p300 Regulates Androgen Receptor–Independent Expression of Prostate-Specific Antigen in Prostate Cancer Cells Treated Chronically with Interleukin-6

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Abstract
Prostate cancer is the most frequent non–skin cancer in men. Although the mechanisms involved in the progression of prostate cancer are not entirely understood, androgen receptor has been shown to play an important role. Androgen receptor is expressed in both early and late-stage prostate cancer. Also, androgen-regulated pathways are thought to be active as evidenced by elevated levels of prostate-specific antigen (PSA). In addition, several androgen receptor co-activators and cytokines are involved in prostate cancer progression. In this regard, we have shown previously that the coactivator p300 plays a major role in the androgen-independent activation of PSA by interleukin 6 (IL-6), a cytokine involved in late-stage prostate cancer. In this study, we investigated the role of p300 and its homologue CREB-binding protein in prostate cancer cells treated chronically with IL-6. We found that p300 but not CREB-binding protein induced activation of PSA in these cells and that the histone acetyltransferase activity of p300 was critical. This effect was independent of the presence of androgens or antiandrogens. Moreover, we found markedly reduced levels of androgen receptor in these cells and p300 transfection did not affect those levels, suggesting that the p300 effect on PSA could be bypassing the androgen receptor. Transfection with exogenous androgen receptor showed minimal response of PSA to androgens but higher response to p300. We found similar effects of p300 on the androgen response element III, which mediates the androgen receptor–dependent activation of PSA. Finally, we showed that p300 alone regulates expression of the endogenous PSA gene in the IL-6–treated cells. These findings reveal a new insight in the progression of prostate cancer, suggesting that coactivators, such as p300, play more important roles in late-stage prostate cancer, and could regulate androgen-dependent genes in the absence or with very low levels of androgen receptor. (Cancer Res 2005; 65(13): 5965-73)

Introduction
Prostate cancer is the second leading cause of cancer-related death in men (1). The majority of prostate cancers are androgen-dependent at diagnosis, thus, they will respond to androgen-ablation therapy. However, most tumors will relapse in an androgen-independent manner following this therapy (2). Currently, there is no successful treatment for the androgen-independent stage of prostate cancer. The androgen receptor is thought to play an important role in both androgen-dependent and -independent prostate cancer because expression of the androgen receptor protein is present at both stages. Moreover, the androgen receptor is thought to be active as evidenced by levels of the androgen-regulated protein, prostate-specific antigen (PSA), in sera of patients with prostate cancer (3). Indeed, the androgen receptor is important for the proliferation of prostate cancer cells and is expressed after progression from androgen-dependent to -independent disease (4, 5).

Generally, the androgen receptor is activated by androgens in a ligand-dependent manner. However, in prostate cancer, the androgen receptor can be activated in a nonandrogenic fashion. Some of the proposed mechanisms by which this occurs are based on androgen receptor mutations, androgen receptor amplification, alterations of growth factors and/or cytokines, and differential regulation of androgen receptor coactivators (6). A cytokine that seems to play a major role in prostate cancer progression is interleukin 6 (IL-6). Elevated levels of IL-6 are found in the sera of patients with prostate cancer when compared with non–prostate cancer patients, and IL-6 levels are even higher in patients with androgen-independent prostate cancer when compared to those with androgen-dependent disease (7, 8).

IL-6 can induce androgen-independent activation of the androgen receptor and can promote or inhibit prostate cancer cell proliferation depending on the context of the cell milieu (9–11). In addition, one study showed that IL-6 can inhibit androgen receptor activation (12).

In order to better understand the role of IL-6 in late-stage prostate cancer, we have attempted to mimic the environment found in late-stage prostate cancer by growing LNCaP cells (which are prostate cancer cells that express androgen receptor) in the presence of IL-6 and androgen receptor and can promote or inhibit prostate cancer cell proliferation depending on the context of the cell milieu (9–11). In addition, one study showed that IL-6 can inhibit androgen receptor activation (12).

