

***p300* (Histone Acetyltransferase) Biomarker Predicts Prostate Cancer Biochemical Recurrence and Correlates With Changes in Epithelia Nuclear Size and Shape**

Sumit Isharwal,¹ Michael C. Miller,² Cameron Marlow,¹ Danil V. Makarov,¹ Alan W. Partin,¹ and Robert W. Veltri^{1*}

¹The James Buchanan Brady Urological Institute, The Johns Hopkins University School of Medicine, Baltimore, Maryland
²Quakertown, Pennsylvania

BACKGROUND. *p300* impacts the transcription of several genes involved in key pathways critical to PCa progression. Therefore, we evaluated the prognostic value of *p300* expression and its correlation with nuclear alterations seen in tumor cells in men with long-term follow-up after radical prostatectomy (RP).

METHODS. NCI Cooperative Prostate Cancer Tissue Resource tissue microarray cores of 92 RP cases (56 non-recurrences and 36 PSA recurrences) were utilized for the study. *p300* expression was assessed by quantitative immunohistochemistry and nuclear alterations in Feulgen-stained nuclei were evaluated by digital image analysis using the AutoCyte™ Pathology Workstation. Cox proportional hazards regression, Spearman's rank correlation, and Kaplan–Meier plots were employed to analyze the data.

RESULTS. *p300* expression significantly correlated with nuclear alterations seen in tumor cells; specifically with circular form factor ($P = 0.012$) and minimum feret ($P = 0.048$). *p300* expression in high grade tumors (Gleason score ≥ 7) was significantly higher compared to low grade tumors (Gleason score < 7) [17.7% versus 13.7%, respectively, $P = 0.03$]. TNM stage, Gleason score, and *p300* expression were univariately significant in the prediction of PCa biochemical recurrence-free survival ($P \leq 0.05$). *p300* expression remained significant in the multivariate model ($P = 0.03$) while Gleason score showed a trend toward significance ($P = 0.06$). Patients with a Gleason score ≥ 7 and *p300* expression $> 24\%$ showed the highest risk for PCa biochemical recurrence ($P = 0.002$).

CONCLUSIONS. *p300* expression correlates with nuclear alterations seen in tumor cells and has prognostic value in predicting long-term PCa biochemical recurrence-free survival. *Prostate* © 2008 Wiley-Liss, Inc.

KEY WORDS: prostate cancer; biochemical recurrence; *p300* expression; prognosis

This article contains supplementary material, which may be viewed at The Prostate website at <http://www.interscience.wiley.com/jpages/0270-4137/suppmat/index.html>.

Sumit Isharwal's present address is Department of Urology, Johns Hopkins University, School of Medicine, 600 North Wolfe Street, Baltimore, MD 21287.

Michael C. Miller's present address is Covered Bridge Road 8032, Quakertown, PA.

Cameron Marlow's present address is Johns Hopkins University, School of Medicine, Department of Urology, 600 North Wolfe Street, Baltimore, MD 21287.

Danil V. Makarov's present address is Johns Hopkins University, SOM, Department of Urology, 600 North Wolfe Street, Baltimore, MD 21287.

Alan W. Partin's present address is Johns Hopkins University, SOM, Department of Urology, 600 North Wolfe Street, Baltimore, MD 21287.

*Correspondence to: Dr. Robert W. Veltri, PhD, Associate Professor, James Buchanan Brady Urological Institute, The Johns Hopkins University School of Medicine, Baltimore, MD 21287.
E-mail: rveltri1@jhmi.edu

Received 1 February 2008; Accepted 6 March 2008
DOI 10.1002/pros.20772

Published online in Wiley InterScience
(www.interscience.wiley.com).

INTRODUCTION

Prostate cancer (PCa) is the second leading cause of cancer death among men in the United States, with an anticipated 218,890 newly diagnosed cases and nearly 27,000 deaths in 2007 [1]. In a series of nearly 2,000 patients treated with radical prostatectomy at Johns Hopkins Hospital, 304 men developed PSA recurrence (15%) and were monitored without hormone therapy until demonstration of metastasis [2]. Of these men, 34% developed distant metastases over a median period of 8 years from the time of the first postoperative PSA elevation [2]. Han et al. [3] updated this study cohort, reporting 360 recurrences (17%) in 2,091 men with PCa. They used three preoperative or postoperative variables to create nomograms to assess biochemical recurrence-free survival probabilities. This study demonstrated the overall actuarial PSA-free survival probabilities at 5, 10, and 15 years to be 84%, 72% and 61%, respectively.

Clearly, the accumulation of repeated insults to the prostate over time through diet, infection, inflammation and aging results in a cascade of biological and molecular events which can result in malignancy. Therefore, PCa is a heterogeneous malignant disease where its' development and progression depends upon the biology of inflammation of the prostate as well as hereditary (genetic susceptibility), epigenetic and somatic gene defects. Many of these alterations are permanent and reflect transition to malignancy and progression to metastasis.

