p300 Modulates Nuclear Morphology in Prostate Cancer

Jose D. Debes,¹,² Thomas J. Sebo,³ Hannelore V. Heemers,¹ Benjamin R. Kipp,³ De Anna L. Haugen,³ Christine M. Lohse,⁴ and Donald J. Tindall¹,²

Departments of ¹Urology and ²Biochemistry/Molecular Biology, ³Laboratory of Medicine/Pathology, and ⁴Health Sciences Research, Mayo Clinic College of Medicine, Rochester, Minnesota

Abstract

Alterations in nuclear structure distinguish cancer cells from noncancer cells. These nuclear alterations can be translated into quantifiable features by digital image analysis in a process known as quantitative nuclear morphometry. Recently, quantitative nuclear morphometry has been shown to predict metastasis and biochemical recurrence of prostate cancer. However, little is known about the cellular mechanisms underlying these nuclear morphometric changes. Alterations of nuclear matrix proteins are frequently involved in changes of nuclear structure. A number of co-activators interact with these nuclear structure--related proteins, suggesting that they might be involved in quantitative nuclear morphometry changes. We have shown previously that the transcriptional co-activator p300 is involved in prostate cancer progression. However, the ability of a transcriptional regulator like p300 to modulate nuclear morphology has not been described previously. In the present study, we show that p300 expression in prostate cancer biopsy tissue from 95 patients correlates with quantifiable nuclear alterations. Moreover, we show that transfection of p300 into prostate cancer cells in culture induces quantifiable nuclear alterations, such as diameter, perimeter, and absorbance among others, as assessed by digital image analysis. These alterations correlate individually with aggressive features in prostate cancer, such as expression of the proliferation marker Ki-67 and extraprostatic extension of the tumor. Finally, we found that transfection of p300 into prostate cancer cells specifically increases mRNA and protein levels of nuclear matrix peptides lamin A and C, suggesting that these proteins mediate the p300--induced effects. These findings reveal a new insight into the transcriptional and structural regulation of prostate cancer. (Cancer Res 2005; 65(3): 708-12)

Introduction

Cancer cells are notably distinguished from noncancer cells by alterations in the nuclear structure (1). In particular, changes in nuclear size, shape, and the nuclear-to-cytoplasmic ratio are common features of cancer (2). These changes are thought to reflect the dynamic mobility of cancer cells. Most of these nuclear features can be translated into quantifiable measures by digital image analysis (DIA), a method that utilizes the ability of a microscope to capture nuclei in a digital form for analysis (3). This process is known as quantitative nuclear morphometry. Nuclear morphometry has been used as a tool to predict progression of different types of cancer, thus supplementing diagnostic and prognostic information in clinical and experimental oncology (3). Recently, quantitative nuclear morphometry has been shown to predict metastasis and biochemical recurrence of prostate cancer (4, 5). Although these quantitative nuclear alterations are useful to assess cancer progression, little is known regarding the rationale for their clinical utility, as well as the mechanisms involved in the alteration of nuclear morphometry. Alterations in nuclear structure frequently involve deregulation of nuclear matrix proteins (2). Several nuclear matrix proteins, such as lamins, PC-1, and NMP149, show differential expression patterns in cancer tissue (6, 7). Indeed, lamins A and C (lamin A/C), which are part of the network of filaments that comprise the nuclear lamina, have been shown to interact with the hypophosphorylated form of Rb, a protein deregulated in many cancers (8, 9).

Only a few transcriptional co-activators are known to interact with actin-binding and nuclear structure--related proteins, suggesting that co-activators are involved in the modulation of nuclear structure (2). We have shown previously that the transcriptional co-activator p300 is involved in prostate cancer proliferation and that p300 expression in prostate biopsies correlates with progression of tumors following surgical treatment (10, 11). p300 is a ubiquitous co-activator that has been shown to play a variety of roles in the transcription process depending on the model or system. p300 can serve as a bridge between the general transcription factors and the basal machinery or can act as a scaffold to bring specific transcription factors together with general transcription factors. Also, p300 induces histone acetylation through its histone acetyltransferase activity, thus altering chromatin structure (12). The ability of transcriptional regulators with chromatin-altering properties, like p300, to directly modulate or influence nuclear structure has not been described previously.

