ABSTRACT

The early diagnosis of bladder cancer is central to the effective treatment of the disease. Presently, there are no methods available to easily and specifically identify the presence of bladder cancer cells. The prevailing method for the detection of bladder cancer is the identification of bladder cancer cells by morphological examination of exfoliated cells or biopsy material by a pathologist. A hallmark of the malignant or transformed phenotype is an abnormal nuclear shape, the presence of multiple nucleoli, and altered patterns of chromatin organization. Nuclear structural alterations are so prevalent in cancer cells that they are commonly used as markers for transformation for many types of cancer. Nuclear shape is determined by the nuclear matrix, the dynamic skeleton of the nucleus. The nuclear matrix is the structural component of the nucleus that determines nuclear morphology, organizes the DNA in a threedimensional fashion that is tissue specific, and has a central role in the regulation of a number of nuclear processes, including the regulation of DNA replication and gene expression. Previous investigations into prostate and breast cancer have revealed that nuclear matrix protein (NMP) composition undergoes alterations with transformation and that the nuclear matrix can serve as a marker for the malignant phenotype. In this study, we have identified six proteins present in all tumor samples that are not present in the corresponding normal samples and three proteins that are unique to the normal bladder tissues in comparison with the tumor samples. Five of the six bladder cancer-associated proteins were also identified in three human bladder cancer cell lines examined (253J, UMUC-2, and T24). Therefore, we have demonstrated that nuclear matrix composition is able to differentiate bladder cancer from normal bladder tissue and may provide useful tools for early detection and recurrence of the disease. Importantly, these markers may provide valuable tools for cytopathological screening for bladder carcinoma.

INTRODUCTION

The nuclear matrix is the framework of the nucleus and consists of the peripheral lamins and pore complexes, an internal ribonucleic protein network, and residual nucleoli (1). The NMPs represent ~10% of all nuclear proteins and are virtually devoid of lipids, DNA, and histones (2). It has been demonstrated that the nuclear matrix plays a central role in the regulation of important cellular processes, such as DNA replication and transcription (3).

These structural components of the nucleus are known to have a central role in the specific topological organization of DNA. DNA in the nucleus is not randomly organized, and although ~10% of the DNA actually encodes proteins, only specific genes are positioned in a manner that permits the expression of both housekeeping and cell type-specific genes. The DNA has many forms of higher-order structure, including topological organization by the nuclear matrix, which gives rise to a tissue-specific pattern of organization that results in the expression of appropriate tissue-specific genes.

A cellular hallmark of the transformed phenotype is an abnormal nuclear shape and the presence of abnormal nucleoli. Nuclear structural alterations are so prevalent in cancer cells that they are commonly used as pathological markers of transformation in many types of cancer. Nuclear shape is thought to reflect the internal nuclear structure and processes and is determined, at least in part, by the nuclear matrix (4).

Most known NMPs are common to all cell types and physiological states. Numerous NMPs, which may be unique to certain cell types or states, have been identified (reviewed in Ref. 3). It has been demonstrated that mitogenic stimulation and the induction of differentiation alter the composition of NMPs and structure (5, 6). The nuclear matrix contains a number of associated proteins that have been found to be involved in transformation. Berezney et al. (7) first showed by examining hepatoma NMPs that the nuclear matrix is altered in transformation. Fey and Penman (8, 9) demonstrated that tumor promoters induce a specific morphological signature in the nuclear matrix-intermediate filament scaffold of kidney cells, and that the pattern of NMPs differed between normal and tumorigenic cell lines.

We have previously characterized NMPs that are able to serve as markers to differentiate prostate cancer from the normal prostate in both a rat model system (10) and in human prostate samples (11). In addition, NMP composition was able to distinguish between metastatic and nonmetastatic tumors (10). NMPs that are associated with human breast cancer and are not found in normal breast tissue have also been identified (12). Recently, an antibody to a NMP, termed NM-200.4, was raised from the breast carcinoma cell line T-47D (13). This antibody reacts strongly with human breast carcinoma specimens as well as specimens from lung, thyroid, and ovarian cancers, but does not react with normal epithelial cells of similar origin, suggesting that certain anti-NMP antibodies may be developed as diagnostic tools. The strong evidence linking changes in the nuclear matrix with cancer has led to the suggestion that the nuclear matrix might serve as an excellent target for anticancer agents (14).

