Primary Prostate Stromal Cells Modulate the Morphology and Migration of Primary Prostate Epithelial Cells in Type 1 Collagen Gels

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Abstract

The effects of human primary prostatic stromal cells on the migration and morphogenesis of human prostatic epithelial cells, derived from tumor or benign prostatic hyperplasia tissue, were studied using a three-dimensional coculture system. Epithelial cells from tumor or benign tissue migrated efficiently into collagen gels populated with stromal cells from benign tissue. Only epithelial cells from benign prostate formed acinus-like structures that exhibited differentiated prostatic function and strong expression of membrane-associated E-cadherin. In gels populated by stromal cells from tumor tissue, migration of primary prostatic epithelial cells did not occur. In the absence of stromal cells, primary epithelial cells were unable to proliferate. This three-dimensional culture system allows closely controlled manipulation and analysis in vitro of interactions between prostatic epithelial and stromal cells.

Introduction

CaP is the second most common cause of cancer-related deaths among men in the United States (1). The American Cancer Society estimates that ~198,100 new cases of CaP will be diagnosed during 2001. Although the lifetime chance of developing CaP is 1 in 6, only 1 in 30 will die of the disease. Identification of the tumors that will progress to metastasis requires an increase in our knowledge of the factors contributing to the development of the disease.

Because most prostate tumors arise in the glandular epithelial cells, these have been a major focus of research. However, it is clear that interactions between stromal, epithelial, and extracellular matrix components play an important role in defining the malignant phenotype, as shown in animal models and xenografts (2, 3). In vitro models provide a complex environment that makes the isolation of modulating factors difficult. In vitro studies have produced conflicting data, although the influence of stroma on epithelial cell invasion and motility has been established (4, 5). The progression to metastatic cancer is linked in pathological terms to a loss of acinar differentiation, but little is known about the cell-cell and cell-extracellular matrix interactions that are important in the regulation of acinar morphogenesis and progression to CaP. Olumi et al. (6), in an in vivo model, described how cancer-associated fibroblasts can direct the progression to tumorigenesis of initiated human prostate epithelium but do not have the same effect on normal primary prostate epithelium.

Recent studies have used collagen type 1, a major structural component of the stromal microenvironment, to generate a three-dimen-
Analysis of Morphology and Migration of Epithelial Cells. Collagen gels were mounted in OCT (BDH, Poole, United Kingdom) and snap-frozen in liquid nitrogen. Care was taken to maintain the orientation of the gels. Transverse 10-μm frozen sections were cut using a Leica cryostat (Cryocut 1800) and mounted on Superfrost Plus microscope slides (BDH). Sections were air dried overnight and then fixed for 10 min in 1:1 methanol:acetone. Cells within the gels were then visualized by hematoxylin staining.

Immunohistochemistry. Serial sections were also cut for immunohistochemical characterization and used unfixed. Sections were blocked with either 1:20 rabbit serum or 1:20 swine serum for 20 min. Primary antibodies and secondary procedures, sources, and dilutions used are shown in Table 1. Antibodies and blocking solutions were prepared in 1% BSA/PBS. Primary antibody incubations were carried out for 1 h, and secondary antibody incubations were carried out for 30 min at room temperature. After each step, three washes with PBS were carried out. Counterstaining of cell nuclei was carried out using 1 μg/ml 4',6-diamidino-2-phenylindole-2HCl (Sigma Chemical Co.). To establish the basal expression levels of each marker, the same procedure was carried out on monolayers of PECs. Samples were observed using a Nikon Eclipse TE300 fluorescent microscope, and images were captured using Openlab image analysis software (Improvision, Coventry, United Kingdom). The intensity of staining was scored using the exposure times and contrast settings.

