p300 Acetyltransferase Regulates Androgen Receptor Degradation and PTEN-Deficient Prostate Tumorigenesis

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Abstract
Overexpression of the histone acetyltransferase p300 is implicated in the proliferation and progression of prostate cancer, but evidence of a causal role is lacking. In this study, we provide genetic evidence that this generic transcriptional coactivator functions as a positive modifier of prostate tumorigenesis. In a mouse model of PTEN deletion–induced prostate cancer, genetic ablation of p300 attenuated expression of the androgen receptor (AR). This finding was confirmed in human prostate cancer cells in which PTEN expression was abolished by RNA interference–mediated attenuation. These results were consistent with clinical evidence that the expression of p300 and AR correlates positively in human prostate cancer specimens. Mechanistically, PTEN inactivation increased AR phosphorylation at serine 81 (Ser81) to promote p300 binding and acetylation of AR, thereby precluding its polyubiquitination and degradation. In support of these findings, in PTEN-deficient prostate cancer in the mouse, we found that p300 was crucial for AR target gene expression. Taken together, our work identifies p300 as a molecular determinant of AR degradation and highlights p300 as a candidate target to manage prostate cancer, especially in cases marked by PTEN loss. Cancer Res; 74(6); 1870–80. ©2014 AACR.

Introduction
Androgens and the androgen receptor (AR) are paramount for prostate cancer growth (1, 2). Androgen-deprivation therapy (ADT) is the mainstay of treatment for patients with advanced/disseminated prostate cancer. Unfortunately, this treatment is palliative, and the majority of prostate cancers evolve into a disease termed castration-resistant prostate cancer (CRPC). In virtually all cases, castration-resistant progression is accompanied by an increase in the level of prostate-specific antigen (PSA), a well-studied AR transactivated gene (3), indicating that the AR is aberrantly activated under castration conditions. AR protein is expressed in nearly all metastatic CRPC. The importance of persistent AR signaling in CRPC is further demonstrated by the antitumor activity of next-generation androgen–AR axis inhibitors, including abiraterone and enzalutamide (4, 5). However, resistance to these drugs has been observed in many patients (6, 7). Thus, there is an unmet need for the development of new strategies to eliminate AR protein and functions in prostate cancer.

The tumor-suppressor gene PTEN is often mutated, deleted, or transcriptionally downregulated in human prostate cancers. Genome-wide integrative genomic profiling analysis showed that PTEN loss and activation of the phosphoinositide 3-kinase (PI3K) pathway occur in up to 70% of metastatic prostate cancers (8). PTEN loss or PI3K activation results in AKT phosphorylation and activation. Importantly, homozygous deletion of Pten invariably induces AKT activation and prostate tumorigenesis in mice (9–11). However, the downstream signaling pathways that contribute to PTEN loss or AKT activation–induced prostate cancer pathogenesis are poorly understood.

P300 was originally identified as a histone acetyltransferase, which acts as a coactivator for many transcription factors, including AR, p53, and NF-κB (12). Thus, the role of p300 in cancer is not entirely clear and may depend on the pathophysiological milieu of the tumors. P300 seems to have an oncogenic potential because its expression is upregulated during human prostate cancer progression (13, 14). Moreover, p300 has been demonstrated to be an essential coactivator of the AR, a key prostate cancer promoter. P300 interacts with and acetylates AR in vitro and in vivo (15–17). Not only is p300 important for androgen-dependent and independent transactivation of the AR (15, 18–20), but also its expression is associated with human prostate cancer proliferation (13). However, the precise roles of p300 in prostate cancer development and progression remain elusive. In the present study, we demonstrate that p300 functions as a key regulator of AR protein degradation and PTEN-deficient prostate tumorigenesis.

