Trans-repression of β-Catenin Activity by Nuclear Receptors*

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The signaling/oncogenic activity of β-catenin can be repressed by the activation of nuclear receptors such as the vitamin A, vitamin D, and androgen receptors. Although these receptors directly interact with β-catenin and can sequester it away from its transcription factor partner T-cell factor, it is not known if this is the mechanism of trans-repression. Using several different promoter constructs and nuclear receptors and mammalian two-hybrid and mutation analyses we now show that interaction with the co-activator, p300, underlies the trans-repression of β-catenin signaling by nuclear receptors and their ligands.

By definition, the term chemoprevention refers to the treatment of individuals with premalignant lesions, individuals who are predisposed to the development of malignant lesions, and individuals early in the process of carcinogenesis, with the intention of preventing the later stages of carcinogenesis from developing (1). Although chemopreventative agents such as vitamins A and D are often effective in this context there is no de facto reason to assume that the same treatment would not be effective in more advanced stages of carcinogenesis. Nevertheless, this is often not the case because many advanced cancers in vivo and de-differentiated carcinoma cells in vitro are refractory to the anti-tumor, growth inhibitory activities of vitamins A and D. Thus, not only do more malignant tumors and cells have defects in the expression and function of several molecules characteristic of differentiated epithelial cells; they are also less responsive to differentiating agents. An obvious question arises from this realization; what are the molecular mechanisms that underlie resistance to vitamins A and D? In some cases, resistance is because of a defect in particular nuclear receptor isoforms. However, some cells, which are not growth-inhibited by vitamin A, express normal retinoic acid receptors (RARs)/RXRs and retain the ability to up-regulate retinoid-sensitive genes (2). These data indicate that other molecules such as components of the cadherin/β-catenin-based signaling and adhesion system may also need for retinoic acid to exert its anti-proliferative effects (3, 4).

Recently, several studies show that the signaling/oncogenic activity of β-catenin is trans-repressed by the activation of nuclear receptors such as RAR, vitamin D receptor (VDR), and androgen receptors (AR) (3, 5). In addition to its role in cell-cell adhesion β-catenin acts as a co-activator for the TCF/lymphoid enhancer factor (LEF) family of transcription factors. Although it is possible that all of these receptors directly interact with β-catenin and sequester it away from TCF, it is not known if this is the mechanism of trans-repression. Nuclear receptors also trans-repress AP-1, serum response factor (SRF), and NFκB activity by a mechanism likely involving interaction with a common pool of co-activators rather than a direct interaction with transcription factors (6–8). On the other hand, in contrast to AP-1, high levels of β-catenin markedly potentiate RARE, vitamin D receptor element, and androgen receptor element reporter activity as well as the expression of retinoid-sensitive genes, a phenomenon that is not likely to be a result of co-activator competition (3, 5, 9). Using several different promoter constructs and nuclear receptors, mammalian two-hybrid analyses, and other biochemical methods we now show that interaction with the histone acetyl transferase, p300, underlies the trans-repression of β-catenin signaling by nuclear receptors. Taken together these data suggest that the failure of retinoic acid to inhibit the growth of some colon cancer cells is because of defects in trans-repression of β-catenin/TCF activity in addition to or instead of a failure to trans-activate retinoid-sensitive genes.