The mechanisms related to the IL-6-mediated regulation of the androgen receptor involve coactivators of the androgen receptor. p300 and its homologous protein, CBB-binding protein (CBP), are ubiquitous coactivators that interact with the androgen receptor and are involved in tumorigenesis (15–17). Both p300 and CBP play important roles in the androgen-dependent activation of the androgen receptor (18, 19). p300 alters chromatin structure via its
histone acetyltransferase activity by weakening histone-DNA interactions, thus inducing nucleosome displacement and favoring access of different transcription factors to the DNA template (20). We have shown previously that p300 mediates the androgen-independent activation of the androgen receptor by IL-6 in prostate cancer cells and that the histone acetyltransferase activity of p300 is required in this context (21). In that study, we showed that although p300 alone is not sufficient to induce androgen receptor activation, it is a necessary component of the IL-6 pathway because p300 suppression impeded androgen receptor activation by IL-6. Moreover, we found that IL-6 increases the proliferation of prostate cancer cells and that the silencing of p300 through small interfering RNA reduces the effect, indicating the importance of this coactivator for the androgen-independent proliferation of prostate cancer cells (17). In addition, we have shown that protein levels of p300 correlate with prostate cancer progression following surgical treatment of the tumor (17).

In the present study, we investigated the role of p300 in prostate cancer cells treated long-term with IL-6 (LNCaP-IL-6+). We found that p300 plays a differential role in the regulation of androgen receptor–dependent genes in these cells. Moreover, we show that p300 can induce activation of PSA in what seems to be an androgen receptor–dependent manner in IL-6-treated cells. These findings reveal a new insight into the regulation of androgen receptor–dependent genes in prostate cancer.

Materials and Methods

Cell culture. LNCaP cells were purchased from the American Type Culture Collection (Rockville, MD). LNCaP-IL-6+ cells were maintained in RPMI 1640 (Celox, St. Paul, MN) containing 5% fetal bovine serum (Biosource International, Rockville, MD) and 5 ng/mL of IL-6 (R&D Systems, Minneapolis, MN). LNCaP-IL-6+ cells were derived from the same stock of cells as LNCaP-IL-6+ and maintained in RPMI 1640 containing 5% fetal bovine serum for an equivalent number of passages. All cells used in our experiments were cultured for >100 passages in the presence of IL-6.

Transfections and luciferase assays. LNCaP-IL-6– and -IL-6+ cells were plated (2.5 \times 10^5 in six-well plates) and 24 hours later transfected with plasmids at quantities described in the figure legends using the Gene Porter Transfection System (GTS, Inc., San Diego, CA). Plasmids containing full-length p300 (p300), mutant histone acetyltransferase–negative derivative (p300-HAT), PSA promoter (PSA-prom), androgen response elements (hK2-ARE), androgen receptor, and CBP have been described previously (19, 22–24). Empty vectors PGL3 and pcDNA3.1 were purchased from Promega (Madison, WI) and Invitrogen (Carlsbad, CA), respectively. Twenty-four hours following transfection, cells received fresh corresponding medium or that containing charcoal-stripped serum (to remove androgens). Steady-Glo Luciferase Assays (Promega) were done according to the manufacturer’s instructions. Transfection efficiency was monitored by cotransfection with a plasmid containing green fluorescence protein (Promega), and visualized with a Zeiss fluorescent microscope at 499 nm. Transfection efficiencies of −40% were obtained routinely. In early experiments, cotransfection of renilla constructs were used to normalize values. However, after finding constant transfection efficiency, experiments were done with the firefly luciferase reporter alone.

Western blot analysis. Cells were washed once with PBS and lysed in cold radioimmunoprecipitation assay buffer [50 mmol/L Tris-HCL (pH 7.4), 1% NP40, 0.25% sodium deoxycholate, 150 mmol/L NaCl, and 1 mmol/L EDTA], plus Complete Protease Inhibitor (Roche, Indianapolis, IN). Western blotting was done using antibodies to androgen receptor (N-20, 441), PSA (A67-B/E3), both from Santa Cruz Biotechnology (Santa Cruz, CA) and androgen receptor (Biogenex, The Hague, the Netherlands). Immunodetection of ERK-2 (D-2, Santa Cruz Biotechnology) was used as a control for...
equal protein loading. Nuclear and cytoplasmic protein extraction reagents were purchased from Pierce (Rockford, IL).

