Growth of Human Urological Tumors on Extracellular Matrix as a Model for the in Vitro Cultivation of Primary Human Tumor Explants


Roswell Park Memorial Institute, Grace Cancer Drug Center, Buffalo, New York 14263

ABSTRACT

The goal of this study was to establish an optimal in vitro growth assay system for human urological tumor explants. Bovine corneal endothelial cell extracellular matrix (ECM) coated dishes were evaluated as a growth substrate for tumor cultures. Growth success for different urological carcinoma (prostatic, bladder, kidney, and testicular) tumors was compared after seeding fresh surgical explants onto bovine corneal endothelial cell ECM and plastic culture flasks. Tumor samples were disaggregated enzymatically, and 1 × 10^6 cells were seeded onto the different substrates using RPMI 1640 medium containing 10% fetal calf serum and/or different growth factors, nutrients, and hormones. Cell growth on ECM was quantitated on days 7–15 by [3H]thymidine uptake, cell counting, and total protein. Tumor cells were characterized by flow cytometry and cytology. It was observed that ECM provides superior culture conditions for urological carcinomas. By increasing the initial number of cells plated on ECM and by adding different growth factors or hormones, the growth rate for specific tumor types was increased significantly. Several tumors (11 cases) grown on ECM were examined under the light microscope, and in all cases pre- and post-cytology confirmed malignancy. Tumor cell lines maintained on ECM and transplanted into nude mice retained their tumorigenic and morphological characteristics. Clinically aggressive tumors were associated with extensive ECM degradation. In addition, the growth of fresh human tumors on ECM provides a biologically relevant model system (for assessing the invasiveness of tumors in vitro) and should also be useful for drug evaluation studies.

INTRODUCTION

Problems encountered in the primary culture of human tumors using soft-agar or plastic culture vessels include low plating efficiencies and poor subsequent cell growth. These problems are related closely to the type of substrate upon which cells must attach and proliferate and to the question of the nutritional adequacy of the culture medium. It was found that cells placed in contact with a natural substratum, rather than an artificial one, adopt growth properties, a morphological appearance, and biological responsiveness which are not expressed when the same cells are maintained on plastic or glass, whether or not these substrates are coated with fibronectin or one of the collagen types found in extracellular matrix (1–3). These problems led us to seek an alternative substrate for the cultivation of cells in vitro from solid human tumors. Vlodavsky et al. (2), as well as other investigators (4, 5), reported superior success when growing solid tumors in vitro on ECM. ECM is produced by BVEC, and as a result serves as a natural substrate upon which tumor cells derived from the epithelium can attach, migrate, proliferate, and differentiate in vitro. ECM is composed of different types of collagen (6), glycosaminoglycans, proteoglycans (7), and glycoproteins (8).

Our working hypothesis has been that the cloning efficiency and growth rate of primary human urological tumor explants could be improved with adjustments in tissue culture medium components, including the addition of appropriate growth factors and hormones and the use of natural substrates. To test this hypothesis we have (a) evaluated different growth substrates, plastic and bovine corneal endothelial extracellular matrix for culturing human urological tumors in vitro; and (b) evaluated the effect of various hormone and growth factors in serum-free or serum containing media, for optimizing tumor cell growth in vitro. The results of these studies suggest that ECM may be a superior substrate for culturing fresh human urological tumors in vitro.