EXPERIMENTAL PROCEDURES

Plasmids—Vectors encoding either wild type or a degradation-resistant mutant of β-catenin (S37A) have been described previously (10). Wild type and dominant negative c-Fos and c-Jun vectors were provided by Dr. Charles Vinson (11). Wild type E1A and its mutant forms are described in Hecht et al. (12). TCF reporter (TOPFLASH) and mutated TCF reporter (FOPFLASH) were provided by Marc van de Wetering (13). The mammalian two-hybrid system was used to assay protein-protein interactions in transfected cells. To construct the VP16/β-catenin fusion protein expression vector, full-length β-catenin from pDNA-β-catenin-FLAG was cloned into pCMX-VP16 vector using 5'-GAGCTCCTGATCACCAGCGGTAGTACC-T3' and 5'-ACTAGTGGAATCCTTTAGGCCTGTAGCGGGA-A3' as forward and reverse primers with BamHI sites at both ends. The PCR-amplified β-catenin fragment was cloned into pCMX-VP16 at the BamHI site. The orientation and reading frame of the resultant vector was confirmed by sequencing. To generate Gal4-β-catenin and Gal4/ARM-β-catenin expression vectors, we used PCR to amplify the full-length and armadillo repeat regions of β-catenin using 5'-GAGCTCCTGATCACCAGCGGTAGTACC-T3' and 5'-ACTAGTGGAATCCTTTAGGCCTGTAGCGGGA-A3' and 5'-ATCCCGGAAAATATCAAGATGATGCGGAA-A3' and 5'-ATGGATCCTTGTGGCTTG-T3' as forward and reverse primers with BamHI sites at both ends. The PCR-amplified β-catenin fragment was cloned into pCMX-VP16 at the BamHI site. The orientation and reading frame of the resultant vector was confirmed by sequencing. To generate Gal4-β-catenin and Gal4/ARM-β-catenin expression vectors, we used PCR to amplify the full-length and armadillo repeat regions of β-catenin using 5'-GAGCTCCTGATCACCAGCGGTAGTACC-T3' and 5'-ACTAGTGGAATCCTTTAGGCCTGTAGCGGGA-A3' and 5'-ATCCCGGAAAATATCAAGATGATGCGGAA-A3' and 5'-ATGGATCCTTGTGGCTTG-T3' as forward and reverse primers, respectively. Both full-length β-catenin with the BamHI site and the ARM region of β-catenin with Smal and BamHI sites were cloned downstream of Gal4 DNA binding sequences of pCMX-Gal4 vector (both pCMX VP16 and GAL4 vectors were kindly provided by Dr. Ronald Evans). To clone...
full-length RARs into pcMX-Gal4 and pcMX-VP16, a standard PCR procedure was followed using 5'-ACTCTGCTGATCCATCPTCGACT-3' as forward and 5'-GCTTATAATAAGATCTCGATCCG-3' as reverse primers. All the newly constructed vectors were sequenced using appropriate primers to confirm the reading frame. In addition, all vectors received from collaborators were sequenced before use in the experiments.

Cell Culture, Treatments, and Antibodies—SKBR3 cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen) plus 10% fetal bovine serum (FBS), SW480 cells were grown in DMEM with 5% FBS, and human embryonic kidney 293 cells were grown in DMEM with 10% horse serum. To study the effects of retinoic acid (RA) cells were treated with 1 μM 9-cis-RA or ethanol for 48 h. Several experiments were repeated using all-trans-retinoic acid with similar results. An anti-β-catenin monoclonal antibody (Transduction Labs) (1:1000), RARα (1:500) and RARβ (1:500) antibodies (Oncogene Research Products) were used for Western blotting. All antibodies were diluted in 5% skim milk in phosphate-buffered saline.

Transient Transfection and Reporter Gene Assays—Cells were seeded in 12-well plates at 1 × 10^5 cells/well and transiently transfected using FuGENE 6 (Roche Applied Science). For luciferase assays cells were transfected with 50 ng of the expression vectors along with 100 ng of TCF reporter or control and mutated TCF reporter, and 10 ng of pCMV-Renilla luciferase (Promega). RA treatment was initiated 24 h post-transfection. Luciferase activity was monitored using the DUAL-luciferase assay system (Promega). The experimental reporter activity was controlled for transfection efficiency by comparison with the constitutively expressed Renilla luciferase. For mammalian two-hybrid analyses, Gal4-RARα and VP16-β-catenin were transiently transfected along with Renilla luciferase as an internal control.

