Nuclear Matrix Proteins in Normal and Breast Cancer Cells

Parvinderjit S. Khauja, Jeffrey E. Lehr, Herbert D. Soule, Suresh K. Gehani, Anthony C. Noto, Sajal Choudhury, Ruey Chen, and Kenneth J. Pienta

The progression from normal breast epithelium to a malignant phenotype may depend on changes in genetic events as well as failure of host mechanisms. Intermediate biomarkers are needed to more effectively identify malignant progression as well as to develop the potential for more specific treatments and prevention strategies. The nuclear matrix is the RNA-protein network which forms the skeleton of the nucleus and participates in DNA organization as well as multiple cellular functions. Nuclear matrix proteins have been demonstrated to be tissue and cell type specific as well as to reflect the state of cell differentiation and/or transformation. We prepared nuclear matrices from normal and cancer breast tissue from 10 patients with infiltrating ductal carcinoma of the breast as well as the MCF-10 mortal, immortal, and transfected breast cell lines. Nuclear matrices derived from normal human breast tissue and tumor tissue share common nuclear matrix proteins as well as demonstrate specific changes which appear to occur with the acquisition of the cancer phenotype. The MCF-10 cell lines demonstrate a phenotype that is intermediate between the normal and cancer tissue. These data suggest that the nuclear matrix may be an important biomarker in the pathogenesis of breast cancer.

INTRODUCTION

The pathogenesis of breast cancer, newly diagnosed in about 180,000 women per year in the United States, remains unclear (1-3). The progression from normal breast epithelium to a malignant phenotype may depend on changes in genetic events as well as failure of host mechanisms. With better understanding of the biology of breast cancer pathogenesis, intermediate biomarkers can be identified which will allow more emphasis to be placed on prevention of the disease as well as to develop the potential for more specific treatments. The documented molecular and structural changes which occur within the cancer cell during transformation still have not yielded a clear picture of the transformation process. The processes by which DNA organization is controlled and how this organization is changed by cell transformation is not well defined. The nuclear matrix contributes to the structural and functional organization of DNA and is a RNA-protein framework scaffolding which forms the superstructure of the nucleus and consists of peripheral laminis and pore complexes, an internal ribonucleic protein network, and residual nucleoli (4-9). The nuclear matrix provides the framework on which the DNA is organized into loop domains of approximately 60 kilobases, and the bases of these loops have been identified as the regions of the DNA that are attached to the nuclear matrix and also have been identified as the location of actively transcribed genes (10-15). This dynamic nuclear skeleton plays a central role in the topological arrangement of DNA and is, therefore, a critical component of multiple cell functions (16-18).

Several observations indicate that the nuclear matrix may be an important modulator of DNA organization and cell function. Nuclear matrix proteins vary in a cell type-specific manner, suggesting that the nuclear matrix may play an important role in the tissue-specific three-dimensional organization of DNA (19-21). The nuclear matrix interacts with steroid receptors to help modulate cell function, and in cancer cells, transformation proteins appear to be associated with the nuclear matrix (4, 21-23).

It has been reported that the nuclear matrices of the Dunning R3227 rat prostate adenocarcinoma cell lines G, AT2.1, and MAT-LyLu (MLL, Metastatic, Anaplastic, Tumor to Lymph nodes and Lungs) share common proteins with the dorsal rat prostate and have unique, cell type-specific nuclear matrix proteins (24). These differences in nuclear matrix proteins between normal tissues and transformed cells appear to be demonstrative of cell protein alterations which occurred during the establishment of the tumor phenotype; however, the significance of these changes has not been established. This study was undertaken to determine the nuclear matrix protein composition of normal human breast and breast tumors. These data were then analyzed in conjunction with the nuclear matrix composition from the human breast cell lines MCF-10 mortal, immortal, and transfected human breast cell lines (25, 26). The use of the human tumors, combined with the data from the MCF-10 cell lines, provides a clearer picture of nuclear matrix changes which occur during the process of cell transformation. These studies suggest that the protein composition of the human breast nuclear matrix may provide insight into the biological etiology of breast cancer as well as to provide a biological marker of growth and gene expression in the human breast.