MATERIALS AND METHODS

Materials. Falcon plastic culture dishes (6 and 10 cm in diameter) and 1-cm × 24-well plates were obtained from Becton Dickinson (Oxnard, CA). N-2-Hydroxyethyl-piperazine-N-2-ethanesulfonic acid, spermidine, and dextran were purchased from Sigma Chemical Co. (St. Louis, MO). Trypsin-EDTA, HIFCS, calf serum, RPMI 1640 medium, i-glutamine, fungizone, and gentamicin were purchased from Grand Island Biological Co. Laboratories (Grand Island, NY); and [methyl-3H]thymidine (specific activity, 20 Ci/mmol), [5,6-3H]uridine (specific activity, 38.9 Ci/mmol), and [4,5-3H](N)leucine (specific activity, 50 Ci/mmol) were from New England Nuclear (Boston, MA). Testosterone was obtained from Upjohn Company (Kalamazoo, MI), platelet derived growth factor, insulin-like growth factor II, and epidermal growth factor from Collaborative Research, Inc. (Lexington, MA), endothelial cell growth factor and fibroblast growth factor from Seragen (Boston, MA), insulin was from Novo (Copenhagen, Denmark), and human growth hormone was from Hoechst (San Diego, CA). SGF-7 and SGF-9 was obtained and purchased from Scott Laboratories (Providence, RI). Substance immunologically cross-reactive with insulin, SICRI (M, 120,000), an autocrine growth factor, was derived from murine melanoma B16. It was found to stimulate DNA synthesis of melanoma cells in vitro and in vivo (9–12) and the growth of murine L1210 leukemia cells, murine myeloid leukemia cells, bovine corneal endothelial cells, and human 205 colon carcinoma cells. Collagenase II and DNAse were obtained from Worthington Biochemicals (Freehold, NJ).

Tumor Specimens. Fresh surgical specimens from human bladder, kidney, prostatic, and testicular carcinoma were washed with RPMI 1640 medium containing 10% HIFCS and defatted, and their malignant nature was confirmed by frozen section pathological and cytological examination prior to processing.

Specimens were disaggregated into cell suspensions enzymatically as described by Slocum et al. (13). Briefly, tumor tissue slices 0.5 mm in thickness were incubated with a mixture of 0.8% collagenase II and 0.002% DNase. Disaggregated cells were characterized by trypsin blue exclusion (viability), cytological examination, and flow cytometric DNA ploidy determination. Following these procedures cell suspensions were seeded and cultured on different substrates.

Preparation of Plates Coated with Extracellular Matrix. Primary

Received 1/21/86; accepted 3/21/86.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This research was partially supported by National Cancer Institute Program Grants CA-13038, CA-21071 and CA-42898.

To whom requests for reprints should be addressed.

The abbreviations used are: ECM, extracellular matrix; BVEC, bovine corneal endothelial cells; HIFCS, heat-inactivated fetal calf serum; SICRI, substance immunologically cross-reactive with insulin; SGF-7, Scott Growth Factor 7 (1 µg EGF, 50 µg transferrin, 500 ng selenious acid, 50 µg bovine insulin, 50 mg fetuin, 50 µg oleic acid-bovine serum albumin, 50 µg linoleic acid-bovine serum albumin in 100 ml medium).


3653
cultures of bovine corneal endothelial cells which synthesize ECM were established from steer eyes (14). BCEC cells were plated at an initial density of 1 x 10^5 cells/ml per well in Dulbecco's modified Eagle's medium supplemented with 5% fetal calf serum, 5% calf serum, 5% Dextran T-40, and fungizone (1%) and gentamycin (2%) in a 24-well tissue culture plate. Epidermal growth factor (1 ng/ml) was added every other day. Once the cultures reached confluency (ordinarily within 6 days) the media were renewed, and the cultures were incubated for another 6 days. Following these procedures the cultures were washed with phosphate-buffered saline and exposed for 5 min to 0.02 M NH_4OH in distilled water to remove the BCEC cells. Once the underlying ECM became visible, the culture plates were washed 3 times with phosphate-buffered saline to prepare stock plates coated with ECM (15).