Western Blot Assays—The Bio-Rad DC protein assay kit was used to measure protein content in the samples. 25 μg of protein were separated on a 4–10% reducing polyacrylamide mini-gel (Novex), transferred onto nitrocellulose (Protran), and blocked overnight in 5% skim milk, washed two times in phosphate-buffered saline, and blocked in 5% milk before re-probing.

RESULTS

Differential Response of β-Catenin/TCF and Retinoic Acid-responsive Promoters to Retinoic Acid in Colon Cancer Cells—In earlier studies we showed that β-catenin/TCF signalizing activity could be inhibited by RA in Caco2 and HT29 colon cancer cells and in β-catenin-transfected SKBR3 and MCF-7 breast cancer cells (3). All these cell lines respond to retinoic acid by decreased proliferation and increased activation of retinoic acid-sensitive genes and RARE reporters (14, 15). However, not all colon cancer cells respond to retinoic acid by decreasing growth even though RA-treatment continues to activate RARE reporters in these cells (2). We wanted to investigate whether the failure of these cells to respond to RA by decreased proliferation was associated with loss of trans-repression of β-catenin signaling. We chose three colon cancer cell lines that had previously been shown to be only modestly responsive (SW480) or unresponsive (SW620, HCT116) to the growth inhibitory effects of RA (2). Like Caco-2 and HT29 cells, SW480 and SW620 cells make large amounts of β-catenin protein as a result of a mutation in the adenomatous polyposis coli gene (APC); HCT116 cells have normal APC but an activating mutation of β-catenin. We first confirmed that all these cells did activate a RARE reporter after treatment with RA (Fig. 1A). SW480 and Caco-2 cells were somewhat more responsive than SW620 and HCT116 cells even though all four cell lines express similar levels of RARα and RARβ (Fig. 1A, A and B, and see Siciński and Weinstein (16)). β-Catenin signaling (TCF reporter activity) was also unaffected by RA treatment in HCT116 and SW620 cells; however, a small but consistent response was observed in SW480 cells (Fig. 1C). These data indicate that the reduced ability of these cells to stop cell growth in response to RA is associated with the failure of RA to trans-repress β-catenin signaling.

The Ability of RAR/RA to Trans-repress β-Catenin Signaling Is Not Associated with Changes in β-Catenin or TCF4 Protein Levels—We showed earlier that trans-repression of β-catenin signaling by RA does not depend upon changes in the levels of β-catenin protein or on the phosphorylation status of the N-terminal serine residues important for targeting for ubiquitination (4). Although Western analysis did not reveal any effect of RA on TCF4 levels (not shown), it is possible that RA might affect the activity or localization of TCF4 and indirectly regulate β-catenin signaling. In addition, dominant negative TCF4 stimulates the ability of β-catenin to potentiate the activity of RARE reporters (Fig. 1D) and vitamin D reporters (5). In contrast, overexpression of TCF4 does not affect the ability of RA or RA to trans-repress β-catenin signaling (Fig. 1E). These data show that in SW480 cells, the ability of RAR/RA to trans-repress β-catenin signaling is not associated with changes in β-catenin or TCF4 protein levels.