MATERIALS AND METHODS

Cell Lines

The MCF-10 cell lines, mortal (MCF-10M) and immortal (MCF-10A), were derived and maintained as previously reported (25-27). The MCF-10 transfected cell lines MCF-10 H-RAS and C-NEU were obtained from Dr. Linda Watkins (Michigan Cancer Foundation, Detroit, MI).

Tumor Samples

Normal human breast tissue and breast tumors were obtained from biopsy or mastectomy specimens at the time of surgery. Normal and cancer tissue samples (1-2 g) were identified by the pathologist (S. K. G., A. C. N., S. C., R. C.), then placed in phosphate-buffered saline containing freshly made 1 mm PMSF3 to inhibit serine proteinases, and processed to isolate nuclear matrix proteins within 4 h. A section of tissue adjacent to the sample was stained as a standard hematoxylin and eosin E section and read by the pathologist to ensure histological accuracy.

Analysis of Nuclear Matrix Proteins

Nuclear Matrix Preparation. Nuclear matrices were prepared according to the methodology of Fey and Penman (19) and Getzenberg and Coffey (21). Cells (5 x 10^6) growing in culture were detached utilizing trypsin. Trypsin was neutralized by adding media with 10% fetal calf serum. Cells were then spun at 800 x g for 10 min to produce a pellet and then resuspended on ice with 0.5% Triton X-100 to release the lipids and soluble proteins in a buffer solution containing 2 mm vanadyl ribonucleoside, an RNAase inhibitor.

Received 12/28/92; accepted 5/11/93.

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1 This work was supported by Physician Scientist Award CA-60156.

2 To whom requests for reprints should be addressed, at Section of Molecular Oncology, Michigan Cancer Foundation, Rm 213, 110 E. Warren, Detroit, MI 48201.

The abbreviation used is: PMSF, phenylmethylsulfonyl fluoride.
proteins could be identified in all normal and tumor samples (Table 2). An ethanol precipitation step was carried out for about 4 h at 12.5 mA/gel. The slab gels were fixed in a solution of 10% acetic acid-50% methanol overnight. The following proteins (Sigma, St. Louis, MO) were used as markers for protein identification: carbonic anhydrase (29,000), and lysozyme (14,000). These standards appear as horizontal lines on the silver-stained 10% acrylamide slab gel (29). The gels were then dialyzed out, and the intermediate filaments were then reassembled (assembly buffer consists of 0.15 M KCl-25 mM imidazole hydrochloride-5 mM MgCl2-2 mM dithiothreitol-0.125 mM ethyleneglycol bis-(β-aminopropyl)ethane sulfonic acid (pH 6.6)-1 mM ethyleneglycol bis-(β-aminopropyl)ether-N,N,N’,N’-tetraacetic acid-1 mM PMSF-0.1 mM MgCl2-1% 2-mercapto-ethanol), and the insoluble components, which consisted principally of carboxydrates and extracellular matrix components, were pelleted. The urea was then dialyzed out, and the intermediate filaments were then reassembled (assembly buffer consists of 0.15 M KCL-25 mM imidazole hydrochloride-5 mM MgCl2-2 mM dithiothreitol-0.125 mM ethyleneglycol bis-(β-aminopropyl)ether-N,N,N’,N’-tetraacetic acid-0.2 mM PMSF) and separated out by centrifugation. The soluble nuclear matrix proteins were ethanol precipitated. All solutions contained freshly made 1 mM PMSF to inhibit serine proteases. The protein composition was determined by resuspending the proteins in 0.1 M sodium hydroxide and using the BCA protein assay (Pierce, Rockford, IL), with bovine serum albumin as a standard.

