

p300 in Prostate Cancer Proliferation and Progression

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Abstract

Although prostate cancer (PCa) is the most frequently diagnosed cancer in males, little is known about the mechanisms involved in its progression. Recent *in vitro* studies suggest that coactivators of the androgen receptor play an important role in PCa progression. We have shown previously that p300 is involved in androgen receptor transactivation. In the present work, we studied 95 patients with biopsy-proven PCa who underwent prostatectomy as treatment of their tumors between 1995 and 1998. We found that p300 correlated with *in vivo* proliferation ($P = 0.009$) as determined by MIB-I expression. Moreover, high levels of p300 in biopsies predicted larger tumor volumes ($P < 0.001$), extraprostatic extension ($P = 0.003$), and seminal vesicle involvement ($P = 0.002$) at prostatectomy, as well as PCa progression after surgery ($P = 0.01$).

Furthermore, we found that the disruption of p300 transcripts through small interfering RNA inhibited PCa cell proliferation both at the basal level and on interleukin 6 stimulation.

We conclude that p300 plays an important role in PCa cell proliferation, as well as PCa progression.

Introduction

PCa⁴ is a leading cause of cancer-related death in men, being second only to lung cancer. The mechanisms by which PCa progresses, both before and after hormonal treatment, are not entirely understood, thus limiting therapeutic possibilities. Several reports suggest that the AR coactivators are involved in androgen-dependent and androgen-independent PCa (1). Indeed, the AR is present in most PCa, both androgen-dependent and -independent, and remains functionally evident by high levels of PSA (2). AR coactivators have been widely studied at the molecular level to understand PCa progression (3, 4). Some coactivators have been shown to play a tumor suppressor role, whereas others are believed to be positive regulators of cancer progression. p300 is a transcriptional coactivator that has been related to a number of tumors (5–7). We have shown previously that p300 is involved in the IL-6-mediated transactivation of the AR in the absence of androgens in PCa cells (8). Others have shown a similar role of p300 in the presence of androgens (9). Whether p300 is involved in the growth of PCa has not been established. In the present study, we show that p300 is involved in PCa growth and is a predictor of aggressive features of PCa. Moreover, we show that p300 plays a major role in PCa cell proliferation.

Materials and Methods

Cell Culture. LNCaP cells were purchased from the American Type Culture Collection (Rockville, MD) and maintained in RPMI 1640 (Celox, St. Paul, MN) containing 9% fetal bovine serum (Biosource International, Rockville, MD).

Proliferation Assays. Cells were plated in six-well plates and transfected with siRNA oligonucleotides designed to target p300 (p300-siRNA) or control sequence as described previously (8). Twenty-four h after transfection cells were plated in 96-well plates and treated with IL-6 (50 ng/ml) or vehicle alone. At indicated time points cell viability was assessed using Cell Titer 96 (Promega, Madison, WI) according to the manufacturer's instructions. Additionally proliferation was measured using Cell Proliferation ELISA, bromodeoxyuridine (Roche, Mannheim, Germany).

Study Population. Ninety-five patients were randomly selected from a sample of 454 patients with biopsy-proven PCa that were treated with radical retropubic prostatectomy between January 1995 and December 1998 without neoadjuvant therapy. After surgery, serum PSA measurements were made every 3 or 4 months for the first 2 years, every 6 months for the next 3 years, and annually thereafter. Systemic progression was determined by bone scan or computed tomography, and local recurrence was determined by clinical examination or needle biopsy. Cancer progression was defined as postoperative levels of PSA >0.4 ng/ml, local recurrence, or systemic progression of PCa. Additional details regarding the sample of 454 patients is described elsewhere (10).

Protein Expression. The expression of p300 was studied using formalin-fixed, paraffin-embedded tissue from needle biopsies by immunohistochemistry using a specific antibody for this protein (Santa Cruz Biotechnology, Santa Cruz, CA) and quantified by DIA using a CAS 200 image analyzer (Bacus Laboratories, Lombard, IL). MIB-I expression was determined similarly and as described previously (11). In addition, all of the samples were visually graded in a blind fashion by the study pathologist (T. J. S.) to assess for the accuracy of quantification by DIA.

Apoptosis Detection. LNCaP cells were transfected with p300-siRNA or control-siRNA. Forty-eight and 72 h after transfection apoptosis was detected using Annexin V Apoptosis detection kit (Santa Cruz Biotechnology), following the manufacturer's instructions.

Statistical Methods. The associations of p300 expression analyzed as a continuous variable with biopsy features were assessed using Spearman rank correlation and Wilcoxon rank sum test. The univariate associations of p300 expression with outcomes at prostatectomy and PCa progression after prostatectomy were assessed using linear, logistic, and Cox proportional hazards regression. Statistical analyses were performed using the SAS software package (SAS Institute, Cary, NC). P s < 0.05 were considered statistically significant.