In the search for new molecular biomarkers to predict biochemical recurrence-free survival in men with PCa, several potential serologic and histological biomarkers have been evaluated [4–8]. At the tissue level, Gleason score and pathological stage are significant predictors of biochemical recurrence and metastasis [9,10]. Further, investigators have used nuclear structure alterations i.e. change in nuclear size, shape, DNA content and chromatin structure, to predict stage, biochemical recurrence and metastasis in men with PCa [11–15]. Recently, Seligson et al. [16] showed that the levels of acetylated histones correlate with increasing tumor grade and global histone modification pattern is able to identify disease subtypes with distinct risks of tumor recurrence in men with PCa.

There are numerous transcriptional coactivators involved in transcription and chromatin remodeling in androgen dependent and independent PCa. *p300*, a transcriptional coactivator that acetylates histones found in the nucleosome, has been shown to be differentially expressed in a number of tumors [17–19]. Debes et al. [20] demonstrated that *p300* is involved in the IL-6-mediated transactivation of the androgen receptor (AR) in the absence of androgens in

PCa cells. Others have shown a similar role of *p300* in the presence of androgens [21]. In addition, Debes et al. [22] showed that *p300* plays a key role in PCa epithelial cell proliferation.

The National Cancer Institute (NCI) engaged multiple institutions to prepare the Cooperative Prostate Cancer Tissue Resource (CPCTR) tissue microarrays (TMAs). We obtained TMAs from this resource that included tumor tissue from a unique patient cohort of 92 men with long-term follow-up to assess biochemical recurrence after surgical treatment for PCa. Using the TMAs from this patient cohort, we recently demonstrated the ability of nuclear morphometry determined by digital image analysis to predict biochemical recurrence with an AUC-ROC of 80% compared to pathology with an AUC-ROC of 67% [23]. Using the same patient cohort, we asked if expression levels of *p300*, which acetylates core histone residues, could predict biochemical recurrence-free survival in men with PCa. We also evaluated the association between *p300* expression, nuclear structure alterations, Gleason score and pathologic stage.

MATERIALS AND METHODS

Prostate Tissue Specimens Dataset

The CPCTR-TMA is the result of a project funded by NCI RFA released in April, 2000 and four academic institutions [George Washington University Medical Center (Washington DC); Medical College of Wisconsin (Milwaukee, WI); New York University School of Medicine (New York, NY); and the University of Pittsburgh (Pittsburgh, PA)] were funded to form a national prostate cancer tissue resource, CPCTR. The resource is entirely funded by an individual Cooperative Agreement Grant from the NCI to each of the four participating sites [24,25]. The CPCTR resource functions as a "virtual tissue bank" with a central database with all four participating sites working jointly with the NCI. Additionally, the methods for TMA construction employ a standardized protocol, a database containing standardized common data elements, and a supporting bioinformatics database with outcome results are also provided in a manuscript [26]. Information about the NCI-CPCTR project and how to obtain these bioreagents can be found on the web at <http://cpctr.cancer.gov>.

NCI-CPCTR Patient Cohort

Pathological material from a total of 299 PCa chronologically consecutive radical prostatectomy patients were arrayed over four blocks with a single focus of tumor from each patient tumor represented in duplicate 0.6 mm core spots. For determination of PSA

recurrence, an algorithm was defined where the PSA values needed to increase >0.4 ng/dl (single value) or a PSA values >0.2 ng/dl with additional subsequent increasing values [27]. The date of initial PSA rise (either the date of the single value >0.4 ng/ml or the date of the PSA value >0.2 ng/ml, before subsequent rising PSA values) was subtracted from the date of initial PSA nadir to determine the months to PSA recurrence. A total of 92 PCa cases ($n=56$ non-recurrence and $n=36$ recurrence) contained complete information for the study (Table I).

Measurement of p300 Protein Expression

Immunohistochemistry for p300 expression in PCa was performed on formalin-fixed paraffin biopsy sections using a DAKO AutoStainer. After dewaxing and dehydration, sections were placed in a rice steamer with citrate buffer (pH 6.0) for 20 min. The 6 μ m sections were pretreated with 0.3% hydrogen peroxide for 10 min, washed with deionized water and phosphate buffer (PBS, pH 7.4), and incubated with 0.5% Triton X-100 and 0.5% milk in PBS for 5 min at room temperature. The DAKO EnvisionPlus IHC kit was used for immunostaining. Briefly, the sections were blocked with 5% milk in PBS containing 0.1% Triton X-100 for 20 min and then incubated with the specific antibody for this protein (Santa Cruz Biotechnology, Santa Cruz, CA) at pre-determined dilutions with PBS containing 0.5% milk and 0.1% Triton X-100 at

room temperature for 1 hr in a humidified chamber. After washing, the sections were sequentially incubated with biotinylated Envision secondary antibody, streptavidin–HRP, and freshly prepared DAB chromogen substrate. The p300 immunohistochemistry (IHC) stained tissues were counterstained with hematoxylin for 1 min and mounted (supplementary Fig. 1). The stained TMAs were scanned with a BLISS virtual slide scanner [Bacus Laboratories, Lombard, IL] at 40 \times magnification using the WebSlide[®] digital microscope slide format. This creates a database input file that lists information on every CPCTR-TMA core and provides an automatic link to the WebSlide[®] Net Viewer ActiveX Control (Bacus Labs, Lombard, IL) for a visual TMA core database. These BLISS virtual slide images were processed using a TMA score software program [Bacus Laboratories, Lombard, IL] that quantified p300 expression by measuring percentage of tumor area positive for the p300 antigen in each PCa case.