**p300 and CREB-binding protein Western blots.** Nuclear extracts were prepared from 2 × 10⁶ cells according to the manufacturer's instructions. Cell passages 100 and higher were used. Samples for Western analysis were prepared with addition of NuPage sample buffer (Invitrogen) and boiled for 10 minutes at 70 °C. Nuclear fractions were loaded onto a 3% to 8% Tris-acetate gel, and Western analysis was carried out as described previously. The monoclonal anti-CBP antibody (C-1, Santa Cruz Biotechnology) and the polyclonal anti-p300 (C-20, Santa Cruz Biotechnology) were diluted 1:100. Western blots for β-actin (Chemicon International, Hofheim/TSGermany) were done as a control for equal protein loading.

**Immunocytochemistry.** Cells were plated on coverslips in six-well plates (1 × 10⁶). After 24 hours, immunocytochemistry was done using primary antibodies to p300 (C-20) or androgen receptor (441, both Santa Cruz Biotechnology), followed by incubation with fluorescent secondary antibodies (Molecular Probes, Eugene, OR). Cells were counterstained with bis-benzimide (Sigma, St. Louis, MO), and images were visualized with an LSM510 confocal microscope (Carl Zeiss, Inc., Oberkochen, Germany). Cells were transfected with p300 24 hours before immunocytochemistry as described in the figures.

**Real-time PCR.** cDNA was isolated from cells using Trizol (Invitrogen). A two-step real-time PCR was done using cDNA prepared from RNA using a First Strand cDNA Synthesis Kit (Roche) and a SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) on an ABI PRISM 7700 SDS instrument following the manufacturer's instructions. Both forward and reverse primers were used at a final concentration of 900 nmol/L. PCR products (100-150 bp) were electrophoresed in 1.2% agarose gels to check for nonspecific amplification. The fold-change in expression levels (using glyceraldehyde-3-phosphate dehydrogenase as control) was determined by the comparative Cₜ method using the formula 2⁽ΔΔCₜ⁾; where Cₜ is the threshold cycle of amplification.

**Conventional PCR.** cDNA was isolated and cDNA prepared as described above. cDNA was amplified for up to 30 cycles with the GC-RICH PCR System (Roche) following the manufacturer's instructions. PCR products were electrophoresed on a 1.5% agarose gel. β-Actin (Promega) primers were used as controls.

**Plasmid construction.** sPSA, a luciferase reporter construct harboring the minimal core enhancer and promoter regions of the PSA gene (25) was a gift from Dr. Leland Chung (University of Virginia). S-All, containing the PSA core enhancer with androgen response elements V, IV, III, and IIIA replaced with GAL4-binding sites (26) was a gift from Dr. Michael Carey (University of California at Los Angeles). The PSA enhancer core region was amplified from S-All with the primers FWD (5'-CAAGATGATATCTCTCTCAG) and REV (5'-GGGTGACCTCGGACATCTGAGGGTG). The PCR product was subsequently digested with EcoRV/XhoI and ligated to EcoRV/XhoI-digested sPSA, thus generating mutant sPSA. All plasmids were sequenced to verify integrity.

**Results.** In order to investigate the role of IL-6 in prostate cancer, LNCaP cells were grown in the presence of IL-6 (5 ng/mL) to mimic the environment found in late-stage prostate cancer. Levels of p300 and its homologue CBP were assessed in these cells. We found that mRNA levels of p300 and CBP, measured by real-time PCR, remained unchanged in LNCaP-IL-6+ cells when compared with LNCaP-IL-6− cells (Fig. 1A). Surprisingly, the protein levels of both coactivators were decreased in LNCaP-IL-6+ cells of high passage (>100; Fig. 1B). Considering the importance of these coactivators for androgen receptor activity, we investigated the effect of reintroducing p300 or CBP into the cells. LNCaP-IL-6− and -IL-6+ cells were cotransfected with expression constructs of either p300 or CBP together with a luciferase reporter construct containing an androgen receptor–responsive PSA promoter. In parental LNCaP cells (LNCaP-IL-6−), CBP increased PSA promoter activity, whereas p300 had no effect (Fig. 2A). In contrast, in LNCaP-IL-6+ cells, p300 substantially increased the activity of the PSA promoter, whereas CBP had no effect (Fig. 2B). Cells transfected with an empty PGL3 vector exhibited no reporter activity, thus verifying that the effect of p300 was mediated via the promoter and not through the vector.

