Distinctive Protein Pattern in Two-Dimensional Electrophoretograms of Cancerous Prostatic Tissues

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ABSTRACT

In this report, we describe methods used to analyze the protein composition of sectioned frozen prostatic tissues by two-dimensional gel electrophoresis. Our results show a high degree of homology in two-dimensional electrophoretograms of proteins extracted from frozen sections of normal and cancerous prostate glands. Such homology was not apparent in protein patterns of benign hypertrophic prostatic tissue sections. Typically, 600 discrete proteins were resolved on two-dimensional electrophoretograms and 9 proteins were present in all patterns of prostatic adenocarcinomatous tissues. These nine proteins were not observed in any of the protein electrophoretograms developed from nonmalignant prostate tissue. Three proteins were found common to nonmalignant prostate glands but were not present in prostatic adenocarcinoma.

INTRODUCTION

Our laboratory, in collaboration with others, has used 2-dimensional gel electrophoresis to resolve proteins excreted in urine (1, 2) and secreted in prostatic fluids (3) in efforts to identify protein alterations which may correlate with the presence of cancer. We have also extended these studies to include analysis of proteins extracted from prostatic tissues. Our earlier studies involving the analysis of proteins extracted from whole tissue pieces were unproductive since contaminating plasma proteins increased background staining of 2-D2 electrophoretogram. Further, tissue obtained for research purposes was usually remote from the site used by the pathologist for histological diagnosis.

A process for analyzing protein extracted from a single frozen section of tissue by 2-D gel electrophoresis was reported by Giometti et al. (4, 5). In those studies, protein in a single frozen section of muscle tissue were 14C-carbamidomethylated in situ prior to 2-D gel electrophoresis and fluorography. This approach made possible the analysis of the protein components of tissue immediately adjacent to that examined histologically.

We report results of the analysis of protein compositions of frozen prostatic tissue sections performed with high resolution, high sensitivity, silver stained 2-D gel electrophoresis (6, 7).

MATERIALS AND METHODS

Materials. Highly purified water (approximately 17 megohm/cm resistivity) was obtained by double distillation and filtration through a Millipore system, (Millipore Corp., Bedford, MA). Nunc vials (Southland Cryogenic, Inc., Carrollton, TX) were used for low-temperature storage of samples. Tissue-Tex II, O.C.T. compound embedding medium was purchased from Miles Laboratories, Naperville, IL. Absolute methyl alcohol, ACS, and ethyl ether anhydrous, ACS, were obtained from Fisher Scientific, Houston, TX. Hematoxylin and eosin were obtained from Lipshaw Manufacturing, Detroit, MI.

Tissue Acquisition and Preparation. Patient tissue samples were obtained by resection or needle biopsy for histological examination by the Pathology Department at University of Texas M. D. Anderson Hospital and Tumor Institute (Table 1). Tissues were sent directly to the pathology frozen-section suite unfixed in normal (0.9%) saline and were selected for this study. A piece of tissue was placed on a metal holder, covered by Tissue-Tex II, O.C.T.-compound embedding medium, frozen in a container using dry ice and absolute ethyl alcohol, and maintained at −70°C. The embedded tissue was positioned on an International Equipment Co. microtome contained in a Harris cryostat and maintained at a constant temperature of −20°C. Tissue sections were then cut at thicknesses ranging from 4 to 6 μm.

The initial tissue section was mounted on a standard glass slide and fixed using an equal mixture of ether and absolute methyl alcohol. The tissue preparation was stained using a standard hematoxylin and eosin system. Once the desired section was obtained, as determined by the pathologist, 11 additional frozen sections were cut. Ten of these 11 frozen sections were placed in labeled Nunc vials, submerged in liquid nitrogen, and stored at −80°C until required. Section 12, the last frozen section, was mounted on a glass slide, fixed, and stained in the same manner as was the first histological slide. This last section was then reviewed to confirm the continued presence of the desired representative tissue. The remaining frozen tissue sections were submitted for routine paraffin-embbedded histological examination.

