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Nuclear Matrix Protein Patterns in Human Benign Prostatic Hyperplasia and Prostate Cancer

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Abstract

The nuclear matrix represents the structural component of the nucleus that determines nuclear shape and higher order DNA organization. We have previously shown tissue specificity in nuclear matrix proteins (NMP), in rat sex accessory tissues, and in a rat model of prostate cancer. This study compares NMP patterns for fresh human normal prostate, benign prostatic hyperplasia (BPH), and prostate cancer for 21 men undergoing surgery for clinically localized prostate cancer or BPH. NMP patterns were compared using high resolution two-dimensional polyacrylamide gel electrophoresis. We identified by molecular weight and isoelectric point 14 different proteins that were consistently present or absent among the various tissues. One protein (PC-1), a $M_r$ 56,000 protein with an isoelectric point of 6.58, appeared in 14 of 14 different nuclear matrix preparations from prostate cancer and was not detected in normal prostate (0 of 13) or BPH (0 of 14). The NMP patterns are consistent with a model of disease progression in which BPH shares many of the nuclear matrix changes observed in prostate cancer.

Introduction

The nuclear matrix consists of the insoluble structural framework of the nucleus that includes the nuclear lamina and pore complex, an internal ribonucleoprotein network, and the residual nucleolus (1-16). Nuclear matrix protein patterns analyzed with two-dimensional gel electrophoresis were first reported by Fey and Penman (7) to be cell type specific in human cultured breast carcinoma cell lines (7). Subsequently, we have shown nuclear matrix protein patterns to have tissue specificity in rat sex-accessory tissues (8) and characteristic differences among a series of rat prostate cancer cell lines and tumors (9). Others have shown relationships between nuclear matrix proteins and matrix-associated proteins during differentiation (13, 14), transcription (15), and transformation (16). This study reports differences in the nuclear matrix protein patterns among normal prostate, BPH, and prostate cancer from fresh human tissue collected from 21 men undergoing surgery for either clinically localized prostate cancer or BPH. Fourteen different nuclear matrix protein changes are identified by molecular weight and isoelectric point that are specific for BPH and prostate cancer.

Materials and Methods

Patients. Fresh prostatic tissue was studied from 21 men undergoing radical retropubic prostatectomy for clinically localized (Stage B, T2) prostate cancer ($n = 19$) (Gleason Grade 5-9) or open prostatectomy for BPH ($n = 2$).

Tissue Preparation. Fresh tissue was obtained within 15 min of surgical removal. Approximately 1 g of gross tumor was taken from a palpable tumor nodule from 14 specimens. One g of normal prostate tissue was obtained from the prostatic lobe contralateral to the tumor nodule in 13 specimens. One g of BPH tissue was obtained from the perirethral region of the contralateral lobe in 12 specimens and 25-30 g from each of the 2 open prostatectomy specimens. All tissues removed were histologically confirmed with hematoxylin and eosin sections on both the proximal and distal ends of the section.

Purification of Nuclear Matrix Proteins. Nuclear matrix proteins were isolated according to the methodology of Fey and Penman (7). Briefly, fresh human prostate tissue was minced into small (1-mm$^3$) pieces and homogenized with a Teflon pestle on ice with 0.5% Triton X-100 in a solution containing 2 mM vanadyl ribonucleoside (RNase inhibitor) containing 1 mM phenylmethylsulfonyl fluoride (serine protease inhibitor) to release the lipids and soluble proteins. Extracts were then filtered through a 350 $\mu$m nylon mesh and extracted with 0.25 M ammonium sulfate to release the soluble cytoskeletal elements. DNase treatment at 25°C is used to remove the soluble chromatin. The remaining fraction contains intermediate filaments and nuclear matrix proteins. This fraction is then disassembled with 8 M urea, and the insoluble components, which consist principally of carbohydrates and extracellulur matrix components, are pelleted. The urea is dialyzed out, and the intermediate filaments are allowed to reassemble and are removed by centrifugation. The nuclear matrix proteins are then ethanol precipitated. Protein concentrations were determined with the Coomassie Plus protein assay reagent kit (Pierce, Rockford, IL) with bovine serum albumin as a standard. For preparation for gel electrophoresis, the nuclear matrix proteins were redissolved in a sample buffer consisting of 9 M urea, 65 mM 3-[(3-cholamidopropyl)dimethylamino]-1-propanesulfonate, 2.2% amylolysates, and 140 mM dithiothreitol.

