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MODIFICATIONS OF THE INTERMEDIATE FILAMENT AND NUCLEAR MATRIX NETWORKS BY THE EXTRACELLULAR MATRIX+

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The tissue matrix system is a dynamic, interacting structural network directly linking the nuclear matrix, cytoskeleton and the extracellular matrix. We report here that interaction of normal rat kidney epithelial cells (NRK) and Kirsten-ras transformed rat kidney cells, with an extracellular matrix secreted by tumor cells, causes modifications to the protein composition of the intermediate filament and nuclear matrix networks. The matrix networks are different between normal and transformed cells; however, these alterations by the tumor extracellular matrix are similar in both cell types. These data represent the first report that modification of the extracellular matrix environment can have an effect on the protein composition of the nuclear matrix. © 1991 Academic Press, Inc.

The accompanying paper from our laboratory describes that various extracellular matrix substrata can alter the morphological structure of cells which interact with them. Plating the normal rat kidney cells (NRK) on the tumor extracellular matrix components in matrigel is able to bring about a transformed phenotype to the normal rat kidney cells. Many laboratories have demonstrated the concept that the extracellular matrix can have a direct role in cell shape, differentiation, gene expression and DNA replication (reviewed in 1). The concept of a interactive tissue matrix which organizes cell structure and function from the cell periphery through to the DNA was originally proposed by Bissell (2) and Isaacs (3) and was supported by studies from Fey et al. (4). The ability of the extracellular matrix to alter the components of the tissue matrix and subsequently which may play a role in the transformed phenotype demonstrated in the previous paper, was examined here by determining the protein components of the intermediate filament and nuclear matrix fractions from normal rat kidney and Kirsten-ras transformed rat kidney cells plated on both glass and the extracellular substrata, matrigel.

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MATERIALS AND METHODS

Materials

The Engelbreth-Holm-Swarm (EHS) mouse sarcoma extracellular matrix product termed matrigel was obtained from Collaborative Research (Bedford, MA). The immortalized normal rat kidney epithelial cell line NRK-52E (ATCC-CRL 1571) and the Kirsten-ras transformed rat kidney epithelial cell KNRK (ATCC CRL 1569) were obtained from the American Type Culture Collection (Rockville, MD). The cell lines were maintained in DMEM supplemented with 5% fetal bovine serum.

Nuclear Matrix and Intermediate Filament Isolation

The nuclear matrices proteins were isolated according to the methodology of Fey and Penman (4). The cells are treated 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 RNAase inhibitor. Salt extraction with 0.25 M ammonium sulfate with vanadyl ribonucleoside is added to release the soluble cytoskeletal elements. DNAase-I and RNAase-A treatment at 25°C. are used to remove the soluble chromatin and RNA. The remaining insoluble fraction contains intermediate filaments and nuclear matrix proteins, is then disassembled with 8 M urea, and the insoluble components which consist principally of carbohydrates and extracellular matrix components, are pelletted. 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 phenylmethylsulfonylfluoride to inhibit serine proteases. The protein composition is determined by resuspending the proteins in 0.1 N 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 and the intermediate filament proteins, are redissolved in a sample buffer consisting of 9 M urea, 65 mM CHAPS, 2.2% ampholytes and 140 mM DTT.

Two-Dimensional Electrophoresis

High resolution two-dimensional gel electrophoresis was carried out utilizing the Investigator 2-D gel system (Milligen/Biosearch, Bedford, MA) with modifications. One dimensional isoelectric focusing was carried out for 18,000 volt-hours 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 constant temperature regulation. The gels were then fixed with 50% methanol and 10% acetic acid. The gels were then thoroughly rinsed and treated with 5% glutaraldehyde and 5 mM dithiothreitol after proper buffering. After rinsing overnight, the gels were stained with silver stain using the methodology of Wray (5) (Accurate Chemical Co., Inc., Westbury, NY). Thirty micrograms 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 Pharmacia (Piscataway, NJ). Multiple gels were run for each sample, and multiple samples were run at different times. The gels shown in the manuscript 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 differences in the nuclear matrix and intermediate filament components. Quantitative alterations in protein composition, while noted, are not demonstrated here.