Histological evaluation included (a) identification of the tissue site; (b) diagnosis; and (c) estimation of the percentages of the tissue occupied by malignant cells, nonmalignant epithelial cells, and connective tissue (Table 2). Prostate adenocarcinomas were histologically graded by the criteria established by Brawn et al. (8) and Gleason et al. (9, 10).

Two-Dimensional Electrophoresis. Prostatic tissue proteins extracted from frozen sections 2 through 11 were analyzed by the 2-D gel electrophoresis Iso-Dalt system as described by Tollaksen et al. (11). An appropriate number of tissue sections were solubilized in 100 μl of 9.0 M urea, 5.0% dithiothreitol, 1% Nonidet P-40 surfactant, 20% glycerol, and 2% ampholytes (0.4%, pH 4-6; 1.6%, pH 3.5-9.5). The volume of each tissue slice was estimated after a ×10 photoprint of slice 1 was made and the surface area was determined by planimetry. Planimetric values were multiplied by 4, 5, or 6 μm based on tissue slice thickness. The average volume for sections from resected tissue was approximately 350 nl/section, whereas sections from tissue obtained via needle biopsy averaged about 90 nl/section. Protein content per section was determined using the method of Bradford (12). Estimates of total protein content ranged from 8 μg/section obtained by needle biopsy to 95 μg/section obtained by resection. Two-dimensional analysis was performed on aliquots that contained between 6.5 and 38 μg protein. Patterns and staining intensity were reproducible within this range as determined visually, and 35 μg was considered optimal.

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2 The abbreviation used is: 2-D, 2-dimensional.
proteins found only in nonmalignant prostate glands. We identified prostate tissue proteins that were present in every sample, and a third template of the shared spots was produced. The procedure was performed by overlaying two templates on a low-intensity light box representing proteins were used for indexing. Comparison of templates are created on transparent polyvinyl sheets. Dots on the anode end of a Dalt-gel at approximately 6000 daltons and indicative of proteolytic activity was not visible in our gels.

RESULTS

Two-dimensional electrophoretogram patterns were analyzed from tissue samples from seven patients who had adenocarcinoma of the prostate and from seven patients who had no histological evidence of prostate cancer. Mounted tissue sections 1 and 12 were used to estimate: approximate volume; total protein content; and composition of glandular and connective cells in the intermediate slices, which were then subjected to 2-D gel analysis (Table 2). Volume determinations for frozen sections of tissues obtained via needle puncture were deemed unreliable because slide mounts of these specimens appeared shredded and smeared. Therefore, total protein per section as determined by the Bradford method (12) was used as an index for loading each first dimension gel. Electrophoretograms (Fig. 2a) were prepared from proteins that were solubilized from tissue slices; proteins in Fig. 2a were derived from tissue located within 30 µm of tissue slice (Fig. 2b), composed of 80% malignant epithelial cells and 20% stromal connective cells, with no nonneoplastic epithelium. Nonmalignant tissue sections were composed of approximately 45% normal epithelium and 55% stroma (Fig. 3) (Table 2; sample 14).

For pattern comparison 35 µg protein per gel in 40 µl of solution were applied to 3.75% polyacrylamide iso-gels (pH gradient 3.5 to 8.5) for separation of proteins in the first dimension. Second-dimension electrophoresis and staining of proteins with silver were performed as described by Guevara et al. (6). Consideration was given to the use of protease inhibitors; however, due to constraints and urgency of diagnosis required of the Pathology Department it was not practical or possible to include protease inhibitors in our protocol. All samples were handled in a standardized fashion. The peptide band that appears across the anode end of a Dalt-gel at approximately 6000 daltons and indicative of proteolytic activity was not visible in our gels.

Qualitative Analysis of 2-D Protein Patterns. Protein patterns on 2-D electrophoretograms were visually analyzed to assess qualitative differences in protein composition. Color-coded templates for each electrophoretogram were created on transparent polyvinyl sheets. Dots representing proteins were used for indexing. Comparison of templates was performed by overlaying two templates on a low-intensity light box and a third template of the shared spots was produced. The procedure used in these comparisons is shown in Fig. 1. From these comparisons we identified prostate tissue proteins that were present in every sample, malignant or nonmalignant. These templates were also used to identify proteins apparently unique to malignant prostate tissue as well as proteins found only in nonmalignant prostate glands.

