 20 (1999) 321–344.
[9] J. Torchia, D.W. Rose, J.Y. Inostroza Kamei, S. Westin, C.K. Glass, M.G. Rosenfeld, The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function, Nature 387 (1997) 677–684.
[10] J.J. Voegel, M.J. Heine, M. Tini, V. Vivat, P. Chambon, H. Gronemeyer, The coactivator TIF2 contains three nuclear receptorbinding motifs and mediates transactivation through CBP binding-dependent and -independent pathways, EMBO J. 17 (1998) 507–519.
[11] H. Chen, R.J. Lin, R.L. Schiltz, D. Chakravarti, A. Nash, L. Nagy, M.L. Privalsky, Y. Nakatani, R.M. Evans, Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300, Cell 90 (1997) 569–580.
[12] J.C. Blanco, S. Minucci, J. Lu, X.J. Yang, K.K. Walker, H. Chen, R.M. Evans, Y. Nakatani, K. Ozato, The histone acetylase PCAF is a nuclear receptor coactivator, Genes Dev. 12 (1998) 1638–1651.
[13] D. Chen, S.M. Huang, M.R. Stallcup, Synergistic, p160 coactivator-dependent enhancement of estrogen receptor function by CARM1 and p300, J. Biol. Chem. 275 (2000) 40810–40816.
[14] H.G. Wang, Z.Q. Huang, L. Xia, Q. Feng, H. Erdjument-Bromage, B.D. Strahl, S.D. Briggs, C.D. Allis, J. Wong, P. Tempst, Methylation of histone H4 at arginine 3 facilitating transcriptional activation by nuclear hormone receptor, Science 293 (2001) 853–857.
[15] A.J. Horlein, A.M. Naar, T. Heinzel, J. Torchia, B. Gloss, R. Kurokawa, A. Ryan, Y. Kamei, M. Soderstrom, C.K. Glass, M.G. Rosenfeld, Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor, Nature 377 (1995) 397–404.
[16] J.D. Chen, R.M. Evans, A transcriptional co-repressor that interacts with nuclear hormone receptors, Nature 377 (1995) 454–457.
[17] M.R. Kang, S.W. Lee, E. Um, H.T. Kang, E.S. Hwang, E.J. Kim, S.J. Um, Reciprocal roles of SIRT1 and SKIP in the regulation of RAR activity: implication in the retinoic acid-induced neuronal differentiation of P19 cells, Nucleic Acids Res. 38 (2010) 822–831.
[18] E.J. Kim, J.S. Park, S.J. Um, Identification and characterization of HIPK2 interacting with p73 and modulating functions of the p53 family in vivo, J. Biol. Chem. 277 (2002) 32020–32028.
[19] Y.S. Cho, E.J. Kim, U.H. Park, H.S. Sin, S.J. Um, Additional sex comb-like 1 (ASXL1), in cooperation with SRC-1, acts as a ligand-dependent coactivator for retinoic acid receptor, J. Biol. Chem. 281 (2006) 17588–17598.
[20] H.S. Youn, E.J. Park, S.J. Kim, Um, PTOV1 antagonizes MED25 in RAR transcriptional activation, Biochem. Biophys. Res. Commun. 404 (2011) 239–244.
[21] Y. Kong, K. Nan, Y. Yin, Identification and characterization of CAC1 as a novel CDK2-associated cullin, Cell Cycle 8 (2009) 3544–3553.
[22] X. Hu, M.A. Lazar, The CoRNR motif controls the recruitment of corepressors by nuclear hormone receptors, Nature 402 (1999) 93–96.
[23] D.M. Heery, E. Kalkhoven, S. Hoare, M.G. Parker, A signature motif in transcriptional co-activators mediates binding to nuclear receptors, Nature 387 (1997) 733–736.
[24] L. Xu, C.K. Glass, M.G. Rosenfeld, Coactivator and corepressor complexes in nuclear receptor function, Curr. Opin. Genet. Dev. 9 (2) (1999) 140–147.
[25] A.D. Weston, B. Blumberg, T.M. Underhill, Active repression by unliganded retinoid receptors in development: less is sometimes more, J. Cell Biol. 161 (2) (2003) 223–228.
[26] P.L. Jones, Y.B. Shi, N-CoR-HDAC corepressor complexes: roles in transcriptional regulation by nuclear hormone receptors, Curr. Top. Microbiol. Immunol. 274 (2003) 237–268.
[27] M.A. van der Heyden, L.H. Defize, Twenty one years of P19 cells: what an embryonal carcinoma cell line taught us about cardiomyocyte differentiation, Cardiovasc. Res. 58 (2003) 292–302.
[28] E.M. Jones-Villeneuve, M.W. McBurney, K.A. Rogers, V.I. Kalnins, Retinoic acid induces embryonal carcinoma cells to differentiate into neurons and glial cells, J. Cell Biol. 94 (1982) 253–262.
[29] Y. Yan, J. Yang, W. Bian, N. Jing, Mouse nestin protein localizes in growth cones of P19 neurons and cerebellar granule cells, Neurosci. Lett. 302 (2001) 89–92.