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Materials and Methods

Plasmids and reagents

HA-p300 and HA-p300AHAT (lacking amino acids 1430–1504) were provided by Dr. R. Janne, the University of Oklahoma College of Medicine (Oklahoma City, OK). AR mutants S81A, S213,791A, K630,632,633A, and K630,632,633Q were generated by mutagenesis (Stratagene). V5-tagged cyclin-dependent kinase 1 (CDK1) and cyclin B1 were described previously (21). Antibodies: AR (N20), CDK1, ERG (C20), ERK2, E2F1 (C20), Mme/CD10 (F4), p53 (DO-1), p300 (C20), Pb (R15), SP1 (PEP2; Santa Cruz Biotechnology), AKT-p (Serine 473 phospho-specific), RB-p (Serine 795 phospho-specific), PTEN (Cell Signaling Technology), V5 (Invitrogen), acetyl-lysine, AR-p (Serine 81 phospho-specific), Pten-p (Serine 81 phospho-specific), p300-p (Serine 81 phospho-specific; Millipore), smooth muscle actin (SMA) monoclonal (Dako), Ki-67 (Novocastra), and Nkx3.1 (Novus Biological).

Generation of prostate-specific p300 single- and p300 and Pten double-deletion mice

p300 conditional knockout (P300<sup>Loxp/Loxp</sup>) mice were reported previously (22) and provided by Dr. Jan van Deursen at Mayo Clinic (Rochester, MN). Pten conditional knockout (Pten<sup>Loxp/Loxp</sup>) mice were generated originally in the laboratory of Dr. Hong Wu, University of California Los Angeles (Los Angeles, CA; ref. 9), and purchased from The Jackson Laboratory. Pb-Cre+ transgenic mice were generated originally in the laboratory of Dr. Pradip Roy-Burman, University of Southern California (Los Angeles, CA; ref. 23), and acquired from the National Cancer Institute (NCI) Mouse Repository. Cohorts of p300<sup>Loxp/Loxp</sup>−/−/Pten<sup>Loxp/Loxp</sup>−/−, p300<sup>Loxp/Loxp</sup>−/−/Pten<sup>Loxp+/-</sup>, p300<sup>Loxp+/-</sup>/Pten<sup>Loxp+/-</sup> mice were generated from p300<sup>Loxp+/-</sup>/Pten<sup>Loxp+/-</sup> males and p300<sup>Loxp+/-</sup>/Pten<sup>Loxp+/-</sup> females, which were obtained by cross-breeding Pb-Cre+ males with p300<sup>Loxp+/-</sup> and Pten<sup>Loxp+/-</sup> females. All procedures were approved by the Mayo Clinic Institutional Animal Care and Use Committee and conform to the legal mandates and federal guidelines for the care and maintenance of laboratory animals.

Human prostate cancer specimens and IHC scoring

Thirty-two prostate cancer tissues [Gleason score (GS10), 1 case; GS9, 2 cases; GS8, 4 cases; GS7 14 cases; and GS6, 11 cases] were selected randomly from patients with biopsy-proven prostate cancer that have been treated at the Mayo Clinic by radical retropubic prostatectomy between January 1995 and December 1998 without neoadjuvant therapy. The age of the patients ranged from 47 to 73 years (mean 62.9). The study was approved by the Mayo Clinic Institutional Review Board. Immunohistochemistry (IHC) with antibodies for p300 (C20) and AR (N20; Santa Cruz Biotechnology) was performed as described above. A staining index (SI) was calculated as follows: Staining intensity and staining percentage of each slide were graded accordingly (intensity: 0, no staining; 1, low staining; 2, media staining; and 3, strong staining; percentage: 0%, 1–5%, 5–25%, 25–50%, 50–75%, 75–100% positive cells). A final SI score for each staining was obtained by multiplying values obtained from staining percentage and intensity and used for correlation analysis.

Cell lines, cell culture, and transfection

Cell lines 22Rv1, PC-3, DU145, and 293T were purchased from the American Type Culture Collection. 22Rv1, PC-3, and DU145 cells were cultured in RPMI-1640 medium supplemented with 10% FBS. 293T cells were maintained in Dulbecco’s Modified Eagle Medium (Invitrogen) supplemented with 10% FBS. LAPC-4 was kindly provided by Dr. Charles Sawyers, Memorial Sloan Kettering Cancer Center (New York, NY), and was grown in Iscove’s Modified Eagle Medium supplemented with 10% FBS. These cell lines have been tested and authenticated (karyotyping, AR expression, and PTEN mutation status) for fewer than 6 months before the first submission of the article. Transfections were performed by electroporation using an Electro Square Porator ECM 830 (BTX; ref. 24) or by using Lipofectamine 2000 (Invitrogen). Approximately 75% to 90% transfection efficiencies were routinely achieved.