These data provide a strong rationale for investigation of the differences in NMP composition that may be discerned in normal versus transformed bladder tissue. We have compared the protein composition of the nuclear matrix in bladder tumors with that in adjacent normal bladder tissue from individuals with these tumors as well as with the composition in normal bladder samples obtained from organ donors. In addition, we examined the NMP composition of several human bladder cancer cell lines. We identified six NMPs that are found in the tumor samples but are not present in the normal tissues and three NMPs that are identified in the normal tissue but are absent in the tumors. Five of the six cancer-specific NMPs were also found in all of the bladder cancer cell lines.

Received 10/20/95; accepted 1/25/96.

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.

1 This work was supported in part by developmental funds of the University of Pittsburgh Cancer Institute and NIH Grant P30 CA47904 (University of Pittsburgh Cancer Institute).
2 To whom requests for reprints should be addressed, At University of Pittsburgh Cancer Institute, 200 Lothrop Street, BST E-1056, Pittsburgh, PA 15213-2582. Phone: (412) 383-8923; Fax: (412) 383-8928.
3 The abbreviations used are: NMP, nuclear matrix protein; BPH, benign prostatic hyperplasia.

Robert H. Getzenberg,2 Badrinath R. Konety, Theresa A. Oeler, Martha M. Quigley, Ardeshir Hakam, Michael J. Becich, and Robert R. Bahnsen


Bladder Cancer-associated Nuclear Matrix Proteins

Robert H. Getzenberg,2 Badrinath R. Konety, Theresa A. Oeler, Martha M. Quigley, Ardeshir Hakam, Michael J. Becich, and Robert R. Bahnsen


ABSTRACT

The early diagnosis of bladder cancer is central to the effective treatment of the disease. Presently, there are no methods available to easily and specifically identify the presence of bladder cancer cells. The prevailing method for the detection of bladder cancer is the identification of bladder cancer cells by morphological examination of exfoliated cells or biopsy material by a pathologist. A hallmark of the malignant or transformed phenotype is an abnormal nuclear shape, the presence of multiple nucleoli, and altered patterns of chromatin organization. Nuclear structural alterations are so prevalent in cancer cells that they are commonly used as markers of transformation for many types of cancer. Nuclear shape is determined by the nuclear matrix, the dynamic skeleton of the nucleus. The nuclear matrix is the structural component of the nucleus that determines nuclear morphology, organizes the DNA in a threedimensional fashion that is tissue specific, and has a central role in the regulation of a number of nuclear processes, including the regulation of DNA replication and gene expression. Previous investigations into prostate and breast cancer have revealed that nuclear matrix protein (NMP) composition undergoes alterations with transformation and that the nuclear matrix can serve as a marker for the malignant phenotype. In this study, we have identified six proteins present in all tumor samples that are not present in the corresponding normal samples and three proteins that are unique to the normal bladder tissues in comparison with the tumor samples. Five of the six bladder cancer-associated proteins were also identified in three human bladder cancer cell lines examined (253J, UMUC-2, and T24). Therefore, we have demonstrated that nuclear matrix composition is able to differentiate bladder cancer from normal bladder tissue and may provide useful tools for early detection and recurrence of the disease. Importantly, these markers may provide valuable tools for cytopathological screening for bladder carcinoma.

INTRODUCTION

The nuclear matrix is the framework of the nucleus and consists of the peripheral lamins and pore complexes, an internal ribonucleic protein network, and residual nucleoli (1). The NMPs represent ~10% of all nuclear proteins and are virtually devoid of lipids, DNA, and histones (2). It has been demonstrated that the nuclear matrix plays a central role in the regulation of important cellular processes, such as DNA replication and transcription (3).

