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THE EFFECT OF EXTRACELLULAR MATRIX INTERACTIONS ON MORPHOLOGIC TRANSFORMATION IN VITRO[+]

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There is emerging evidence that the structure and function of a cell is dependent in part on the contacts that cells make with the extracellular matrix. We report here the effect of extracellular matrices secreted from both normal and tumor cells have on the structure of normal rat kidney epithelial cells. Normal rat kidney cells plated on the basement membrane secreted by tumor cells adopt a morphology and phenotype which closely resembles a Kirsten-ras transformed normal rat kidney cell. This morphologic transformation was not observed for cells plated on individual extracellular matrix components or on basement membrane secreted by normal placenta cells. This suggests that tumor derived basement membrane has unique characteristics which may cause morphologic transformation of normal rat kidney cells.

The extracellular matrix which a cell touches has been shown to play a major role in determining how a cell functions. Several studies have demonstrated that the extracellular matrix can alter cell morphology and effect DNA synthesis and gene expression (1-7). Furthermore, Martin and Kleinman have pioneered studies which demonstrate that matrigel, a reconstituted basement membrane derived from the Engelbreth-Holm-Swarm sarcoma, is capable of increasing the in vivo tumorigenicity of several different cell lines when injected into athymic mice (8-10). However, it remains unclear how these extracellular matrix mediated alterations in cell structure, function, and tumorigenicty are generated (11-13).

The extracellular matrix is a complex three-dimensional array composed of proteins which include laminin, collagen IV, and entactin, as well as proteoglycans and sequestered growth factors. We, therefore, undertook a study to quantitate the morphologic effects on the normal rat kidney cell of basement membranes and their individual components.

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MATERIALS AND METHODS

Materials: Murine extracellular matrix products matrigel, laminin, type IV collagen, entactin, and human placenta matrix and dispase were obtained from Collaborative Research (Bedford, MA, 01730). Lab-Tek double well chamber slides (2cm x 2cm) were obtained from Nunc Inc., Naperville, Il. Preparation of substrata for the morphology assay was as follows: laminin 1-2 micrograms/cm^2, Matrigel 125 micrograms/cm^2, type IV collagen 15-20 micrograms/cm^2, and placenta matrix 125 micrograms/cm^2 substrata were formed on double well chamber slides according to Collaborative Research specifications. Glass double well chamber slides were used for the glass substratum. All experiments performed with matrigel were also done with E-C-L Attachment Matrix, the Upstate Biotechnology Inc. (UBI) product (Lake Placid, NY 12946) that is derived from the same tumor line as matrigel and similar results were obtained. Entactin, laminin, as well as basic fibroblast growth factor and transforming growth factor beta antibodies were all obtained from UBI.

Cell culture: 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 (Parklawn Drive, Rockville, MD 20852.) The cell lines were maintained in DMEM supplemented with 5% fetal bovine serum.

Morphometric analysis: Cells were plated on substrata prepared double well chamber slides at a concentration of 2 x 10^4 cells per well. Cells were incubated at 37^0c for 24 hours. After incubation, morphometric analysis and computation was performed as previously described using the Zeiss Dynacell system (14). In brief, area in square microns and perimeter in microns are measured for each nucleus. Form factors 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 as the nuclear contour deviates from a perfect circle. The formula for the roundness form factor is $\{ [\text{Perimeter} / 2 \times \text{pi} / (\text{area} \times \text{pi})^{1/2}] - 1 \} \times 1000$. The nuclear/cytoplasmic ratio is calculated by dividing the projected area of a nucleus by the projected area of the cell and multiplying the result by 100.

Cell Motility Assay: Cell motility was measured using a time-lapse videomicroscopy assay as previously described (15). Briefly, translational motility of each cell was measured from the TV monitor over two hours by directly measuring the total path distance each cell travelled. A minimum of 25 cells were measured on each substratum.

Statistics: Statistical analysis was performed using Statgraphics v.4.0 (Statistical Graphics Corp.). Statistical significance was determined using the Student's t test.