Table 1 Immunohistochemical characterization of collagen gel cocultures

<table>
<thead>
<tr>
<th>Marker and antigen</th>
<th>Clone</th>
<th>Source</th>
<th>Dilution</th>
<th>Secondary procedures</th>
<th>PNT1A</th>
<th>PC3</th>
<th>bPEC</th>
<th>tPEC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ML</td>
<td>bPS</td>
<td>tPS</td>
<td>ML</td>
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<tr>
<td>Luminal epithelial</td>
<td>Cytokeratin 8</td>
<td>M20</td>
<td>Sigma</td>
<td>1:100</td>
<td>RT</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Basal epithelial</td>
<td>Cytokeratin 1, 5, 10, 14</td>
<td>34bE12</td>
<td>Dako</td>
<td>1:50</td>
<td>RB, SF</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Prostate specificity and function</td>
<td>PSA</td>
<td>Gf1</td>
<td>1:100</td>
<td>RT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>PNT1A</td>
<td>Dako</td>
<td>1:50</td>
<td>SB, SF</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-ve</td>
</tr>
<tr>
<td></td>
<td>Cell adhesion</td>
<td>E-cadherin</td>
<td>HECD-1</td>
<td>R&amp;D</td>
<td>10μg/ml</td>
<td>RB, SF</td>
<td>+</td>
<td>-ve</td>
</tr>
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</table>

Table 2 Immunohistochemical characterization of stromal cells

<table>
<thead>
<tr>
<th>Marker</th>
<th>Antigen</th>
<th>Clone</th>
<th>Source</th>
<th>Dilution</th>
<th>Secondary procedures</th>
<th>% positive (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>smooth muscle actin</td>
<td>IA4</td>
<td>Sigma</td>
<td>1:400</td>
<td>RB, SAB, DAB</td>
<td>50%</td>
</tr>
</tbody>
</table>

Results

Growth of Stromal Cells in Collagen Gels. By phase microscopy, bPS and tPS seeded into the collagen matrix were seen to have established growth, developed elongated morphology, and formed a dense three-dimensional matrix in the gels within 5 days, although after the gels had been sectioned, the stromal cells were only visible when the plane of cut was directly through the nucleus. Stromal cell counts revealed that, in the coculture medium, neither bPS nor tPS proliferated, although they remained viable. This contrasted with the situation found when stromal cells were cultured in stromal growth combinations on the extent of collagen gel contraction. For example, more contraction was observed when PNT1A cells were cocultured with bPS than with tPS (Fig. 1, E and M), whereas PC3 cells with bPS or tPS contracted to the same extent (Fig. 1, F and N).

In the absence of PECs, the extent of contraction seen in the collagen gels was similar in bPS- and tPS-containing gels. However, the tissue source of stromal cells had a marked effect on the extent of gel contraction when combined with PECs. In cocultures of bPECs, more gel contraction was observed on bPS than on tPS gels (Fig. 1, G and O). In contrast, when tPECs were added to the collagen gels, the situation was reversed; less contraction was seen on bPS than on tPS gels (Fig. 1, H and P).
Benign or Tumor-derived Epithelial Cells into Collagen Type 1

A–D bPS and tPS are presented (morphology after 5 days of PNT1A, PC3, bPEC, and tPEC growing on gels containing bPS and tPS are presented (A–D and I–J). The extent of gel contraction is shown in photographs of individual wells from a 24-well plate (E–H) and (M–P). The images are representative of coculture experiments that were repeated three times. Bars: A–D and I–J, 50 μm; E–H and M–P, 500 mm.

Benign Tissue-derived Stromal Cells Allow the Migration of Benign or Tumor-derived Epithelial Cells into Collagen Type 1 Gels. Observations using phase light microscopy suggested that migration of cells into the gels had taken place. This was confirmed by sectioning and hematoxylin staining of frozen gels.

Fig. 1. Representative inverted phase light microscopy images demonstrating the morphology after 5 days of PNT1A, PC3, bPEC, and tPEC growing on gels containing bPS and tPS are presented (A–D and I–J). The extent of gel contraction is shown in photographs of individual wells from a 24-well plate (E–H) and (M–P). The images are representative of coculture experiments that were repeated three times. Bars: A–D and I–J, 50 μm; E–H and M–P, 500 mm.

A striking result in primary cell cocultures was obtained after staining with anti-E-cadherin. In both bPEC/bPS gels and Gleason score 6 tPEC/tPS gels, staining was strong and concentrated at cell-cell junctions (Fig. 3, E and J). Diffuse cytoplasmic staining was observed in cocultures of bPEC/tPS and Gleason score 6 tPEC/bPS. However E-cadherin staining was absent when Gleason score 8 tPECs were grown on either bPS or tPS gels.