RESULTS AND DISCUSSION

The mechanism by which the extracellular matrix alters cells function is unknown but it is believed that cell structural changes may be involved (1). The placement of cells on matrigel

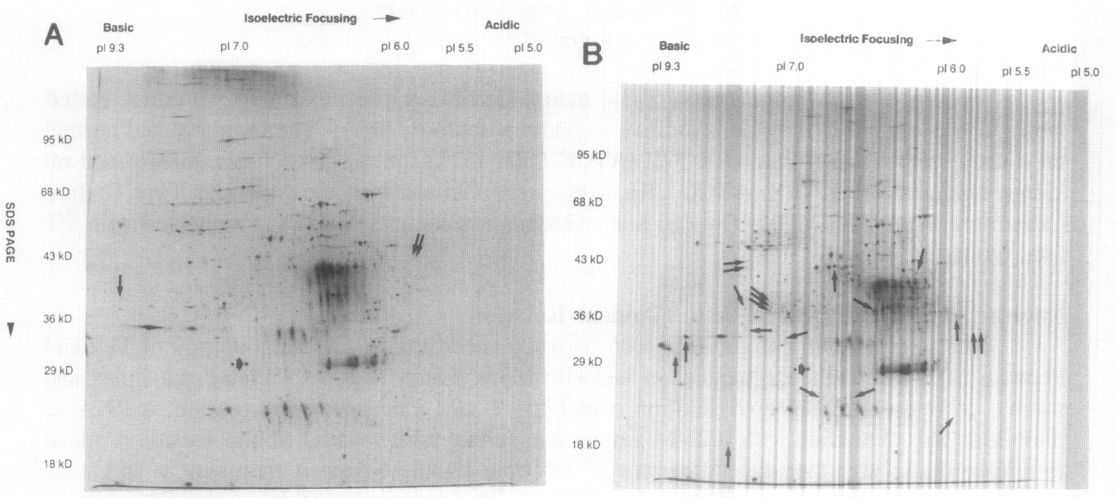

Figure 1. High Resolution Two-Dimensional Gel Electrophoresis of Nuclear Matrix Proteins of Normal Rat Kidney Cells.

Nuclear matrix proteins isolated from normal rat kidney cells after plating for 24 hours on either (A) glass or (B) matrigel. The arrows represent the proteins which are unique for the substrata. The * indicates the protein (30 kD, pI 6.96) which is unique to normal rat kidney cells and disappears in the K-ras transformed normal rat kidney cells.

has a dramatic effect on the structure of the cell and nucleus as reported in the previous manuscript. One of the determinants of the structure of the cell is the intermediate filament-nuclear matrix network (4). In this study, we report that the protein composition of both the nuclear matrix and intermediate filament networks are qualitatively altered by the substratum on which the cells grow. When normal rat kidney cells are plated on matrigel, many proteins appear in the nuclear matrix fraction which were not present when the cells were plated on glass. There also are several nuclear matrix proteins which disappear when comparing the matrigel plated cells with the glass plated controls (Figure 1). In comparison, Kirsten-ras transformed rat kidney cells plated on the same substrata undergo similar modifications to their nuclear matrix protein composition. The same proteins either appear or disappear when comparing the matrigel plated with the glass plated controls (Figure 2). In addition, as noted in figure 1, there is only one major protein (30 kD, pI 6.96) that can be identified as being different between the normal rat kidneys and the transformed Kirsten-ras transformed rat kidneys. This protein is not present in the Kirsten-ras transformed rat kidney cell nuclear matrices on any surface and is present in both the matrigel and glass normal rat kidney fractions.