Measurement of Nuclear Alterations

Using ~ 5 - μ m sections prepared from the TMA blocks, Feulgen DNA-staining was performed per the manufacturer's instructions (TriPath Imaging Inc., Burlington, NC). Next, a minimum of 125 intact, Feulgen-stained cancer nuclei were captured from the 0.6 mm spots for each case using an AutoCyte Pathology Workstation (APW) [TriPath Imaging Inc., Burlington, NC] and the QUIC-DNA software

TABLE I. Prostate Cancer Patients Demographics

Variable Description	No Biochemical Recurrence (N = 56)	Biochemical Recurrence (N = 36)	P value
Median age in years (range)	65.5 (47–76)	64 (42–77)	0.274 ^a
Pathologic stage (%)			
T2a	9 (16.1)	2 (5.6)	
T2b	32 (57.1)	17 (47.2)	0.010 ^b
T3a	13 (23.2)	9 (25.0)	0.028 ^c
T3b	2 (3.6)	8 (22.2)	
Gleason score (%)			
5	6 (10.7)	0 (0)	
6	21 (37.5)	10 (27.8)	0.024 ^b
7	27 (48.2)	23 (63.9)	0.106 ^c
8	1 (1.8)	2 (5.6)	
9	1 (1.8)	1 (2.8)	
Race (%)			
White	51 (91.1)	32 (88.9)	0.574 ^b
Black	1 (1.8)	3 (8.3)	0.505 ^c
Others	3 (5.3)	1 (2.8)	
Unknown	1 (1.8)	0 (0)	

^aMedian test.

^bWilcoxon ranksum test.

^cFisher's exact test.

[11,12,28]. The QUIC-DNA software calculated a total of 40 nuclear alterations [listed in Ref. [28]], including nuclear size, shape, DNA content and chromatin texture features (at a step size of one pixel), for each nuclei captured. For each case, the variance of each nuclear alteration was determined, thereby reducing the complexity of the nuclear alteration database to a single set of 40 variables for each case.

Statistical Methods

All data were analyzed using Stata™ v10.0 statistical analysis software (Stata Corporation, College Station, TX). A non-parametric *k*-sample chi-squared test for equality of medians was used to evaluate differences in the non-normally distributed ages. Wilcoxon's ranksum test was used to test for distribution differences and Fisher's exact test was used to test for differences in proportions between patients with and without biochemical recurrence. Correlations of *p300* expression with Gleason score, pathologic stage and nuclear alterations were evaluated using Spearman's rank correlation coefficients. Univariate Cox proportional hazards regression was used to identify significant prognostic factors for PCa biochemical recurrence. Ties were handled by the Breslow method, and the proportional hazard assumption was verified by examination of residual plots. We determined optimal cut-point for dichotomized *p300* expression data using classification and regression tree analysis. Kaplan–Meier survival plots were created to demonstrate the ability of the *p300* expression, pathologic stage and Gleason score to predict PSA recurrence-free survival. Univariately significant variables were further considered in multivariate model. Statistical significance in this study was set as $P \leq 0.050$.

RESULTS

The demographic and pathologic information for the biochemical (PSA) recurrence and non-recurrence groups of PCa patients are shown in Table I. This table shows that patients with biochemical recurrence tended to have higher Gleason scores and higher pathologic stages. The mean *p300* expression levels (% area positive for *p300* immunostaining) in the biochemical (PSA) recurrence and non-recurrence groups of men were $18.69 \pm 9.03\%$ and $14.40 \pm 6.53\%$, respectively ($P = 0.009$).

The *p300* protein expression was significantly higher in high grade tumors (Gleason score ≥ 7 : $17.70 \pm 7.50\%$) compared to low grade tumors (Gleason score < 7 : $13.67 \pm 7.83\%$) ($P = 0.03$). The mean *p300* expression in pathologic stage T2 and T3 patients was $15.48 \pm 7.16\%$ and $17.20 \pm 9.01\%$, respectively ($P = 0.43$). We observed significant associations between *p300* protein expres-

sion and nuclear alterations seen in tumor cells in these CPCTR-TMA radical prostatectomy tissue samples. Of particular interest, it was noted that the circular form factor ($\rho = -0.26$; $P = 0.012$) and minimum feret ($\rho = 0.21$; $P = 0.048$) exhibited statistically significant correlations with *p300* protein expression. An assessment of other nuclear features, such as area ($\rho = 0.16$; $P = 0.12$), excess of gray value ($\rho = 0.17$; $P = 0.10$) and standard deviation of gray value ($\rho = -0.17$; $P = 0.10$), showed a trend toward statistical significance for correlation with *p300* expression levels.