RESULTS AND DISCUSSION

A hallmark of transformation from a normal to a cancer phenotype is an alteration in cell and nuclear morphology that produces variation in abnormal shapes and volumes termed pleomorphism (16). To investigate whether matrigel could alter the normal rat kidney cell phenotype we plated normal rat kidney cells on glass or matrigel and compared their morphology (see Table 1). We found that cells in contact with matrigel demonstrated significantly decreased cell area ($p < 0.001$), altered cell morphology ($p < 0.01$), an increased nuclear/cytoplasmic ratio, increased cell motility ($p < 0.01$), and loss of contact inhibition. All of these parameters are consistent with those exhibited by the transformed counterpart of the normal rat kidney cell, the Kirsten-ras transformed normal rat kidney cell. The transformed rat kidney cell plated on glass

Table 1. The basement membrane secreted by the Engelbreth-Holm-Swarm tumor (Matrigel) alters normal rat kidney (NRK) cell morphology to resemble a cancer phenotype (T-KNRK)

CELL TYPE / SURFACE	CELL AREA (μm^2)[A]	CELL SHAPE[B]	N/C RATIO[C]	MOTILITY ($\mu m/hr$)[A,D]	CONTACT INHIBITED
NRK[E]/ GLASS	2130 +/- 200	1394 +/- 216	7+/-1	6 +/- 1	+
NRK/ MATRIGEL	594 +/- 51	3405 +/- 315	16+/-1	33 +/- 4	-
T-KNRK[F]/ GLASS	535 +/- 15	2632 +/- 218	19+/-1	22 +/- 5	-
T-KNRK/ MATRIGEL	640 +/- 28	6694 +/- 402	22+/-2	55 +/- 7	-

[A] All values for cell area, cell shape, N/C ratio, and motility are represented as mean +/- standard error. All values are significantly different as compared to NRK/Glass controls ($p<0.001$).
[B] Shape is measured as a form factor: $\{[perimeter/2xpi / (areaxpi)^{1/2}] - 1\}x1000$. A perfect circle has the value of zero.
[C] N/C ratio = (projected nuclear area/projected cytoplasmic area) x 100.
[D] Motility is measured as the total path length a cell travels in one hour utilizing a time-lapse videomicroscopy assay.
[E] NRK cells are immortalized rat kidney epithelial cells.
[F] T-KNRK cells are Kirsten-ras transformed NRK cells.

exhibits a cell size, shape, nuclear/cytoplasmic ratio, and motility pattern that is very similar to the normal rat kidney cell on matrigel. When the transformed cell is plated on matrigel, it becomes even more out of shape and demonstrates an even faster baseline motility. These results suggest that matrigel causes the normal rat kidney cells to adopt static and dynamic morphologic characteristics which resemble those of transformed cells.

To dissect the components which might be involved in causing this morphologic transformation, we examined the morphologic characteristics of normal rat kidney cells plated on the three major structural protein components derived from matrigel; collagen IV, laminin, and entactin (see Figure 1). Normal rat kidney cells plated on these individual components assumed a more spread out morphology and subsequently demonstrated an increased cell area. Laminin and entactin had no effect on cell shape but collagen IV caused the cells to adopt a significantly more round morphology ($p<0.001$). The individual components had no effect on nuclear/cytoplasmic ratio.

These results suggested that the observed matrigel associated morphologic transformation of normal rat kidney cells was not mediated by one of these proteins. However, it was still possible that it was the three dimensional conformation of these components that was important. To investigate this phenomenon normal rat kidney cells were plated on a basement membrane