Discussion

The primary goal of any valid in vitro model of tissue differentiation and disease must be to replicate the in vivo situation. Thus, the

Phenotype of Cell Lines Grown on Stromal Cell-populated Collagen Gels. PNT1A showed similar immunohistochemical characteristics in monolayers and on bPS and tPS gels. Staining for luminal cytokeratin was stronger than for basal cytokeratin and PSA and PSMA confirmed prostatic specificity and function. PNT1A were negative for E-cadherin when grown on bPS gels; however, they did show weak cytoplasmic staining on tPS gels. Fluorescent immunohistochemistry results obtained from PC3 cocultures showed no difference between monolayers and bPS and tPS gels (Tables 1 and 2).

Phenotype of PECs on Stromal Cell-populated Collagen Gels. In monolayers and on gels, both bPECs and tPECs stained more strongly for basal cytokeratin than luminal cytokeratin (Fig. 3, A, B, F, and G). This differential was even more pronounced when they were grown on tPS (Tables 1 and 2). PSA and PSMA expression was maintained (Fig. 3, C, H, D, and I).

A significant and reproducible result was the formation of acinus-like structures within the collagen gel when bPECs were cocultured with bPS (Fig. 2C). No such acinus-like structures were observed in the absence of stromal cells. In contrast, in coculture experiments of bPEC/tPS, the cells formed a loosely associated layer at the surface of the gel (Fig. 2G), similar to that seen with PNT1A/tPS.

In coculture experiments using PECs derived from tumor tissue, the results reflected the Gleason score of the original tumor from which the PECs were derived. On bPS gels, both Gleason score 6 and 8 PECs migrated to a similar extent into the gels (Fig. 2D). In contrast, Gleason score 6 tPECs cultured on tPS gels formed a tight monolayer at the surface of the gel (Fig. 2H). When sections were cut, this layer easily lifted intact from the surface of the gels. In the case of the Gleason score 8 tPECs, migration into the gel did take place, as observed in PC3 cocultures (results not shown because they are identical to Fig. 2F).

Fig. 2. Photomicrographs of hematoxylin-stained, 10-μm, frozen, 15-day collagen gel sections. A and E show that more migration of PNT1A cells occurred on bPS-populated gels. The extent of migration of PC3 cells was not affected by the tissue origin of the stromal cells (B and F). C is representative of the acinus-like structures (arrow), which developed on each occasion that bPEC were cocultured with bPS; such structures did not develop on tPS gels (G). D is the migration of tPECs cells from Gleason score 6 tissue into bPS gels. These gels developed a fragmented structure. H is representative of tPECs derived from Gleason score 6 tissue grown on tPS gels. The tPECs remained as a monolayer on the gel surface. Bar, 50 μm.

The highly invasive PC3 cells migrated rapidly into all gels, irrespective of the presence of either bPS or tPS or the absence of stromal cells (Fig. 2, B and F). Individual cells were clearly visible within the gels by inverted phase light microscopy after 3 days when cocultured with either bPS or tPS. A similar amount of migration was seen on both bPS and tPS gels (Fig. 2, B and F).

Five days after seeding the cells onto the gel surface, it was possible, by focusing through the gels, to see that PNT1A cells had migrated into bPS gels but not into tPS gels. Hematoxylin staining showed that PNT1A cells had migrated into bPS gels as single cells without forming apparent structures (Fig. 2A). On tPS gels, PNT1A cells formed a loosely associated layer at the surface of the gel (Fig. 2E). In the absence of stromal cells, PNT1A cells remained at the surface of the collagen gel.