Cell Attachment Assay. Cell attachment assays were performed using ECM coated and uncoated plastic culture dishes. Tumor cell suspensions were prelabeled for 24 h at 37°C using [H]thymidine (0.5 μCi/ml) in medium supplemented with SFG-7 and HIFCS. Subsequently, all labeled cells were removed from ECM coated plates using trypsin-EDTA (0.2 g EDTA/l, 5 min at 37°C) and washed several times in RPMI 1640 containing 20% HIFCS. Radiolabeled cells were then added to the various culture vessels. Following incubations at 37°C for 30, 60, and 180 min unattached cells were removed and incubated at the medium and rinsing each well several times with phosphate-buffered saline (0.2 g KCl, 0.2 g KH_2PO_4, 8 g NaCl and 1 g Na_2HPO_4 per liter, pH 7.2). Attached cells were then harvested on filter paper using an automated cell harvester. Radioactivity of incorporated [H]thymidine was measured by use of scintillation counting methods (16).

Plating Efficiency. In order to determine the colony forming efficiency of human urological carcinoma cells, preliminary experiments were performed to determine optimal conditions for this assay. In general, cells from suspension were diluted in complete RPMI 1640 medium to concentrations between 25 and 3125 cells/ml. A 4.0-ml part of these suspensions was then transferred into 6-cm plastic culture dishes and incubated in a humidified 5% CO_2 atmosphere at 37°C. After 7 days incubation, plates were inspected using a phase microscope. The number of colonies per dish was counted, and colony forming efficiency was calculated by dividing the number of colonies by the total number of cells plated and expressed as a percentage.

Tumor Cell Growth Assay. Tumor cells (10^5) were seeded on day 0 on various substrates (plastic and ECM); cell proliferation was determined between day 7 and day 15. DNA synthesis was measured in cells grown on plastic or ECM substrate by measuring [methyl-^3]H]thymidine (specific activity, 20.0 Ci/mmol) incorporation. Cells were harvested after 6-8 h using a Bเขโอlico cell harvester. RNA and protein synthesis were measured by counting cells in approximately 30% of total cases. Fig. 5 shows a typical cytology confirmed malignancy. Tumor cell ploidy measured with these tumor cells cultured on ECM was evaluated by cytology and flow cytometry. In all 12 cases examined pre- and post-culture varied however, with only 4 of 10 tumors retaining their original DNA ploidy.

Table 1 Growth success for urological carcinomas growing on ECM and plastic

<table>
<thead>
<tr>
<th>In vitro system</th>
<th>Tumor</th>
<th>Growing/total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular matrix</td>
<td>Bladder</td>
<td>19/30 (63)</td>
</tr>
<tr>
<td></td>
<td>Prostatic</td>
<td>8/9 (89)</td>
</tr>
<tr>
<td></td>
<td>Renal</td>
<td>16/17 (94)</td>
</tr>
<tr>
<td></td>
<td>Testicular</td>
<td>2/3 (67)</td>
</tr>
<tr>
<td>Plastic</td>
<td>Bladder</td>
<td>0/11 (0)</td>
</tr>
<tr>
<td></td>
<td>Prostatic</td>
<td>0/7 (0)</td>
</tr>
<tr>
<td></td>
<td>Renal</td>
<td>1/9 (11)</td>
</tr>
<tr>
<td></td>
<td>Testicular</td>
<td>0/1 (0)</td>
</tr>
</tbody>
</table>

To determine whether attachment of these tumor cells to different substrates plays a critical role in growth success, several cell attachment studies were performed. Prostatic, kidney, and bladder carcinoma cells were observed to attach very well onto ECM coated dishes, while less than 5% of the renal and bladder carcinoma cells and none of the prostatic carcinoma cells attached under similar conditions to plastic alone (Fig. 3). It became obvious that if tumor cells could not attach they could not grow and subsequently did not form colonies on plastic and that colony forming efficiency was higher for cells seeded on ECM as compared to plastic or soft agar, as demonstrated in Fig. 4. Cells with higher attachment rates also demonstrated better colony forming efficiencies.