Immunoprecipitation and Western blotting

Protein immunoprecipitations were carried out using an immunoprecipitation kit (Roche Applied Science) as described previously (24), and Western blotting was performed as described previously (24). Antibodies used for Western blotting were diluted at 1:1,000 to 1:2,000.

Statistical analysis

Experiments were carried out with three or more replicates unless otherwise stated. Statistical analyses were performed by the Student t test for most studies. For the analysis of correlation between p300 and AR protein expression in human prostate cancer specimens, nonparametric Spearman rank correlation was used. Values with P < 0.05 are considered statistically significant.

Additional methods

Additional methods are provided in Supplementary Materials and Methods.

Results

p300 is required for PTEN-deficient prostate tumorigenesis in mice

The tumor suppressor PTEN is frequently lost in advanced human prostate cancer (8), and overexpression of p300 is associated with human prostate cancer progression (13, 14). To test whether PTEN-deficient tumors require a functional p300, we investigated whether deletion of p300<sup>Loxp/Loxp</sup> impairs Pten knockdown-induced tumorigenesis in the mouse prostate. By cross-breeding probasin-Cre transgenic mice (Pb-Cre; ref. 23) with p300 conditional (p300<sup>Loxp/Loxp</sup>, p300<sup>Loxp+/-</sup>, ref. 22) and Pten conditional (Pten<sup>Loxp+/-</sup>, Pten<sup>Loxp+/-</sup>) mice (9), we generated four cohorts of animals: prostate-specific Pten knockout (Pb-Cre; Pten<sup>Loxp+/-</sup>, Pten<sup>Loxp+/-</sup>, hereafter termed as Pten<sup>Loxp+/-</sup>), Pten<sup>Loxp+/-</sup>/p300 double knockout (Pb-Cre4Pten<sup>Loxp+/-</sup>/p300<sup>Loxp+/-</sup>, hereafter termed as Pten<sup>Loxp+/-</sup>; p300<sup>Loxp+/-</sup>), p300 knockout (Pb-CreAp300<sup>Loxp+/-</sup>), and Cre-negative Pten<sup>Loxp+/-</sup>/p300 control mice (hereafter termed as "wild-type"). Similar to the knockout of other genes such as Pten<sup>Loxp+/-</sup>, the p300 gene was robustly deleted in the different lobes of the prostate in mice (Supplementary Fig. S1). As demonstrated by IHC, Pten protein was readily detected.
in the prostates of "wild-type" and p300+/− mice, but little or no Pten protein was detected in Pten−/− and Pten−/−:p300−/− prostates (Fig. 1A, i). In accordance with Pten deletion, Akt phosphorylation robustly increased in the prostates of both Pten−/− and Pten−/−:p300−/− mice compared with the Pten-positive counterparts (Fig. 1A, ii). Similar to the genomic DNA deletion (Supplementary Fig. S1), p300 protein was effectively depleted in p300−/−:Pten−/− mice (Fig. 1A, iii, column 3). As expected, p300 protein was detected in the prostates of Pten−/− and "wild-type," but not in Pten−/−:p300−/− mice (Fig. 1A, iii). These data indicate that both Pten and p300 proteins were effectively deleted in the prostates of mice studied.

