ELSEVIER

NUCLEAR SHAPE AND NUCLEAR MATRIX PROTEIN COMPOSITION IN PROSTATE AND SEMINAL VESICLES

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ABSTRACT

Objectives. The nucleus controls cell function and behavior. The nuclear matrix determines internal nuclear changes. Two-dimensional gel electrophoresis is the reference standard for the analysis of nuclear matrix protein (NMP) composition. Differences in NMP composition should therefore be reflected by changes in nuclear shape. We investigated the differences in NMP composition and nuclear morphometry of the prostate and seminal vesicles. Both tissues are androgen-dependent sex accessory organs with completely different biologic behavior.

Methods. High-resolution two-dimensional gel electrophoresis and silver staining were used to evaluate NMP composition from histologically normal prostate and seminal vesicle epithelial cells. Nuclear morphometry, performed using a computer-assisted image analysis system, described the distribution, variability, and extremes of nuclear shape.

Results. NMP composition analysis demonstrated that both tissues have a similar NMP composition, and tissue-specific NMPs that were consistently present in all specimens of each tissue could not be demonstrated. Nuclear morphometry showed a significantly greater heterogeneity in nuclear shape in the seminal vesicles than in the prostate.

Conclusions. The striking similarity of the NMP composition demonstrates the close biologic relationship between prostate and seminal vesicle tissue. The similar NMP composition does not correlate with the marked alterations in nuclear shape and structure between these tissues. Therefore, nuclear morphometry may depict differences in the functional state of a similar set of NMPs, shown by two-dimensional gel electrophoresis, which may be responsible for the different biologic behavior of these tissues.

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The nucleus is the most important site for regulation of cellular function and behavior. The nuclear matrix regulates nuclear shape and function.[1] The nuclear matrix is the insoluble skeletal framework of the nucleus.[2] It plays an important role in DNA replication, transcription,[3] and tissue-specific patterns of DNA organization.[4] Thus, changes in nuclear shape and function are thought to be a result of the internal nuclear structural changes, which are determined by the nuclear matrix.[5] A number of nuclear matrix proteins (NMPs) seem to be common to all cell types within a species, yet a smaller set of NMPs are said to be specific for each tissue and state of differentiation.[6] Furthermore, NMP composition in normal cells and cancer cells of the prostate has been reported to differ.[7] The standard tool for the analysis of the nuclear matrix is two-dimensional gel analysis.[5] Changes in nuclear function should result in changes in NMP composition.

Since the replication and organization of DNA in the cell nucleus are closely involved in the regulation of cell structure and function and because the nuclear matrix and nuclear shape changes should mirror the functional and structural state of the nucleus, we compared the results of two-dimensional gel electrophoresis of NMPs with the nuclear morphometry.

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Diamond and associates[8] were the first to develop a computerized image analysis system for the evaluation of nuclear shape. Additional improvements in the computerized digitization system led to a reproducibility and accuracy of nuclear shape measurements of more than 90%.[9–11]

Prostate and seminal vesicles both belong to the male sex accessory organs. Both tissues are androgen dependent and share the same blood supply, innervation, and exposure to carcinogens.[12] Despite these similarities, the growth behavior of the prostate and seminal vesicles is vastly different. Although benign prostatic hyperplasia is the most frequent cause for surgical procedures in men in the United States, hyperplasia of the seminal vesicles is rarely reported. Moreover, prostate cancer is the most common noncutaneous cancer in men today.[13] In contrast, less than 60 cases[14–16] of adenocarcinoma of the seminal vesicles have been described. Thus, comparison of the two-dimensional gel electrophoresis results and the nuclear morphometry of these tissues should demonstrate whether the analysis of nuclear shape and NMP composition can reveal differences in nuclear function in these closely related organs with completely different biologic behavior.