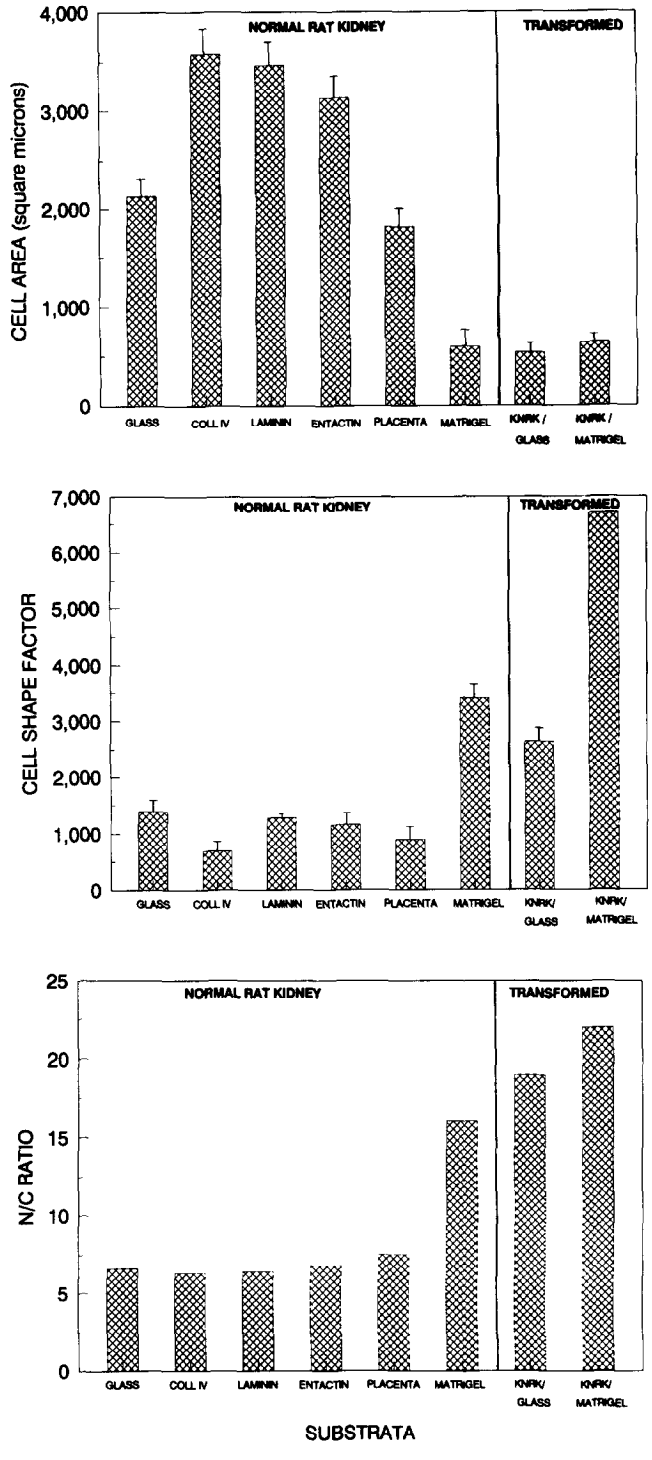

Figure 1. The morphologic characteristics of normal rat kidney cells (NRK) plated on various extracellular matrices as compared to the morphologic characteristics of the Kirsten-ras transformed rat kidney cells (KNRK). Matrigel caused the normal rat kidney cells to assume a transformed morphologic phenotype (see text). Normal rat kidney epithelial cells plated on individual matrigel components or a basement membrane derived from normal human placenta did not exhibit this morphologically transformed phenotype.

secreted by human placenta. This placenta matrix should present the extracellular matrix components in a three dimensional array and is reported to have a similar protein compositon as matrigel by Collaborative Research. However, normal rat kidney cells plated on placenta matrix exhibited similar morphologic patterns as compared to cells plated on glass or the individual components (see Figure 1). Therefore, matrigel, as a basement membrane secreted by tumor cells, appears to have unique transforming properties that are not associated with a basement membrane derived from nontumorigenic placenta cells. The transformation determinants of the tumor derived extracellular matrix have not been resolved, but may involve specific three dimensional conformation, growth factors, proteoglycans, or other unknown determinants.