As expected, Pten−/− mice at 4 months of age displayed histologic evidence of high-grade prostatic intraepithelial neoplasia (HGPIN) in most acini (Fig. 1B and Supplementary Fig. S2), with occasionally diseased acini suggestive of microinvasive cancer in which periglandular SMA-positive stroma was disrupted or absent (Fig. 1C). In contrast, Pten−/−:p300−/− mice developed primarily low-grade PIN (LGPIN) but not invasive cancer (Fig. 1B and C and Supplementary Fig. S2). P300 deletion alone had no overt impact on the morphology of prostate epithelium in p300−/− mice (Fig. 1B). Quantitative analysis of malignant acini in Pten−/− prostates revealed approximately 90% HGPIN/cancer and approximately 10%
Pten deletion decreases cell proliferation and AR protein level in PTEN-null prostate cancer in mice

To determine the molecular mechanism underlying p300 deletion–imposed inhibition of Pten knockout tumor progression, we examined cell proliferation in the prostates of Pten knockout and/or p300 knockout mouse. Similar to previous reports (9), Ki-67 staining significantly increased in the prostates of Pten knockout mice compared with the normal prostatic epithelium in "wild-type" mice (Fig. 2A and B). Importantly, p300 deletion markedly decreased Ki-67 staining in Pten-deficient prostate (Fig. 2A and B). To our knowledge, this is the first demonstration of the causal role of p300 in prostate cancer cell proliferation in vivo, which is consistent with a previous finding that p300 knockdown significantly inhibits proliferation of PTEN-null human LNCaP prostate cancer cells in culture (26). P300 deletion alone had no overt effect on Ki-67 staining (Fig. 2A and B). Because the background level of Ki-67 staining was quite low in wild-type mice at the age examined (Fig. 2A and B), which is consistent with previous reports (25, 27), our data cannot rule out the possibility that p300 loss alone affects prostatic cell proliferation in mice at different ages or with different genetic backgrounds, and thus further investigation is warranted. Because Pten-deleted murine prostate cancer cells depend on the AR for growth in vitro and in vivo (28), we examined whether p300 deletion affects AR protein levels in both Pten-deficient and wild-type prostate. As evident by IHC and Western blot analyses, AR protein was substantially downregulated in prostatic cells in Pten knockout mice compared with "wild-type" counterparts (Fig. 2C and D), which is consistent with previous reports (27, 29). Strikingly, p300 deletion further decreased AR protein in the vast majority of prostatic epithelial cells in Pten knockout mice compared with Pten knockout counterparts (Fig. 2C), and this result was confirmed by Western blot.
analysis (Fig. 2D). These data suggest that p300 is required for prostate cancer cell proliferation and maintenance of AR protein levels in PTEN-deficient mouse prostate tumors.

**Expression of p300 positively correlates with AR protein levels in human prostate cancer specimens**

To support the clinical relevance of p300 regulation of AR protein observed in p300/Pten knockout mice, we sought to determine if p300 and AR expression correlates in human prostate cancer specimens. To this end, we examined the expression of these proteins by IHC in prostate cancer specimens obtained from a cohort of 32 patients. Examples of both strong and weak staining of p300 and AR and hematoxylin and eosin (H&E) staining are shown in Fig. 3A–C. The p300 and AR protein expression data are summarized in Fig. 3D. Nonparametric Spearman rank correlation analysis indicated that there is a strong correlation between p300 and AR protein expression in the human prostate cancer specimens examined ($r = 0.64; P < 0.001$).

**p300 precludes complete degradation of AR protein in PTEN-deficient prostate cancer cells**

Human prostate cancer cell lines were used to determine mechanistically how p300 regulates AR protein levels. Similar to the results seen in mice (Fig. 2C and D), PTEN knockdown by siRNAs decreased AR protein expression and concomitant knockdown of p300 further reduced AR protein levels in both LAPC4 and 22Rv1 cell lines (Fig. 4A and Supplementary Fig. S3A). Of note, the effect of PTEN and p300 knockdown on AR levels was much greater than that on other cancer-relevant transcription factors examined, including p53, E2F1, ERG, and SP1 (Supplementary Fig. S3B), suggesting a specific impact of PTEN and p300 on AR protein levels in prostate cancer cells. P300 knockdown alone also decreased AR protein in LAPC4 and 22Rv1 cells (Fig. 4A and Supplementary Fig. S3A). PTEN and/or p300 knockdown–mediated decrease in AR protein was largely blocked by the proteasome inhibitor MG132 (Fig. 4A and Supplementary Fig. S3A), suggesting a proteasome-dependent mechanism. Furthermore, PTEN depletion increased AR protein instability, and this effect was further enhanced by p300 silencing in LAPC4 cells (Supplementary Fig. S3C and S3D). Neither PTEN nor p300 knockdown had any overt effect on AR mRNA levels in these cell lines (Supplementary Fig. S3E). These data indicate that p300 is a key regulator of proteasome-mediated AR degradation in both PTEN-proficient and -deficient prostate cancer cells.

