β-Catenin Affects Androgen Receptor Transcriptional Activity and Ligand Specificity

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Abstract

β-Catenin is a multifunctional molecule with important roles in intercellular adhesion and signal transduction. We reported previously that β-catenin is mutated in human prostate cancer. In this study, we investigated the role of β-catenin mutations on androgen receptor (AR) signaling. β-Catenin significantly enhanced androgen-stimulated transcriptional activation by the AR. β-Catenin also increased AR transcriptional activation by androstenedione and estradiol and diminished the antagonism of bicalutamide. Coinmunoprecipitation of β-catenin with AR from LNCaP prostate cancer cells showed that the two molecules are present in the same complex. The amount of β-catenin in complex with AR was increased by androgen. These findings implicate β-catenin in the regulation of AR function and support a role for β-catenin mutations in the pathogenesis of prostate cancer.

Introduction

Androgens have an essential role in the development, growth, and function of the prostate gland. Androgens are also promoters of prostate cancer, and all experimental models of prostatic adenocarcinoma require androgen for tumor induction (1). Furthermore, most malignant prostate tumors are androgen dependent during the first part of their natural history, and treatment with androgen ablation leads to tumor regression in the majority of patients with disseminated disease (2, 3). However, prostate cancer recurs despite continued androgen deprivation, and no therapy has been shown to increase survival once androgen deprivation is no longer effective (4).

Most androgen-independent prostate cancers continue to express AR after becoming hormone-independent. In fact, one-third of androgen-independent prostate carcinomas show amplification and overexpression of the AR gene, suggesting that maintenance of a functional AR signaling pathway despite castrate levels of testosterone favors cancer cell growth (5). The notion that the AR may play a role in androgen-independent prostate cancer progression has been strengthened by the discovery of AR mutations in patients with androgen-independent tumors (6, 7). Moreover, some of these mutations were shown to alter the binding specificity of receptors for steroid hormones, such that mutant ARs can bind and be activated by estrogens, progesterone, adrenal steroids, and even by androgen antagonists like hydroxyflutamide (8). Altered ligand specificity can also result from interaction between WT AR and certain AR coactivators (9, 10). Potentially, coactivator expression could result in activation of the AR and the progression of prostate cancer despite therapeutic androgen deprivation.

In this report, we describe alteration of AR activation and steroid hormone specificity by interaction with the oncogene β-catenin. β-Catenin plays a pivotal role in cell-cell adhesion by linking the cytoplasmic tail of cadherins to α-catenin and the actin cytoskeleton. In addition, β-catenin is a key downstream effector in the Wnt/Wingless signaling pathway that governs developmental processes such as cell fate specification, proliferation, polarity, and migration (11). The Wnt pathway has also been implicated in oncogenesis. The activation of the Wnt/β-catenin pathway leads to the formation of a free, signaling pool of β-catenin that enters the nucleus and forms a complex with members of the TCF/LEF family of transcription factors, initiating transcription of new genes (12). In colon cancer, up to 80% of tumors harbor defects in the APC gene that lead to β-catenin up-regulation and constitutive signaling by the β-catenin-TCF complex (12). Moreover, 50% of colon tumors that express WT APC have mutations in the β-catenin gene that up-regulate the levels of the protein by preventing its degradation (13). Recent studies have shown that β-catenin is mutated in a variety of human cancers such as medulloblastoma, hepatocellular carcinoma, endometrial cancer, and ovarian cancer (12). We reported previously that 5% of primary prostate cancers contained mutations in exon 3 of the β-catenin gene, a region that controls the stability of the protein (14). In this report, we show that β-catenin enhances androgen-dependent transactivation and that mutant β-catenin can relieve the suppression of antiandrogens on androgen-dependent transcription and change the sensitivity of the AR to ligands.

