Identification of Nuclear Matrix Proteins in the Cancer and Normal Rat Prostate

Robert H. Getzenberg, Kenneth J. Pienta, Edwin Y. W. Huang, and Donald S. Coffey

ABSTRACT

The nuclear matrix is the structural component of the nucleus that determines nuclear morphology and organizes the DNA in a threedimensional fashion that is tissue specific. Previously, some of the nuclear matrix proteins have been reported to be both tissue and cell type specific and are altered with the state of differentiation and transformation. This study demonstrates that the nuclear matrix is specific for the individual lobes of the normal rat prostate and that the nuclear matrix undergoes changes in protein composition in the Dunning prostate cancer tissue. Additionally, in the Dunning rat prostate adenocarcinoma cell lines, there is a range of tumor phenotypes and the nuclear matrix varies in composition in each tumor cell type. These differences in the nuclear matrix proteins are associated with quantitative changes in nuclear morphology that form the pleomorphic state of the cancer nucleus.

INTRODUCTION

The nuclear matrix is the insoluble structural framework of the nucleus and includes an internal ribonucleoprotein network, residual nucleoli, peripheral lamins and the nuclear pore complexes (reviewed in Refs. 1–6). Several studies have shown that the nuclear matrix is both tissue (7) and cell type specific (8) and undergoes changes with differentiation (9–11) and transformed tissue culture cells (8, 12). In addition, Fey and Penman (8) demonstrated in cell culture that the nuclear matrix is unique to various cancer cell lines and suggested that these could be used as tumor markers. These studies have all demonstrated the presence of some nuclear matrix proteins which are common to all cell types and which compose a majority of the demonstrated proteins, and a smaller set of nuclear matrix proteins which are specific for the cell type and/or the state of differentiation. The nuclear matrix is the scaffolding framework of the nucleus, which specifically organizes the DNA threedimensionally in a tissue specific manner and is responsible, in part, for tissue specific gene expression since many studies have reported that active genes are associated with the nuclear matrix and inactive genes are not (reviewed in Refs. 1 and 2).

The rat prostate is a suitable system in which to study tissue specificity since it is composed of several lobes and can be compared to a spectrum of cancer cell lines all of which originally arose spontaneously in a rat prostate. The rat prostate is composed of three distinct anatomical lobes, the ventral, dorsal, and lateral, each of which has been demonstrated to differ morphologically and functionally, with the dorsal and lateral lobes more closely resembling each other than the ventral prostate (13). The different prostatic lobes were also shown to differ in their hormone sensitivity and the regulation of lobe specific gene expression (14–16). Thus, we test here whether the nuclear matrix protein pattern can distinguish these closely related lobes and will then test how these nuclear matrix proteins change in the cancer state.

The prostate cancer cells studied are the transplantable Dunn-ning rat prostatic adenocarcinomas which originated from a spontaneous tumor in the dorsal prostate of an aged rat (17), and variant cell lines have been established with a wide variety of transformed phenotypes. Three of the Dunning sublines were studied: the G-cell tumor subline that is androgen sensitive but is not metastatic and can grow to greater than the body weight of the animal without killing it; the AT2 cell line, which also has a low metastatic ability, is faster growing than the G-cell, and has low to moderate metastatic potential; the MLL, a highly metastatic cell line which will rapidly metastasize and kill the animal while the primary tumor size remains relatively small. All of these Dunning tumors have previously been well characterized (18) and have been reported to have different total cellular protein expression patterns and also differ significantly in gene expression from the dorsal prostate from which they originated (18, 19). It was concluded that major alterations in total protein expression have occurred in going from the normal rat dorsal prostate to the transformed Dunning R3327 adenocarcinoma.

It has been shown that genes are organized in different threedimensional patterns between tissues from the same animal (20, 21) and it has been proposed that this specificity is defined in part by the proteins of the nuclear matrix (5). Since the prostate cancer cells have protein expression patterns that differ from those of the normal prostate, it is possible that the DNA is organized differently in the cancer cell than the normal cell. Therefore, it was of interest to determine whether differences in nuclear matrix proteins existed in the Dunning cancer cell lines and to compare these with quantitative changes in their nuclear morphometry since the nuclear matrix is believed to be responsible for determining the nuclear shape, which is commonly altered in transformation.