In this study, we show that protein expression of p300 correlates with quantifiable nuclear alterations in tissue sections of prostate cancer biopsies. Moreover, we show that transfection of p300 into prostate cancer cells in culture induces quantifiable nuclear alterations. These alterations are of clinical importance and correlate with more aggressive prostate cancer. Finally, we show that levels of the nuclear matrix peptides lamin A/C increase upon p300 transfection, suggesting that the nuclear alterations occur at least in part through regulation of these proteins. This study shows for the first time a direct modulation of nuclear structure by a transcriptional co-activator in prostate cancer, revealing a new insight in the transcriptional and structural regulation of cancer.

Materials and Methods

Study Population. Ninety-five patients were randomly selected from a sample of 454 patients with biopsy-proven prostate cancer. Additional details regarding the sample of 454 patients are described elsewhere (13).
digital image analysis. DNA content and quantifiable nuclear morphometry were determined using CAS 200 image analyzer (Bacus Laboratories, Lombard, IL) with a 6-μm-thick section from paraffin-embedded needle biopsy tissue blocks containing cancer. All images were captured with a 40× lens. The results were reviewed by the study pathologist (T.J.S.). Sections cut for nuclear DNA and morphometry were stained with Feulgen dye. To assess quantitative nuclear morphometry, digitized nuclear images from each case were retrieved and analyzed using the Cell Sheet software program of CAS 200. Over 16,000 nuclei representing 95 cancer specimens were evaluated for specific nuclear features.

**Tissue Section 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 (C-20, Santa Cruz Biotechnology, Santa Cruz, CA) and quantified by DIA using CAS 200 image analyzer as described previously (11). All samples were graded visually in a blind fashion by the study pathologist (T.J.S.).

**Cell Culture.** C4-2 cells were purchased from American Type Culture Collection (Rockville, MD) and maintained in RPMI 1640 (Celoz, St. Paul, MN) containing 9% fetal bovine serum (BioSource International, Camarillo, CA), and transfected with p300 (pLipofectamin: ref. C14), or empty vector (pDNR3.1, Promega, Madison, WI). Twenty-four hours after transfection, cells received fresh media containing 9% fetal bovine serum. Transfection efficiency was monitored by co-transfection with pGFP (2 μg/10 mm plate, Promega). Routinely, transfection efficiencies of ~40% were obtained. Forty-eight hours after transfection, cells were collected by cyto-spin onto positively charged glass slides. Feulgen staining and DIA analysis were done as described for prostate cancer biopsy tissue sections. An average of 200 nuclei was analyzed per group of cells.

**Real-Time PCR.** Twenty-four hours after transfection with p300 (as described above), RNA was isolated from cells using TRZol (Invitrogen, Carlsbad, CA). A two-step real-time PCR was done using cDNA prepared from RNA using a First Strand cDNA Synthesis kit (Roche, Indianapolis, IN) and a SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) on an ABI PRISM 7700 SDS instrument following the manufacturer’s instructions. PCR products (100–150 bp) were electrophoresed in 1.2% agarose gels to check for nonspecific amplification. The fold change in expression levels (using GAPDH as control) was determined by a comparative Ct method using the formula 2^ΔΔCt, where Ct is the threshold cycle of amplification.

**Conventional PCR.** RNA was isolated and cDNA prepared as described above. cDNA was amplified for up to 30 cycles with the GC-RICH PCR System (Gene Therapy Systems, Inc., San Diego, CA) with primers containing 9% fetal bovine serum. Transfection efficiency was monitored by co-transfection with pGFP (2 μg/10 mm plate, Promega). Routinely, transfection efficiencies of ~40% were obtained. Forty-eight hours after transfection, cells were collected by cyto-spin onto positively charged glass slides. Feulgen staining and DIA analysis were done as described for prostate cancer biopsy tissue sections. An average of 200 nuclei was analyzed per group of cells.

**Western Blot Analysis.** Forty-eight hours after transfection, cells were lysed for protein extraction. Protein extraction reagents were purchased from Pierce (Rockford, IL). Nuclear and cytoplasmic extracts were prepared according to manufacturer’s instructions. Proteins were loaded onto a NuPage 10% Bis-Tris gel (Invitrogen), and Western blot analysis was done using specific antibody to lamin A/C (clone 14, Upstate Biotechnology, Lake Placid, NY). Immunodetection was done with ECL Plus (Amersham Pharmacia Biotech, Little Chalfont, United Kingdom). Immunodetection of extracellular signal-regulated kinase 2 (D-2 SCBT, Santa Cruz Biotechnology) was used as control for equal protein loading.