Although the effect of CBP, as well as lack of effect of p300, on androgen receptor transactivation in LNCaP cells has been reported previously (19, 21), the ability of p300 to induce transactivation of the androgen receptor after long-term treatment...
with IL-6 was unexpected. p300 is a coactivator of the androgen receptor, and thus is necessary, but not sufficient, to induce transcriptional activity. In order to determine whether the presence of exogenous IL-6 contributes to the p300 effect, LNCaP-IL-6+ cells were depleted of IL-6 for 5 days and then cotransfected with p300 along with the PSA-promoter. PSA promoter activity was increased by p300 even in the absence of IL-6 in the medium (Fig. 2C), suggesting that the p300 effect is not dependent on exogenous IL-6. Moreover, treatment with additional IL-6 decreased the ability of p300 to induce PSA-promoter activity (data not shown). This finding suggests that the p300 effect is due to changes in the phenotype of the cells after long-term IL-6 treatment or endogenous production of IL-6 from the cells.

p300 contains histone acetyltransferase activity, which could alter chromatin structure and induce activation of a number of transcription factors, including the androgen receptor. In order to determine if the histone acetyltransferase activity of p300 is important for its effects on the PSA promoter activity, we cotransfected cells with a mutant p300 that lacks histone acetyltransferase activity (p300-HAT). This mutant p300 failed to induce PSA promoter activation in the presence (data not shown) or absence of IL-6 (Fig. 2C), suggesting that the histone acetyltransferase activity is important for the biological effect of p300 in this context.

In order to assess whether androgens contribute to the p300-mediated induction of the PSA promoter, LNCaP-IL-6+ cells were grown in charcoal-stripped serum, which removes androgens (normal serum level <1 pmol/L), and cotransfected with p300 and the PSA-prom. The response of the PSA promoter to p300 was the same whether cells were grown in normal serum (Fig. 3A, lane 2), or in charcoal-stripped serum (Fig. 3A, lane 4), indicating that the effect of p300 is independent of the presence of androgens. Another group of cells were treated with the antiandrogen bicalutamide (casodex; Fig. 3A, lane 5). Cells treated with bicalutamide exhibited a marginal decrease of activation of the PSA promoter by p300, again supporting the concept that the p300 effect is independent of androgen-mediated pathways.

The fact that treatment with antiandrogens did not alter the effects of p300 raised the question of whether the androgen receptor was mediating the p300 induction of PSA activity. In order to determine this, we measured androgen receptor mRNA levels by real-time PCR and confirmed the results with semiquantitative PCR. We found that the mRNA levels of androgen receptor were considerably lower in LNCaP-IL-6+ cells compared with LNCaP-IL-6− cells (Fig. 3B). Furthermore, LNCaP-IL-6+ cells showed undetectable levels of androgen receptor protein (Fig. 3C) by Western blot, and very low levels of androgen receptor were detected by immunocytochemistry (Fig. 3D). These results were surprising because activation of the PSA promoter usually requires androgen receptor activity. To exclude any possibility that the antibody was not recognizing the androgen receptor protein, these experiments were repeated with three different antibodies against the androgen receptor.