MATERIALS AND METHODS

Animals. Mature intact male Sprague-Dawley rats (300–350 g) were obtained from Charles River (Wilmington, MA). All animals were untreated and cared for in accordance with the Guidelines for Care and Use of Experimental Animals.

Cell Lines. The G, AT2.1, and MLL sublines of the Dunning R3327 rat prostate adenocarcinoma cell line were previously characterized and obtained from J. T. Isaacs, Johns Hopkins University School of Medicine (18). These cells were cultured in RPMI 1640 containing 10% fetal bovine serum, 250 ng dexamethasone, penicillin G (100 units/ml), and streptomycin (100 units/ml) as described previously (18).

Tumor Samples. Dunning R-3327 AT2.1 rat prostate tumors were transplanted s.c. into male Copenhagen rats and harvested when weights reached 3–4 g (generously provided by J. T. Isaacs).

Nuclear Matrix. The nuclear matrix proteins were isolated from fresh rat prostates, tumor cells, or tumors according to the methodology of Fey and Penman (8). Briefly, the tissues were chopped into small pieces in ice-cold buffer. The tissue pieces are then homogenized with a Teflon pestle on ice with 0.5% Triton X-100 to release the lipids and soluble proteins, in a buffered solution containing 2 mM vanadyl ribonucleoside, a RNase inhibitor. The extract is filtered through a nylon mesh to remove connective tissue and large debris. Salt extraction with 0.25 M ammonium sulfate with 2 mM vanadyl ribonucleoside is added to release the soluble cytoskeletal elements. DNase I and RNase

Received 7/17/91; accepted 10/10/91.

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 by Grants DK-22000 and CA 15416 from the National Cancer Institute, from the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, Department of Health and Human Services.

2 The abbreviation used is: MLL, Mat-LyLu.
A treatment at 25°C is used to remove the soluble chromatin and RNA. The remaining fraction contains intermediate filaments and nuclear matrix proteins, and this fraction is then disassembled with 8 M urea, and the insoluble components, which consist principally of carbohydrates and extracellular matrix components, are pelleted. The urea is then dialyzed out, and the intermediate filaments then reassemble and are removed by centrifugation. The nuclear matrix proteins that remain soluble are then ethanol precipitated. All solutions contain freshly prepared 1 mM phenylmethylsulfonyl fluoride to inhibit serine proteases. The protein composition is determined by resuspending the proteins in 0.1 M sodium hydroxide and utilizing the BCA protein assay (Pierce, Rockford, IL) with bovine serum albumin as a standard. For gel electrophoresis, the ethanol precipitated nuclear matrix proteins were redissolved in a sample buffer consisting of 9 M urea, 65 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, 2.2% ampholytes, 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) (22). One dimensional isoelectric focusing was carried out for 18,000 V-h using 1-mm x 18-cm tube gels after prefocusing. The tube gels were extracted and placed on top of 1-mm 10% sodium dodecyl sulfate-polyacrylamide electrophoresis slab gels and the gels were electrophoresed with 12°C constant temperature regulation. The gels were then fixed with 50% methanol and 10% acetic acid. The gels were thoroughly rinsed and treated with 5% glutaraldehyde and 5 mM dithiothreitol after buffering with 50 mM phosphate (pH 7.2). After overnight rinsing, the gels were stained with silver stain using the methodology of Wray et al. (23) (Accurate Chemical Co., Inc., Westbury, NY). Forty µg of nuclear matrix protein were loaded for each gel. Protein molecular weight standards were Silver Standards from Diversified Biotechnology (Newton Centre, MA). Isoelectric points were determined using carbamylated standards from

Fig. 1. Nuclear matrix protein composition of rat normal prostate lobes. High resolution two-dimensional gel electrophoresis of nuclear matrix preparations isolated from (A) ventral, (B) dorsal, and (C) lateral rat prostate. Arrows, lobe specific protein components identified with the use of multiple gels. Only spots consistent on three repetitive samples and gels were identified as lobe specific. Arrows in all of the figures represent only the presence and absence of proteins. Quantitative differences were also noted but are not described here. kD, molecular weight in thousands; SDS PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
Pharmacia (Piscataway, NJ). Multiple gels were run for each sample, and multiple samples were run at different times. The gels shown in this paper are representative of at least three gels produced for each sample. Only protein spots clearly and reproducibly observed in all gels of a sample type were counted as actually representing the nuclear matrix components; quantitative variations were not scored.