Results and Discussion

We have shown previously the importance of p300 in the ligand-independent transactivation of the AR in PCa cells, indicating that p300 may be involved in PCa pathogenesis and/or progression (8). To assess whether p300 is involved in the clinical progression of PCa we evaluated 95 patients with biopsy-proven PCa. The dataset used represent a unique population of patients who have had clinical, biopsy, and prostatectomy findings reviewed. Thus, it allows for a detailed correlative analysis between p300 expression and a variety of clinicopathological parameters. The expression of p300 was quanti-

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⁴The abbreviations used are: PCa, prostate cancer; AR, androgen receptor; PSA, prostate-specific antigen; IL, interleukin; siRNA, small interference RNA; DIA, digital image analysis; CI, confidence interval.

fied by DIA and visual grade. The mean p300 value, expressed as the percentage of total nuclear area positive for this marker, was 14%, and the median value was 10.5% (range, 0.03–56.4%).

First, we assessed the relationship between p300 expression levels and MIB-I expression, an *in situ* marker of cell proliferation. The Spearman rank correlation coefficient was 0.27 ($P = 0.009$), indicating that p300 expression on PCa biopsy correlated positively with MIB-I expression (Fig. 1A). An association between p300 and cell proliferation index suggests that tumor volume at the time of surgery and other aggressive features including extraprostatic extension and seminal vesicle involvement might also be associated with p300 expression. The Spearman rank correlation coefficient for the association of p300 and tumor volume was 0.39 ($P < 0.001$), indicating that higher levels of p300 on biopsy correlated with larger tumors at prostatectomy (Fig. 1B). We also found a significant association between p300 expression and extraprostatic extension of PCa (odds ratio, 1.06; 95% CI, 1.02–1.10; $P = 0.003$; Fig. 1C), as well as with seminal vesicle involvement (odds ratio, 1.07; 95% CI, 1.03–1.12; $P = 0.002$; data not shown). The association between p300 and aggressive features of PCa suggests that this coactivator might be involved in some of the genotypic and phenotypic cellular changes associated with PCa. Therefore, we compared p300 expression with DNA ploidy content, as nondiploid DNA has been related to more aggressive cancers (10), and with Gleason score, because Gleason score is independent of nuclear grade or DNA content. The mean p300

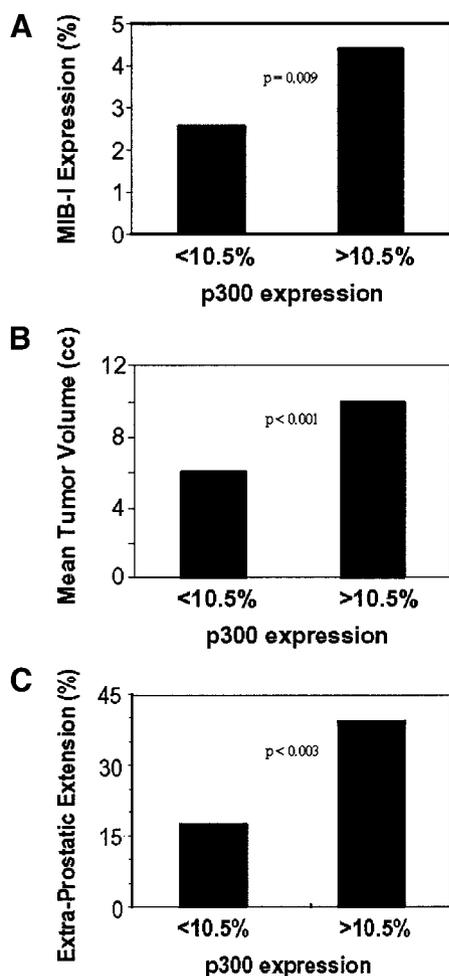


Fig. 1. p300 correlation with aggressive surgical features and cancer progression. Expression of p300 (above the median expression) correlates positively with higher MIB-I expression (A), tumor volume (B), and extraprostatic extension (C). p300 values were categorized above and below its median value for illustration purposes only.