**Statistical Methods.** The associations of p300 expression with clinical, pathologic, and nuclear morphometry features and the associations of MB-1 (Ki-67) with nuclear morphometry features were summarized with adjusted R^2 from linear regression models. The associations of extraprostatic extension with nuclear morphometry features were summarized with odds ratios from logistic regression models. These associations were done using the SAS software package (SAS Institute, Cary, NC). In tissue culture cells, the values of the Nuclear Grade program were recorded into an electronic database and analyzed with SPSS statistical software (version 11, SPSS, Inc., Chicago, IL). The nonparametric Mann-Whitney U test was used to compare the medians of the selected nuclear features among the transfected and nontransfected control groups from each run. Values are statistically significant at P < 0.05.

**Results**

Quantifiable nuclear features of prostate cancer cells have been shown to correlate with prostate cancer progression. However, the mechanisms involved in this process have not been elucidated. We have shown previously that the transcriptional co-activator p300 correlates with prostate cancer progression following prostatectomy. To determine whether p300 expression correlates with quantifiable nuclear alterations, we evaluated 95 patients with biopsy-proven prostate cancer. Definitions of the most important nuclear features are found in Supplementary data. We found significant associations between p300 expression and several nuclear alterations in prostate cancer biopsy tissue samples (Table 1). Of particular interest, area (P = 0.018), perimeter (P = 0.032), minimum diameter (P = 0.016), Feret Y (P = 0.018), DNA mass (P = 0.006), and DNA index (P = 0.002) exhibited statistically significant correlations with p300 expression. Alterations of these features are common manifestations of neoplasia and are suggestive of a more aggressive cancer phenotype. In addition, we found that p300 correlates with the amount of tumor at biopsy (%cores and %surface positive for cancer). There was no correlation between p300 expression and Gleason score, a grading system based on the architectural growth of the malignant glands. This finding suggests that p300 correlates with morphologic variations of nuclei that are independent of alterations in the gland architecture (Gleason pattern).

| Table 1. Association of p300 with clinical, biopsy, and DIA features |
|--------------------------|----------------------|-------|
| DIA features             | Mean (range)         | P*    |
| DNA index                | 1.2 (0.83-1.86)      | 0.002 |
| DNA mass                 | 8.4 (5.75-13.34)     | 0.006 |
| Minimum diameter         | 6.7 (5.42-8.65)      | 0.016 |
| Area                     | 48.5 (28.3-79.3)     | 0.018 |
| Feret Y                  | 8.6 (6.79-11.6)      | 0.018 |
| Summed optical density   | 104.1 (68.9-167.4)   | 0.019 |
| Perimeter                | 27.1 (20.1-35.4)     | 0.032 |
| Maximum diameter         | 9.8 (7.29-12.69)     | 0.066 |
| Feret X                  | 8.9 (6.72-11.1)      | 0.121 |
| Elongation               | 1.4 (1.35-1.68)      | 0.184 |
| Shape                    | 14.3 (13.3-15.3)     | 0.444 |
| Density                  | 0.18 (0.13-0.25)     | 0.844 |
| Average optical density  | 0.4 (0.28-0.58)      | 0.890 |
| Pathological features    |                      |       |
| %Cores positive for cancer| 43.9 (11.1-100.0)   | <0.001 |
| %Surface area positive for cancer | 15.7 (10.0-65.0) | <0.001 |
| Gleason score            | 6.5 (4-9)            | 0.138 |
| Clinical features        |                      |       |
| Age                      | 64 (43-76)           | 0.593 |
| Preoperative prostate-specific antigen | 9.1 (0.7-0.502) | 0.006 |