The Promoters of the β-Catenin-regulated Genes Cyclin D1 and siamois Are Also Regulated by RA in an RAR-dependent Manner—We next investigated the effects of RA and retinoid receptors on the activity of the β-catenin-sensitive cyclin D1 and siamois gene promoters. In a previous study we showed that RA-regulation of a cyclin D1 promoter fragment (−163 bp) was mediated through its TCF site in SW480 cells (4). In the present study we used a much larger cyclin D1 promoter reporter (−1745 bp). We transfected SW480 cells with RARα and then treated them with RA. The expression of RARα alone significantly reduced the activity of the cyclin D1 and siamois promoters (Fig. 2) but did not affect the activity of cytomegalovirus-Renilla luciferase (not shown). It is interesting to note in Fig. 2 that the exogenous expression of RARα could decrease β-catenin signaling even in the absence of added RA. This is probably because of the small amount of endogenous RA present in serum, which is difficult to remove even after charcoal stripping. However, both promoters exhibited a dramatic increase in sensitivity to RA after transfection with RARαs. In the presence of exogenous RARs, cyclin D1 reporter activity was reduced to background levels by RA treatment, and the siamois promoter only became retinoid-sensitive after transfection with RARα. As was the case with the cyclin D1 promoter, the ability of the siamois reporter to be trans-repressed by RAR/RA depended on the presence of its TCF sites (Fig 2D). These data confirm that the trans-repressive effects of RA on β-catenin signaling are dependent upon RAR and are mediated through TCF binding elements.

The Ability of RAR and Other Nuclear Steroid Receptors to Trans-repress β-Catenin Signaling Depends on Their Co-activator Interacting Regions—Ligand-activated vitamin D and androgen receptors can also trans-repress β-catenin signaling (5, 17). Like RAR these receptors directly interact with β-catenin and can sequester it away from TCF, but it is not known if this is the mechanism of trans-repression. Nuclear receptors also trans-repress AP-1, SRF, and NFκB activity by a mechanism likely involving competition for a common pool of co-activators rather than or as well as a direct interaction with Fos or Jun (18, 19). In view of these observations we examined whether nuclear receptor trans-repression of β-catenin signaling involves structures that are required for co-activator recruitment. In most nuclear receptors, full transcriptional activity depends upon regions at their N- and C-terminal domains. Deletion of either domain has a profound effect on the ability of the RAR, VDR, and AR to activate target genes (20–22). We transfected cells with RARα,
RARαΔA/B, RARαΔF-2, and RARΔ403, a naturally occurring oncogenic mutant missing 60 amino acids at the C terminus (Fig. 3A). Unlike RARα, expression of RARα, RARαΔA/B, RARαΔF-2, and RARΔ403 did not reduce β-catenin signaling measured by TCF reporter activity (Fig. 3B). In addition none of these RAR mutants supported the trans-repressive effects of RA; rather, when compared with RAR, all mutants hyperactivated β-catenin signaling (Fig. 3B). Similar results were obtained using the β-catenin-responsive cyclin D1 and siamosi reporters (Fig. 2A and C). These data indicate that the ability of RARα to inhibit β-catenin signaling is associated with trans-activation in general and with the regions that recruit co-activators such as members of the steroid receptor coactivator (SRC) family and p300 (23). In addition, this explanation is also consistent with hyper-activation of β-catenin signaling by mutants of RARα. It is known that the all RARα mutants used in this study are able to hetero-dimerize with RXR, which means when RARα mutants are overexpressed they can sequester

**Fig. 1. Differential TCF reporter and RARE activity in colorectal cells.** A, cells were transiently transfected with RARE-Luc reporter construct (100 ng) along with Renilla luciferase (10 ng) as an internal control. Luciferase activity was measured 24 h after RA treatment. In this and all subsequent reporter assays values are plotted as relative light units. B, Western blot of RARα and RARβ in the indicated cells. Cells were lysed in Nonidet P-40 lysis buffer, and 40 μg of protein was subjected to SDS-PAGE (4–12% denaturing gradient gels) and transferred to nitrocellulose membranes. RARα and RARβ were detected using ECL. C, cells were transiently transfected with TCF reporter or a mutated TCF reporter (10 ng/well) and Renilla luciferase (10 ng). Luciferase activity was measured 24 h after RAR treatment. D and E, effects of TCF on RARE activity in SW480 and SKBR-3 cells. D, SKBR-3 cells were transfected with β-catenin, TCF4, and dominant negative TCF4 (dnTCF4) along with RARE-luc reporter. E, SW480 cells were transiently transfected with TCF reporter, wild type TCF4, and RARα plasmids along with Renilla luciferase as an internal control. TCF reporter activity was measured after 24 h of RA treatment. Wt, wild type. dn, dominant negative.
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In these experiments the full-length receptor trans-repressed β-catenin signaling; however, the deletion of AF2 from VDR and A/B from AR completely impaired the trans-repression ability of these receptors. Taken together these data strongly indicate that the ability of nuclear receptors to bind co-activators and/or co-repressors is necessary for their effects on β-catenin signaling.