Characterization of Tumor Cells Grown on ECM. The nature of the tumor cells cultured on ECM was evaluated by cytology and flow cytometry. In all 12 cases examined pre- and post-culture varied however, with only 4 of 10 tumors retaining their original DNA ploidy. These tumor cultures on ECM were virtually fibroblast free during the first 10-15 days. However, fibroblasts began to proliferate and eventually overgrew the urological carcinoma cells in approximately 30% of total cases. Fig. 5 shows a typical situation in which fibroblast contamination of a prostatic carcinoma was noted. The initial culture was fibroblast-free for 10
Fig. 1. Primary bladder carcinoma cells grown on ECM after mechanical (A) and enzymatic (B) disaggregation of tumor tissue. Photographs were made on day 9 after seeding (600x). C and D, enzymatically disaggregated renal carcinoma on plastic (C), or ECM (D), day 10 (650x).

Days (Fig. 5A, after which fibroblasts began to proliferate (Fig. 5B). Twenty-five days later the culture containing only fibroblastic cells (Fig. 5C).

Tumor Cell Growth Kinetics on ECM. Tumor cells seeded onto ECM may have three different fates. In some cases (15%) cells did not grow at all. In other cases (about 40%) cells grew
Fig. 2. Different patterns of human urological tumor growth on ECM. A, bladder carcinoma, day 10 (700×); B, bladder carcinoma, day 10 (650×); C, bladder carcinoma, day 15 (700×); D, renal carcinoma, day 12 (750×); E, testicular carcinoma, metastases from inguinal lymph nodes, day 12 (550×); F, renal carcinoma, day 12 (400×).

exponentially under standard conditions (RPMI 1640 medium supplemented with 10% fetal calf serum) and in the third (Fig. 6A) cells grew exponentially for about 15 days at which time cell growth decreased and eventually stopped (45% cases). In both cases, where bladder carcinoma cells grew on ECM they were not able to grow on plastic substrate.
renal carcinoma cells obtained from fresh tumor tissue were grown on ECM was evaluated with nude mice. As an example, nations were made, and each point represents the mean. Bars, SD.

and Methods." % Attachment refers to the percentage of total cellular radioactivity added to the dish which became firmly adherent with tumor. Triplicate determi

Drop cortisone. By using SICRI as a medium supplement we derived from melanoma B16), insulin, triiodo-L-thyronine, pro-

drugs of the original renal cell carcinoma (clear cell type) of right kidney and the tumor from the nude mouse are shown in Fig. 8. The histological pattern of the original renal cell carcinoma was maintained after passage into the nude mouse.

Invasion. Clinically aggressive tumors seeded on ECM were observed to extensively degrade ECM (Fig. 9). Degradation of ECM adjacent to growing cells or colonies was observed in all 12 invasive and poorly differentiated renal or prostatic carcinomas. Typical degradation patterns are shown in Fig. 9.

Tumor cells from different urological locations degraded ECM. Following ECM degradation cells were less able to grow on plastic.

DISCUSSION

Major problems exist with in vitro cultivation of human tumor explants, including low colony formation efficiencies and subsequent poor tumor cell growth rates. These problems have plagued the development of a predictive in vitro antitumor drug sensitivity test, as well as limiting other biochemical assays which require relatively large numbers of actively growing tumor cells. The aim of this present study was the evaluation of an extracellular matrix, produced by bovine corneal endothelial cells, as a biochemically complex, biologically relevant substrate for studying the attachment and growth of freshly isolated, human urological tumors in vitro.

A variety of metastatic and primary urological cancers, including prostatic, bladder, kidney, and testicular, were evaluated for growth in vitro using various substrates. Results of this and our previous studies suggest that ECM provides superior culture conditions for human urological tumors as compared with plastic or double layer and capillary soft agar. The highest