MATERIAL AND METHODS

Nuclear Morphometry

Patients. Paraffin-embedded histologic sections were taken from histologically normal seminal vesicles of 10 men who underwent radical prostatectomy for localized prostate cancer (n = 8) or radical cystectomy for infiltrating bladder cancer (n = 2) without any previous hormonal treatment or radiation. Ten specimens from prostates without histologic evidence of cancer were evaluated.

Morphometric Measurements. Hematoxylin-eosin-stained histologic slides were reviewed. In all specimens, the presence of tumor was excluded by a pathologist (J.I.E.). In all prostate and seminal vesicle specimens, the luminal epithelial cells were analyzed. A total of 100 epithelial nuclei per specimen was chosen and analyzed by a random standardized morphometric method, as previously described.[9,10] The histologic sections were studied using a Zeiss microscope (Carl Zeiss, Thornwood, NY) at a total magnification of ×2440. For image analysis, the DynaCELL Motility Morphometry Measurement workstation (JAW Associates, Annapolis, Md) was used.

Morphometric Shape Descriptors. Fifteen shape descriptors were calculated for each of the digitized nuclei. Measurements were performed using the DynaCELL system. A detailed description of the different factors has been previously published.[17]

Statistical Analyses. A detailed description of the statistical methods used has been previously published.[17] Statistical analysis to determine whether individual shape descriptors differed between prostate and seminal vesicle samples was done using the Mann-Whitney-Wilcoxon (2 sample) rank sum test for the analysis of the separation of the two groups. The Mann-Whitney-Wilcoxon test proved significant when the *P* value was less than 0.05.

NMP Analysis

Patients. Samples of histologically confirmed normal prostate tissue and seminal vesicles from 10 men undergoing radical cystectomy, radical prostatectomy for low-volume localized prostate cancer, or open prostatectomy for benign disease were studied.

Tissue Preparation. Fresh tissue was obtained within 15 minutes of surgical removal and was either processed immediately or shock frozen and stored at −70°C until the final analysis. Approximately 0.5 g of tissue per specimen was used. All specimens were histologically confirmed with hematoxylin-eosin sections from their proximal and distal ends.

Preparation of NMPs. NMPs were isolated according to the method of Fey and Penman.[18] In brief, the tissue was minced into small pieces (~1 mm^3) and homogenized in a solution containing 0.5% Triton X-100 and 2 mM vanadyl ribonucleoside (RNase inhibitor) to release the soluble proteins and lipids. Extracts were filtered and extracted with 0.25 M ammonium sulfate to release the soluble cytoskeletal elements. DNase I and RNase A treatment at 25°C was used to remove RNA and the soluble chromatin. The remaining fraction was disassembled with 8 M urea, and the insoluble components were pelleted. The urea was removed by dialysis, and the intermediate filaments were removed by centrifuge. All solutions contained freshly prepared 1 mM phenylmethylsulfonyl fluoride as a protease inhibitor. The NMPs were then precipitated with ethanol, and the protein concentrations were determined using the Coomassie Plus protein assay reagent kit (Pierce, Rockford, Ill). Before gel electrophoresis, the NMPs were dissolved in a sample buffer containing 9 M urea, 65 mM 3-[(3-cholamidopropyl) dimethylammino]-1-propanesulfonate, 2.2% ampholytes, and 140 mM dithiothreitol.