**RA-mediated Trans-repression of the Cyclin D1 Promoter Is Mediated via Changes in β-Catenin/TCF, Not AP-1, in Colon Cancer Cells**—We showed recently that the trans-repressive effects of RA on a short fragment of the cyclin D1 promoter (−163-bp CD1) expressed in SW480 colon cancer cells required the presence of a TCF binding element (4). However, RA trans-repression of the same promoter expressed in SKBR3 breast cancer cells was independent of the TCF site. This promoter fragment contains adjacent AP-1 and TCF sites, and we reasoned that RA-mediated trans-repression of its activity was mediated by β-catenin/TCF in colon cancer cells, which have high levels of β-catenin signaling activity, and by the AP-1 site in SKBR3 cells, which have high AP-1 activity (4). Consistent with this, the basal activity of −163-bp CD1 was also markedly inhibited by mutation of the TCF site in SW480 cells but not in SKBR3 cells. To study this further we used another cyclin D1 promoter (−1745-bp CD1), which contains many potential regulatory elements including several AP-1 sites. The basal activity of −1745-bp CD1 is less dependent on the TCF site (Fig. 4). Expression of a dominant negative form of fos (to inhibit AP-1) actually increased the basal activity of −1745-bp CD1 but markedly reduced the basal activity of −1745-bp CD1ΔTCF. This experiment indicates that in the absence of TCF binding, the activity of the cyclin D1 promoter is regulated by AP-1. Nevertheless, like −163-bp D1, the ability of RA and RAR to trans-repress the activity of −1745-bp CD1 in SW480 cells depends on the TCF site.

**RA Trans-repression of β-Catenin Signaling Can Occur on a Heterologous Promoter and Depends on the N- and C-terminal Regulatory Domains of β-Catenin**—The preceding experiments indicate that the ability of nuclear receptors to trans-repress β-catenin signaling depends upon their N- and C-terminal co-activator recruitment domains. If interaction with a common co-activator is involved in the mechanism of trans-repression one would expect that the co-activator recruitment N- and/or C-terminal domains of β-catenin would also be necessary for trans-repression to occur. Other studies have demonstrated that β-catenin can activate heterologous promoters such as Gal-4 if it is fused to the appropriate DNA binding domain. However no studies have investigated whether the trans-repressive effects of nuclear receptor ligands can occur in this situation. To test this we made various β-catenin-Gal4 fusion constructs and expressed them in 293 cells (Fig. 5). We first confirmed that 293 cells made RARs and were responsive to RA/RAR−ΔAF-2

much of RXR in the cell and can deprive other nuclear receptors from the formation of dimers with the RXR. The impairment of nuclear receptor activation, due to a limited amount of RXR, could free up components of transcriptional machinery, which in turn might be available to augment the β-catenin signaling. Other experiments performed using the VDR and AR also showed trans-repression of β-catenin signaling (Fig. 3, C and D). In these experiments the full-length receptor trans-repressed β-catenin signaling; however, the deletion of AF2 from VDR and A/B from AR completely impaired the trans-repression ability of these receptors. Taken together these data strongly indicate that the ability of nuclear receptors to bind co-activators and/or co-repressors is necessary for their effects on β-catenin signaling.
hyperactivated β-catenin-Gal4 activation of the Gal-4 promoter and completely prevented RA-mediated trans-repression. These data show that RA/RAR effects on β-catenin signaling activity can be exerted on a heterologous promoter and confirm that neither TCF nor TCF binding elements are required for the trans-repressive effects of RA. Deletion of the N-terminal of β-catenin-Gal4 only reduced basal activity of the Gal4 promoter by approximately 50% but completely abrogated response to RA/RAR (Fig. 6A). In contrast to β-catenin-Gal4, RARΔAF-2 did not hyperactivate N-terminal-deleted β-catenin-Gal4. Deletion of the C-terminal of β-catenin-Gal4 reduced basal activity of the Gal4 promoter by approximately 80%, but this activity could be further reduced by the addition of RA/RAR (Fig. 6B). Although the degree of trans-repression was small (20–50%), it was consistently observed. In contrast to ΔN-β-catenin-Gal4, RARΔAF-2