We suggest that these effects of β-catenin on AR transcriptional activity could contribute to prostate cancer progression and that the role of β-catenin in oncogenesis may not be limited to its ability to act as a transcriptional activator in conjunction with the TCF/LEF proteins.

Materials and Methods

Plasmids. Human AR expression vector and MMTV-luc plasmid were a gift from M. Danielsen (Georgetown University, Washington, D.C.). AR transcriptional activity was measured using luc reporter gene assays with reporter genes having either a MMTV long terminal repeat promoter (MMTV-luc) or the probasin promoter (Prob-luc). Prob-luc was obtained from R. Matsukis (Vanderbilt University, Nashville, TN). The full-length SRC-I plasmid was a gift from Bert O’Malley (Baylor College of Medicine, Houston, TX). Plasmid pcDNA3.1 with human β-catenin has been described previously (15). The human β-catenin mutants S33F and D32Y, corresponding to mutations found in prostate cancer (14), were constructed by site-directed mutagenesis. The host plasmid pcDNA3.1(+), into which β-catenin was cloned, is designed for high-level constitutive expression in mammalian cell lines and was purchased from Invitrogen (Carlsbad, CA). This empty vector was added in transfections when necessary to keep the amount of DNA constant. The pRL-TK and pRL-CMV Renilla vectors used to normalize for transfection efficiency were purchased from Promega (Madison, WI). PAI-110 and PAI-111A rabbit antibody to AR were purchased from Affinity Bioreagents, Inc. (Golden, CO). Mouse monoclonal anti-β-catenin antibody was purchased from Transduction Laboratories (Lexington, KY).

Cell Culture and Transient Transfections. All cell lines were grown in Improved MEM with 5% fetal bovine serum. Transient transfection of LNCaP...
cells was done using the Fugene reagent (Boehringer Mannheim, Indianapolis, IN) as per the manufacturer’s instructions. Briefly, LNCaP cells were plated in Improved MEM without phenol red with 5% CSS at 2 × 10⁵ cells/well in 22-mm wells 3 days before transfection. At 16–18 h after transfection, cells received fresh medium containing 5% CSS with or without hormone. The cells were harvested 36 h after transfection. pRL-CMV Renilla luc (10 ng) was cotransfected in each sample as an internal control for transfection efficiency. The luc activities were detected using the Dual-luc assay (Promega). TSU-Pr1 and CV-1 cells were transfected using calcium phosphate precipitation. Twenty-four h before transfection, either 1.25 × 10⁵ TSU-Pr1 cells or 2 × 10⁵ CV-1 cells were plated in 35-mm wells. At 16–18 h after transfection, cells were washed three times in PBS, and new media with 5% CSS with or without hormone were added. Twenty-four h later, cells were harvested and assayed as described above. pRL-TK Renilla (10 ng) was cotransfected to normalize for transfection efficiency. Where indicated, cells were treated with 10⁻⁹ M R1881, a synthetic androgen, or with varying concentrations of DHT, testosterone, DHEA, androstenedione (all from Sigma, St. Louis, MO), or bicalutamide [a gift from the Zeneca Corp. (now AstraZeneca, Wayne, PA)]. All hormones were dissolved in ethanol. Control cells received a corresponding volume of ethanol only. The total amount of DNA was kept constant in each transfection by adding empty pcDNA3.1 vector when appropriate. Cell extracts were assayed for luc activity at 48 h after transfection. All experiments were performed in triplicates and repeated at least twice. Results are expressed as fold transactivation relative to cells transfected with the reporter gene alone, in the absence of hormone (basal activity), and are presented as the mean ± SD of triplicate cultures.