Gleason score showed significant correlation with several nuclear alterations seen in the tumor cells, including skewness of OD ($\rho = 0.24$; $P = 0.0212$), excess of OD ($\rho = 0.22$; $P = 0.0337$), DNA ploidy ($\rho = 0.25$; $P = 0.0155$), variance ($\rho = -0.20$; $P = 0.049$), sum average-AC ($\rho = -0.23$; $P = 0.0280$), sum variance-AC ($\rho = -0.31$; $P = 0.0023$), cluster shade ($\rho = -0.28$; $P = 0.0064$) and second diagonal moment ($\rho = -0.27$; $P = 0.0084$). The pathologic stage also showed significant correlation with several nuclear alterations seen in tumor cells including skewness of gray value ($\rho = 0.22$; $P = 0.0323$), DNA ploidy ($\rho = 0.28$; $P = 0.0078$), variance ($\rho = -0.22$; $P = 0.0397$), cluster shade ($\rho = -0.23$; $P = 0.0249$), and second diagonal moment ($\rho = -0.21$; $P = 0.0440$).

Upon univariate analyses, *p300* expression as a continuous variable was a significant prognosticator ($P = 0.021$) for PCa biochemical recurrence. A dichotomized population for *p300* expression was then defined with an optimal cutoff of 24% (85th percentile), specifically patients were categorized as having either low ($\leq 24.0\%$) or high ($> 24.0\%$) *p300* expression. Dichotomized pathologic stage, Gleason score and *p300* expression were univariately significant (Table II) for prediction of biochemical recurrence. However, when these three variables were considered together in a multivariate Cox proportional hazards model, only *p300* expression was significant (Table II). Figure 1A–C shows Kaplan–Meier survival curves for prediction of PCa biochemical recurrence-free survival using pathologic stage, Gleason score, and *p300* expression, respectively.

Additionally, we stratified the NCI-CPCTR patients based upon Gleason score and *p300* expression status. Table II and Figure 1D show the ability of Gleason score and *p300* expression status combined to predict PCa biochemical recurrence-free survival. Because there were only four patients with a Gleason score < 7 and high *p300* expression, this subcategory was merged with cases having Gleason score ≥ 7 and low *p300* expression for these analyses. Patients with Gleason score ≥ 7 and high *p300* protein expression had a significantly higher risk of PCa biochemical recurrence ($P = 0.002$) (Table II and Fig. 1D).

TABLE II. Cox Proportional Hazards Regression

Variable	N	Univariate		Multivariate		Stratification based upon Gleason score and p300 status	
		HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
TNM stage							
T2	60	1.00	0.034	1.00	0.170		
T3	32	2.03 (1.06–3.92)		1.60 (0.82–3.15)			
Gleason score							
<7	37	1.00	0.013	1.00	0.060		
≥7	55	2.54 (1.22–5.30)		2.07 (0.97–4.43)			
p300							
≤24%	78	1.00	0.013	1.00	0.032		
>24%	14	2.47 (1.21–5.03)		2.19 (1.07–4.47)			
Gleason p300							
1 ^a	33					1.00	
2 ^b	49					2.81 (1.19–6.66)	0.018
3 ^c	10					5.18 (1.88–14.30)	0.002

^aGleason score <7 and p300 ≤ 24%.

^bGleason score <7 and p300 > 24%/Gleason score ≥7 and p300 ≤ 24%.

^cGleason score ≥7 and p300 > 24%.