Improvement of Culture Conditions Using ECM. By use of different media supplements we were able to increase the growth success of various urological tumors on ECM. Table 2 shows effect of different growth factors and hormones on [3H]thymidine incorporation into kidney or bladder carcinomas growing on ECM. Experiments were performed with serum free or serum supplemented RPMI 1640 medium. Cells were grown on ECM in the presence of various supplements for 24 h and [3H]thymidine (0.2 µCi/ml) for another 24 h. Bovine insulin, SICRI, endothelial cell growth factor, epidermal growth factor, and spermidine caused significant increases in the growth rate of renal carcinoma cells in serum free medium. In the second group of experiments tumor cells were cultivated in RPMI 1640 medium supplemented with 10% fetal calf serum. In a third group of experiments, we used different growth factors and hormones to increase the growth of tumor cells on ECM, including spermidine, an autocrine growth factor SICRI (derived from melanoma B16), insulin, triiodo-L-thyronine, progesterone, o-phosphoryl-ethanolamine, aminoethanol, and hydrocortisone. By using SICRI as a medium supplement we increased the growth of a number of human urological tumors. Nine of 22 tumors (41%) plated on ECM and treated with SICRI demonstrated a significantly higher growth rate, as measured by [3H]thymidine utilization.

Testosterone, a target hormone for prostatic and testicular tissue, was postulated to be a good growth supplement for prostatic or testicular carcinomas in vitro. Our results indicated that five of six (83%) prostatic tumors grew significantly better on ECM after treatment with testosterone (Fig. 7). It was observed that testosterone stimulated DNA, RNA, and protein synthesis of primary prostatic cells maintained in serum-free medium.

Tumorigenicity. Tumorigenicity of several human tumors grown on ECM was evaluated with nude mice. As an example, renal carcinoma cells obtained from fresh tumor tissue were maintained on ECM substrate for 14 days and implanted s.c. into nude mice (5 x 10⁶ cells/mouse). One month later mice were sacrificed and tumor was examined histologically. There were morphological similarities between tumor from nude mice and the original tumor tissue removed surgically from the patient (Fig. 8), indicating that tumor cells proliferated in vitro on ECM and retained their tumorigenicity in vivo. Light micrographs of the original renal cell carcinoma (clear cell type) of right kidney and the tumor from the nude mouse are shown in Fig. 8. The histological pattern of the original renal cell carcinoma was maintained after passage into the nude mouse.

Fig. 4. Colony forming or plating efficiency by prostatic carcinoma cells on ECM (O) or plastic (¶). Cells were grown on 6-cm Petri dishes. Seven days later colonies were counted under phase contrast inverted microscope. Results are presented as the mean of three parallel samples. Bars, SD.
success level was obtained with renal tumors grown on ECM, 16 of 17 or 95%, as compared with 40% in double layer soft agar and 60% in capillary soft agar. Similar success rates were obtained with prostatic carcinomas (89% on ECM versus 59% in double layer soft agar or 60% in capillary soft agar). Bladder and testicular carcinomas also grew very well using ECM as a substrate, with growth success exceeding 60%. In our hands, plastic alone was not a good substrate for culturing primary urological explants. In addition, the use of enzymatic disaggregation of tumor tissues, optimizing the number of cells plated, and selection of an appropriate nutrient medium all resulted in even better growth responses of human urological tumors on ECM.

Studies in our laboratory and others have demonstrated a much faster and firmer attachment for both tumor and normal cells (2, 5, 18–20) to ECM than to plastic or an intact endothelial or mesothelial cell monolayers (5, 18, 19, 21, 22). The nature and specificity of the adhesive components in ECM are being studied extensively (20, 23–25). Depending on the cell type and its surface properties, cells can either interact directly with collagen (20) or via large glycoproteins, such as fibronectin and laminin. Following cell attachment to ECM a substrate-
induced proliferative response is observed which may be mediated by changes in cell shape and corresponding changes in cell surface receptors. These events may induce sensitivity in the cell to physiological agents (growth factors, etc.) (26, 27) present in serum. A number of in vitro studies have demonstrated that the substrate upon which cells are cultured can alter both the shape and proliferative response to various mitogens in an interrelated manner (2). This might also suggest an increased sensitivity to various hormones as well as to therapeutic agents. These effects might be mediated by the collagenous matrix, as suggested by the involvement of collagen and mucopolysaccharides in epithelial morphogenesis (28). In addition, contact between cells could have a permissive influence on cell proliferation.