The finding that LNCaP-IL-6+ cells have decreased expression of the androgen receptor suggests that either p300-mediated activation is independent of androgen receptor or that p300 is inducing transcription of PSA at very low levels of androgen receptor. To determine whether the p300 effects involve the androgen receptor, luciferase reporter experiments were repeated in cells cotransfected with androgen receptor. When androgen receptor was transfected into the LNCaP-IL-6+ cells, there was no increase in PSA promoter activity by the synthetic androgen milboline (Fig. 3E). However,
when androgen receptor was cotransfected together with p300, there was a marked increase of the PSA promoter activity (Fig. 3E). The increase in activity was higher when p300 was cotransfected with androgen receptor than that found with p300 alone (Fig. 3E). This finding suggests a mechanism by which, after long-term treatment with IL-6, androgen-dependent genes in prostate cancer cells become less responsive to androgen but become more responsive to coactivators like p300.

The activation of androgen-dependent genes in both the normal and malignant prostate is mediated by androgen response elements (24, 25). In particular, the promoters of the androgen-responsive genes PSA and human kallikrein 2 (hK2) have been shown to be transcriptionally activated by androgens. To assess whether p300 activation of the PSA promoter involves androgen response elements or is directed through a different mechanism we performed luciferase reporter experiments using a hK2-ARE-promoter construct with a luciferase reporter (hK2-ARE; ref. 24). The synthetic androgen mibolerone induced hK2-ARE activity ∼8-fold in LNCaP-IL-6 cells cotransfected with the androgen receptor (Fig. 4A), whereas p300 had no effect whether in the absence or presence of androgen receptor. When the same experiment was done in LNCaP-IL-6 cells, p300 increased hK2-ARE activity only marginally in the absence of androgen receptor but induced a 6-fold activation when cotransfected with the androgen receptor (Fig. 4B). When we used a reporter encoding a mutated androgen response element, p300 did not induce activation (Supplementary Data 1). In contrast, mibolerone had only a marginal (1-fold) effect in cells cotransfected with the androgen receptor (Fig. 4B). Moreover, cells transfected with p300 alone and treated with mibolerone showed no induction of the promoter (data not shown) demonstrating that levels of endogenous androgen receptor in these cells are too low to activate gene expression. These results are similar to those in Fig. 3E, where mibolerone alone could not induce a strong reporter activity from the full-length PSA promoter. This suggests that although part of the p300-regulated activation of androgen-responsive genes is mediated through the androgen response elements (thus through the androgen receptor), additional activation is mediated through an androgen receptor–independent mechanism.

Many transcriptional coactivators like p300 can act directly on viral vectors themselves, thereby inducing transcriptional activity of the reporter. Most of the vectors used in our experiments are of viral origin. In order to verify that the p300 effect was due to a direct effect on the PSA promoter rather than an indirect effect via the viral vector, we studied the effect of p300 on expression of endogenous PSA. LNCaP-IL-6 cells were transfected with p300 or empty vector, and RNA was extracted 48 hours later. Cells transfected with p300 showed a marked increase in PSA mRNA, determined by both real-time and semiquantitative PCR (Fig. 5A). Furthermore, we measured the protein levels of PSA in...
LNCaP-IL-6+ cells transfected with p300 alone or cotransfected with p300 and the PSA promoter (in order to mimic the experimental conditions in our previous experiments). PSA protein expression increased 48 hours after transfection of p300 (Fig. 5B) confirming that the endogenous PSA gene is regulated by p300. Because PSA is a well-characterized androgen receptor–dependent gene and is often used to study androgen receptor activity, we examined the effect of p300 on another androgen receptor–dependent gene. NKX3.1 is a member of the NKX family of transcription factors that is highly expressed in prostate cells, and is known to be regulated by androgens (27–29). We measured RNA levels of NKX3.1 in LNCaP-IL-6+ cells transfected with p300 or empty vector. Levels of NKX3.1 RNA increased in cells transfected with p300 (Fig. 5C), suggesting that the effect of p300 extends to other androgen-responsive genes.