**Fig. 3. Effects of nuclear receptor transactivation domains on β-catenin signaling in SW480 cells.** A, schematic representation of the RARα constructs. WT, wild type. B, 50 ng of the indicated RARα constructs were transfected either with or without RA (10⁻⁶ M). TCF reporter activity was measured as described above. C, control. C, SW480 cells were transiently transfected with VDR or AB domain-deleted VDR constructs along with TCF reporter. Luciferase activity was measured 24 h after vitamin D₃ treatment. D, SW480 cells were transiently transfected with AR or AB domain-deleted AR constructs along with TCF reporter. Luciferase activity was measured 24 h after treatment with the androgen receptor ligand, R1881.

**Fig. 4. Role of AP-1 in RA-mediated trans-repression of cyclin D1 reporter.** A, SW480 cells were transiently transfected with RARα, and dominant negative AP-1 (dnAP-1), and CD1-luciferase reporter activity was measured in the absence (−) and in the presence (+) of RA. B, same as in A except CD1-luciferase with its TCF site mutated was used as reporter.

These data show that RA/RAR effects on β-catenin signaling activity can be exerted on a heterologous promoter and confirm that neither TCF nor TCF binding elements are required for the trans-repressive effects of RA. Deletion of the N-terminal of β-catenin-Gal4 only reduced basal activity of the Gal4 promoter by approximately 50% but completely abrogated response to RA/RAR (Fig. 6A). In contrast to β-catenin-Gal4, RARΔAF-2 did not hyperactivate N-terminal-deleted β-catenin-Gal4. Deletion of the C-terminal of β-catenin-Gal4 reduced basal activity of the Gal4 promoter by approximately 80%, but this activity could be further reduced by the addition of RA/RAR (Fig. 6B). Although the degree of trans-repression was small (20–50%), it was consistently observed. In contrast to ΔN-β-catenin-Gal4, RARΔAF-2
FIG. 5. Mammalian two-hybrid assays for protein-protein interactions. A, to perform mammalian two-hybrid assay in 293 cells, we measured TCF and RARE reporter activity. Cells were transfected with TCF or RARE reporter plasmids along with Renilla luciferase as an internal control. Reporter activity was measured at 24 h after RA treatment. B, Western blot of RARα and RARβ in 293 cells. C, schematic representation of β-catenin and Gal4-DNA binding domain. D, to confirm the activity of various Gal4-β-catenin constructs, the assay was performed using VP16-LEF and VP16-LEF missing the β-catenin binding site. 293 cells were transfected with the indicated plasmids, and Gal4 reporter activity was measured after 24 h.
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**A**

![Graph showing Gal4 Reporter activity](image)

- 9cRA
- Gal4-βcat
- Gal4-ΔN-βcat
- RARα
- RARα-ΔAF-2

**B**

![Graph showing Gal4 Reporter activity](image)

- 9cRA
- Gal4-ΔN-βcat
- RARα
- RARα-ΔAF-2

**C**

![Graph showing TCF Reporter activity](image)