RESULTS

Two-dimensional electrophoretogram patterns were analyzed from tissue samples from seven patients who had adenocarcinoma of the prostate and from seven patients who had no histological evidence of prostate cancer. Mounted tissue sections 1 and 12 were used to estimate: approximate volume;
Fig 2. Two-dimensional electrophoretogram (a) of proteins in a frozen tissue section (b) of prostatic adenocarcinoma. Proteins from a single tissue slice were solubilized, resolved, and stained with silver as described in “Materials and Methods.” Proteins designated A through I were identified by template comparison and were observed in all samples of prostate cancer but were not observed in normal prostate tissue or benign prostatic hypertrophy samples. Prostatic tissue slice shown in Fig. 2b (×100) is composed of 80% malignant epithelial cells and 20% stromal connective cells. No nonneoplastic epithelium was observed in this tissue section. KD (ordinate), molecular weight in thousands.
ELECTROPHORETIC PROTEIN PATTERN IN CANCEROUS TISSUE

Fig. 3. Two-dimensional electropherogram (a) of proteins in a frozen tissue section (b) of nonmalignant prostate. Proteins, denoted by 1, 2, and 3, were identified by exhaustive template comparison and were observed in all samples free of malignant cells only. These nonmalignant prostate tissue sections (X 100) were composed of about 4% normal epithelium and 95% stroma. KD (ordinate), molecular weight in thousands.
The heterogeneity of prostatic tissue makes it necessary that a more accurate method for selecting tissue for analysis be developed (13-16). By using histological frozen section controls of prostate tissue in which we can describe tissue composition and continuity, we have demonstrated that a distinct protein pattern is expressed in prostatic adenocarcinoma. Other investigators have reported data that indicate the possible existence of such a pattern (17, 18). This observation warrants further investigation as it may present a useful added diagnostic and prognostic parameter.

Characteristics of proteins we identified as unique to patterns of prostate cancer have been compared with those of other established markers for prostate cancer. Proteins, designated A through I in Table 3, and indicated on Fig. 2a, ranged from molecular weights of approximately 21,500 to 81,000 with relative isoelectric points of 4.8 to 8.3. Proteins D, E, and F resolve to the same region as proteins seen in region 2 of prostatic fluid proteins from men with benign prostatic hyper trophy (3). More stringent comparison of proteins D, E, and F with proteins of region 2 indicate significant differences in characteristics. Markers of prostatic cancer such as prostate acid phosphatase and prostate specific antigen have molecular weights of 55,000 and 34,000 with isoelectric points of 4.2–5.5 and 6.0, respectively (12). The characteristics are distinct from proteins shown in Table 3 as common to the malignant prostate. We did observe multielectrophoretic variants of prostate acid phosphatase in some samples; however, their presence did not correlate with malignancy. Our results corroborate other reports which have shown that immunoreactivity of prostate acid phosphatase varied within a prostatic tumor as well as among samples (14). No attempts were made to identify prostate specific antigen on 2-D electrophoretograms immunologically on “Western” transfers.

Certain oncogene-related proteins have been shown to correlate with the presence of malignancy in the prostate (19, 20). Using semiquantitative immunohistochemical methods, Viola et al. (19) demonstrated that levels of p21ras antigen correlated with prostatic tumor grade. It is interesting to speculate that our marker protein, designation I, with a molecular weight of 21,500 and a relative pl of 8.3 may be the ras oncogene product. In a separate report, Niman et al. (20) showed that certain oncogene-related peptides were excreted in urine of individuals with cancer. Their results indicate that patients with prostatic cancer excreted sis-related peptides with molecular weights of 56,000, 31,000, and 25,000 in their urine. Our marker protein H, with a molecular weight of 26,500 and a relative pl of 5.6 may prove to be a sis oncogene product. Further studies involving Western transfer of proteins from 2-D electrophoretograms immunologically on “Western” transfers.

Electrophoretic Protein Pattern in Cancerous Tissue

Table 3 Marker proteins of malignant and nonmalignant prostatic tissues

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<th>Protein designation</th>
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REFERENCES


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