**p300 inhibits AR polyubiquitination via its acetyltransferase activity**

Next, we sought to determine whether p300 regulates AR protein polyubiquitination. PTEN knockdown increased AR polyubiquitination in LAPC4 cells (Fig. 4B), which is consistent with the finding that AR polyubiquitination is regulated by AKT (30). P300 knockdown alone also increased AR polyubiquitination, and this effect was further enhanced by conknockdown of PTEN (Fig. 4B). Lysine (K) residues 630, 632, and 633 (K630, 632, and 633) on AR are three known p300 acetylation sites (15). Interestingly, conversion of these residues to alanine (K630/632/633A) diminished AR polyubiquitination (Fig. 4C). This result could be due to the possibility that these lysine residues are
ubiquitin-accepting sites or that blockage of acetylation of these sites affects AR ubiquitination through mechanisms other than site competition. To test the second possibility, the lysine residues were converted into glutamine (Q), which structurally mimics the acetylation state of lysine (31). Polyubiquitination on the K630/632/633Q mutant was further reduced compared with the K630/632/633A mutant, although the expression levels of these mutated proteins were comparable (Fig. 4C). Thus, these experiments identified AR acetylation at K630, 632, and 633 residues as an important inhibitory mechanism of AR polyubiquitination in prostate cancer cells. Consistent with these observations, we further showed that ectopic expression of wild-type p300, but not the histone acetyltransferase (HAT)-deficient mutant (p300ΔHAT), diminished AR polyubiquitination (Fig. 4D). In contrast, overexpression of p300 had little or no effect on polyubiquitination of the acetylation-resistant K630/632/633A mutant of AR (Fig. 4D). We conclude that p300 inhibits AR polyubiquitination and that this effect is mediated by its acetyltransferase activity.

Serine 81 phosphorylation in AR is required for the p300–AR interaction

Overexpression of active AKT triggers AR degradation (30). As expected, PTEN knockdown by siRNAs downregulated AR...
protein in LAPC4 cells and this was blocked by the AKT inhibitor LY294002 (Fig. 4E, left). Similarly, AR degradation was inhibited by LY294002 in PTEN and p300 co-knockdown cells (Fig. 4E, right). When two AKT phosphorylation sites in AR (serine 213 and 791; ref. 30) were mutated to alanine, the steady-state level of the S213,791A mutant was higher than the wild-type AR (AR-WT) in DU145 cells (Fig. 4F). However, these mutations failed to block AR degradation induced by co-knockdown of PTEN and p300 (Fig. 4F). These data suggest that the effect of p300 on AR degradation is AKT dependent but independent of AKT-mediated phosphorylation of AR at multiple serine/threonine sites, including serine 81 (Ser81; ref. 32). PTEN loss or AKT activation promotes cell-cycle progression by increasing the activity of CDKs (33–36). We therefore examined the role of AR Ser81 phosphorylation in the regulation of p300 and PTEN deficiency–induced AR protein degradation. PTEN knockdown increased RB phosphorylation, an indicator of CDK activity, and AR phosphorylation at Ser81 (Fig. 5A). Next, we examined whether AR Ser81 phosphorylation affects the p300–AR interaction. The interaction between these two proteins was readily detected, but was largely attenuated by the S81A mutation (Fig. 5B). Accordingly, overexpression of cyclin B1 and CDK1 greatly enhanced p300 interaction with AR-WT but not the S81A phosphorylation of AR by CDK1 is crucial for p300–AR interaction, AR acetylation, and p300 regulation of AR ubiquitination. A, LAPC4 cells were transfected with indicated siRNAs for 72 hours followed by Western blot analysis. B, 293T cells were transfected with wild-type and S81A-mutated AR for 24 hours followed by treatment with 10 nmol/L mibolerone (Mib) for additional 24 hours. Cell lysates were subjected to immunoprecipitation and Western blot. C, 293T cells were transfected with AR-WT and S81A mutant in combination with or without CDK1 and cyclin B1 for 24 hours followed by treatment with 10 nmol/L Mib for additional 24 hours. Cell lysates were subjected to immunoprecipitation and Western blot. D, 293T cells were transfected with the indicated plasmids for 24 hours followed by treatment with 10 nmol/L Mib in combination with or without 10 μmol/L roscovitine for 24 hours. Cell lysates were subjected to immunoprecipitation and Western blot. E, 293T cells were transfected with the indicated plasmids for 24 hours and treated with 10 nmol/L Mib for 24 hours. Cell lysates were subjected to immunoprecipitation and Western blot analysis. F, 293T cells were transfected with the indicated plasmids and siRNAs as indicated for 24 hours and treated with 10 nmol/L Mib for 24 hours. Cell lysates were subjected to immunoprecipitation and Western blot. G, LAPC4 cells were transfected with indicated siRNAs for 72 hours followed by Western blot analysis.
mutant (Fig. 5C and Supplementary Fig. S4). Furthermore, p300–AR interaction was completely blocked by the pan CDK inhibitor roscovitine (Fig. 5D). We conclude that loss of PTEN increases CDK1-mediated phosphorylation of AR at Ser81, which enhances the interaction between p300 and AR.