Two-Dimensional Electrophoresis. High-resolution two-dimensional gel electrophoresis was carried out utilizing the Investigator 2-D gel system (Milligen/Biosearch, Bedford, MA) (17). One-dimensional isoelectric focusing was carried out for 18,000 V-h using 1-mm × 18-cm tube gels after 1.5 h of prefocusing. The tube gels were extruded and placed on top of 1-mm precast 10% Tris-acetate-sodium dodecyl sulfate Duracryl (Millipore, Bedford, MA) high tensile strength polycrylamide electrophoresis slab gels, and the gels were electrophoresed with 12°C constant temperature regulation for approximately 5 h. Gels were fixed with 50% methanol and 10% acetic acid. After thorough rinsing and rebhydration, gels were treated with 5% glutaraldehyde and 5% dithiothreitol after buffering with 50 mM phosphate (pH 7.2). Gels were stained with silver stain using the methodology of Wray et al. (18) (Accurate Chemical Co., Inc., Westbury, NY). Fifty $\mu$g of nuclear matrix protein were loaded for each gel. Protein molecular weight standards were determined with the Coomassie Plus protein molecular weight marker kit ($M_r$ 12, 400-97, 400) (Pierce). Isoelectric points were determined using carbamylated creatine kinase standards (pI 7.0-4.95), (BDH Limited, Poole, England). Only protein spots clearly and reproducibly observed or absent in all samples from the various tissues were considered when determining variations in nuclear matrix proteins between tissues. Quantitative differences in protein composition from the different tissues, while noted, are not elaborated on in this text.

Results

The nuclear matrix protein patterns for fresh human normal prostate, BPH, and prostatic adenocarcinoma were compared using high resolution two-dimensional electrophoresis. There were approximately 150 different and identifiable protein spots seen on each gel from the various types of fresh prostate tissue. There was a high degree of similarity between nuclear matrix protein patterns from patient to patient regardless of type of prostate tissue examined with only 10-15 protein spots that varied in an inconsistent manner among various patients, and these protein spots were specifically marked and

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2 The abbreviation used is: BPH, benign prostatic hyperplasia.
NUCLEAR MATRIX PROTEINS IN HUMAN PROSTATE

Fig. 1. Nuclear matrix protein composition of normal human prostate (A), benign prostatic hyperplasia (B), and prostate cancer (C). High-resolution two-dimensional gel electrophoresis of nuclear matrix protein preparations isolated and analyzed as described in “Materials and Methods.” Arrowhead (A), variable group of proteins that were inconsistently present on various types of tissue. Arrows, proteins that are consistently changed in all tissues and are identified by molecular weights and isoelectric point in Table 1. LA, lamin A; LB, lamin B; LC, lamin C; A, actin; NP, normal prostate; NPB, normal prostate and BPH; BPC, BPH and prostate cancer; PC, prostate cancer; kD, molecular weight in thousands; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Table 1 Nuclear matrix proteins from fresh normal prostate, BPH, and prostate cancer tissue which were identified by two-dimensional gel electrophoresis

<table>
<thead>
<tr>
<th>Protein</th>
<th>M, kD</th>
<th>pl</th>
<th>Normal (n = 13)</th>
<th>BPH (n = 14)</th>
<th>Cancer (n = 14)</th>
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<tr>
<td>NPB-1*</td>
<td>17,000</td>
<td>6.91</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>NPB-2</td>
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</tr>
<tr>
<td>NPB-4</td>
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<td>+</td>
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<tr>
<td>NPB-5</td>
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<tr>
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</tr>
<tr>
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<tr>
<td>NP-1</td>
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<tr>
<td>NP-2</td>
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<tr>
<td>PC-1</td>
<td>56,000</td>
<td>6.58</td>
<td>-</td>
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<td>+</td>
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* NP, normal prostate; B, BPH; PC, prostate cancer.

Fig. 2. Specific nuclear matrix proteins in BPH and prostate cancer. Schematic of major tissue specific nuclear matrix proteins of normal prostate, BPH, and prostate cancer. kD, molecular weight in thousands; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

The designation of each protein corresponds to the identified proteins in Fig. 1. Molecular weights and isoelectric points were identified as stated in “Materials and Methods.”