Two-Dimensional Electrophoresis. High-resolution two-dimensional gel electrophoresis was performed using the Investigator 2-D gel system (Milligen/Biosearch, Bedford, Mass).[19] Each specimen was examined separately. Fifty micrograms of NMP was loaded for each gel. One-dimensional isoelectric focusing was carried out for 18,000 volt-hours using 1-mm × 18-cm acrylamide tube gels. The tube gels were then placed on top of precast 1-mm, 10% Tris-acetate sodium dodecyl sulfate Duracryl, high-tensile strength polyacrylamide electrophoresis slab gels (ESA, Chelmsford, Mass). The slab gels were electrophoresed with 12°C constant temperature regulation for approximately 5 hours. Gels were fixed with 50% methanol and 10% acetic acid. After thorough rinsing and rehydration, gels were treated with 5% glutaraldehyde and 5 mM dithiothreitol after buffering with 50 mM phosphate (pH 7.2). Gels were stained with silver stain using the method of Wray *et al.*[20] (Accurate Chemical, Westbury, NY). Protein molecular standards were determined with the Prestained Protein Molecular Weight Standards (M_r 14,300 to 200,000) (Gibco BRL, Gaithersburg, Md). Isoelectric points were determined using the two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis standards (pI 4.5 to 8.5) (Biorad, Richmond, Calif). High-resolution two-dimensional gel electrophoresis was performed at least twice on each specimen.

Evaluation of NMP Compositions. The silver-stained gels were scanned and digitized using previously described techniques.[21] For the scanning, we used the Bio Image 2D Analyzer software (Bio Image, Ann Arbor, Mich) on a UNIX-based workstation (Sun Microsystems, Mountain View, Calif). Using the two-dimensional gel data base GELLAB II software of the National Cancer Institute,[21] gel images were segmented. The images were then compared by aligning putative corresponding subregions of two rapidly alternating images (flickering) across the Internet using the World Wide Web.[22] This technique allowed a more precise comparison of the gels than visual comparison.[22]

TABLE I. *Comparison of different shape descriptors for prostate and seminal vesicle epithelial cells*

Shape Descriptor	Prostate	Seminal Vesicle	*P* Value of Means
Area	41.20 ± 10.70 (13.60–83.60)	43.20 ± 13.80 (12.60–158.00)	0.0005
Roundness	1.04 ± 0.03 (1.00–1.37)	1.07 ± 0.05 (1.01–1.31)	0.00001
Perimeter	23.50 ± 3.15 (14.10–36.70)	24.60 ± 3.91 (13.00–49.90)	0.0001
Form factor	1.09 ± 0.74 (1.01–1.88)	1.15 ± 0.11 (1.02–1.71)	0.00001
Ellipticity (feret)	1.04 ± 0.05 (1.00–1.85)	1.06 ± 0.05 (1.00–1.36)	0.00001
Ellipticity (inertia)	1.06 ± 0.05 (1.01–1.86)	1.09 ± 0.06 (1.01–1.63)	0.00001
Ellipticity (Fourier)	1.06 ± 0.05 (1.01–1.86)	1.09 ± 0.06 (1.01–1.44)	0.00001
Convexity	0.43 ± 0.68 (0.00–8.69)	1.06 ± 1.85 (0.00–22.30)	0.00001
Bending energy	0.87 ± 0.13 (0.61–2.45)	0.85 ± 0.13 (0.57–1.55)	0.023
Chain code range	0.10 ± 0.03 (0.03–0.25)	0.13 ± 0.04 (0.04–0.33)	0.00001
Chain code sum of squares	0.08 ± 0.05 (0.01–0.32)	0.13 ± 0.09 (0.01–0.7)	0.00001
Chain code maximum	0.05 ± 0.02 (0.01–0.19)	0.07 ± 0.03 (0.01–0.26)	0.00001
Chain code standard deviation	0.03 ± 0.01 (0.01–0.05)	0.03 ± 0.01 (0.01–0.09)	0.00001
Chain code variance ($\times 10^{-3}$)	0.84 ± 0.53 (0.01–3.40)	1.30 ± 0.90 (0.10–6.40)	0.00001
Suboptimal circle fit	18.40 ± 7.15 (3.66–42.90}	22.73 ± 8.30 (5.20–52.50)	0.00001

Data presented as the mean ± SD, with the range in parentheses, unless otherwise noted.

RESULTS

Nuclear Morphometry

Shape Descriptor Analysis. A total of 17 different statistical tests were used to evaluate the distribution, variability, and extremes of each of the 15 shape descriptors calculated for each of the 100 nuclei measured in each specimen. Of the 272 statistical tests, 104 showed significant differences ($P < 0.05$) between the prostate and seminal vesicle specimens.