- 9cRA
- RARα
- Δ1-553-β-Cat
- Δ 575-781-β-Cat

potentiates the activity of ΔC-β-catenin-Gal4. Deletion of both the N and C termini of β-catenin-Gal4 (ARM-β-catenin Gal4) also reduced basal activity of the Gal4 promoter by ~80% compared with wild type β-catenin. Although the basal activity of ARM-β-catenin-Gal4 was similar to the ΔC-β-catenin-Gal4, it was not trans-repressed by RA/RAR and was not hyperactivated by RARΔAF-2. Taken together these data show that although both N- and C-terminal regulatory domains of β-catenin-Gal4 are required, for full trans-activation of the Gal4 promoter and for the trans-repressive effects of RA/RAR, the N-terminal region is absolutely essential.

To further characterize the N- and C-terminal domains of β-catenin we generated C-terminal (N-terminal and ARM repeats 1–9 deleted (Δ1–553-β-cat)) and N-terminal (ARM repeats 10–12 and C-terminal deleted (Δ575–781-β-cat)) β-catenin. We found that overexpression of either of these constructs could abrogate the ability of RA to inhibit β-catenin signaling (Fig. 6C). These results indicate that these regions of β-catenin mediate the repressive effects of RA on β-catenin signaling. Because important binding sites for p300 are present in both the N- and C-terminal regulatory domains of β-catenin, we next asked if RA/RAR trans-repression of β-catenin signaling involves p300 (12).

**RA/RAR Trans-repression of β-Catenin Signaling Involves p300**—The N and C termini of β-catenin interact with co-activators and with the basal transcriptional machinery (12, 25, 26). Our demonstration that the co-activator recruitment domains of both β-catenin and RAR are involved in RA trans-repression of β-catenin activity suggests that interaction with a common co-activator may occur. The histone acetyltransferase, p300, is important in the regulation of RAR and β-catenin activity (25). To test the involvement of p300 we transfected cells with the p300 inhibitor E1A. Because wild type E1A has effects in addition to those exerted on p300, we used a mutant that retains p300 inhibitory effects but has lost other activities (25). Inhibition of p300 by mutant E1A repressed β-catenin activity to the same extent as RA/RAR, whereas expression of p300 slightly increased basal β-catenin activity and completely prevented RA/RAR-mediated trans-repression (Fig. 7A). A mutated E1A that cannot bind to CBP/p300 but retains its other activities did not affect β-catenin activity. Expression of E1A also markedly inhibited the hyperactivation of the TCF reporter observed when RARΔAF-2 mutants were expressed instead of RAR (Fig 7B). Our earlier results indicate that both the N- and C-terminal regulatory domains of β-catenin are required to mediate RA/RAR trans-repression (Fig. 6). Consistent with this we found that expression of p300 did not potentiate the activity of the ΔNΔC forms of β-catenin but did significantly increase the activity of ΔC-β-catenin and to a lesser extent ΔN-β-catenin (Fig. 7C). Taken together, these data demonstrate that RA/RAR trans-repression of β-catenin activity is mediated through both the N- and C-terminal domains of β-catenin and is due to interaction with p300.

**Trans-repression of N-terminal-mutated Forms of β-Catenin**—In addition to its role in transactivation, the N terminus of β-catenin is important for phosphorylation and degradation (10). In many cancers, these phosphorylation sites are mutated.
which leads to the accumulation of β-catenin in the cytoplasm and the nucleus (27). To test if phosphorylation mutants can be repressed by RA we performed transient transfection assays using S37A and ΔN mutants of β-catenin. We found that the RA treatments can trans-repress S37A but not ΔN forms of β-catenin (Fig. 7D). This data indicate that the properties of activating mutations are quite different from those of β-catenin missing the N terminus and that the tumors with certain mutations may still be susceptible to trans-repression with retinoids.