Table 1 demonstrates the molecular weight and isoelectric points of the 14 different protein spots found to be consistently present or absent when comparing nuclear matrix proteins from normal prostate, BPH, and prostate cancer tissue for this group of 21 patients. Fig. 1 demonstrates the typical high-resolution two-dimensional gel electrophoresis patterns for nuclear matrix proteins isolated from normal human prostate (Fig. 1A), human BPH (Fig. 1B), and human prostate cancer (Fig. 1C). Gel spots differing between normal prostate, BPH, and prostate cancer (in all specimens examined) have been marked with arrows and identified with labels corresponding to those in Table 1. Fig. 2 summarizes the location of the protein spots that differed among the various tissues.

We found no nuclear matrix proteins that were present only in BPH but were absent in normal prostate and prostate cancer. Likewise, we observed no nuclear matrix proteins that were present in both normal prostate and prostate cancer but were absent in BPH. PC-1 (M, 56,000...
and isoelectric point 6.58) represents a nuclear matrix protein seen only in human prostate cancer tissue and was consistently absent in all normal prostate and BPH samples. Table 1 summarizes the two-dimensional gel electrophoresis results for PC-1 observed for all of the patients studied.

In summary, there was marked similarity seen in the nuclear matrix protein patterns between patients with approximately 120 of 150 proteins spots consistently seen from patient to patient. Fourteen nuclear matrix proteins were identified that were consistently present or absent when comparing normal prostate BPH and prostate cancer. A Mr 56,000 protein (PC-1) represents a nuclear matrix protein that appeared in all (14 of 14) human prostate cancer specimens studied but was not detected in any normal prostate (0 of 13) or BPH tissue (0 of 14).

Discussion

In this work, we describe the characterization of nuclear matrix protein patterns for fresh human normal prostate, BPH and prostate cancer for 21 men undergoing surgery for either clinically localized prostate cancer or BPH. Fourteen different nuclear matrix proteins were identified by molecular weight and isoelectric point that were specific for the various regions of the prostate and prostate cancer. PC-1 (Mr 56,000 and isoelectric point 6.58) represents a nuclear matrix protein that appears in 14 of 14 prostate cancer nuclear matrix preparations and no (0 of 13) normal prostate or (0 of 14) BPH nuclear matrix protein preparations.

Although the precise molecular and/or environmental events necessary for the development of prostatic disease are largely unknown, it has been well established that the development of prostate cancer is a multistep process (19). Epidemiological studies based on the original work of Ashley (20) and Armitage and Doll (21), using age specific incidence rates for prostate cancer and BPH in the United States, demonstrate that development of BPH is most likely a two-step process while the development of clinically evident prostate cancer most likely involves a multistep (greater than 2-event) process (19).

Whether or not BPH cells found in the periurethral region of the prostate undergo early events similar to those of cancer cells found in the periphery of the prostate is unknown. Two different models can be postulated for the progression of a normal prostatic epithelial cell to either BPH or prostate cancer. The first model [Model I (Fig. 3)] predicts that the early events for progression from either normal to BPH or normal to prostate cancer are similar (events A–B in Model I). The second model [Model II (Fig. 3)] predicts that progression for BPH and cancer would undergo different events (events A–B versus events E–H). Using the presence of absence of nuclear matrix proteins as a phenotypic marker to test these models, it would be predicted that Model I would be satisfied if a specific group of protein spots were either absent or present in both BPH and prostate cancer (NP 1–3, BPC 1–3) and additional protein spots were present or absent in only prostate cancer (NPB 1–7 or PC-1). Thus all of the differences we observed in the nuclear matrix proteins satisfied Model I. In order to satisfy Model II, a protein(s) need be present or absent in BPH only, and this was not observed in any samples. Thus these data support Model I in which similar phenotypic expressions are occurring in the nuclear matrix of cells progressing to BPH as those cells progressing to prostate cancer.

In summary, we have identified the nuclear matrix proteins in fresh human normal prostate, BPH, and prostate cancer with two-dimensional polyacrylamide gel electrophoresis and demonstrated 14 different protein spots that consistently differ between the various tissues for the 21 patients studied. One protein, PC-1, represents a Mr 56,000 protein with an isoelectric point of 6.58 that was found in 14 of 14 prostate cancer nuclear matrix preparations and not in BPH or normal prostate. PC-1 is a candidate nuclear matrix marker for prostate cancer.

References

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