Normal cell lines also grow better on ECM. We have observed that the growth rate of normal human mesothelial cells is increased when cultured on ECM and that a more normal cellular morphology is maintained on ECM (5). Others have shown that when the proliferation of bovine granulosa cells on plastic is compared to that on ECM, the cultures maintained on plastic and exposed to serum proliferated, but when on an ECM they proliferated quite actively and no longer required fibroblast growth factor in order to become confluent. In contrast, when maintained on plastic, cells exhibited an absolute requirement for fibroblast growth factor in order to reach confluence.

Fig. 6. Different growth kinetics of bladder carcinomas seeded onto ECM. Tumor cells (1 × 10⁶) were seeded on day 0 on each substrate. Each point represents the mean of 3 parallel samples. Cells were grown in RPMI 1640 media supplemented with 10% heat inactivated fetal calf serum. Inset, total protein content in cells growing on two different substrates on day 11.

Fig. 7. Growth of prostate carcinoma on plastic substrate. Tumor cells (1 × 10⁶) were initially plated on ECM. After 15 days cells were trypsinized, and the same number of cells were seeded on plastic. Each point represents the mean of four samples. Bars, SD.

Table 2 Effect of various supplements on growth of human urological tumors on ECM

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Media supplement</th>
<th>Concentration range</th>
<th>Optimal</th>
<th>Growth rate (% of control)</th>
<th>Significance (Student's t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney carcinoma*</td>
<td>None</td>
<td>0.1-100 μU/ml</td>
<td>10</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Insulin</td>
<td>0.1-100 μU/ml</td>
<td>40</td>
<td>165</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>SICR1</td>
<td>0.1-1000 μg/ml</td>
<td>1</td>
<td>113</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>ECGF</td>
<td>1.0-100 ng/ml</td>
<td>10</td>
<td>121</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>EGF</td>
<td>1.0-20 ng/ml</td>
<td>—</td>
<td>NS*</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>IGF II</td>
<td>1.0-25 ng/ml</td>
<td>—</td>
<td>NS*</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>FGF</td>
<td>0.5-25 μg/ml</td>
<td>—</td>
<td>NS*</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>HGH</td>
<td>1.0-100 μg/ml</td>
<td>5</td>
<td>120</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>PDGF</td>
<td>0.5-100 μg/ml</td>
<td>—</td>
<td>NS*</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Spermidine</td>
<td>0.5-25 μM</td>
<td>—</td>
<td>NS*</td>
<td>—</td>
</tr>
</tbody>
</table>

Kidney carcinoma*:
- HIFCS — 10%: 100
- SGF-7: 71
- SICR1: 44
- Spermidine: 45
- SICR1, spermidine: 90

Bladder carcinoma*:
- HIFCS — 10%: 100
- HIFCS, SGF-7: 138
- HIFCS, SICR1: 110
- HIFCS, spermidine: 101
- HIFCS, SICR1, spermidine: 190

* Concentrations at which [³H]thymidine incorporation was maximal.
* 1 × 10⁶ cells/ml.
* RPMI 1640 medium supplemented with transferrin (5 mg/l) and selenous acid (5 μg/l).
* No mitogenic activity was observed at the concentrations listed above.
* ECGF, endothelial cell growth factor; EGF, epidermal growth factor; IGF II, insulin-like growth factor II; FGF, fibroblast growth factor; HGH, human growth hormone; PDGF, platelet-derived growth factor.
* NS, not significant.
* 5 × 10⁶ cells/ml.

3659
Fig. 8. Photomicrographs of the original surgical specimens and of the tumor transplanted from ECM into the nude mouse. A and C, original renal cell carcinoma. (hematoxylin and eosin, 300×). B and D, the same tumor after growth in the nude mouse. (hematoxylin and eosin, 900×).