Morphometric Analysis. Standard hematoxylin and eosin stains of paraffin sections of normal prostate lobes and Dunning tumors were analyzed utilizing the quantitative morphometric system of the Zeiss DynaCell Program. Two hundred nuclei, and their respective nucleoli, of each specimen were manually digitized and the X-Y coordinates of the nuclear boundary were stored using DynaCell software and a digitizer tablet (SummaSketch Model MM-1201). This system digitized standard contours with an accuracy of greater than 95% (24).

Morphometric Shape Descriptors. Seventeen shape descriptors including area, perimeter, form factors, and ellipticity were calculated for each nucleus along with statistical variations. A detailed description of the morphometric shape descriptors and the mathematical equations used in DynaCell has been published previously (25, 26). In brief, area in μm^2 and perimeter in μm are measured for each nucleus. The nuclear roundness form factors reported here are calculated for both the measured area and perimeter of the nucleus which represent a dimensionless, size-invariant shape descriptor that yields a value of zero for a perfect circle and increases in value as the nuclear contour deviates from a perfect circle. The formula for the roundness form factor is

\[ \left( \frac{\text{Perimeter}}{2 \times \pi} \right) \left( \frac{\text{Area} \times \pi}{ \text{Area} \times \pi \left( \text{Area} \times \pi \right)^n - 1} \right) \times 1000 \]

Statistics. Statistical analysis was performed using Statgraphics v.4.0 (Statistical Graphics Corp.). Statistical significance was determined using a one-way analysis of variance test and a Tukey multiple comparisons test.

RESULTS

Previous results from our laboratory have demonstrated that two sex accessory tissues in the rat, the ventral prostate and seminal vesicle, are composed of tissue specific nuclear matrix proteins (7), and it is proposed that the nuclear matrix may be involved in regulation of gene expression in these tissues (2, 5). Even though the lobes are closely related, examination of the nuclear matrix protein composition of the three lobes of the rat prostate reveals the presence of differences in the nuclear matrix patterns between these prostate lobes (Fig. 1; Table 1). The dorsal and lateral prostates are relatively similar, exhibiting only one major unique nuclear matrix protein for the rat dorsal prostate and four specific nuclear matrix proteins in the lateral prostate. The ventral prostate was significantly different from the other prostate lobes and consists of at least nine unique nuclear matrix proteins.

We then compared how the nuclear matrix is altered in cancer and if these matrix protein patterns could distinguish closely related sublines of the same Dunning tumor. The Dunning adenocarcinoma cell lines were originally derived from a spontaneous rat dorsal prostate tumor (17). With transplantation, this original tumor has given rise to a number of cell lines with differing degrees of transformed phenotype that have been well characterized (18). The nuclear matrix proteins in several Dun-ning cell lines were examined and compared with the nuclear matrix protein composition of the dorsal prostate, the original tissue from which this tumor was derived. Using a high resolution two-dimensional gel electrophoresis technique, the nuclear matrix proteins of the Dunning cell lines were found to be significantly different from the rat dorsal prostate. A minimum of ten abundant spots were identified as unique to the rat dorsal prostate when compared with the Dunning lines (Fig. 2); i.e., they were absent in the tumor cells. Similarly, there were several proteins which were unique to the Dunning cell lines that were not found to be present in the dorsal prostate nuclear matrix. This is demonstrated with the G cell nuclear matrix protein pattern (Fig. 2B).

When the nuclear matrix protein patterns of the Dunning cell lines were compared with one another, they appeared relatively similar in protein composition. The AT2 and MLL cell lines were alike in their abundant protein composition. These two cell lines did contain two proteins which were not found in the G-cell line. The nuclear matrix of the G-cell line exhibited two unique proteins which were not present in the AT2 or MLL (Table 2).

To demonstrate that these changes found in the Dunning cell lines actually represent changes which occur with transformation, an AT2.1 tumor was removed from a rat and its nuclear matrix protein components were identified with high resolution two-dimensional gel electrophoresis (Fig. 3). This tumor contains all of the Dunning specific proteins found in the cell lines and only a few of the proteins that were identified as dorsal prostate specific. NP-1, NP-2, and NP-3 were common in both the AT2.1 tumor nuclear matrix and the normal dorsal prostate nuclear matrix. In addition, the AT2 tumor contains both of the proteins that were shown to be AT2 and MLL specific and neither of the two G-cell specific proteins. The AT2 tumor does contain a number of proteins that are different from the dorsal prostate that were not found in the cell lines.