These structural components of the nucleus are known to have a central role in the specific topological organization of DNA. DNA in the nucleus is not randomly organized, and although ~10% of the DNA actually encodes proteins, only specific genes are positioned in a manner that permits the expression of both housekeeping and cell type-specific genes. The DNA has many forms of higher-order structure, including topological organization by the nuclear matrix, which gives rise to a tissue-specific pattern of organization that results in the expression of appropriate tissue-specific genes.

A cellular hallmark of the transformed phenotype is an abnormal nuclear shape and the presence of abnormal nucleoli. Nuclear structural alterations are so prevalent in cancer cells that they are commonly used as pathological markers of transformation in many types of cancer. Nuclear shape is thought to reflect the internal nuclear structure and processes and is determined, at least in part, by the nuclear matrix (4).

Most known NMPs are common to all cell types and physiological states. Numerous NMPs, which may be unique to certain cell types or states, have been identified (reviewed in Ref. 3). It has been demonstrated that mitogenic stimulation and the induction of differentiation alter the composition of NMPs and structure (5, 6). The nuclear matrix contains a number of associated proteins that have been found to be involved in transformation. Berezney et al. (7) first showed by examining hepatoma NMPs that the nuclear matrix is altered in transformation. Fey and Penman (8, 9) demonstrated that tumor promoters induce a specific morphological signature in the nuclear matrix-intermediate filament scaffold of kidney cells, and that the pattern of NMPs differed between normal and tumorigenic cell lines.

We have previously characterized NMPs that are able to serve as markers to differentiate prostate cancer from the normal prostate in both a rat model system (10) and in human prostate samples (11). In addition, NMP composition was able to distinguish between metastatic and nonmetastatic tumors (10). NMPs that are associated with human breast cancer and are not found in normal breast tissue have also been identified (12). Recently, an antibody to a NMP, termed NM-200.4, was raised from the breast carcinoma cell line T-47D (13). This antibody reacts strongly with human breast carcinoma specimens as well as specimens from lung, thyroid, and ovarian cancers, but does not react with normal epithelial cells of similar origin, suggesting that certain anti-NMP antibodies may be developed as diagnostic tools. The strong evidence linking changes in the nuclear matrix with cancer has led to the suggestion that the nuclear matrix might serve as an excellent target for anticancer agents (14).

These data provide a strong rationale for investigation of the differences in NMP composition that may be discerned in normal versus transformed bladder tissue. We have compared the protein composition of the nuclear matrix in bladder tumors with that in adjacent normal bladder tissue from individuals with these tumors as well as with the composition in normal bladder samples obtained from organ donors. In addition, we examined the NMP composition of several human bladder cancer cell lines. We identified six NMPs that are found in the tumor samples but are not present in the normal tissues and three NMPs that are identified in the normal tissue but are absent in the tumors. Five of the six cancer-specific NMPs were also found in all of the bladder cancer cell lines.
MATERIALS AND METHODS

Cell Lines and Tissue Processing. Matched normal and tumor bladder tissue samples were obtained from the same patients undergoing surgery for bladder cancer at the University of Pittsburgh Medical Center. All patients had transitional cell carcinoma. The tumor samples collected were of the following clinical stages according to the American Joint Committee on Cancer (15). Six of the tumors were T_1, eight were T_2, and three were T_4 (Table 1). The TNM histopathological grade of the lesions revealed 4 G_1 tumors, 2 G_2 tumors, and 11 G_3 lesions (16, 17). In addition to TNM staging and grading, the tumors were graded according to the classification of Bergkvist et al. (16). None of the lesions was grade 1, seven were grade 2, nine were grade 3, and one was grade 4. Normal bladders were obtained from the Center for Organ Recovery and Education. Only samples that could be clearly identified by the pathologist as containing approximately pure populations of the stated tumor grade were used. The cell lines (253J, UMUC-2, and T24) were kindly provided by Dr. Monica Liebert (University of Texas M. D. Anderson Cancer Center, Houston, TX) and grown in RPMI 1640 (Life Technologies, Inc., Rockville, MD) with 10% fetal bovine serum (Life Technologies).