Description of Nuclear Shape in Seminal Vesicles and Prostate. The mean form factor, ellipticity factor (calculated by the feret-diameter method, the moment of inertia method, and the Fourier method), suboptimal circle fit, convexity, roundness, perimeter, chain code calculations, and area were significantly higher in seminal vesicle cells than in normal prostate epithelial cells (Table I). Furthermore, the standard deviation for most parameters (Table I) was higher in the seminal vesicle cells than in the prostate cells, indicating a tendency toward a higher variance of the nuclear shape descriptors in seminal vesicle epithelial cells. All shape descriptors describe the degree of deviation of the nuclear shape from an ideal circle or an ideal ellipsoid; thus, normal seminal vesicle epithelial cells seem to have a broader variety of nuclear shape than do prostate cell nuclei.

NMP Patterns

Typical examples of the NMP patterns of the examined normal seminal vesicle and prostate tissue are depicted in Figures 1 and 2. Surprisingly, there was no constant NMP pattern within the seminal vesicle tissues. Visual comparison and computer-assisted analysis (flickering) of the NMP composition of these tissues revealed differences within the seminal vesicle gels. Although most of the NMPs were detected in most of the seminal vesicle gels, not all NMPs were constantly expressed in all seminal vesicle gels. Thus, although there were some proteins found that were mainly expressed either in the prostate or the seminal vesicles, there was no single protein that was constantly present in one tissue and constantly absent in the other tissue. To ensure reproducibility of the NMP patterns, two electrophoresis gels were run on every specimen. No major differences were found within the two gels of an individual specimen. The group of prostate gels also did not reveal intraindividual differences, but, in accordance with the seminal vesicle gels, not all NMPs were constantly expressed in all prostate gels, although most of the NMPs were detected in most of the prostate gels.

COMMENT

The nucleus plays a crucial structural, as well as functional, role in normal cellular function. It is involved in DNA organization, DNA replication, RNA synthesis, and nuclear regulation.[1] As nuclear shape is said to be, at least in part, controlled by the nuclear matrix,[2] we combined the morphometric measurement of nuclear shape and NMP composition to investigate the differences in cellular shape and matrix protein composition, which may reflect differences in nuclear function between the pros-

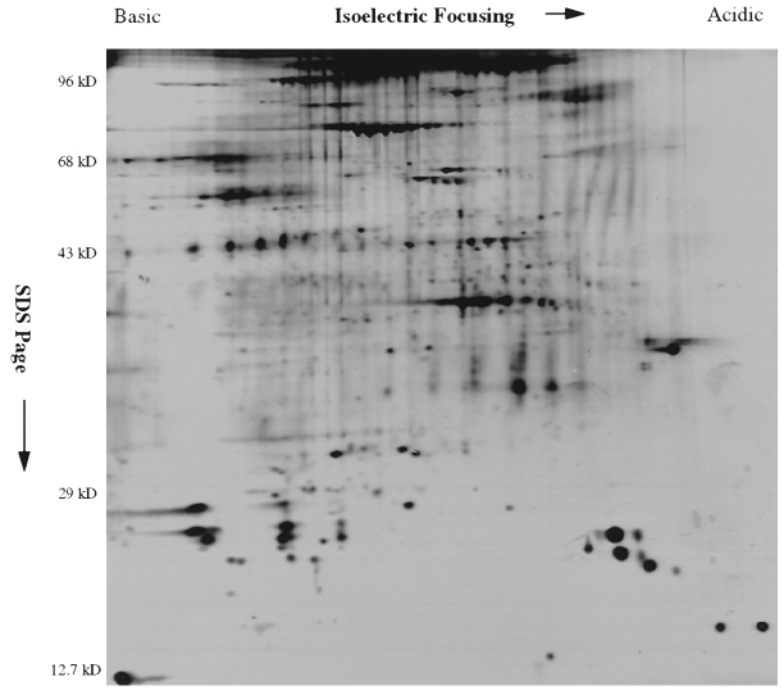

FIGURE 1. *NMP composition of normal seminal vesicle tissue. High-resolution two-dimensional gel electrophoresis of nuclear matrix preparations from human seminal vesicles. kD = molecular weight in kilodaltons; SDS Page = sodium dodecyl sulfate-polyacrylamide gel electrophoresis.*