In summary, these results demonstrate that the nuclear matrix protein pattern is specific in the various lobes of the rat prostate. In addition, transformed cell lines differ significantly from their tissue of origin and while these transformed cell lines are composed of almost entirely a common set of nuclear matrix proteins, there are differences which can distinguish cell lines of different degrees of the transformed phenotype from one another. These results are further supported by evidence from a AT2 tumor demonstrating the same changes found in the cell lines.
A cellular hallmark of the transformed phenotype is abnormal nuclear shape and the presence of multiple nucleoli. Nuclear structural alterations are so prevalent in cancer cells that they are commonly used as a pathological marker of transformation for many types of cancer. Nuclear shape is thought to reflect internal nuclear processes and is determined, at least in part, by the nuclear matrix. We therefore studied the quantitative changes in nuclear morphometry in the normal and cancer prostate cells. Examination of the nuclear morphology of the normal prostate cells reveals that the nuclei of the ventral, lateral, and dorsal prostate have similar size and morphological attributes (Table 3). When these same nuclear shapes are investigated in the Dunning prostate tumors, it is evident that the cancer cells are very different from the normal prostate nuclei. All of the values for the area and nuclear shape increase when comparing the tumor cells to the normal prostate lobes including, an increase in nuclear area and variance, nuclear shape, nuclear shape variance, and nucleolar number and area. A characteristic of pleomorphism is variation in nuclear shape. The Dunning tumor cells have a larger variance in both nuclear area and shape in comparison to the normal prostate cells. Since the nuclear matrix is the organizing framework of the nucleus, it is possible that changes in nuclear matrix composition are a possible determinant of the alterations observed in the Dunning prostate tumors.
nuclear morphology. These results cannot be explained simply on the basis of DNA content alone as is demonstrated by chromosome number (Table 3). A Dunning subline, AT6, while demonstrating alterations in both nuclear and nucleolar morphological parameters, has a diploid number of chromosomes. While the chromosome content of the AT6 cells is not normal, there are both additions and subtractions to the normal chromosome pool; the total number of chromosomes is diploid. Thus, DNA content alone cannot be responsible for these morphological alterations with transformation.

**DISCUSSION**

The protein components of the nuclear matrix are not only tissue and lobe specific but also tumor specific. The Dunning cell lines which were originally derived from the rat dorsal prostate are all substantially different in nuclear matrix composition than the original tissue and there appear to be at least ten nuclear matrix proteins which are missing when comparing the dorsal prostate to the Dunning cells and three nuclear matrix proteins which are novel in the Dunning cell lines. These alterations are significant in magnitude and the number of protein changes is in the range of protein patterns previously found between tissues (7).

When comparing the AT2.1 tumor nuclear matrix with the nuclear matrix of the normal dorsal prostate, most of the differences in protein composition that were found between the normal tissue and the cell lines were also noted. The cell lines had at least 10 major proteins (NP-1 through NP-10) which were present in the normal dorsal prostate and are absent in all of the cell lines. All but three of these proteins were absent in the AT2.1 tumor nuclear matrix. The proteins NP-1, NP-5, and NP-10 were all found in the AT2.1 tumor sample. The three proteins (D-1, D-2, and D-3) that were found in all of the cell lines but not in the original tissue were all found in the AT2.1 sample and the AT2.1 tumor had the two AT2 and MLL cell line specific proteins while not containing the G-cell line specific proteins. These differences could possibly be due to several factors including alterations caused by transformation, a difference in the extracellular environment of the tumor, or an inflammation in the tumor area. Utilizing the cell lines helps to narrow in on the possible determinants of these changes in nuclear matrix proteins by providing a single cell type and extracellular environment and eliminating the possibility of inflammation. Recently, we have shown that the extracellular environment can alter both cellular morphology (27) and the protein composition of both the cytoskeleton and nuclear matrix (28). Thus, the extracellular matrix is connected to other cellular components including the nuclear matrix and may in turn have an effect on DNA organization and gene expression. These results raise the possibility that the three proteins that are found to be common to both the normal dorsal prostate and the AT2.1 tumor but are not found in the Dunning cell lines may be a result of the *in vivo* extracellular environment of both the tumor and the organ. Under appropriate conditions, the tumor may be able to express an extracellular matrix that is similar in some components to that in the normal prostate and would then result in the proteins common between the normal prostate and the AT2.1 tumor.