**Immunoprecipitation and Immunoblotting** Whole cell extracts were prepared using a lysis buffer containing 20 mM Tris-HCl (pH 7.8), 140 mM NaCl, 1 mM EDTA, 0.5% NP40, 1 mM phenylmethylsulfonyl fluoride, and complete protease inhibitors mixture (Boehringer Mannheim). Where indicated, cells were grown in the presence of 10⁻⁹ M DHT for 24 h before protein extraction. Cell lysates were passed several times through a 30.5-gauge needle to disrupt the nuclei. Protein extract (500 µg) was incubated with PA1–110 anti-AR antibody for 1 h at 4°C, followed by a 1-h incubation with protein A/G-Sepharose (Calbiochem, La Jolla, CA). Control immunoprecipitations were performed using rabbit preimmune serum or a control rabbit anti-JNK1 antibody (Santa Cruz Biotechnology, Inc.). After four washes with 0.5 ml of lysis buffer, the pellets were resuspended in electrophoresis sample buffer, boiled for 5 min, and analyzed on an 8% Tris-glycine acrylamide gel. Proteins were transferred to a nitrocellulose membrane and blotted with mouse anti-β-catenin antibody. Immunoblotting of the AR was performed with rabbit anti-AR antibody PA1–110.

**Results**

**β-catenin Enhances AR-mediated Transcription.** The ability of β-catenin to modulate AR-mediated transcription was initially studied in the LNCaP prostate cancer cell line that expresses AR. In the presence of androgen, expression of a mutant β-catenin (S33F) in LNCaP cells increased transcription of the MMTV-luc reporter 2.5-fold over that in basal levels (Fig. 1A). In the absence of androgen, β-catenin had no effect on transcription from the MMTV long terminal repeat. Similar results were obtained using the probasin promoter, indicating that the effect of β-catenin on AR function was not specific for a single androgen-responsive promoter (Fig. 1B). Although transient overexpression of WT β-catenin also enhanced AR-dependent transcription, the mutant proteins (S33F and D32Y) had a more pronounced and consistent effect (Fig. 1B). Because it has been shown previously that the S33F mutant increases the half-life of the protein and enhances β-catenin-regulated transcription, the S33F mutant was chosen for additional experiments.

The AR in the LNCaP cells has a mutation (T877A) that alters its ligand binding affinity (16). To evaluate the effect of β-catenin on the transcriptional activity of WT AR, we used the AR-negative TSU-Pr1 and PC-3 prostate cell lines in which an exogenous WT AR plasmid was transiently transfected to provide AR activity. Cotransfection of AR with β-catenin in the presence of androgen lead to a 2–4-fold increase in the transcriptional activity of the reporter. In the absence of AR, β-catenin had no effect on the activity of the reporter, confirming that the increase in transcription was not due to a nonspecific interaction between β-catenin and the reporter. The data with TSU-Pr1 cells are shown in Fig. 1C. The ability of β-catenin to enhance AR-dependent transcription was identical with that of the prototypical NRC SRC-1 (Fig. 1C, Lanes 6 and 7). The effect of β-catenin on AR transactivation was seen across a wide range of androgen concentrations (Fig. 1D).

Because the effect of steroid hormone coactivators on transcription can depend on the cellular milieu, we studied the interaction between β-catenin and AR in several additional cell lines including CV-1 kidney epithelial cells and Cos-7 cells and SKBr-3 human breast cancer cells. A 2–3-fold induction of AR-dependent transcription was seen in all cases. Data with CV-1 cells are shown in Fig. 1E. The effects of β-catenin on AR transcriptional activity were specific and did not reflect a general increase in cellular transcription that may have been induced by β-catenin. For example, cotransfection of β-catenin with another reporter gene containing a nuclear factor κB-responsive promoter did not lead to an increase in the reporter gene expression (Fig. 1F).