**Electrophoresis.** Two-dimensional electrophoresis was performed by Kendrick Labs, Inc. (Madison, WI) according to the method of O’Farrell (28) as follows. Isoelectric focusing was carried out in glass tubes of inner diameter 2.0 mm using 2.0% Ph 4-8 ampholines (BDH from Hoefer Scientific Instruments, San Francisco, CA) for 9600 V-h. An isoelectric focusing internal standard (40 ng), tropomyosin protein (M, 33,000) and P (isolectric point 5.2) was added to the samples. This standard is indicated with an arrow on the two-dimensional gel pattern. After equilibration for 10 min in buffer “O” (10% glycerol-50 mM dithiothreitol-2.3% sodium dodecyl sulfate-0.0625 M Tris, pH 6.8), the tube gel was sealed to the top of a stacking gel which was on top of a 10% acrylamide slab gel (420,000, phosphorylase A (94,000), catalase (60,000), actin (43,000), carbonic anhydrase (29,000), and lysozyme (14,000)). These standards appear as horizontal lines on the silver-stained 10% acrylamide slab gel (29). The gels were then dried between sheets of cellulose paper with the acid edge to the left. Only protein spots clearly and reproducibly observed in all gels of a sample type were counted as actually representing the nuclear matrix component. At the end of the experiment, samples were pooled as “normal” and “cancer,” and three composite gels were run to ensure accuracy of protein spots identified in all normal and tumor samples (Table 2). MW, molecular weight (in thousands).

**RESULTS**

Although work has been done to investigate changes in the nuclear matrix in cell transformation, it has not been demonstrated that similar changes in nuclear matrix composition occur within a given breast tumor type. This study investigated 10 normal breast tissue nuclear matrices as well as tumor specimens derived from patients with infiltrating ductal carcinoma of the breast. The patient characteristics are summarized in Table 1. Nine of the 10 patients were postmenopausal. Seven of eight patients who underwent mastectomy had positive lymph nodes, and estrogen receptor/progesterone receptor reported as fmol/mg protein, positive >15; PR, progesterone receptor reported as fmol/mg protein, positive >15; MRM, modified radical mastectomy; P, positive, Simp. Mast, simple mastectomy.

Specific differences did exist between the normal and cancer tissue. Review of the tumor samples revealed that at least four proteins, marked W, X, Y, and Z, appeared to be expressed in tumor tissue but not in normal tissue (Table 3 and Fig. 3B). These proteins were found as a pair of closely migrating spots, marked A and B, in the normal samples of M, 24,000 and 26,000 and may represent specific differences did exist between the normal and cancer tissue. Review of the tumor samples revealed that at least four proteins, marked W, X, Y, and Z, appeared to be expressed in tumor tissue but not in normal tissue (Table 3 and Fig. 3B). These proteins were found as a pair of closely migrating spots, marked A and B, in the normal samples of M, 24,000 and 26,000 and may represent a single protein and a modified (e.g., phosphorylated) variant of the same protein. These tissue specific proteins, A, B, W, X, Y, and Z, were not found in the soluble protein fractions extracted by the nuclear matrix purification procedure (data not shown). These changes in nuclear matrix composition between normal and cancer tissue could represent stromal or epithelial cell component changes. To determine whether these changes occur in the nuclear matrix composition of epithelial cells, we next investigated the composition of the nuclear matrix proteins of the MCF-10 cell lines. The MCF-10 mortal line, the spontaneously immortalized MCF-10A, and the transfected variants of the immortalized line, H-RAS-MCF10A and C-NEU-MCF10A, were all investigated. These cultured cells demonstrated a nuclear matrix protein composition phenotype that...
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Fig. 2. A schematic representation of the high resolution two-dimensional gel electro-phoresis of the nuclear matrix proteins from normal breast as well as from infiltrating ductal breast carcinoma. Proteins found in all tissues are numbered 1–10. Cancer tissue-specific proteins are labeled W, X, Y, and Z. Normal tissue-specific proteins are labeled A and B. MW, molecular weight (in thousands); pi, isoelectric point.

Table 2 Common nuclear matrix proteins found in both normal and cancerous breast tissue

<table>
<thead>
<tr>
<th>Designation</th>
<th>$M_w$</th>
<th>Isoelectric point</th>
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<tr>
<td>NMB1</td>
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<td>6.8</td>
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<td>5.7–5.9</td>
</tr>
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<td>NMB9</td>
<td>76,000</td>
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</tr>
<tr>
<td>NMB10</td>
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Table 3 Nuclear matrix proteins found in tumor samples.