These differences in nuclear matrix proteins between the normal tissue lobes and transformed cell lines and tumor and within the transformed lines could be demonstrative of cell alterations which have occurred during the establishment of the tumor phenotype; however, cause and effect are not established. Some of these differences may correlate with alterations in DNA organization between the normal and transformed cell types which could correspond with the differences in protein

<table>
<thead>
<tr>
<th>Specific to normal dorsal prostate and not present in all Dunning tumors</th>
<th>$M_r$</th>
<th>$pI$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDP-1</td>
<td>95,000</td>
<td>6.74</td>
</tr>
<tr>
<td>NDP-2</td>
<td>57,000</td>
<td>8.33</td>
</tr>
<tr>
<td>NDP-3</td>
<td>57,000</td>
<td>8.0</td>
</tr>
<tr>
<td>NDP-4</td>
<td>47,000</td>
<td>5.26</td>
</tr>
<tr>
<td>NDP-5</td>
<td>47,000</td>
<td>5.80</td>
</tr>
<tr>
<td>NDP-6</td>
<td>41,000</td>
<td>6.83</td>
</tr>
<tr>
<td>NDP-7</td>
<td>37,200</td>
<td>7.05</td>
</tr>
<tr>
<td>NDP-8</td>
<td>36,900</td>
<td>7.35</td>
</tr>
<tr>
<td>NDP-9</td>
<td>35,000</td>
<td>6.25</td>
</tr>
<tr>
<td>NDP-10</td>
<td>32,500</td>
<td>5.46</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Specific to G</th>
<th>$M_r$</th>
<th>$pI$</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-1</td>
<td>55,000</td>
<td>6.48</td>
</tr>
<tr>
<td>G-2</td>
<td>52,000</td>
<td>6.93</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Specific to all Dunning tumors and not present in normal dorsal prostate</th>
<th>$M_r$</th>
<th>$pI$</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-1</td>
<td>63,000</td>
<td>8.55</td>
</tr>
<tr>
<td>D-2</td>
<td>40,000</td>
<td>5.91</td>
</tr>
<tr>
<td>D-3</td>
<td>33,000</td>
<td>6.97</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Specific to AT2 and MLL</th>
<th>$M_r$</th>
<th>$pI$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM-1</td>
<td>40,000</td>
<td>6.73</td>
</tr>
<tr>
<td>AM-2</td>
<td>36,000</td>
<td>8.33</td>
</tr>
</tbody>
</table>
expression found in transformed cell lines. Thus, the nuclear matrix which is the determinant of DNA organization (2) may play a role in the tissue specific organization of DNA as well as in the transformed phenotype. The determination of the nuclear matrix protein patterns of these cell types and the proposed resulting specificity in three-dimensional DNA organization is a possible mechanism, in part, by which cancer cells are able to express different proteins than the normal cell counterpart.

Changes in nuclear morphology in general are a hallmark of cancer cytopathology and diagnosis. In this regard, the Dunning tumor nuclear shapes are highly variant (pleiomorphic) and have significantly different values for all of their morphological descriptors than the original dorsal prostate from which they spontaneously arose. It is unknown what causes these changes in tumor nuclear morphology but, since the nuclear matrix is the paramount structural component of the nucleus, it would appear to be a primary candidate. While morphological factors may provide good visual discriminants for describing the state of transformation, they do not provide insight into tumor nuclear function. Resolution of DNA organization dictated in transformation, they do not provide insight into tumor nuclear function. ACKNOWLEDGMENTS

We would like to thank Dr. John T. Isaacs of Johns Hopkins for generously providing the chromosome numbers for the Dunning tumors and the Dunning cell lines, tumor sections, and the tumor.

REFERENCES


Identification of Nuclear Matrix Proteins in the Cancer and Normal Rat Prostate


Updated version Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/51/24/6514

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.