A Rapid in Vitro Assay for Quantitating the Invasive Potential of Tumor Cells

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ABSTRACT

We have reconstituted a matrix of basement membrane onto a filter in a Boyden chamber and assessed the ability of various malignant and nonmalignant cells to penetrate through the coated filter. Cells from all the malignant cell lines tested were able to cross the matrix in 5-6 h, whereas human fibroblasts as well as mouse 3T3 and 10T½ cell lines, which are not tumorigenic, were not invasive. In addition, normal primary prostate epithelial cells and benign prostatic hyperplasia cells were not invasive when tested in this assay, whereas malignant prostate carcinoma cells were highly invasive. Parallel experiments with these prostatic cells using the intrasplenic assay for metastasis detection in the nude mouse confirmed the benign behavior of the former cells and the metastatic phenotype of the latter ones. These results suggest that this in vitro test allows the rapid and quantitative assessment of invasiveness and a means to screen for drugs which alter the invasive phenotype of tumor cells.

INTRODUCTION

Basement membranes are thin continuous sheets which separate epithelial tissues from adjacent stroma (1). The predominant components of basement membranes are laminin, a large multifunctional glycoprotein, collagen IV, and a heparan sulfate proteoglycan (2, 3). Additional common components have been identified, as well as other components with a more limited distribution (4, 5). Basement membranes form barriers that block the passage of cells and macromolecules but become permeable during tissue development and repair, at inflammatory sites, and are resorbed in areas where basement membranes contact invasive neoplasms (6). Tumor invasion of basement membranes is a crucial step in the complex multistage process which leads to the formation of a metastasis (6). Tumor cells cross basement membranes as they initially invade the lymphatic or vascular beds during dissemination, and when they penetrate into their target tissue (7). It seems likely that the penetration of the tumor cell into basement membranes involves distinct events which include: (a) attachment of the tumor cells to the basement membrane via cell surface receptors (8–12); (b) secretion of enzymes by the tumor cells that cause the degradation of the adjacent basement membrane (9, 13–15); and (c) migration of the cells into the target tissue in response to specific chemotactic stimuli (16–19).

A variety of in vitro systems have been developed to assess the invasiveness of tumor cells (for a review, see Ref. 20). Several of these assays utilize tissues which contain basement membranes, such as bladder wall (21), amnion (22), lens capsule (23), and chick chorioallantoic membranes (24). In addition, a pressed disc composed of lyophilized collagen IV and laminin has also been used to assay for the invasiveness of tumor cells (25) in Boyden chambers. The behavior of human HT-1080 fibrosarcoma cells on reconstituted basement membrane (i.e., matrigel) (5) in Petri dishes has recently been reported (26). The cells were found to invade this matrix, leaving tunnels and passages and ultimately degrading the gel.

Here we have used matrigel as a coating on the top of a porous filter as a barrier in Boyden chambers to test the invasive behavior of tumor cells. Fibroblast-conditioned medium, which contains known (i.e., fibronectin and collagen) as well as unknown chemotactic factors (27), was placed in the lower well beneath the filter to stimulate rapid penetration of the cells. Some 20 normal and tumorigenic cell lines were studied and the malignant cells were found to be the more invasive. Malignoma cells recovered after invading the matrix were found to be over 2-fold more invasive when retested in the assay, probably due to the selection of a more invasive subpopulation of cells. Striking differences in morphology were noted between malignant and nonmalignant cells cultured on the matrigel.

MATERIALS AND METHODS

Cell Lines. Murine melanoma cell lines B16F1 and B16F10 were provided by I. J. Fidler, Houston, TX. B16F1 cells are a subline with low metastatic potential and B16F10 cells are a high metastatic variant of the same tumor (28). B16B1 is a line of melanoma cells derived from a brain metastasis, which is highly invasive in vivo and in vitro (25). Two lines derived from the K-1735 UV-induced melanoma, C110 (nonmetastatic) (29) and M2 (highly metastatic) (30), were also provided by I. J. Fidler.

Nontumorigenic cells, MRC-5 (31) human embryonic fibroblasts (CCL 171), and C3H/10T½ (32) mouse fibroblasts (CRL 226), were obtained from the American Type Culture Collection. Several cell lines known to form malignant tumors in vivo were also obtained from the American Type Culture Collection: ME-180 (HTB 33), an invasive squamous cell epidermoid carcinoma of the ovary which had metastasized to the omentum (33); Melme 3M (HTB 64), a lung metastasis from a human melanoma (34); A204 (HTB 82), a human rhabdomyosarcoma (35); SW 620 (CCL 228), a grade III–IV colon adenocarcinoma (36); and PA-1 (CCL 1572), an ovarian teratocarcinoma (37) adapted to ascitic culture. The line HT-1080 (CCL 121) was derived from a metastatic lesion of a human fibrosarcoma (38). All cells were maintained in DMEM supplemented with 10% fetal calf serum, glutamine, vitamins, nonessential amino acids, and antibiotics.