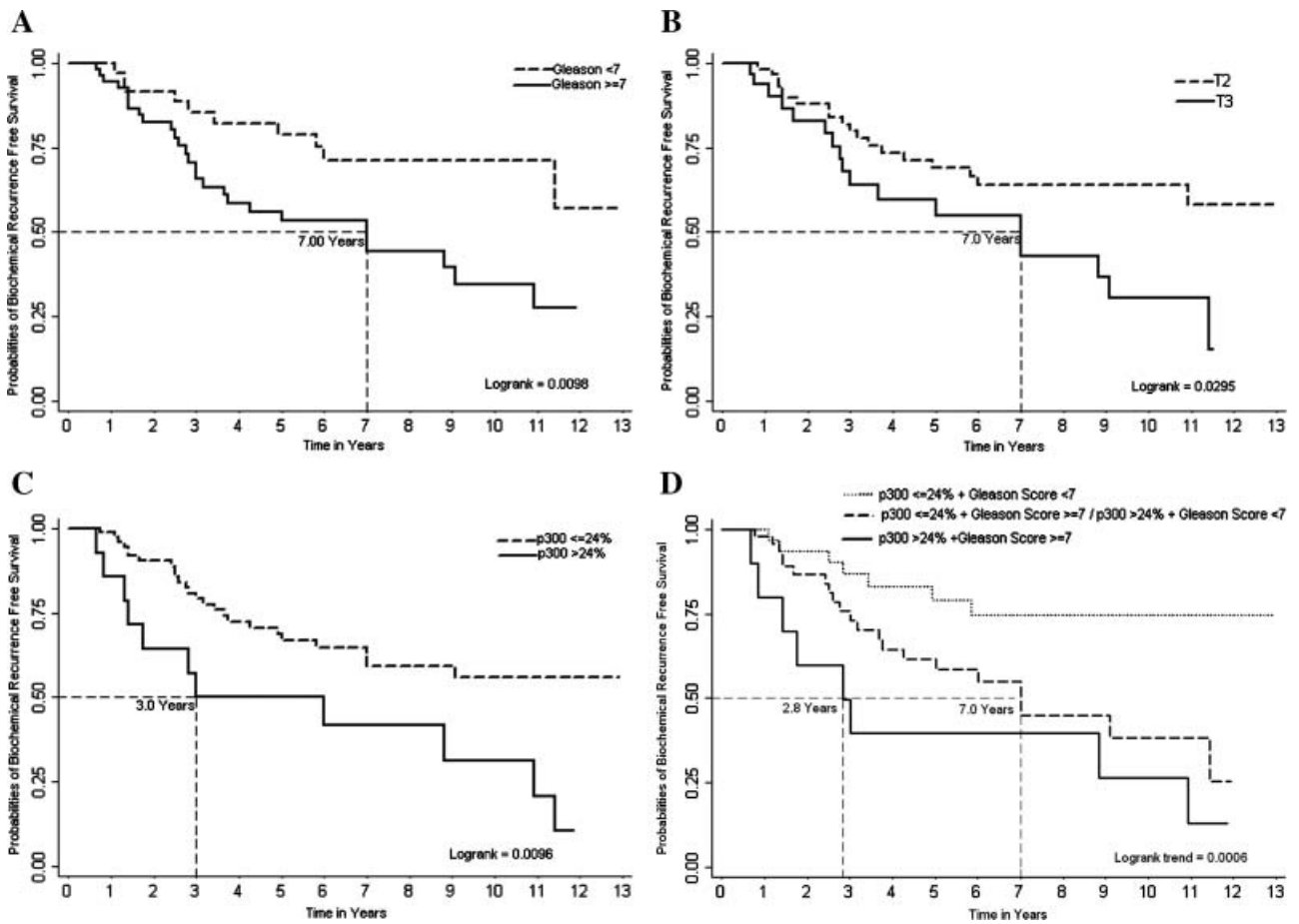


Fig. 1. Kaplan–Meier plots showing ability of Gleason score (A), pathological stage (B), p300 expression (C), and Gleason score and p300 combined (D) to predict biochemical recurrence-free survival. Logrank test and Logrank trend test were used to test equality of survivor functions across two groups and three ordered groups, respectively.

DISCUSSION

The nucleosome, i.e. the fundamental unit of chromatin organization, is composed of 146 base pairs of DNA wrapped in 1.65 turns around an octamer of the four core histones, H2A, H2B, H3, and H4 [29]. Chromatin remodeling directly influences the activity of DNA as it relates to transcription, replication, and recombination and is regulated by two highly conserved mechanisms, post-translational modifications of histone residues (e.g. acetylation, methylation) and ATP-dependent nucleosome position reorganization.

Seligson et al. [16] showed that PCa cells have global level modifications in individual histones and that altered patterns of these modifications are predictive of clinical outcome. Polycomb group protein EZH2 causes methylation of histone H3 lysine 9 and histone H3 lysine 27 and its overexpression is associated with poor prognosis [30–32]. The *p300*/CBP histone acetyltransferase (HAT) causes acetylation of all four core histone residues of the nucleosome. Hence, modifications of the nucleosome's net charge by neutralizing the positive charge of lysine ϵ -amino group alters DNA–histone interactions (cross-talk), which then modify transcriptional activity of the cell [33]. Also, other nucleosome assembly proteins functionally interact and augment the activity of *p300*/CBP, and the presence of core histones appears to regulate the interaction between *p300* and key nucleosome assembly proteins that establish various chromatin organization states, impacting nuclear structure (nuclear importins and Lamins A and C) and functions (i.e. cell proliferation, DNA repair, etc.) [34].

The *p300* HAT domain is essential for physiological processes of cell proliferation, differentiation and apoptosis [35–37]. Mammals lacking *p300* gene exhibit defects in neurulation, cell proliferation and heart development [38]. In addition to histone modifications, *p300*/CBP can acetylate and modify activity of several non-histone proteins [reviewed in Ref. [39]] including p53 [40,41], HMG I(Y) [42], HMG14 [43], GATA-1 [44,45], c-Myb [46], E2F-1 [47], EKLF [48], ACTR, TIF2, SRC-1 [49], Tat [50,51], TCF [52], TFIIE and TFIIF [53]. Further, *p300*/CBP depletion causes cyclin E down-regulation [17], which in association with CDK2, controls DNA replication, centrosome duplication and histone gene expression [54].

