The Mucosal Invasion Model

A Novel In Vitro Model for Evaluating the Invasive Behavior of Mucocutaneous Malignancies

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Background: Prevention of regional and metastatic spread of cutaneous malignancies requires understanding the physiologic mechanism of tumor cell invasion. In vitro models are convenient for studying the in vitro invasive phenotype of normal cells, tumor cell lines, or genetically altered cells in a 3-dimensional matrix, but they should attempt to recapitulate the complex in vivo submucosal environment. A new acellular extracellular matrix, porcine submucosal matrix (PSM), is thought to accurately recapitulate the submucosal matrix. A novel in vitro model using PSM to assess mucocutaneous tumor cell invasion was studied.

Methods: The morphologic characteristics, growth, and invasive behavior of human head and neck squamous cell carcinoma (UM-SCC-1, UM-SCC-5, UM-SCC-17B, and OSC-19) cell lines were assessed on the PSM gel and compared with commonly used in vitro invasion models (type I collagen and Matrigel matrices). The invasive phenotype of canine kidney cells was also assessed on PSM, because this cell line is known to demonstrate a characteristic in vitro invasive phenotype.

Results: The PSM-supported head and neck squamous cell carcinoma tumor cell line growth and single cell invasion were seen under stimulated conditions, similar to type I collagen gels. The invasive phenotype of canine kidney cells behaved similarly on PSM and collagen. Matrigel did not support growth well, and invasion occurred only superficially in isolated areas.

Conclusion: The PSM is a good in vitro model for assessment of pharmacologic and genetic manipulations of head and neck squamous cell carcinoma tumor cell lines and has several advantages over other commonly used matrices.


Original Article

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Patient survival in cutaneous and mucocutaneous malignant tumors of the head and neck is largely limited by failure to control regional and distant metastatic disease. Central to tumor cell invasion and metastasis is the ability of malignant cells to migrate through the normal barriers presented by components of the extracellular matrix (ECM). Determining the mechanisms tumor cells use to traverse the ECM may lead to therapies directed against regional and metastatic spread of disease, thereby improving patient survival.

Understanding of the mechanisms of tumor cell invasion and metastasis has been expanded by use of in vitro models that allow subtle manipulations of tumor cell environments. Currently, there is not a model to assess the submucosal matrix that head and neck tumor cells must traverse in vivo. In vitro models used to assay malignant cell invasion usually use isolated ECM components, such as type I collagen, fibrin, or mixtures of ECM components such as the extract Matrigel (Becton Dickinson and Company, Bedford, Mass.). A matrix of porcine submucosal intestine has recently been introduced that retains the convenience of other commonly used in vitro invasion materials but more closely recapitulates the in vivo stroma milieu of mucosal surfaces.

The porcine submucosal matrix (PSM), also called small intestinal submucosa, has been used in numerous preclinical animal studies to restore damaged tissue structures. In its most basic compositional form, small intestinal submucosa consists primarily of type I collagen and other typical components of the submucosal ECM including fibronectin, glycosaminoglycans, and growth factors.

Many common in vitro substrates used to study mechanisms of tumor cell invasion and metastasis fail to adequately reproduce the complexity of the submucosal matrix. In vivo, metastatic epithelial tu-
MATERIALS AND METHODS

CELL CULTURE

The human head and neck squamous cell carcinoma (HNSCC) lines UM-SCC-1, UM-SCC-5, and UM-SCC-17B (provided by Thomas Carey, PhD, University of Michigan, Ann Arbor), OSC-19 (provided by Toshio Yokoi, MD, Kanazawa University, Kanazawa, Japan) and MDCK cells (provided by Stephen Weiss, MD, University of Michigan, Ann Arbor) were routinely maintained in minimal essential medium supplemented with L-glutamine (2mM), penicillin (100 U/mL), and streptomycin (100 µg/mL; all from Life Technologies Inc, Gaithersburg, Md) and 10% heat-inactivated fetal bovine serum (Hyclone Laboratories Inc, Logan, Utah). Cells were cultured at 37°C in humidified 5% carbon dioxide–95% air.