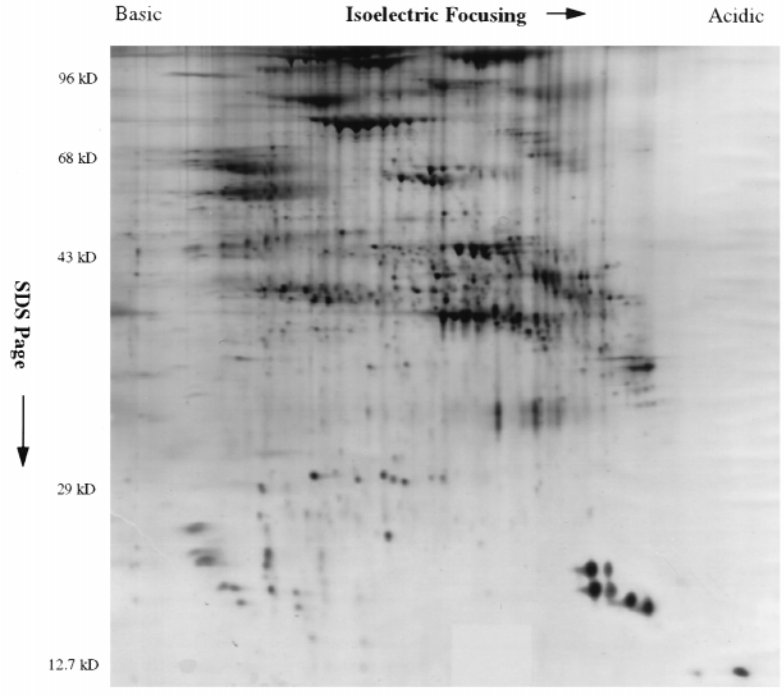

FIGURE 2. *NMP composition of normal prostatic tissue. High-resolution two-dimensional gel electrophoresis of nuclear matrix preparations from human prostates. kD = molecular weight in kilodaltons; SDS Page = sodium dodecyl sulfate-polyacrylamide gel electrophoresis.*

tate and seminal vesicles. Some investigators explain the differences in biologic behavior of the prostate and seminal vesicles by their different embryologic origin, as the prostate derives from the urogenital sinus, and the seminal vesicles develop from the wolffian duct.[23] Both organs, however, belong to the group of accessory sex tissues and both are known to be androgen dependent. Animal studies revealed that in postnatal animals, dihydrotestosterone, as well as testosterone, provides a stimulus for cell proliferation not only in the prostate but also in the seminal vesicles.[24] Thus, the different androgen receptors do not seem to be the major cause of the different growth patterns of the two tissues.

Berezney and Coffey[25] first demonstrated a residual nuclear structure, termed the nuclear matrix, that remained after a series of hypertonic salt and detergent extractions. The nuclear matrix organizes the DNA at a structural and functional level.[26] Furthermore, the nuclear matrix plays an important role in RNA transcription and in the

regulation of gene expression.[27] DNA replication, organization, transcription, and gene regulation are crucial for the regulation of cellular function.[28]

We were not able to detect a single protein that was either constantly expressed or constantly absent in all gels of one tissue (Figs. 1 and 2). The similarity of the nuclear protein composition of prostate and seminal vesicle tissue evaluated by two-dimensional gel electrophoresis in this study demonstrates the close relationship between the NMPs of these tissues. Unlike previous studies comparing NMPs of different tissues,[4,18,26,29] we were not able to demonstrate any significant differences between these two organs.