**β-Catenin Binds to AR.** Given that β-catenin has several transcriptional domains and can act as a transcriptional activator when bound to TCF (12), we hypothesized that β-catenin enhanced AR-dependent transcription by directly interacting with the receptor. We performed coimmunoprecipitation with lysates from LNCaP cells that constitutively express high levels of AR and also express β-catenin. A polyclonal anti-AR antisera was used for immunoprecipitation. Rabbit preimmune serum and a rabbit polyclonal antibody against JNK1 were used as negative controls to confirm that we did not have nonspecific binding of β-catenin to the antisera. The immune complexes were subjected to SDS-PAGE/immunoblot analysis using an antibody to β-catenin. β-Catenin was detected in association with anti-AR immune complexes in the LNCaP cells (Fig. 2, Lanes 1 and 2), whereas no β-catenin was detected in the immune complexes prepared using the control sera (Lanes 3 and 4). Unlike some SRCs, where binding to the receptor depends on the presence of hormone, β-catenin was coimmunoprecipitated with AR in the absence of hormone. However, the addition of DHT increased the amount of β-catenin in the immune complex.

**β-Catenin Affects AR Response to Adrenal Androgens and Antiandrogens.** In late-stage prostate cancer, AR mutations may occur in tumor cells after prolonged treatment with antiandrogens. In certain cases, these mutations change the ligand affinity of the receptor such that adrenal androgens are seen as potent agonists by the mutant receptors (8). AR-interacting proteins such as receptor coactivators can also change the ligand affinity of both WT and mutant AR. This prompted us to examine the effect of mutant β-catenin on the ligand specificity of AR. Androstenedione and DHEA are weak androgens, exerting their effects mainly via peripheral conversion to testosterone. As shown in Fig. 3, in the TSU-Pr1 cells, β-catenin increased AR-induced transcription in response to 1 nM androstenedione to a level comparable to that of 1 nM testosterone (Fig. 3A, Lane 7 versus Lane 3). At 10 nM androstenedione, AR activity in the presence of β-catenin increased 9.8-fold over basal levels, more than twice the activity of AR alone in the presence of 1 nM testosterone (Fig. 3A, Lane 8 versus Lane 3). In the absence of β-catenin, 10 nM androstenedione also increased the transcriptional activity of AR 2.7-fold over the basal level, probably because some androstenedione was converted to testosterone and DHT in TSU-Pr1 cells. In contrast to the response to androstenedione, DHEA at concentrations from 1–10 nM had no effect on AR transcriptional activity, irrespective of the presence of β-catenin (Fig. 3A, Lanes 11 and 12 versus Lanes 9 and 10).

Proteins that interact with hormone receptors, such as receptor coactivators, may also affect the specificity of steroid hormone recep-
Fig. 1. Enhancement of AR transcriptional activity by β-catenin. A, LNCaP cells were transfected with MMTV-luc reporter (1 μg) and 1 μg of plasmid coding for mutant β-catenin (S33F) or the expression vector pcDNA3.1. Eighteen h after transfection, the cells received fresh media with 1 nM R1881 or ethanol, the diluent. Cell extracts were assayed for luc activity at 48 h after transfection. Reporter gene activities are expressed as fold transactivation relative to cells transfected with reporter in the absence of hormone (basal activity), and data are the mean ± SD of three experiments. B, transient transfection of LNCaP cells similar to that described in A, except that the probasin-luc reporter was used. Wild-type β-catenin and two mutant β-catenin constructs, S33F and D32Y, were tested. C, TSU-Pr1 cells were transfected with 0.1 μg of MMTV-luc reporter plasmid, 0.1 μg of WT AR expression plasmid, and 1 μg of β-catenin S33F plasmid, WT β-catenin or SRC-1 plasmid. Results are expressed as fold transactivation relative to the activity of the reporter alone in the absence of hormone. D, β-catenin enhancement of AR-driven transcription is hormone dependent. LNCaP cells were transfected with 1 μg of MMTV-luc reporter and 1 μg of either β-catenin or empty vector. Eighteen h after transfection, cells were stimulated with DHT at concentrations ranging from 0.01–10 nM or ethanol. E, transient transfection and reporter assay in CV-1 cells, as described in C. F, TSU-Pr1 cells were transfected with 0.5 μg of nuclear factor κB-luc reporter, 0.01 μg of Ren-TK plasmid, and 1 μg of either empty pcDNA3.1 vector, β-catenin S33F plasmid, or SRC-1. Results are expressed as relative light units of luc activity normalized to the Renilla activity.
tors for heterologous ligands. For example, the AR coactivator ARA70 was shown to activate mutant as well as WT AR in the presence of either estrogens or antiandrogens (9). To evaluate the effect of β-catenin on AR-dependent transcription in the presence of estrogens, we used the TSU-Pr1 cell line that does not express estrogen receptor (17). Although estradiol at either 1 or 10 nM had no detectable effect on WT AR, the addition of mutant β-catenin increased transcription 2.3-fold at 1 nM estradiol and 7-fold at 10 nM estradiol. In the presence of 10 nM estradiol, AR activity in the presence of β-catenin was higher than that induced by 1 nM R1881 or 1 nM testosterone in the absence of β-catenin (Fig. 3B, Lane 7 versus Lane 2). We further studied the effect of β-catenin on the AR response to the AR antagonist bicalutamide, a drug commonly used in the treatment of prostate cancer. TSU-Pr1 cells were transfected with the MMTV-luc reporter, the AR expression plasmid, and either the mutant β-catenin plasmid or the empty vector in the presence of 0.1 nM R1881 and increasing concentrations of bicalutamide. As shown in Fig. 4, at each concentration of bicalutamide, the inhibitory effect of the antiandrogen was diminished by the mutant β-catenin.