CELL GROWTH AND MOTILITY

Dishes were coated with 0.8 mL of either type I rat tail collagen, 3.0 mg/mL (Sigma-Aldrich Corp, St Louis, Mo), Matrigel (both normal and growth factor reduced; Becton Dickinson and Company), or PSM (vivoSIS Gel, 3.0-mg/mL collagen concentration; a gift of Cook Biotech, West Lafayette, Ind). Growth assays were performed with cells plated at 1×10⁴ in 6-well dishes coated with matrix (0.8 mL) and assessed during a 6-day incubation period (at 1, 3, and 6 days) by count of cells per high-power field (×40).

CELL INVASION ASSAY

Matrigel was placed in the 12-mm upper portion of the 2-chambered culture system (Transwell dishes, pore size, 3 µm; Corning Costar Corp, Cambridge, Mass) and gelled for 45 minutes at 37°C. The PSM was provided as a neutralized solution of 3.0-mg/mL collagen concentration that was gelled at 37°C for 45 minutes. Type I collagen (0.9 mL) was added to the upper chamber dishes and gelled for 45 minutes at 37°C. The collagen was prepared by means of rat-tail type I collagen dissolved in 0.2% acetic acid at 3.2 mg/mL and gelled by neutralizing the acid with 0.3N sodium hydroxide containing phenol red as a pH indicator. A final concentration of 3.0 mg/mL was chosen to closely match the type I collagen concentration of PSM. Medium was then added to the upper and lower chambers before the addition of 2×10⁵ cells per well. All invasion assays were performed in this serum-containing medium. Cells were grown to confluence (12-24 hours), and if growth factors were used, either recombinant human scatter factor (SF, 0.5nM; Becton Dickinson and Company) or epidermal growth factor (EGF, 3.5nM; Becton Dickinson and Company) was added to the lower chamber. Media were changed every 3 days, and the respective growth factor was re-added to the lower chamber. Invasion occurred during a 7-day incubation period, at which time samples were harvested for histologic analysis and cellular invasion was assessed.

SAMPLE FIXATION AND PROCESSING

Gels were removed from the upper chamber with minimal manipulation and then placed in 2.7% formaldehyde and embedded in paraffin. Sections (3 µm wide) were cut and stained with hematoxylin-eosin. Tumor cell invasion was assessed by light microscopy in a minimum of 4 randomly selected sections for each experimental sample.

RESULTS

MORPHOLOGY AND GROWTH

The HNSCC cell lines (UM-SCC-1, UM-SCC-5, UM-SCC-17B, and OSC-19) were sparsely plated on gels of collagen, Matrigel, and PSM grown on collagen. Tumor cell lines plated on Matrigel consistently failed to reach confluence, and instead cells developed a weblike pattern that persisted throughout the 7-day culture period with some stacking of cells (Figure 1A). The HNSCC tumor cell lines on collagen and PSM displayed single or multiple layering of tumor cells (depending on the tumor cell line) with very similar morphologic characteristics and time to confluence (Figure 1). For all HNSCC cell lines tested, the cell growth rates were lower for Matrigel than collagen or PSM (Figure 2).

TUMOR CELL INVASION

Invasion studies of HNSCC and melanoma cell lines were conducted under EGF-stimulated conditions by means of a 2-chambered culture system during a 7-day incubation period. Invasion was assessed by phase microscopy and confirmed by histologic analysis. Cer-
tain cell lines (UM-SCC-1 and OSC-19) displayed a single cell invasive phenotype beginning at 48 hours into the collagen and PSM throughout the surface of the gel (Figure 3). The UM-SCC-5 and UM-SCC-17B tumor cell lines invaded the matrix at the end of the 7-day incubation period but did not penetrate as deeply into the matrix as UM-SCC-1 and OSC-19. On Matrigel, the HNSCC tumor cell lines under EGF-stimulated conditions demonstrated small, rounded clusters of cell that penetrated into the matrix as small groups of cells after 72 to 96 hours in culture (Figure 3). Tumor cell invasion on Matrigel was limited to distinct areas over the culture surface. The isolated areas of cells on cross-sectional histologic examination were consistent with the inverted light microscopic view (Figure 1A).