Additionally, *p300*/CBP is required for effective ligand-dependent gene activation by nuclear receptor [55]. The *p300* protein acetylates the androgen receptor (AR) at three lysine residues in its DNA-binding domain [21]. Point mutations in these AR acetylation sites selectively prevent androgen-induction of androgen-responsive genes, hampers coactivation of the

AR by SRC-1, p300, Tip60 and Ubc9, and results in a 10-fold increase in the binding of the co-repressor NCoR [56]. High levels of AR are associated with aggressive clinicopathologic parameters and decreased PCa recurrence-free survival [57]. Furthermore, IL-6 cytokine mediated transactivation of AR-dependent genes in the absence of androgens requires *p300* HAT activity, implicating *p300* in PCa progression [20].

The role of *p300* in PCa molecular pathogenesis is an important event that impacts transcription of several genes involved in key pathways critical to PCa recurrence and progression. Hence, our observation on the prognostic clinical value of *p300* protein expression and its potential role in transcription and effects on chromatin organization provide confirmation of results from other laboratories [16,20–22,37,38,43,55,58] and extend our understanding of its role in PCa progression.

In conclusion, *p300* expression in PCa tissue may be a useful biomarker for predicting progression and is one step in a series of finding additional tissue biomarkers that will improve early prognostic decisions on PCa patient management.

ACKNOWLEDGMENTS

Funding was provided by Johns Hopkins University Prostate Cancer Specialized Programs of Research Excellence (SPORE) [Grant number: P50CA58236], the Patana Fund and the Early Detection Research Network (EDRN) of the National Cancer Institute [Grant number: CA086323-06].

REFERENCES

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. *CA: Cancer J Clin* 2007;57(1):43–66.
2. Pound CR, Partin AW, Eisenberger MA, Chan DW, Pearson JD, Walsh PC. Natural history of progression after PSA elevation following radical prostatectomy. *J Am Med Assoc* 1999;281(17):1591–1597.
3. Han M, Partin AW, Zahurak M, Piantadosi S, Epstein JI, Walsh PC. Biochemical (prostate specific antigen) recurrence probability following radical prostatectomy for clinically localized prostate cancer. *J Urol* 2003;169(2):517–523.
4. DeMarzo AM, Nelson WG, Isaacs WB, Epstein JI. Pathological and molecular aspects of prostate cancer. *Lancet* 2003;361(9361):955–964.
5. Nelson WG, De Marzo AM, Isaacs WB. Prostate cancer. *New Engl J Med* 2003;349(4):366–381.
6. Rhodes DR, Sanda MG, Otte AP, Chinnaiyan AM, Rubin MA. Multiplex biomarker approach for determining risk of prostate-specific antigen-defined recurrence of prostate cancer. *J Natl Cancer Inst* 2003;95(9):661–668.
7. Tricoli JV, Schoenfeldt M, Conley BA. Detection of prostate cancer and predicting progression: current and future diagnostic markers. *Clin Cancer Res* 2004;10(12 Pt 1):3943–3953.