Nuclear Matrix Preparation. The nuclear matrix is defined as the residual components of the nucleus, which are insoluble to a series of detergent and salt extractions following DNase treatment (1). The isolation procedure used in these experiments involves the release of cytoskeletal proteins by the use of a mild salt extraction with 0.25 M ammonium sulfate of detergent-treated nuclei that no longer possess their membrane lipid components. This is followed by DNase I and RNase A treatment to remove chromatin structures. This extraction causes minimal disruption of the NMPs and nuclear matrix structure (8, 18).

The NMPs were isolated from bladder tissue and tumors, according to our previously published adaptation of the method of Getzenberg et al. (10) and Fey et al. (18). Briefly, the tissue pieces were 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) to release the lipids and soluble proteins. Extracts were then filtered through a 350-/µM nylon mesh and extracted with 0.25 M ammonium sulfate to release the soluble cytoskeletal elements. DNase treatment at 25°C was used to remove the soluble chromatin. The remaining fractions contained intermediate filaments and NMPs. This fraction was then disassembled with 8 M urea, and the insoluble components, which consist principally of carbohydrates and extracellular matrix components, were pelleted. The urea was dialyzed out, and the intermediate filaments were allowed to reassemble and were subsequently removed by centrifugation. The NMPs were then ethanol precipitated. All solutions contained freshly prepared 1 mM phenylmethylsulfonyl fluoride to inhibit serine proteases, 0.3 µM aprotinin, 1 µM leupeptin, and 1 µM pepstatin.

RESULTS

We have obtained tumor and normal tissue from 17 matched bladder samples. NMPs have been extracted and separated by high-resolution, two-dimensional gel electrophoresis. The NMP compositions of the 17 tumors and their corresponding normal tissue were then analyzed using a computer-based gel analysis system. All tumors contain differences in their nuclear matrix compositions when compared with the nuclear matrix compositions of the matched normal tissue from the same bladders. Consistent differences were noted for all of the samples. An example of the NMP composition of a bladder tumor and normal bladder tissue is presented in Fig. 1. There are several notable differences in the nuclear matrix composition in the bladder tumor when compared with the normal tissue. We have identified six proteins (BLCA-1–BLCA-6) that are present in all of the tumors and are absent in the normal tissue (Fig. 1B) and three proteins (BLNL-1–BLNL-3) that are found in all of the normal bladder tissue samples (Fig. 1A) and are missing in the tumor samples (Fig. 1 and Table 2). These differences seem to be unique to bladder cancer in that the molecular weights and isoelectric points of the proteins do not correspond to those proteins previously reported to be different in prostate and breast cancers (Table 2). In addition, we have now examined several normal human bladders to further our investigations into the NMP composition of normal bladder tissue. We have relatively large numbers of these normal bladders, which will allow us to study, in detail, changes that occur in NMPs during the transformation from normal cells to bladder cancer. Although these comparisons are still in the preliminary stage, it seems that the nuclear matrix composition of these normal bladders contains the three normal specific proteins and does not contain the tumor specific proteins.

The tumor samples that we examined are complex mixtures of epithelial, stromal, immunological, and other cell types. To determine
whether the nuclear matrix changes that we were detecting actually represented changes that were occurring in the neoplastic transitional cell, we examined the NMP compositions of several bladder cancer cell lines. The human bladder cancer lines 253j, T24, and UMUC-2 were grown, and their NMPs were isolated. These NMPs were then separated by two-dimensional electrophoresis and silver stained. As demonstrated in Fig. 2, of the six NMPs determined to be found only in the bladder tumor samples, five of these proteins were identified in the three cell lines. Only the BLCA-5 protein was not identified in the three cell lines. None of the proteins found only in normal bladder samples (BLNL-1–BLNL-3) was found in these lines.

DISCUSSION

The nuclear matrix is the binding site for tumor-associated proteins, including the myc oncogene product, adenovirus E1A-transforming protein, polyoma large T antigen, and Tat protein from HIV-1 (21–24). Changes in the expression of a nuclear matrix-associated protein in NIH3T3 cells have been found in oncogene-transformed cells (25).

It has been demonstrated that the monoclonal antibody Ki-67, typically used to identify proliferating cells, is directed against a NMP (26). It has also been demonstrated that the nuclear matrix is the preferential binding target of the carcinogen benzo(a)pyrene (27).