The protein composition of the intermediate filament network also undergoes modifications when plated on matrigel. There are proteins which both appear and disappear when comparing the matrigel plated versus the glass controls in both the normal rat kidney (Figure 3) and the transformed Kirsten-ras transformed rat kidney cell lines (Figure 4) and these

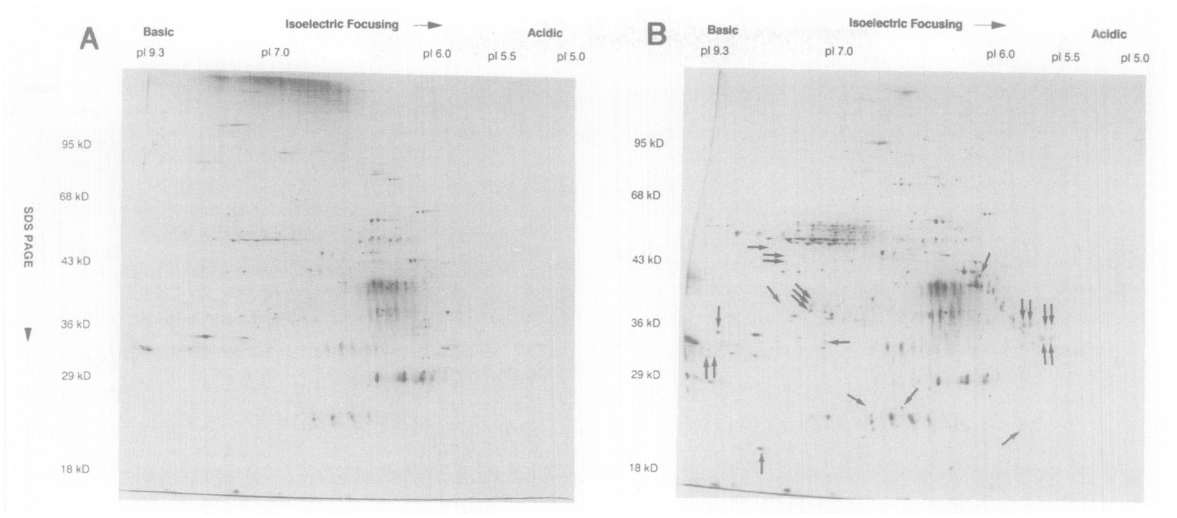

Figure 2. High Resolution Two-Dimensional Gel Electrophoresis of Nuclear Matrix Proteins of K-ras Transformed Normal Rat Kidney Cells.

Nuclear matrix proteins isolated from K-ras transformed normal rat kidney cells after plating for 24 hours on either (A) glass or (B) matrigel. The arrows represent the proteins which are unique for the substrata.

proteins appear to be similar between the cell lines. It is important to note that only a few of the intermediate filament proteins noted are present also in the nuclear matrix changes seen in figures 1 and 2.