*p for association of p300 with each feature listed.
To investigate if this association of p300 with nuclear alterations in prostate cancer biopsy tissue sections is due to an effect of the co-activator on the structure of prostate cancer cells, we transfected a construct containing wild-type p300 or empty vector into the prostate cancer cell line C4-2. After transfection, cells were stained with Feulgen dye for analysis by DIA to assess nuclear alterations. Expression of p300 resulted in increases in area, perimeter, Ferets X/Y, and minimum/maximum diameter in prostate cancer cells (Fig. 1A and B). In addition, expression of p300 resulted in decreases in density and average absorbance (Fig 1B). This suggests that the nuclear alterations found in prostate cancer biopsies that correlate with p300 expression might be a consequence of p300 overexpression. Moreover, nuclear features, such as density and average absorbance, which showed no significant correlations with p300 expression in prostate cancer biopsy tissue sections, did not increase following p300 transfection into cells grown in tissue culture (Fig 1B). This suggests that the nuclear alterations found in prostate cancer biopsies that correlate with p300 expression might be a consequence of p300 overexpression.

We therefore evaluated these specific nuclear features in the complete database of 454 patients with prostate cancer. We found that these features (perimeter, area, minimum/maximum diameter, and Ferets X/Y) correlated independently with the in situ proliferation marker Ki-67 (MIB-1) on biopsy (Table 2). Thus, alterations of these features correlate with a higher proliferation rate of prostate cancer. Moreover, we found that the same nuclear alterations on biopsy correlated significantly with extraprostatic extension of prostate cancer at the time of surgery, indicating a more aggressive cancer phenotype in patients with changes of nuclear area, perimeter, Ferets X/Y, and minimum/maximum diameter (Table 2).

Figure 1. p300 induces nuclear morphometry changes in prostate cancer cells. A, nuclear features that increased upon p300 transfection into C4-2 cells. Color lines, specified feature. Numbers, quantitative measure. Upper panel, cells transfected with empty vector; lower panel, cells transfected with p300. B, three consecutive nuclear morphometry analyses performed on C4-2 cells. Cells were transfected with 5 µg wild-type p300 or empty vector. Forty-eight hours later, cells were harvested and collected via cyto-centrifugation onto charged slides and analyzed by DIA as described in Materials and Methods. The following features are shown: area, perimeter, shape, density, average optical density (Aver. O.D.), summed optical density, Ferets X/Y, maximum diameter (Max. Diam.), and minimum diameter (Min. Diam.). Green, statistically significant increase of the feature in p300 transfected cells compared to empty vector; red, statistically significant decrease of the feature; gray, nonsignificant alteration of the feature.
factors, including the above-mentioned proteins. Through real-time and semiquantitative PCR, we found that mRNA levels of the nuclear matrix peptide lamin A/C were up-regulated following p300 transfection (Fig. 2A). Moreover, nuclear expression of lamin A/C protein was increased in C4-2 cells transfected with p300 (Fig. 2B). These findings suggest that p300 targets lamin A/C to induce nuclear changes in prostate cancer cells. When we assessed the expression of other nuclear matrix proteins (i.e., actin, laminin, and lamina-associated peptide 2), we found no alteration, indicating specificity in the p300 regulation of lamin A/C (data not shown).

Discussion

Morphologic changes in the structure of cells, primarily the nuclei, are characteristic features of cancer cells. In recent years, quantification of these nuclear features has been used to assess progression of different cancers (5, 15, 16). However, the mechanisms underlying these nuclear alterations and their relation to transcription are not clear. Recently, studies have shown that alterations in nuclear structure can predict progression and metastasis in prostate cancer (5). Here, we show that the transcriptional co-activator p300 is involved in the regulation of this process. Furthermore, our data suggest that lamin A/C may mediate these effects.

p300 is a ubiquitous protein that plays a role in cancer progression. Previously, we have shown that p300 protein expression in biopsies correlates with a more aggressive phenotype and with progression of prostate cancer. Moreover, we have shown that p300 is involved in prostate cancer cell proliferation (11). In this study, we show that p300 protein expression in prostate cancer biopsies correlates with alterations in nuclear morphometry. Several of these alterations are considered characteristic of cancer cells. Most of the features we measured are those visible to the naked eye. Moreover, several nuclear features that relate to chromatin texture showed a statistical correlation with p300 expression. Some clinical parameters (e.g., serum prostate-specific antigen) were also associated with p300 expression. Serum prostate-specific antigen is a widely accepted prognostic marker of prostate cancer progression and is used in nomograms for purposes of guiding patient therapy and management (17, 18). Therefore, these results suggest an association of p300 with a more aggressive prostate cancer phenotype.