**Ser81 phosphorylation in AR is crucial for p300-mediated inhibition of AR ubiquitination**

We further examined whether AR Ser81 phosphorylation affects the regulation of AR acetylation and polyubiquitination by p300. S81A mutation decreased AR acetylation (Fig. 5E, lane 1 vs. lane 3). Consistent with CDK1/cyclin B1–enhanced interaction between p300 and AR (Fig. 5C), overexpression of CDK1 and cyclin B1 augmented acetylation of AR-WT but not the S81A mutant (Fig. 5E, lane 3 vs. lane 4). Moreover, overexpression of CDK1 and cyclin B1 substantially decreased polyubiquitination of AR-WT, and this effect was partially reversed by p300 knockdown (Fig. 5F). Consistent with the result that acetylation of S81A was lower than AR-WT (Fig. 5E), polyubiquitination of S81A was greater than AR-WT (Fig. 5F). However, in contrast with the effect on AR-WT, ectopic expression of CDK1/cyclin B1 alone or in combination with p300 knockdown had a negligible effect on polyubiquitination of the S81A mutant (Fig. 5F). In agreement with these results (Fig. 5E and F) and the finding that loss of PTEN increases CDK1 phosphorylation of AR at Ser81 (Fig. 5A), we further showed that knockdown of CDK1 accelerated PTEN loss–induced decrease in AR protein level and that this effect was further enhanced by p300 knockdown (Fig. 5G). Thus, besides its role in mediating the interaction between p300 and AR, Ser81 phosphorylation is crucial for CDK1-enhanced AR acetylation and p300-mediated inhibition of AR polyubiquitination.