**Discussion**

We described a novel mechanism by which activation of β-catenin signaling could contribute to prostate cancer progression. We show here that β-catenin can activate AR-dependent transcription, change the AR specificity to ligands, and relieve the repression of antiandrogens on AR-dependent transcription. AR, like other members of the steroid receptor superfamily, functions as a ligand-activated transcription factor, controlling the expression of genes involved in functions such as cell proliferation, cell growth, differentiation, and cell death. A major challenge has been to define the molecular properties of each hormone receptor that determine its ability to regulate the transcription of specific target genes. Studies within the past few years have lead to the identification of a multitude of cofactors that mediate the transcriptional effects of hormone receptors either as activators or repressors (18). Most coactivators have been shown to possess intrinsic activation domains that enhance nuclear receptor activity. Some coactivators are parts of complexes containing histone acetyltransferase activity that modulates chromatin structure, thereby influencing the accessibility of transcription factors to the chromatin template (18).

A large number of proteins that interact directly with steroid hormone receptors and modulate their activity have been identified recently. Many of the coactivators have a common site of interaction with the steroid hormone receptors and modulate transcriptional activity of nuclear receptors in a ligand-dependent manner. The SRC/NRC subclass represents the prototype of NRCs (18). All three members of this subclass contain two major transactivation domains and three highly conserved motifs that mediate the binding of these coactivators to nuclear receptors. These motifs contain a consensus amino acid sequence, the LXXLL motif (where L is leucine and X is any amino acid), called the NR box that binds to the COOH-terminal end of nuclear receptors (18). β-Catenin contains five LXXLL motifs, all situated in a highly conserved central region of the protein con-
receptor, indicating that β-catenin could function as a coactivator for a variety of steroid hormone receptors (21). We have previously shown that β-catenin mutations occur focally in prostate cancer, suggesting that these mutations are late events in prostate tumorigenesis and could be related to the development of hormone independence. In conclusion, in hormone-dependent cancers, mutations in β-catenin may contribute to disease progression by modulating receptor-dependent signaling.

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Note Added in Proof

We are grateful to E. Wilson (University of North Carolina, Chapel Hill, NC) who pointed out to us that the empty pcDNA3.1 vector may inhibit AR activity in reporter gene assays. We have recently repeated our reporter gene assays using pSV2neo or no DNA to replace pcDNA3.1. We observed no differences in AR transactivation of MMTV-luc.

References


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