8. Veltri R. Molecular biology of serum biomarkers of prostate cancer. In: Kirby R, Partin A, Feneley M, Parsons J, editors. *Prostate cancer: Principles and practice*. London & New York: Taylor & Francis; 2006. p 269–284.
9. Epstein JI, Allsbrook WC Jr, Amin MB, Egevad LL. The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason grading of prostatic carcinoma. *The American journal of surgical pathology* 2005;29(9):1228–1242.
10. Gleason DF. Histologic grading of prostate cancer: A perspective. *Hum Pathol* 1992;23(3):273–279.
11. Veltri RW, Partin AW, Epstein JE, Marley GM, Miller CM, Singer DS, Patton KP, Criley SR, Coffey DS. Quantitative nuclear morphometry, Markovian texture descriptors, and DNA content captured on a CAS-200 Image analysis system, combined with PCNA and HER-2/neu immunohistochemistry for prediction of prostate cancer progression. *J Cell Biochem* 1994;19:249–258.
12. Veltri RW, Partin AW, Miller MC. Quantitative nuclear grade (QNG): A new image analysis-based biomarker of clinically relevant nuclear structure alterations. *J Cell Biochem* 2000;Suppl 35:151–157.
13. Veltri RW, O'Dowd GJ, Orozco R, Miller MC. The role of biopsy pathology, quantitative nuclear morphometry, and biomarkers in the preoperative prediction of prostate cancer staging and prognosis. *Semin Urol Oncol* 1998;16(3):106–117.
14. Mohler JL, Figlewicz WM, Zhang XZ, Partin AW, Maygarden SJ. Nuclear shape analysis for the assessment of local invasion and metastases in clinically localized prostate carcinoma. *Cancer* 1994;74(11):2996–3001.
15. Khan MA, Walsh PC, Miller MC, Bales WD, Epstein JI, Mangold LA, Partin AW, Veltri RW. Quantitative alterations in nuclear structure predict prostate carcinoma distant metastasis and death in men with biochemical recurrence after radical prostatectomy. *Cancer* 2003;98(12):2583–2591.
16. Seligson DB, Horvath S, Shi T, Yu H, Tze S, Grunstein M, Kurdastani SK. Global histone modification patterns predict risk of prostate cancer recurrence. *Nature* 2005;435(7046):1262–1266.
17. Bandyopadhyay D, Okan NA, Bales E, Nascimento L, Cole PA, Medrano EE. Down-regulation of p300/CBP histone acetyltransferase activates a senescence checkpoint in human melanocytes. *Cancer Res* 2002;62(21):6231–6239.
18. Muraoka M, Konishi M, Kikuchi-Yanoshita R, Tanaka K, Shitara N, Chong JM, Iwama T, Miyaki M. p300 gene alterations in colorectal and gastric carcinomas. *Oncogene* 1996;12(7):1565–1569.
19. Fan S, Ma YX, Wang C, Yuan RQ, Meng Q, Wang JA, Erdos M, Goldberg ID, Webb P, Kushner PJ, Pestell RG, Rosen EM. p300 Modulates the BRCA1 inhibition of estrogen receptor activity. *Cancer Res* 2002;62(1):141–151.
20. Debes JD, Schmidt LJ, Huang H, Tindall DJ. p300 mediates androgen-independent transactivation of the androgen receptor by interleukin 6. *Cancer Res* 2002;62(20):5632–5636.
21. Fu M, Wang C, Reutens AT, Wang J, Angeletti RH, Siconolfi-Baez L, Ogryzko V, Avantaggiati ML, Pestell RG. p300 and p300/cAMP-response element-binding protein-associated factor acetylate the androgen receptor at sites governing hormone-dependent transactivation. *J Biol Chem* 2000;275(27):20853–20860.
22. Debes JD, Sebo TJ, Lohse CM, Murphy LM, Haugen DA, Tindall DJ. p300 in prostate cancer proliferation and progression. *Cancer Res* 2003;63(22):7638–7640.
23. Veltri RW, Miller MC, Isharwal S, Marlow C, Makarov DV, Partin AW. Prediction of prostate-specific antigen recurrence in men with long-term follow-up postprostatectomy using quantitative nuclear morphometry. *Cancer Epidemiol Biomarkers Prev* 2008;17(1):102–110.
24. Berman JJ, Datta M, Kajdacsy-Balla A, Melamed J, Orenstein J, Dobbin K, Patel A, Dhir R, Becich MJ. The tissue microarray data exchange specification: implementation by the Cooperative Prostate Cancer Tissue Resource. *BMC Bioinform* 2004;5:19.
25. Melamed J, Datta MW, Becich MJ, Orenstein JM, Dhir R, Silver S, Fidelia-Lambert M, Kadajcsy-Balla A, Macias V, Patel A, Walden PD, Bosland MC, Berman JJ. The cooperative prostate cancer tissue resource: A specimen and data resource for cancer researchers. *Clin Cancer Res* 2004;10(14):4614–4621.
26. Patel AA, Kajdacsy-Balla A, Berman JJ, Bosland M, Datta MW, Dhir R, Gilbertson J, Melamed J, Orenstein J, Tai KF, Becich MJ. The development of common data elements for a multi-institute prostate cancer tissue bank: the Cooperative Prostate Cancer Tissue Resource (CPCTR) experience. *BMC Cancer* 2005;5:108.
27. Liao Z, Datta MW. A simple computer program for calculating PSA recurrence in prostate cancer patients. *BMC Urol* 2004;4:8.
28. Veltri RW, Marlow C, Khan MA, Miller MC, Epstein JI, Partin AW. Significant variations in nuclear structure occur between and within Gleason grading patterns 3, 4, and 5 determined by digital image analysis. *Prostate* 2007;67(11):1202–1210.
29. Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* 1997;389(6648):251–260.
30. Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones RS, Zhang Y. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science (New York, NY)* 2002;298(5595):1039–1043.
31. Czermin B, Melfi R, McCabe D, Seitz V, Imhof A, Pirrotta V. Drosophila enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. *Cell* 2002;111(2):185–196.
32. Varambally S, Dhanasekaran SM, Zhou M, Barrette TR, Kumar-Sinha C, Sanda MG, Ghosh D, Pienta KJ, Sewalt RG, Otte AP, Rubin MA, Chinnaiyan AM. The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature* 2002;419(6907):624–629.
33. Vo N, Goodman RH. CREB-binding protein and p300 in transcriptional regulation. *J Biol Chem* 2001;276(17):13505–13508.
34. Shikama N, Chan HM, Krstic-Demonacos M, Smith L, Lee CW, Cairns W, La Thangue NB. Functional interaction between nucleosome assembly proteins and p300/CREB-binding protein family coactivators. *Mol Cell Biol* 2000;20(23):8933–8943.
35. Goodman RH, Smolik S. CBP/p300 in cell growth, transformation, and development. *Genes Dev* 2000;14(13):1553–1577.
36. Giordano A, Avantaggiati ML. p300 and CBP: Partners for life and death. *J Cell Physiol* 1999;181(2):218–230.
37. Ait-Si-Ali S, Poleskaya A, Filleur S, Ferreira R, Duquet A, Robin P, Vervish A, Trouche D, Cabon F, Harel-Bellan A. CBP/p300 histone acetyltransferase activity is important for the G1/S transition. *Oncogene* 2000;19(20):2430–2437.
38. Yao TP, Oh SP, Fuchs M, Zhou ND, Ch'ng LE, Newsome D, Bronson RT, Li E, Livingston DM, Eckner R. Gene dosage-dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300. *Cell* 1998;93(3):361–372.
39. Sterner DE, Berger SL. Acetylation of histones and transcription-related factors. *Microbiol Mol Biol Rev* 2000;64(2):435–459.
40. Liu L, Scolnick DM, Trievel RC, Zhang HB, Marmorstein R, Halazonetis TD, Berger SL. p53 sites acetylated in vitro by PCAF