These findings emphasize the importance of adequate nutritional support to the maintenance of human tumors in vitro. Tumors arising in different tissue are very likely to require different nutritional factors. The problem is that cells from different sources with the same histology and even different clones within a single tumor may show marked heterogeneity.
HUMAN UROLOGICAL TUMOR GROWTH ON ECM

Fig. 9. Degradation of ECM by clinically invasive (A) bladder carcinoma, day 10 (750×) and (B) prostatic carcinoma, day 15 (700×). Arrows denote areas of ECM degradation.

with respect to nutritional requirements for growth in culture. Results reported herein confirmed the hypothesis that media supplemented with appropriate growth factors and hormones are important for growth of primary human tumor explants in cultures on ECM. By using supplements the growth success was increased by 10–25% in comparison with media supplemented with 10% HIFCS only. Calvo et al. (29) obtained similar results with mammary carcinomas maintained on soft agar.

Fibroblasts are one of the major difficulties in obtaining pure and actively growing urological tumor cells on ECM, especially from prostatic tumors. In our hands all urological tumors in examined cultures were relatively free of fibroblasts in the first 10–15 days, after which fibroblasts began to proliferate and eventually overgrew the carcinoma cells in almost 30% of all urological tumors tested. The reported approaches to overcome this difficulty: poorly supplemented media, 1-h preplating of cultures on plastic, etc., were not satisfactory. Only a few of the renal carcinomas became contaminated with fibroblasts, and these did not exceed 2% of the total tumors tested.

In spite of fibroblast contamination and other limitations of the ECM system, the use of matrix allows one to study tumor cell growth and also invasion in vitro. Twelve tumors tested in the present study degraded matrix. In all cases these tumors were clinically very aggressive and/or poorly differentiated. This is similar to our findings with metastatic human ovarian tumors, which also demonstrated extensive degradation of ECM (30). Other tumor cell lines have been shown previously to degrade the glycoprotein components of a matrix elaborated by rat smooth cells in vitro (31). Human rhabdomyosarcoma, neuroblastoma, and osteogenic sarcoma cells digested mainly the glycoprotein components of the subendothelium, and only a little collagenolytic activity was displayed by these cells (32). Only human fibrosarcoma cells displayed significant collagenolytic activity when grown on glycoprotein-depleted matrices. The presence of collagenase-like enzymes in a human tumor cell line may be significant, since Liotta et al. (33) found that the metastatic potential of mouse melanoma cells correlated with their production of collagenase. Poste and Fidler’s studies (34) have demonstrated that cells with a higher metastatic potential adhere to the endothelial basement membrane more efficiently than clones of low metastatic potential. Other advantages of the use of ECM include: the potential development of a routine procedure for the growth of human individual tumor cells derived from individual patients. This has both basic and therapeutic significance. The definition of appropriate culture conditions also may permit the development of an in vitro predictive assay that will help in adjusting a therapeutic protocol for a given patient (15).

In vitro drug responsive of tumors grown on ECM could lead to early clinical testing of selected investigational drugs in the search for more effective chemotherapy of the more resistant urologic cancers. A relatively high growth success rate of urological tumors maintained on ECM will allow drug sensitivity tests for almost every patient with kidney and prostatic tumor.
REFERENCES


ACKNOWLEDGMENTS

The authors thank M. Hillman, J. Veith, M. Vaughan, L. Kenny, S. Polonskaya, N. Reska, E. Winslow, and P. Wohlhueter for technical help and S. Trafalski for typing. Approval for the use of human tumor material was given by the Clinical Investigation Committee, Roswell Park Memorial Institute.
Growth of Human Urological Tumors on Extracellular Matrix as a Model for the \textit{in Vitro} Cultivation of Primary Human Tumor Explants


\textbf{Updated version}  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/46/7/3653

\textbf{E-mail alerts}  Sign up to receive free email-alerts related to this article or journal.

\textbf{Reprints and Subscriptions}  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

\textbf{Permissions}  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.