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<th>Designation</th>
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<tr>
<td>NMBC-W</td>
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</tr>
<tr>
<td>NMBC-X</td>
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<td>5.3</td>
</tr>
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<td>NMBC-Y</td>
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<td>4.7</td>
</tr>
<tr>
<td>NMBC-Z</td>
<td>80,000</td>
<td>5.5</td>
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Table 3 Nuclear matrix proteins found in tumor samples.

These proteins were not present in nuclear matrix prepared from normal breast tissue.

DISCUSSION

Since the nuclear matrix is the dynamic scaffold through which DNA is structurally and functionally organized, it makes inherent sense that nuclear matrices from different cell types would share at least some common proteins. Stuurman et al. (31) demonstrated that a “minimal nuclear matrix” exists, consisting of proteins found in virtually all mammalian cell types which have been examined. Nakayasu and Berezney (32) further identified specific proteins, termed matrices, which appear to be present in multiple cell types. Partin et al. (33) recently demonstrated that nuclear matrices derived from prostates of different patients share common proteins in their normal, hyperplastic, and cancer tissues. We demonstrate here that the protein components of the breast tissue-derived nuclear matrix express common as well as different components. We arbitrarily chose 10 of these proteins that could be identified in all normal and cancer tissues (labeled 1–10 in figures). Because of differences in isolation and electrophoresis conditions, it is difficult to determine with certainty that these proteins can be exactly identified in the published two-dimensional gels of other tissues (19, 21, 23, 24, 31–34). When two-dimensional gels of prostate cancer cell lines isolated in our laboratory were compared in the same way and run under the same electrophoretic conditions, many of these common nuclear matrix proteins can be identified (data not shown, 30).

In 1979, Berezney et al. (34) demonstrated that the nuclear matrix of hepatoma cells appeared qualitatively different than nuclear matrices derived from normal liver. Since then, multiple investigators have suggested that the study and characterization of nuclear matrix protein composition can lend insight into tissue origin of a cell and how a disease process is altering that cell. For example, Fey and Penman (19, 35), demonstrated that nuclear matrix proteins are cell type specific,
Table 4  Nuclear matrix proteins found in normal breast tissue only.

<table>
<thead>
<tr>
<th>Designation</th>
<th>M_i</th>
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<tbody>
<tr>
<td>NMNB-A</td>
<td>24</td>
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</tr>
<tr>
<td>NMNB-B</td>
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and they also demonstrated that nuclear matrix composition is altered by tumor promoters. Dworetzky et al. (36) further elegantly demonstrated how these cell type-specific matrices undergo specific alterations in protein composition as a cell undergoes differentiation. Also, evidence that the nuclear matrix may play a role in gene expression has been suggested by the observation that protein composition of the nuclear matrix varies within cells of the same organ which have different functions. For example, the nuclear matrix composition of the epithelial cells of the rat ventral prostate are different from the nuclear matrix proteins of the epithelial cells of the dorsal lobe of the rat prostate (24). Dworetzky et al. (37) strengthened these suggestions further by demonstrating that sequence-specific DNA-binding proteins are components of nuclear matrix attachment sites for an actively transcribed gene. We demonstrate here that the nuclear matrices derived from breast cancer tissue are qualitatively different from their normal counterparts, demonstrating both loss and gain of specific proteins. It is unclear whether these proteins correspond to any of the normal counterparts, demonstrating both loss and gain of specific proteins. It is unclear whether these proteins correspond to any of the normal counterparts, demonstrating both loss and gain of specific proteins.

ACKNOWLEDGMENTS

We thank MaryAnn Terranova for her assistance in arranging the manuscript. We thank Dr. Linda Watkins and Dr. Thomas Weise for their help with cell lines. We thank Tracy Accardo and Karen Filipak for their assistance in obtaining the pathological specimens.

REFERENCES

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