**DISCUSSION**

β-Catenin, a multifunctional protein, is responsible for the transduction of Wnt-mediated signals as well as for cell-cell adhesion (28, 29). The signaling function of β-catenin is particularly important in colon cancer, in which activation of β-catenin, as a result of mutations in adenomatous polyposis coli or stabilizing mutations in the N-terminal region of β-catenin, is common (for review see Ref. 30). Activating mutations of β-catenin are also common in many other cancers (31). β-Catenin signaling promotes the G1 to S-phase transition, inhibits anoikis, and allows cells to progress into S-phase after radiation damage (32). Wnt-1 signaling also inhibits apoptosis by activation of β-catenin/TCF-mediated transcription (33), and β-catenin signaling plays an important role in the growth of colon cancer cells (34). Agents that interfere with the oncogenic activity of β-catenin but which do not affect its function in cell-cell adhesion would be candidates for cancer therapies. In this regard, nuclear receptor ligands such as retinoic acid and
vitamin D markedly repress β-catenin signaling activity in breast and colon cancer cells (3, 5). Epidemiological studies have also established a link between circulating vitamins A and D and the incidence of colon cancer (35–38). In certain cells treatment with retinoic acid or vitamin D results in increased cadherin expression (4, 5). Because membrane-associated cadherins can sequester β-catenin away from a signaling pool, it is likely that at least some of the effects of vitamins A and D on β-catenin signaling are indirectly a result of increased cadherin expression (4). However, retinoic acid can still trans-repress β-catenin signaling in cells in which cadherin function is blocked or in cells in which cadherin expression is not induced by RA (4). Because nuclear receptors can interact with β-catenin under certain circumstances we initially hypothesized that RA-mediated trans-repression of β-catenin signaling involved a direct RAR/β-catenin interaction and interference with TCF-mediated transcription (3). The present work shows that the C-and N-terminal trans-activating domains of β-catenin and not the common protein-protein interaction armadillo repeat region mediate RA/RAR trans-repression. Similarly, nuclear receptors with deletions or mutations in their activation function domains (AP-1 and/or AP-2) failed to trans-repress β-catenin signaling. Taken together these results indicate that the trans-repressive effects of RA/RAR might be a result of interaction with a co-activator common to both nuclear receptors and β-catenin. Other experiments go on to show that the ability of RA and other nuclear receptor ligands to trans-repress β-catenin signaling involves the histone acetyltransferase p300. We showed that inhibition of p300 by mutant ETA1A-repressed β-catenin activity to the same extent as RA/RAR, whereas expression of p300 did not affect basal β-catenin activity or completely prevented RA/RAR-mediated transrepression.

As discussed earlier, nuclear receptors and their ligands also trans-repress AP-1, SRF, and NFκB activity by interacting with a common pool of co-activators (7, 8). In the case of RA, trans-repression of AP-1, not activation of retinoid-responsive genes, is the mechanism whereby retinooids inhibit cell proliferation in cells activated AP-1 such as SKBR3 breast cancer cells (24). Consistent with this, RA trans-repression of a cyclin D1 promoter containing both AP-1 and TCF sites in SKBR3 breast cancer cells was independent of the TCF site. However, the trans-repressive effects of RA on the same cyclin D1 promoter expressed in SW480 colon cancer cells is independent of the AP-1 site and required the presence of a TCF binding element (4). Taken together with the present work these data indicate that RA-mediated trans-repression of cyclin D1 and cell proliferation is mediated by β-catenin/TCF in colon cancer cells, which have high levels of β-catenin signaling activity, and by AP-1 in SKBR3 cells, which have high AP-1 activity.

Nevertheless, some colon cancer cells and tumors are resistant to the trans-repressive effects of RA and vitamin D on β-catenin signaling. Even though these cells continue to activate RA-responsive genes and promoters, their growth is not inhibited by RA (Ref. 2 and this study). Our demonstration that p300 is a key intermediary in the trans-repressive effects of RA on β-catenin signaling may provide some insight into the mechanism of RA and vitamin D resistance in these cancers.

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