Nuclear grading systems have been developed to predict cancer features or to assess response to chemopreventive agents (19, 20). Nuclear morphometry has been shown to be an important tool to predict progression as well as biochemical recurrence in prostate cancer (3). The quantitative nuclear grade includes features such as size and shape, DNA content, and chromatin structure. Thus, co-activators with histone-modifying properties like p300 have the potential to induce alterations in chromatin that might lead to changes in nuclear morphometry. We found that several nuclear morphometric features were altered in prostate cancer cells transfected with p300. Many of these changes occurred on those features related to chromatin texture. However, we focused on those features that are visible to the eye and are altered in tumorigenic cells. We cannot rule out variability in nuclear morphology due to expression of exogenous DNA. Changes in nuclear area, perimeter, and diameter are frequent events in cancer cells. Whether any of these features are associated with a more aggressive cancer phenotype has not been shown until now. We found that each of these features correlated with Ki-67 expression in prostate cancer tissue. This finding is important because it suggests a higher proliferation rate in these tumors. Moreover, each of these features

**Table 2. Associations of DIA features with MIB-1 and extraprostatic extension (EPE)**

<table>
<thead>
<tr>
<th>DIA feature</th>
<th>P for association with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIB-1</td>
</tr>
<tr>
<td>Perimeter</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Area</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Minimum diameter</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maximum diameter</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Feret X</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Feret Y</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Density</td>
<td>0.436</td>
</tr>
</tbody>
</table>

Figure 2. p300 induces specific up-regulation of lamin A/C expression. A, quantitative (top) and semiquantitative (bottom) PCR using specific primers for lamin A/C were performed from cDNA of C4-2 cells 36 h following transfection with 5 μg p300 or empty vector. In quantitative PCR, bar graph is normalized to GAPDH control amplification. B, nuclear and cytoplasmic fractions of protein lysates from C4-2 cells transfected with p300 or empty vector were analyzed by Western blot using a specific antibody to lamin A/C. Extracellular signal-regulated kinase 2 protein expression was used as loading control. These experiments were repeated at least three times.
independently predicted extraprostatic extension of the tumor at the time of surgery, indicating a more aggressive phenotype. Therefore, these data indicate that the nuclear morphology changes induced by p300 in prostate cancer cells are of clinical importance and correlate with aggressive prostate cancer.

Changes in nuclear morphology potentially involve deregulation of nuclear matrix proteins (1, 2). Several nuclear matrix proteins have been shown to be involved in cancer pathogenesis and progression (2, 21, 22). Indeed, expression of lamin A has been found altered in undifferentiated neoplastic cells (23). We show that alteration of nuclear morphology by p300 involves up-regulation of lamin A/C. This is a novel finding because it has not been shown before that a co-activator, such as p300, could regulate a nuclear matrix protein like lamin A/C. Interestingly, although mRNA levels of lamin A/C were up-regulated, only the nuclear fraction of the protein showed higher levels following p300 transfection. Moreover, the size of lamin A/C protein in the nuclear fraction was slightly different from that in the cytoplasmic fraction, suggesting different isoforms in these compartments. Whether these different forms affect the protein function or activity remains to be investigated.

In conclusion, our findings show for the first time a direct regulation of quantifiable nuclear features by a transcriptional co-activator. This regulation occurred in cell culture and correlates with data from prostate cancer biopsy tissue sections. We show that regulation of nuclear lamin A/C seems to be involved. The mechanisms by which p300 induce these nuclear changes warrants further investigation.

Acknowledgments


Grant support: NIH grants DK60920, DK65236, CA91956, and CA70892; T. J. Martell Foundation; Belgian American Educational Foundation fellowship (H.V. Heerens).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Dr. Joan Boyes for providing plasmids (p300), Dr. Martin Fernandez-Zapico for helpful discussion, and Kristin Raclaw for help in experimental design.

References

p300 Modulates Nuclear Morphology in Prostate Cancer

Jose D. Debes, Thomas J. Sebo, Hannelore V. Heemers, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/65/3/708

Cited articles
This article cites 23 articles, 8 of which you can access for free at:
http://cancerres.aacrjournals.org/content/65/3/708.full#ref-list-1

Citing articles
This article has been cited by 4 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/65/3/708.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.