Our results showing increased activation of the androgen-dependent gene activity in cells with reduced expression of the androgen receptor suggest that the p300 effects are either bypassing the androgen receptor, activating extremely low levels of the androgen receptor, or increasing expression of androgen receptor. Thus, we investigated androgen receptor levels (mRNA and protein) in LNCaP-IL-6+ cells transfected with p300. Forty-eight hours after transfection of p300, androgen receptor mRNA levels were higher in those cells with p300 but not in the vector group (Fig. 6A). However, protein levels of androgen receptor were still undetectable after transfection with p300 or cotransfection with p300 and PSA-prom (Fig. 6B), indicating that either the p300 effects are mediated through a different pathway than the androgen receptor or that Western blots are not sensitive enough to detect an increase in protein expression. When p300 was transfected into LNCaP-IL-6+ cells, there was no increase in androgen receptor RNA levels (data not shown). Finally, when LNCaP-IL-6+ cells were transfected with either p300 or vector alone and subjected to immunocytochemistry, there was no increase in androgen receptor staining (Fig. 6C). Thus, p300 seems to have little, if any, effect on androgen receptor protein expression in these cells.

Discussion

The androgen receptor is thought to be functional in late-stage prostate cancer, as evidenced by elevated levels of PSA in serum. PSA is a direct target of the androgen receptor. Serum levels of the cytokine IL-6 are also elevated in late-stage prostate cancer. In addition, IL-6 can induce activation of the androgen receptor and modulate prostate cancer cell proliferation (8, 11, 30). This indicates an important role for IL-6 in late-stage prostate cancer. In this study, we show that the PSA promoter can be activated by the coactivator p300 in cells following long-term treatment with IL-6. Interestingly, androgen receptor protein was nondetectable by Western blot in these cells, and immunocytochemistry showed very low levels of the androgen receptor protein. This finding is important because it suggests that coactivators may increase androgen receptor–dependent genes through mechanisms that are independent of the androgen receptor, or through much reduced androgen receptor levels. In this regard, one study has shown the existence of a region within the PSA promoter that seems to be an androgen receptor–independent regulatory element of the promoter in prostate cancer cells (25). Long-term treatment with IL-6 may induce selection of such a region to induce positive regulation of PSA activity independent of androgen receptor. This observation deserves further investigation.

We have shown previously that p300 is necessary for the androgen-independent activation of the androgen receptor by IL-6 (21). The observation that cells which underwent long-term treatment with this cytokine have decreased levels of androgen receptor mRNA and protein was surprising. However, the fact that p300 and CBP proteins were also down-regulated reflects a correlation between the levels of the androgen receptor and some of its coactivators. Whether there is decreased transcription or increased degradation of the androgen receptor should be explored. Interestingly, inhibition of methylation with 5-azacytidine failed to increase androgen receptor mRNA levels (Supplementary Data 2), indicating that methylation is not involved in the down-regulation of the androgen receptor in these cells. We showed previously that LNCaP-IL-6+ cells up to passage 70 express androgen receptor with higher androgen binding than their respective counterpart (13). However, we have noted that LNCaP-IL-6+ passages 80 and higher lack androgen receptor expression. This cannot be attributed simply to high passage number because LNCaP-IL-6− of the same passage are androgen receptor–positive. Although androgen receptor is consistently detected in the majority of prostate cancers, it has been also shown that some cell subpopulations lose androgen receptor expression due to epigenetic changes (31, 32). Modification of androgen receptor in LNCaP-IL-6+ cells may reflect some of these changes. IL-6 is thought to induce differentiation in at least some prostate cancers, (33). However, our finding that several androgen-related genes are down-regulated after long-term treatment with IL-6 suggests that induction of differentiation may be lost after prolonged exposure to IL-6. When IL-6 was removed from the medium for over 5 days there was no reexpression of androgen receptor (Supplementary Data 3). When LNCaP-IL-6+ cells were treated with the antiandrogen casodex, p300 still activated the PSA promoter, suggesting that the androgen receptor is not mediating these effects. Furthermore, when androgen receptor was transfected into LNCaP-IL-6+ cells, the synthetic androgen mibolerone did not induce a strong activation of the PSA promoter, as did p300. These findings suggest that during long-term exposure to IL-6, prostate cancer cells select mechanisms that are less dependent on androgen receptor and more dependent on coactivators to activate androgen receptor–dependent genes. On the other hand, it cannot be ruled out that the very low levels of androgen receptor are more responsive to coactivators than to androgens. Failure of prostate cancer cells to respond to androgens is a complex issue that has been reported previously (34). Whether the androgen receptor is being degraded at a faster rate or if there is a transcriptional repressive complex inhibiting the activation of the exogenous androgen receptor by androgens should be considered and deserves further investigation.

