Nuclear Matrix Proteins in Normal and Breast Cancer Cells

Parvinderjit S. Khanuja, Jeffrey E. Lehr, Herbert D. Soule, Suresh K. Gehani, Anthony C. Noto, Sajal Choudhury, Ruy 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 lamins and pore complexes, 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, 22).

It has been reported that the nuclear matrices of the Dunning R3227 rat prostate adenocarcinoma cell lines G, AT2.1, and MAT-LyLu (ML, 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 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 PMSF to inhibit serine proteases, 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 × 10^6) growing in culture were detached utilizing trypsin. Trypsin was neutralized by re-adding media with 10% fetal calf serum. Cells were then spun at 800 × 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 buffered solution containing 2 mM vanadyl ribonucleoside, an RNAase inhibitor.

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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). Center of Metropolitan Detroit as previously described (30).

ensure accuracy of protein spots on the ISODALT system as part of the molecular biology core facility of the Meyer L. Prentis Comprehensive Cancer ChemC, St. Louis. MO) were added as molecular weight standards (in gels (0.75 mm thick), and sodium dodecyl sulfate slab gel electrophoresis were then dried between sheets of cellophane paper with the acid edge to the horizontal lines on the silver-stained 10% acrylamide slab gel (29). The gels bonic anhydrase (29,000), and lysozyme (14,000). These standards appear as (220,000), phosphorylase A (94,000), catatase (60,000), actin (43,000), car

threitol-2.3% sodium dodecyl sulfate-0.0625 MTris, pH 6.8), the tube gel was added to the samples. This standard is indicated with an arrow on the standard (40 ng), tropomyosin protein (M, 33,000) and P: (isoelectric 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 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 gels (0.75 mm thick), and sodium dodecyl sulfate slab gel electrophoresis 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 Chemical Co., St. Louis, MO) were added as molecular weight standards (in parentheses) to the agarose which sealed the tube gel to the slab gel: myosin (220,000), 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 cellophane 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 compo

ments. At the end of the experiment, samples were pooled as “normal” and we chose 10 of these proteins to serve as markers for the common and Fig. 1; see Fig. 2 for a schematic of the two-dimensional data), and we chose 10 of these proteins to serve as markers for the common set.

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. 3A). One protein was of low molecular weight (18,000), and the others were found at relatively low isoelectric points, between 60,000 and 80,000. Two proteins appeared to be lost in the tumor samples (Table 4 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
NUCLEAR MATRIX IN BREAST CANCER

Fig. 2. A schematic representation of the high resolution two-dimensional gel electrophoresis 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); pl, 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>
<td>12,000</td>
<td>6.8</td>
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<tr>
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<td>NMB7</td>
<td>31,000</td>
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<td>NMB8</td>
<td>64,000</td>
<td>5.6–5.7</td>
</tr>
<tr>
<td>NMB9</td>
<td>76,000</td>
<td>5.7–5.9</td>
</tr>
<tr>
<td>NMB10</td>
<td>92,000</td>
<td>5.9–6.1</td>
</tr>
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* NMB, nuclear matrix breast.

Fig. 3. A, high resolution two-dimensional gel electrophoresis of a breast cancer specimen from a patient with infiltrating ductal carcinoma revealing four nuclear matrix proteins found only in tumor tissue samples. These proteins are marked by arrows and the letters W, X, Y, and Z and correspond to the proteins in Table 3 designated NMBC-W and -Z. B, high resolution two-dimensional gel electrophoresis of normal breast tissue from the same patient revealing two nuclear matrix proteins specifically found in normal breast tissue but not in breast tumor tissue. These proteins are marked by arrows and the letters A and B and correspond to the proteins in Table 4 designated NMNB-A and -B. MW, molecular weight (in thousands); pl, isoelectric point.

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 electrophoretic 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,
NUCLEAR MATRIX IN BREAST CANCER

Table 4 Nuclear matrix proteins found in normal breast tissue only.

<table>
<thead>
<tr>
<th>Designation</th>
<th>M_W</th>
<th>Isoelectric point</th>
</tr>
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<tbody>
<tr>
<td>NMNB-A</td>
<td>24</td>
<td>6.2</td>
</tr>
<tr>
<td>NMNB-B</td>
<td>26</td>
<td>6.4</td>
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These proteins were not present in tumor tissue samples. NMNB, nuclear matrix normal breast.

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REFERENCES

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