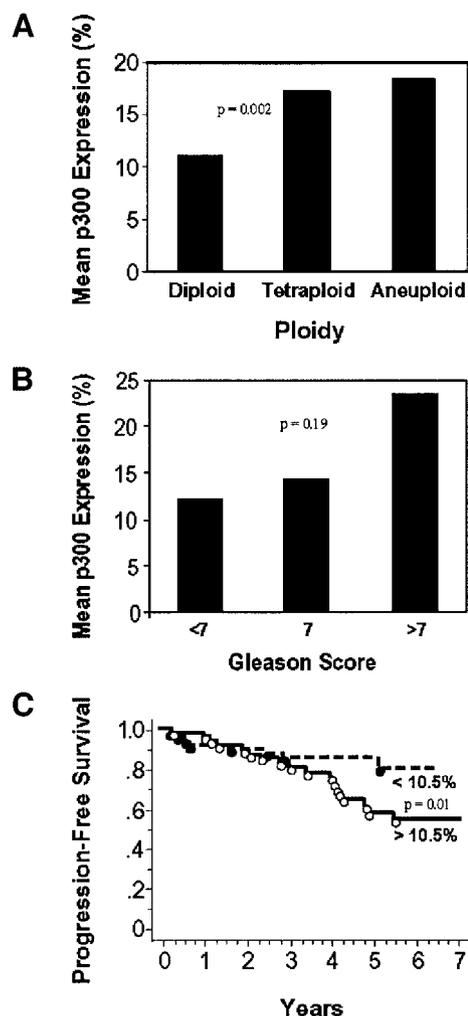


Fig. 2. p300 expression correlates with aggressive phenotype, genotype, and increased susceptibility to PCA progression. p300 levels correlate positively with nondiploid DNA content (A), higher Gleason scores, and PCA progression (C). In C, --- represents p300 expression values below 10.5%, — represents values above 10.5%. p300 values were categorized above and below its median value for illustration purposes only.

expression for diploid tumors was 11.1% compared with 17.8% for nondiploid tumors ($P = 0.002$), indicating that higher levels of p300 correlated positively with nondiploid DNA content (Fig. 2A). The mean p300 expression for PCa with Gleason scores of <7 was 12.2%, of scores equal to 7 was 13.0%, and of cancers with Gleason scores >7 was 23.6% (Fig. 2B). Whereas not statistically significant ($P = 0.19$), likely due to the few patients in the Gleason score >7 category ($n = 9$), there was a trend toward a positive correlation between p300 expression and higher Gleason scores, which correspond to more undifferentiated tumors (12).

Lastly, we assessed the association of p300 expression with PCA progression after prostatectomy. Among the 95 patients studied, 26 (27.4%) experienced cancer progression at a mean of 2.7 years (range, 0.5–5.5 years) after prostatectomy. The mean follow-up for all of the patients was 4.2 years (range, 0.2–7.1 years). We found a significant correlation between p300 expression levels and PCA progression (risk ratio, 1.04; 95% CI, 1.01–1.07; $P = 0.01$) suggesting that patients with higher levels of p300 on biopsy are more susceptible to PCA progression after surgical treatment (Fig. 2C). Thus, p300 could be used to evaluate patient risk of PCA progression, thereby helping treatment and management.

This positive correlation between protein expression and clinical features lead us to address the direct role of p300 in PCa cellular

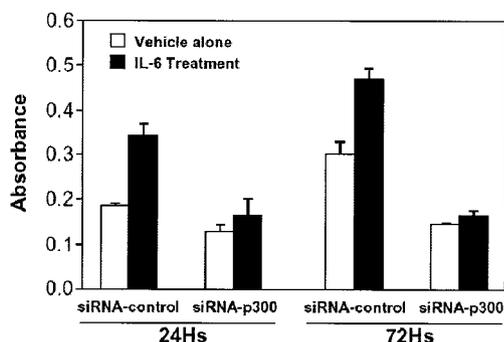


Fig. 3. Decreased PCa cell proliferation on inhibition of p300. LNCaP cells were transfected with siRNA designed to target p300 (*siRNA-p300*) or control siRNA. After transfection cells were treated with IL-6 (■) or vehicle alone (□). Cell proliferation assays were performed 24 and 72 h after siRNA transfection. Results are expressed in absorbance units at 495 nm (absorbance is directly proportional to the number of living cells). This figure represents three experiments.

proliferation. For this purpose, we used the PCa cell line LNCaP. We have shown previously that transfection of p300-siRNA, designed to target and destroy p300, into LNCaP cells disrupts protein expression of this coactivator (8). We transfected p300-siRNA into cells and assessed the effect on cell growth with a proliferation assay. We found that inhibition of p300 through siRNA decreased cell proliferation after 76 h when compared with control cells (control-siRNA transfection). Moreover, we found that androgen-independent induction of cell proliferation by IL-6 was no longer possible in p300-siRNA-transfected cells (Fig. 3). These experiments were confirmed using a bromodeoxyuridine incorporation assay (data not shown). These results suggest that p300 is involved in PCa growth, and, thus, may promote progression of PCa. In addition, the fact that p300 inhibition interfered with IL-6-induced cell proliferation suggests that p300 might play an important role in the androgen-independent progression of PCa. Early apoptosis signs were not detected in cells after p300-siRNA transfection (data not shown), indicating that the role of p300 in proliferation may involve alteration of the cell cycle. In this regard, p300 has been shown to interact with cell cycle-related proteins like the retinoblastoma protein (13) and cyclin B (14).

In summary, we showed that the coactivator p300 is associated with proliferation of PCa both *in vitro* and *in vivo*. Moreover, p300 expression on biopsy is a potential marker to predict aggressive features of PCa at the time of prostatectomy, as well as after surgical treatment. Considering the ubiquitous role of p300 in mediating the activity of a number of transcription factors, it will be important to examine its potential role in the progression of other hormonally driven tumors.

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