- and p300 are acetylated in vivo in response to DNA damage. *Mol Cell Biol* 1999;19(2):1202–1209.
41. Gu W, Roeder RG. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell* 1997;90(4):595–606.
 42. Munshi N, Merika M, Yie J, Senger K, Chen G, Thanos D. Acetylation of HMG I(Y) by CBP turns off IFN beta expression by disrupting the enhanceosome. *Mol Cell* 1998;2(4):457–467.
 43. Bergel M, Herrera JE, Thatcher BJ, Prymakowska-Bosak M, Vassilev A, Nakatani Y, Martin B, Bustin M. Acetylation of novel sites in the nucleosomal binding domain of chromosomal protein HMG-14 by p300 alters its interaction with nucleosomes. *J Biol Chem* 2000;275(15):11514–11520.
 44. Hung HL, Lau J, Kim AY, Weiss MJ, Blobel GA. CREB-Binding protein acetylates hematopoietic transcription factor GATA-1 at functionally important sites. *Mol Cell Biol* 1999;19(5):3496–3505.
 45. Boyes J, Byfield P, Nakatani Y, Ogryzko V. Regulation of activity of the transcription factor GATA-1 by acetylation. *Nature* 1998;396(6711):594–598.
 46. Tomita A, Towatari M, Tsuzuki S, Hayakawa F, Kosugi H, Tamai K, Miyazaki T, Kinoshita T, Saito H. c-Myb acetylation at the carboxyl-terminal conserved domain by transcriptional co-activator p300. *Oncogene* 2000;19(3):444–451.
 47. Marzio G, Wagener C, Gutierrez MI, Cartwright P, Helin K, Giacca M. E2F family members are differentially regulated by reversible acetylation. *J Biol Chem* 2000;275(15):10887–10892.
 48. Zhang W, Bieker JJ. Acetylation and modulation of erythroid Kruppel-like factor (EKLF) activity by interaction with histone acetyltransferases. *Proc Natl Acad Sci USA* 1998;95(17):9855–9860.
 49. Chen H, Lin RJ, Xie W, Wilpitz D, Evans RM. Regulation of hormone-induced histone hyperacetylation and gene activation via acetylation of an acetylase. *Cell* 1999;98(5):675–686.
 50. Kiernan RE, Vanhulle C, Schiltz L, Adam E, Xiao H, Maudoux F, Calomme C, Burny A, Nakatani Y, Jeang KT, Benkirane M, Van Lint C. HIV-1 tat transcriptional activity is regulated by acetylation. *EMBO J* 1999;18(21):6106–6118.
 51. Ott M, Schnolzer M, Garnica J, Fischle W, Emiliani S, Rackwitz HR, Verdin E. Acetylation of the HIV-1 Tat protein by p300 is important for its transcriptional activity. *Curr Biol* 1999;9(24):1489–1492.
 52. Waltzer L, Bienz M. Drosophila CBP represses the transcription factor TCF to antagonize Wingless signalling. *Nature* 1998;395(6701):521–525.
 53. Imhof A, Yang XJ, Ogryzko VV, Nakatani Y, Wolffe AP, Ge H. Acetylation of general transcription factors by histone acetyltransferases. *Curr Biol* 1997;7(9):689–692.
 54. Ewen ME. Where the cell cycle and histones meet. *Genes Dev* 2000;14(18):2265–2270.
 55. Chakravarti D, LaMorte VJ, Nelson MC, Nakajima T, Schulman IG, Juguilon H, Montminy M, Evans RM. Role of CBP/P300 in nuclear receptor signalling. *Nature* 1996;383(6595):99–103.
 56. Fu M, Rao M, Wang C, Sakamaki T, Wang J, Di Vizio D, Zhang X, Albanese C, Balk S, Chang C, Fan S, Rosen E, Palvimo JJ, Janne OA, Muratoglu S, Avantaggiati ML, Pestell RG. Acetylation of androgen receptor enhances coactivator binding and promotes prostate cancer cell growth. *Mol Cell Biol* 2003;23(23):8563–8575.
 57. Li R, Wheeler T, Dai H, Frolov A, Thompson T, Ayala G. High level of androgen receptor is associated with aggressive clinicopathologic features and decreased biochemical recurrence-free survival in prostate: cancer patients treated with radical prostatectomy. *Am J Surg Pathol*. 2004;28(7):928–934.
 58. Yao TP, Ku G, Zhou N, Scully R, Livingston DM. The nuclear hormone receptor coactivator SRC-1 is a specific target of p300. *Proc Natl Acad Sci USA* 1996;93(20):10626–10631.