**p300 is important for AR transcriptional activity in PTEN-null prostate tumors in mice**

To gain insight into the biologic importance of p300 regulation of AR protein degradation in PTEN-deficient prostate cancer cells, we sought to determine how p300 affects AR activity by examining AR target gene expression. Consistent with the observation that p300 knockout induced downregulation of AR protein in PTEN-deleted prostate cancer cells in mice (Fig. 2C and D), mRNA levels of AR-regulated genes, including probasin (Pb), Nkx3.1, and Mme, were significantly reduced in Pten<sup>pc-/pc-</sup>–p300<sup>pc-/pc-</sup>prostates compared with Pten<sup>pc-/pc-</sup>–p300<sup>pc+-/pc+</sup>prostates (Fig. 5H). In contrast, there was little or no difference in expression of Pai-1, a non–androgen-regulated gene, between Pten<sup>pc-/pc-</sup> and Pten<sup>pc+-/pc+</sup>-p300<sup>pc-/pc-</sup>prostates (Supplementary Fig. S3B), arguing that p300 deletion does not result in a general downregulation of gene expression in PTEN-deleted prostate cancer cells in vivo. To determine whether p300 exerts a similar effect on AR activity in human PTEN-deficient prostate cancer cells, LAPC4 cells were transfected with PTEN siRNAs in combination with control or p300-specific siRNAs followed by treatment with vehicle or mibolerone, a synthetic androgen. P300 silencing (Supplementary Fig. S3B) markedly decreased the expression of AR target genes PSA, Nkx3.1, Hk2, and TMPRSS2 in both androgen-untreated and -treated PTEN knockdown cells (Fig. 6D), indicating that p300 is important for AR activation in the presence or absence of androgens in human PTEN-deficient prostate cancer cells. However, no such effects were detected on the expression of p21<sup>WAF1</sup>, E2F1, PLAT, and Pai-1, target genes of p53, E2F1, ERG, and SP1 transcription factors, respectively (Supplementary Fig. S5C). Consistent with the protein expression results (Supplementary Fig. S3B), among the transcription factors examined AR activity seems to be mostly affected by p300 depletion. Together, these results suggest that p300 is essential for the transcriptional activity of the AR in PTEN-deficient prostate cancer cells in vitro and in vivo.

**Discussion**

p300 has been implicated in human prostate cancer proliferation and progression (13, 37, 38), although the direct evidence that p300 is causally involved in prostate oncogenesis is lacking. In the present study, we revealed that prostate-specific homozygous deletion of p300 dramatically inhibits Pten-deficient prostate cancer proliferation and progression and prolongs mouse survival. Thus, in the Pten knockout mouse model, we demonstrate for the first time that p300 functions as a bona fide oncogenic promoter of prostate cancer. The relevance of this observation is further substantiated by our findings that high expression of p300 correlates with AKT phosphorylation, a surrogate of PTEN inactivation, in a cohort of human prostate tumors examined (Supplementary Fig. S6). It is worth noting that our present study cannot rule out the possibility that p300 is also critical for prostate tumorigenesis induced by other oncogenic factors such as RAS and Myc. It is hence important to address this issue in the future using transgenic mouse models.

p300 is a well-known acetyltransferase that catalyzes acetylation of both histone and nonhistone proteins such as AR and p53 (12, 15, 39). It is generally accepted that p300 is a generic transcription coactivator. Moreover, it has been shown that p300 is targeted by oncogenic viral proteins such as E1A and is often mutated or truncated in endometrial, breast, and pancreatic cancers (40), implying that p300 functions as a classic tumor suppressor. However, in contrast with this concept, different studies invariably demonstrate that p300 is overexpressed in human prostate cancers (13, 14). These observations in clinical specimens and our findings in the mouse model clearly support the hypothesis that p300 functions as an oncogene in the prostate. Therefore, our findings about how p300 functions paradoxically as an oncogene in the prostate are of importance from both basic science and translational perspectives.

Development, differentiation, and growth of the prostate rely on androgens and androgen activation of the AR. The same is true for prostate cancer growth and progression. Because of the androgen/AR addiction of prostate cells, ADT is the mainstay of treatment for advanced prostate cancer in the clinic. It is known that p300 promotes AR transactivation by inducing acetylation of AR and histone proteins (15). In the current report, we have discovered another important layer of p300 regulation of the AR by demonstrating for the first time
that p300 plays an essential role in regulation of AR protein degradation. First, we provide evidence that homozygous deletion of p300 decreases AR protein levels in the mouse prostate. Second, we show that expression of p300 correlates positively with AR protein levels in a cohort of human prostate cancer specimens. Third, using human prostate cancer cell lines as a working model, we demonstrate that p300 inhibits AR protein polyubiquitination and proteasome degradation by inducing AR acetylation at lysine residues 630, 632, and 633. Importantly, the effect of p300 on AR degradation seems to be AR-specific because p300 depletion had no overt effect on other cancer-relevant transcription factors examined such as p53, E2F1, ERG, and SP1. Consistent with the essential role of AR in prostate cancer proliferation and growth, we further show that homozygous deletion of p300 significantly decreases prostate cancer cell proliferation in vivo. Together, our findings imply that the oncogenic function of p300 in the prostate could be linked, at least in part, to the role of p300 in regulation of AR protein levels, which is highly relevant to prostate tumorigenesis and progression.