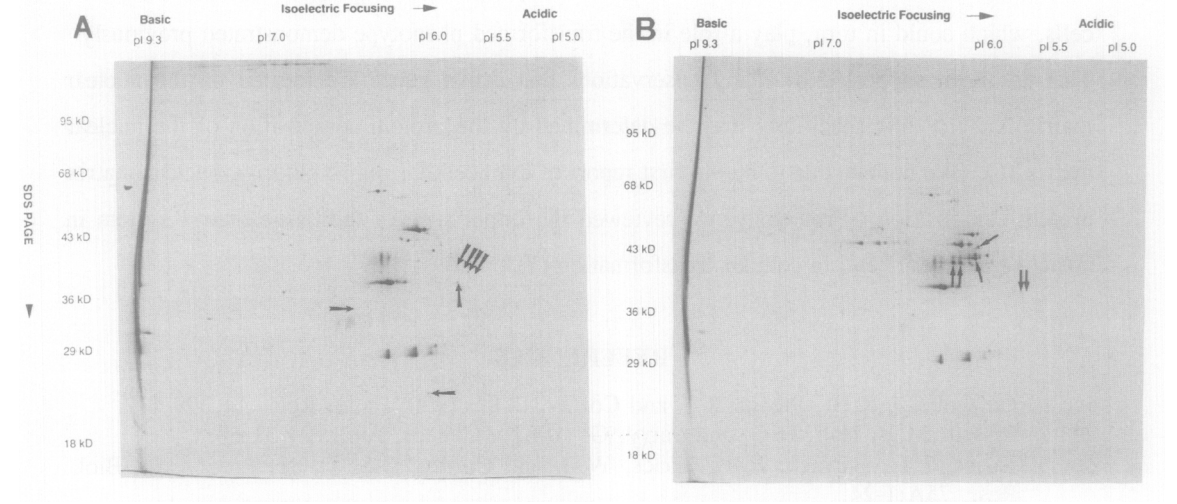

Figure 3. High Resolution Two-Dimensional Gel Electrophoresis of Intermediate Filament Proteins of Normal Rat Kidney Cells.

Intermediate filament proteins isolated from normal rat kidney cells after plating for 24 hours on either (A) glass or (B) matrigel. The arrows represent the proteins which are unique for the substrata.

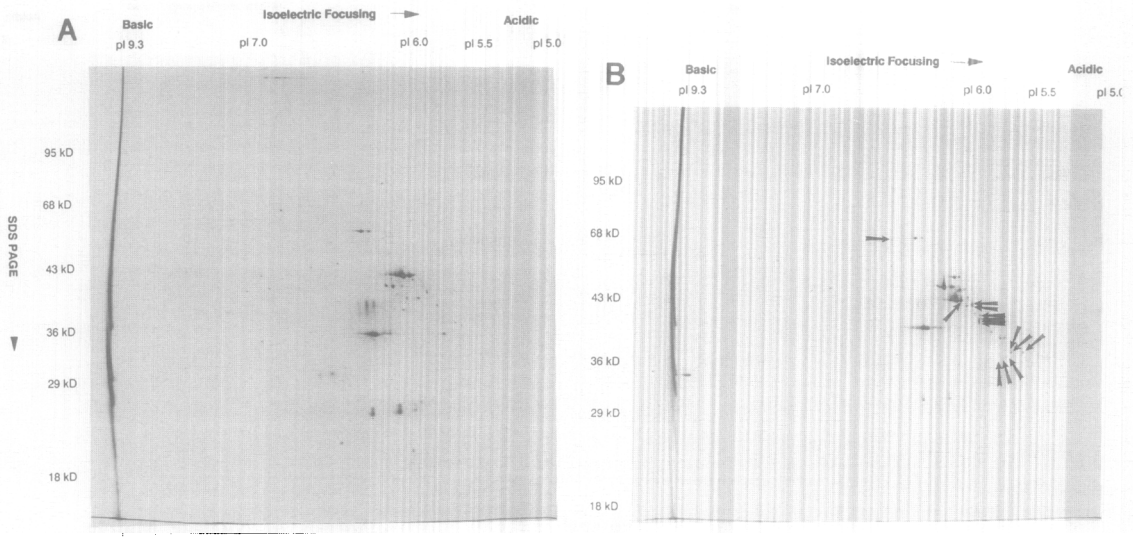

Figure 4. High Resolution Two-Dimensional Gel Electrophoresis of Intermediate Filament Proteins of K-ras Transformed Normal Rat Kidney Cells.

Intermediate filament proteins isolated from K-ras transformed normal rat kidney cells after plating for 24 hours on either (A) glass or (B) matrigel. The arrows represent the proteins which are unique for the substrata.

We have observed many modifications to both the nuclear matrix and intermediate filament networks when cells were plated on matrigel. These results demonstrate that the extracellular matrix environment on which a cell grows has a dramatic effect on its tissue matrix system. These differences in intermediate filament and nuclear matrix protein compositions could have a dramatic effect on the DNA organization and subsequent gene expression of these cells, which could in turn, play a role in the transformed phenotype demonstrated previously. This has been supported by many observations that active genes are located on the nuclear matrix and that this specificity may be determined by the protein composition of the nuclear matrix (6). We believe this to be the first report of extracellular matrix altering nuclear matrix protein composition. Previously, we reviewed the importance of the tissue matrix system in hormone action (1) and in cellular transformation (7).

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