Surprisingly, we found variations in the nuclear matrix patterns of the same tissue, especially within the seminal vesicle tissues. In our study, repeated gel electrophoresis of a certain specimen did not reveal any intraspecimen variations. Thus, the differences between different specimens of the same tissue seem to be caused by tissue-related factors rather than by any variation in method or technique.

Computer-assisted analysis greatly facilitates the comparison of large numbers of different proteins in different gels. In a pilot experiment, we were able to detect subtle differences in nuclear protein patterns that escaped the visual analysis of various experienced researchers by using the gel flickering program (data not shown).

Technical improvements in nuclear morphometric measurements introduced by state-of-the-art computerized image analysis techniques have led to an objective, reproducible, and accurate method for describing nuclear shape.[30] Using this technique, several studies have demonstrated the clinical value of nuclear shape as a predictor of prognosis in a variety of tumors, including prostate cancer.[11,17] To our knowledge, nuclear morphometry of benign tissue has not previously been fully described. Our study demonstrated that nuclear shape in normal prostate tissue is significantly different from that of normal seminal vesicle. Moreover, the variance in the different nuclear shape parameters is more pronounced in the seminal vesicle epithelial cells than in the prostate epithelium or in normal breast tissue, studied in a previous study at this institution.[31] In malignant tissues, an increased heterogeneity of nuclear shape has correlated with a worse prognosis.[11] The implication of these findings for benign tissues is not quite clear.

These findings bear important implications for our understanding of the nuclear matrix, which is said to be tissue specific. Two-dimensional gel electrophoresis separates the NMPs in a two-dimensional manner. Our understanding of the nuclear matrix implies a three-dimensional network that is furthermore linked to other cytoskeletal elements.[32] Therefore, the analysis of the NMP composition may not be able to detect differences that are due to the three-dimensional architecture and the complex interactions that take place between the nuclear skeleton and the cytoskeleton of a cell. Nonaltered, closely related tissues with physiologically similar behavior, such as the prostate and seminal vesicle tissues evaluated in our study, may have the same NMP composition, which may differ only in their three-dimensional structural interactions or their functional state. For example, differences in NMP compositions may be caused by entirely different proteins, differences in posttranslational modification of the same proteins, or different proteins with a limited degree of homology, such as the putative lamin D.[26]

Other genitourinary tissues that are less closely related to the accessory sex organs, such as the urothelium and testes, demonstrate marked differences in nuclear matrix composition when compared with the seminal vesicle and prostate (unpublished data).

CONCLUSIONS

Our study demonstrated that two-dimensional analysis of NMP composition of prostate and seminal vesicle tissue does not reveal significant differences between these tissues. Both organs seem to be equipped with a similar set of NMPs. We demonstrated that normal prostate and seminal vesicle tissue have a distinctly different nuclear shape distribution. As nuclear shape is said to be related to nuclear function, the different biologic behavior of the prostate and seminal vesicles seems to be reflected by the different nuclear morphometry. Therefore, nuclear morphometry may depict differences in the functional state of a similar set of NMPs, shown by two-dimensional gel electrophoresis, which may be responsible for the different biologic behavior of these tissues. The current technique of two-dimensional gel electrophoresis of NMPs does not seem to reveal the complex interactions of cellular regulation. Therefore, the use of two-dimensional gel electrophoresis of NMP composition as a single tool for analysis of the functional state and behavior of a tissue seems to be limited. Moreover, this is the first report demonstrating similar NMP compositions in two different organs. Thus, the theory of a tissue-specific NMP composition evaluated by this technique needs further evaluation. Furthermore, a combination of different techniques (eg, electron microscopy[31]) and improvement of the techniques currently used for the analysis of the nuclear matrix are required to analyze the interactive behavior of the NMPs.

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