AR protein stability is known to be regulated by CDK1 in prostate cancer cells, and this effect is likely mediated by CDK1 phosphorylation of multiple serine/threonine residues in the AR protein, including the major phosphorylation site Ser81 (32). However, the precise mechanism by which Ser81 phosphorylation influences AR protein stability is unclear. Our data demonstrate that loss of PTEN increases AR phosphorylation at Ser81 and that Ser81 phosphorylation acts as a molecular beacon that is required for the binding of p300, a key event that subsequently leads to AR acetylation.
inhibition of AR ubiquitination, and AR stabilization (Fig. 6E). Thus, we identify p300 as a key molecular determinant of AR degradation in PTEN-mutated prostate cancer cells. In addition to its role in regulation of AR stability, Ser81 phosphorylation is important for AR transactivation (41, 42). In the present study, we reveal for the first time that Ser81 phosphorylation is essential for p300–AR interaction and AR acetylation. Given that p300 is a key transcriptional coactivator, beside its role in mediating chromatin binding (42), enhanced p300–AR interaction represents a novel mechanism of action of Ser81 phosphorylation in AR transactivation (Fig. 6E). Another important function of AR Ser81 phosphorylation is its role in regulation of prostate cancer cell growth (41). Consistent with these findings, we provide evidence that p300 is important for prostate cancer cell growth in mice. Thus, it can be speculated that Ser81 phosphorylation may promote prostate cancer cell growth via mechanisms such as increased interaction between AR and p300 and subsequent AR activation (Fig. 6E).

The findings that p300 acetylation of AR is important for AR stabilization and transactivation and prostate cancer cell growth and survival (Fig. 6E; refs. 13, 16, 19, 26, 43, 44) imply that p300 could be a potential target for prostate cancer therapy. P300 is a bromodomain protein, and it could potentially be targeted for cancer treatment because inhibition of other bromodomain proteins can effectively block tumor growth (45–46). A virtual screen for inhibitors of p300 and CBP, a homolog protein of p300, has been conducted, and a selective small molecule inhibitor C646 has been identified. The antitumor effect of C646 was demonstrated in human prostate cancer cells cultured in vitro (26). However, the in vivo application of C646 is unlikely because it can be inhibited by serum (26). Interestingly, curcumin (diferuloylmethane), a phenolic compound derived from the root of the plant Curcuma longa, and procyanidin B3, a compound extracted from grape seeds, have been demonstrated as natural anti-p300 substances (47, 48). Indeed, treatment of the liposome form grape seeds, have been demonstrated as natural anti-p300, and procyanidin B3, a compound extracted from

References


inhibitors are strong candidates for pharmacologic intervention of p300 function in cancers such as prostate cancer, although the specificity of these compounds in inhibiting p300 is an open question because numerous targets of dietary compounds have been reported. Thus, although p300 is an attractive antiprostate cancer target, novel strategies are needed to specifically abolish the oncogenic function of p300 in prostate cancer. In this report, we demonstrate that p300 favors PTEN-deficient prostate cancer progression by protecting AR protein from degradation. Because this role of p300 depends on the interaction between p300 and AR, its blockage of AR degradation can be overcome by disrupting their physical interaction without disturbing p300 regulation of other signaling pathways. Our finding therefore opens a unique avenue for development of novel therapeutics for prostate cancer via specific elimination of p300-mediated protection of AR proteins in prostate cancer, especially those without a functional PTEN.

Disclosure of Potential Conflicts of Interest

D.J. Tindall is a consultant/advisory board member of Medivation. No potential conflicts of interest were disclosed by the other authors.

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Conception and design: J. Zhong, L. Ding, T.J. Sebo, D.J. Tindall, J. van Deursen, H. Huang
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27. Cancer Research


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