Previous investigations from our laboratory have demonstrated that the nuclear matrix is altered in cancer cells in the Dunning rat prostate adenocarcinoma model (10). We have recently completed a study on the NMP composition in human prostate tissue (11). We compared the NMP patterns in fresh normal prostate, BPH, and prostate cancer from 21 men undergoing surgery for clinically localized prostate cancer or BPH. One protein (PC-1), a Mr 56,000 protein with an isoelectric point of 6.58, appeared in all of 14 nuclear matrix preparations from different prostate cancer specimens and was not detected in normal prostates (0 of 13) or BPH (0 of 14). Work from Khanuja et al. (12) has begun to determine the NMP composition of normal human breast tissue as well as breast tumors (12). A review of 10 different tumor samples revealed at least four proteins that were expressed in tumor tissue but not in normal tissue. One protein was a low-molecular-weight (Mr 18,000) protein, and the others were found at relatively low isoelectric points, between Mr 60,000 and 80,000. In addition, two proteins, of Mr 24,000 and 26,000 were found in the normal breast samples but were not detected in the tumor samples. These studies, taken as a whole, suggest that the protein composition of the human breast nuclear matrix may provide insight into the biological etiology of breast cancer and may provide biological markers of growth and gene expression in the human breast.

Presently, the only available marker for bladder cancer identification is morphological examination of cytological samples or cystoscopic biopsies. This method is accurate for high-grade lesions; however, a significant proportion of bladder tumors (25–45%) are low grade or well differentiated and escape detection on cytological examination of exfoliated cells. In general, the diagnostic accuracy of cytological examination alone for the detection of low-grade transitional carcinoma is between 49 and 64% (if suspicious urines are
Fig. 2. NMPs associated with human bladder cancer are identified in three human bladder cancer cell lines. Silver-stained, high-resolution, two-dimensional gel electrophoresis of NMPs of the human bladder cancer cell lines 253J (A), T24 (B), and UMUC-2 (C). Identified proteins, found only in human bladder cancer samples, correspond to those in Fig. 1.

considered positive; Ref. 28). The accuracy of cytological examination can be increased by repeating the study (29); however, this is a costly and time-consuming practice for both the patient and physician. Development of a sensitive screening assay that could specifically detect bladder carcinoma would significantly facilitate patient care and the early detection of bladder carcinoma. Therefore, the identification of NMPs that indicate the presence of bladder cancer in a sample may permit the development of a diagnostic test for bladder cancer. NMPs have been shown to be shed by tumors as cells die, and it has been demonstrated that they are present in the serum and urine of cancer patients (30). Therefore, it is possible that the NMPs described here could be used as markers for bladder cancer in serum or voided urine. These markers could aid not only in the early identification of bladder cancer but also in the detection of recurrent disease. Presently, patients who have had bladder tumors removed must undergo frequent cystoscopic examinations to rule out recurrent disease. The ability to use a blood or urine test to identify patients with disease recurrence would greatly aid in the detection of the disease and would decrease the need for these patients to have repeated cystoscopic evaluations.

We have recently obtained relatively significant quantities of the isolated NMPs BLCA-1–BLCA-6. There proteins are being se-
sequenced and, in addition, are being used to raise antibodies against these proteins. This information will then be used to clone the genes encoding these proteins as well as to begin to understand their role in cancer. We have been successful in developing methods for isolating spots from two-dimensional gels in sufficient quantities for protein sequencing and antibody production. Deciphering the NMPs that are either present or absent in cancer cells will provide novel information about their role in cancer, and because the nuclear matrix plays a central role in DNA organization and the regulation of gene expression, these proteins may be crucial to the transformation process. Finally, these NMPs may serve as possible therapeutic targets by serving not only as cancer-specific markers, but also as tissue-specific addresses, thereby theoretically permitting the targeting of molecules to specific cell types within the body.

REFERENCES


Bladder Cancer-associated Nuclear Matrix Proteins


Cancer Res 1996;56:1690-1694.

Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/56/7/1690

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, use this link http://cancerres.aacrjournals.org/content/56/7/1690. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.