Matrigel is also reported to be rich in growth factors and proteoglycans (9). We attempted to block the morphological transformation of normal rat kidney cells by adding anti-transforming growth factor beta antibodies and anti-basic fibroblast growth factor antibodies to the matrigel substrata prior to and during cell seeding. Neither of these antibodies could block the matrigel effect (see Table 2). We also attempted to pretreat matrigel with heparin and suramin, drugs which reportedly bind and block growth factor functions (17), and could not block matrigel induced transformation. Previously, it had been reported that laminin, as the major protein component of matrigel, could cause morphologic transformation of small cell lung cancer cells (8). Furthermore, these earlier studies demonstrated that YIGSR, a synthetic peptide which is part of the cell attachment site of laminin, could inhibit tumor growth in vivo (8). In this in vitro system, the addition of YIGSR had no effect on matrigel induced morphologic transformation. Entactin and laminin antibodies were also not effective (see Table 2). Therefore, the mechanism of morphologic transformation of nontumorigenic immortal normal rat kidney cells in vitro by matrigel remains an enigma. This morphologic transformation, however, also correlates with the transformation of normal rat kidney cells in vivo. When 500,000 normal rat kidney cells were injected into the flanks of four athymic mice, no tumors formed. When the same amount of cells were co-injected with five milligrams of matrigel, three out of four animals formed tumors by day 28 (data not shown) in a similar manner as had been reported by Fridman and colleagues for many types of other cells (8).

Therefore, matrigel causes morphologic transformation in vitro and enhances tumorigenicity in vivo of the normal rat kidney cell line. Although the mechanisms for this transformation to a tumor phenotype remain unclear we had previously demonstrated that alterations in nuclear morphology could be correlated with acquisition of the cancer phenotype (16,18). Therefore, we postulated that matrigel may cause alterations in nuclear structure that could lead to alterations in nuclear function. Indeed, matrigel induced dramatic alterations in the shape of both the normal and transformed rat kidney nuclei (see Figure 2). It is possible that

Table 2. Matrigel induced morphologic transformation of normal rat kidney cells cannot be blocked by inhibitors to individual matrigel components or growth factors

CELL/ TREATMENT	CELL AREA (μm^2)[A]	CELL SHAPE[B]
NRK CONTROL	2130 +/-200	1394 +/- 216
NRK/ MATRIGEL	594 +/- 51	3405 +/- 315
INHIBITOR		
ANTI-LAMININ AB	545 +/- 25	3632 +/- 226
YIGSR	650 +/- 30	2908 +/- 301
ANTI-ENTACTIN AB	498 +/- 11	3500 +/- 280
HEPARIN	612 +/- 42	2543 +/- 346
SURAMIN	716 +/- 38	3219 +/- 262
ANTI-bFGF AB	522 +/- 18	2314 +/-193
ANTI-TGFb AB	578 +/- 21	2749 +/- 250

[A] All values for cell area and cell shape are represented mean as +/- standard error. All values for cell area and shape are significantly different than NRK control cells plated on glass ($p<0.01$).

[B] Cell shape is measured as a form factor: $\{[perimeter/2xpi / (areaxpi)^{1/2}] - 1\}x1000$. A perfect circle has the value of zero.

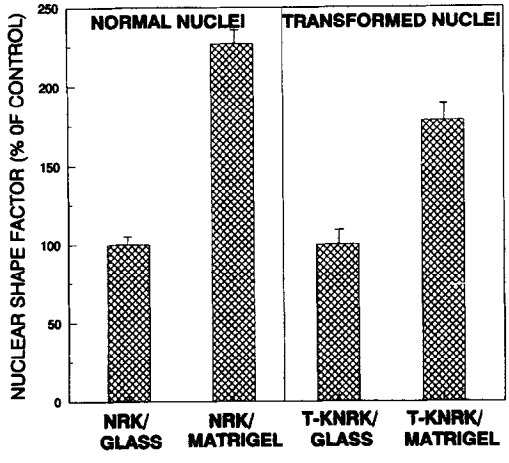

Figure 2. Matrigel alters the nuclear morphology of both normal (NRK) and transformed (T-KNRK) rat kidney epithelial cells. Cells plated on matrigel exhibited nuclei which were significantly more out of shape as compared to control cells plated on glass.

these alterations in nuclear structure may play a role in the enhanced tumorigenicity and morphologic transformation of the normal rat kidney cells by matrigel. In the companion paper we will report matrigel induced changes in nuclear matrix proteins.

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