**MDCK CELL INVASION**

The MDCK cells have a well-defined invasive phenotype on collagen when stimulated withSF–hepatocyte growth factor (SF/HGF). To assess the influence of PSM on the invasive pattern, subconfluent cultures were maintained for 24 hours on Matrigel (normal and growth factor reduced), collagen (3.0 mg/mL), and PSM and then stimulated with SF/HGF. On Matrigel the cells formed a web-like pattern and the invasive phenotype, and there was no significant evidence of invasion at the conclusion of the 7-day incubation period. No invasion occurred in the absence of SF/HGF. Stimulated MDCK cells behaved similarly on collagen and PSM: focal areas of single cell invasion occurred throughout the matrix after 5 days (data not shown). The morphologic character of the invading tumor cells was similar on collagen and PSM.

**COMMENT**

In vitro models of tumor cell invasion are commonly used to evaluate the phenotype of genetically altered (transfected) tumor cells, or tumor cell response to growth factors and/or inhibitors. The invasion models evaluated in this study all have several advantages compared with in vivo models. First, evaluation can be done by inverted phase light microscopy throughout the culture period and cross-sectional histologic analysis can be performed for the purposes of graphic representation. Second, the assays are practical. They are relatively inexpensive and can be conducted in a relatively short time course (2 weeks). Last, these experiments can be conducted with the use of commercially available reagents, which improve reproducibility between laboratories. Currently, there is no invasion model specific for the submucosal matrix. We describe a novel invasion model for HNSCC cell lines using components from porcine submucosal intestine.

All the HNSCC tumors attached and formed a monolayer on PSM, and under EGF-stimulated conditions the tumor cells invaded over the course of 72 hours in a single cell fashion. The morphologic features and growth of HNSCC cell lines on PSM were consistent with the initial report of this matrix that described the growth and morphologic characteristics of epithelial cell lines cultured atop PSM. Matrigel (as a gel form) did not allow variations in morphologic features or support cell growth in the same manner as PSM. The HNSCC cells consistently displayed a web pattern of growth that was not consistent with morphologic findings on the other ECM components assessed in this report. The HNSCC and MDCK cells had little invasive potential when cultured on Matrigel.

Because of its high type IV collagen content, Matrigel is considered a model of the basement membrane, whereas type I collagen and PSM represent the stromal or submucosal matrix, which is predominantly type I collagen. Although investigations often focus on the mechanism by which tumor cells degrade and migrate through the basement membrane, invasion through the stromal ECM is more relevant to development of new therapeu-
assess tumor cell invasion, the PSM contains the matrix tumor has already progressed beyond the basement mem-
tic modalities, since at the time of cancer diagnosis the tumor has already progressed beyond the basement membrane. 
Although PSM or type I collagen can be used to assess tumor cell invasion, the PSM contains the matrix components specific to the submucosal matrix. The value of in vitro models is in comparing phenotypic changes that occur with perturbations in the system, such as addition of a growth factor or transfection with a gene. Moreover, the mechanism of novel molecular and pharmacologic interventions during the HNSCC tumor cell invasive program can be assessed by this model.

In summary, the PSM model has several advantages over other in vitro invasion matrices. Compared with collagen, PSM better recapitulates aspects of the physiologic matrix that HNSCC cells must traverse in vivo. It supports tumor cell growth better, is less expensive, and allows single cell invasion compared with Matrigel. Therefore, the PSM should be considered as an in vitro model for assessment of new pharmacologic molecular genetic modalities of altering HNSCC cell invasion and metastasis.

Accepted for publication August 24, 2001.

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REFERENCES


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