We have shown previously that LNCaP-IL-6+ cells express a more aggressive phenotype, as evidenced by a higher proliferation rate and formation of larger tumors in nude mice (13, 14). Whether p300 plays a role in this more aggressive phenotype needs to be investigated. We found that inhibition of p300 through small interfering RNA results in a decreased proliferation in these cells (data not shown), indicating that p300 plays an important role in the more aggressive behavior of LNCaP-IL-6+ cells.

In the normal prostate, as well as in prostate cancer, the androgen receptor binds to androgen response elements within androgen-responsive gene promoters, and induces transcriptional activation in the presence of androgens (3, 35). In particular, the androgen response elements of the PSA and the hK2 promoter
have been extensively studied. In LNCaP-IL-6+ cells, p300 induced activation of hK2-ARE to a higher degree than the synthetic androgen mibolerone when cotransfected with the androgen receptor. This indicates that p300 induces gene activity to a limited extent through this androgen response element (thus through the androgen receptor). There is a general assumption that higher levels of PSA or hK2 in patients with advanced prostate cancer are due to increased androgen receptor activity (2, 3). Nonetheless, our findings suggest that there are additional pathways involved in the induction of androgen-responsive genes.

The coactivators p300 and CBP are often referred to as if they were the same protein, and indeed they both play major roles in the activation of a number of transcription factors (20). However, p300 and CBP are different proteins and have different functions. For example, p300 is important for the initiation of activation of the estrogen receptor, whereas CBP is responsible for maintenance of the estrogen receptor transcription complex (36). Here, we show the differential effect of these two coactivators where p300 was able to induce PSA promoter activation in LNCaP-IL-6+ cells, whereas CBP induced little or no activity. In contrast, CBP alone induced PSA expression in IL-6− cells, whereas p300 alone had little or no effect. Whether this lack of effect of CBP applies to other transcriptional factors remains to be investigated.

p300 has been shown to have various roles in the transcription process depending on the model or system being examined. p300 could serve as a bridge between the general transcription factors and the basal machinery, or it could be a scaffold to bring specific transcription factors together with general transcription factors. Also, p300 induces histone acetylation through its histone acetyltransferase activity, thus making the DNA template more susceptible to binding by different transcription factors (20). In our experiments, we found that the histone acetyltransferase activity of p300 is critical for activation of the PSA promoter.

Acetylation of androgen receptor by p300 increases the ability of the androgen receptor to induce proliferation in prostate cells (37). However, the ability of p300 to modulate transcription of the androgen receptor has not been shown until now. One mechanism by which p300 activates the PSA promoter in the presence of very
low levels of androgen receptor protein might be through transcription of androgen receptor mRNA. Nonetheless, we found that although there were minimum changes in androgen receptor mRNA levels after p300 transfection, protein levels of the androgen receptor were unaffected. This finding is controversial because it suggests that p300 can elevate androgen receptor RNA levels but not protein. In this regard, it should be noted that androgens have been previously shown to differentially regulate RNA and protein levels in LNCaP cells (35). Also, it is possible that there is an increased degradation of the androgen receptor protein in these cells.

Our experiments show that p300 induces mRNA and protein expression of the endogenous PSA in IL-6+ cells. We also show that p300 induces increased levels of endogenous NKX3.1 RNA. Interestingly, the finding that p300 exerts a differential effect at the transcriptional and translational levels, suggests that p300 regulates different transcriptional factors that ultimately will affect protein function or degradation of androgen receptor–dependent genes.

Taken together, these data provide new insights into the mechanism by which androgen receptor and androgen–dependent genes are regulated in late-stage prostate cancer. They suggest that cytokines and coactivators may have important roles in bypassing androgen receptor function. The mechanism by which p300 can induce PSA activity with low to nondetectable androgen receptor protein levels warrants further investigation.

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References

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