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Discussion

The primary goal of any valid in vitro model of tissue differentiation and disease must be to replicate the in vivo situation. Thus, the
formation of differentiated acinus-like structures only when nonmalignant epithelial and stromal cells were cocultured was an important observation in our three-dimensional collagen culture system. PSA and PSMA expression were clearly observed in the presence of strong intercellular adhesions. Further work will determine the full degree of similarity of these acinus-like structures to those present in normal human prostate tissue. Acinus-like structures were not observed when either the epithelial or stromal cells were derived from tumor tissue or in the absence of stromal cells, emphasizing the importance of normal stromal/epithelial interactions in the maintenance and formation of differentiated acini. Tumor progression is accompanied by the loss of normal glandular morphology and is undoubtedly influenced by the change in stromal/epithelial interactions.

The lack of differentiated acinus-like structures when SV40 immortalized normally derived (PNT1A), PC3, and primary tumor epithelial cells were introduced into the cocultures could be explained by a multitude of factors. In the former case, immortalization by SV40 probably ensures that the ability to differentiate morphologically in vitro is destroyed. The lack of differentiated acinus-like structures when tumor cells were introduced into the cocultures could be explained by changes in cell-to-matrix adhesion, cell-to-cell adhesion, motility, and invasion. It is most likely that a combination of all these factors occurs, because they are interdependent. Our coculture system will allow individual assessments of such factors at a cellular level.

It has been shown recently that integrin-to-matrix interactions are critical for acinar morphogenesis (9). Changes in integrin profiles
associated with the progression of CaP (10, 11) may contribute to the altered morphologies observed here. In cocultures of nonmalignant epithelial and stromal cells, strong intercellular E-cadherin expression was noted, matching that seen in intact prostate tissue. Tissue expression of E-cadherin is, however, progressively lost with increasing Gleason score (10). This was reflected by tumor-derived epithelial cells grown in the presence of tumor stroma. Epithelia derived from Gleason 6 tumors expressed cell-to-cell E-cadherin, whereas those derived from Gleason 8 tumors had lost expression. Clearly, the Gleason score 8 epithelial cells behaved more like PC-3, by migrating into both malignant and nonmalignant stroma, which might be expected because both are derived from poorly differentiated tumors. Therefore, our system will also provide a useful tool for studying the behavior and interactions of cells derived from different grades of cancer and their potential effects on cellular architecture and cancer progression.

The significance of malignant stroma has not been studied widely, although there is some preliminary evidence of genetic changes relative to normal stroma in human prostatic tissue (12). The tissue origin of the stromal cells in our cocultures had a distinct effect on the extent to which prostate epithelial cells migrated into the collagen gels. When stromal cells were derived from nonmalignant tissue, the epithelial cells migrated into the gels. On stromal cells derived from Gleason score 6 CaP, only epithelial cells derived from poorly differentiated cancers migrated into the gels. Further study using stromal cells derived from poorly differentiated tumors would extend our understanding of the role that the stroma plays in the progression to a malignant phenotype.

A number of phenotypic changes have also been assigned to malignant stroma such as increased motility in vitro (13) and increased expression of matrix metalloproteinase 2 in breast tumor stroma (14). In CaP, malignant stroma produces less HGF (or scatter factor) than nonmalignant (15). HGF is an important paracrine growth factor that influences cell differentiation, adhesion, invasion, and motility (16). This finding provides one explanation why epithelial cells preferentially migrated into collagen gels populated with nonmalignant stromal cells. Future work should establish the contribution of HGF.

More recently, Olumi et al. (6) found that in vivo culture of tumor-associated fibroblasts and initiated PECs led to the loss of normal acinus morphology. In vitro, the normal morphology of skin epidermis, recreated using collagen raft cultures similar to our own, was lost in the presence of tumor stroma (17). These results are consistent with those presented here, suggesting that the ability of epithelial cells to differentiate in vitro requires nonmalignant cells.

In prostate and other cancers (5, 18), malignant epithelial cells are known to be more invasive and motile than normal epithelial cells, and such in vitro differences undoubtedly contribute to the results found here. The factors that are responsible for these findings have not been established, although expression of matrix metalloproteinases is of great importance for breast epithelial invasive potential and morphology (19).

In summary, prostate cell reconstitution in three-dimensional collagen gel cocultures has revealed distinct differences between tumor and normal tissue-derived stromal cells, emphasizing the critical role of prostatic stroma in cancer progression.

Acknowledgments

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References

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