T24/3T3 cells were derived from transfection of NIH 3T3 cells with DNA from the human bladder carcinoma T24, and contain an activated H-ras oncogene with a point mutation in the position corresponding to amino acid 12 (39, 40). Hs242/3T3 were obtained by transfecting NIH 3T3 cells with DNA from the human bladder carcinoma T24, and contain an activated H-ras oncogene with a point mutation in the position corresponding to amino acid 12 (39, 40). Hs242/3T3 were obtained by transfecting NIH 3T3 cells with DNA from the human lung carcinoma HaS242, and contain an H-ras oncogene with a mutation in position 61 (41). NIH 3T3 cells and their transfecants were maintained in medium supplemented with 10% calf serum, glutamine, and antibiotics.

Normal prostate epithelial cells, and benign prostate hyperplasia cells were obtained at surgery and were isolated by collagease digestion. Du 145 cells are a human prostate carcinoma cell line established by Stone et al. (42), which only rarely gives rise to metastasis in vivo; Du LM is derived from the rare liver metastasis formed from Du 145 cells injected in nude mice. PC 3 cells are a hormone-resistant line derived from a bone metastasis of a prostatic cancer (43). Primary prostate epithelial cells were grown in WJAC 404 medium (44) (Irving Scientific, Santa Ana, CA), supplemented with glutamine, antibiotics, and the following factors: epidermal growth factor (10 ng/ml); bovine pituitary extract (30 μg/ml); insulin, transferrin, and selenium in lino- lenic acid and bovine serum albumin (10 ml/liter) (Collaborative Re-

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2 The abbreviation used is: DMEM, Dulbecco's minimal essential medium.

J. M. Kozlowski et al., unpublished data.
search, Waltham, MA); cholera toxin (10 ng/ml); prolactin (2 μg/ml); and 2 mg/ml polyvinylpyrrolidone (M, 40,000) (Sigma Chemical Co., St. Louis, MO). DU 145 and DU LM cells were maintained in DMEM supplemented with 4.5 g of glucose/liter, nonessential amino acids, and 10% fetal bovine serum. PC-3 cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum.

Basement Membrane Matrigel. The basement membrane matrigel, an extract of the Engelbreth-Holm-Swarm tumor, was prepared in sterile form by exposure to chloroform and then dialyzed as previously described (5). The amount of protein in the gel was estimated according to Lowry et al. (45). The concentration of protein in the batch used throughout these studies was 10 mg/ml.

Chemoinvasion Assay. Blind well chemotaxis chambers with 13-mm diameter filters were used for the assay (Fig. 1). Polyvinylpyrrolidone-free polycarbonate filters, 8-μm and 12-μm pore size (Nuclepore, California), were coated with varying amounts of basement membrane matrigel (12.5-200 μg/filter). The matrigel was diluted to the desired final concentration with cold distilled water, applied to the filters, dried under a hood, and reconstituted with serum-free medium. In some cases, the homogeneity of the coating was checked by protein stain. The coated filters were placed in Boyden chambers. Cells (2-3 × 10^6), suspended in DMEM containing 0.1% bovine serum albumin, were added to the upper chamber. Under these conditions, few cells died within 24 h as measured by trypan blue exclusion in preliminary experiments.

Conditioned medium was obtained by incubating either mouse or human fibroblasts for 24 h in serum-free medium in the presence of ascorbate. Comparable results were obtained using either source of conditioned medium in preliminary experiments and for convenience 3T3-conditioned medium was used here. This medium was used as a source of chemoattractants and was placed in the lower compartment of the Boyden chambers. There is very little cell migration (less than 5%) in the absence of the chemoattractant over a 6-h period, and no passive diffusion since the pores of the filter are smaller than the cells. Assays were carried out at 37°C in 5% CO₂. The gradient of chemotactic protein is stable for at least 8-10 h as determined by the diffusion of radiolabeled proteins. Over 90% of the cells attach to the filter after a 2-h incubation. At the end of the incubation, the cells on the upper surface of the filter were completely removed by wiping with a cotton swab, as monitored visually under high power. The filters were fixed in methanol and were stained with hematoxylin and eosin. Cells from various areas of the lower surface were counted and each assay was performed in triplicate.

Chemotaxis. For chemotaxis studies, filters were coated with collagen type IV alone (5 μg/filter) to promote cell adhesion. Fibronec tin (25 μg/ml), conditioned medium, and medium alone were used as chemoattractants in the lower compartment of the chamber. Cells were added to the upper chamber and the assay was carried out as previously described (27).

Recovery of Invasive Cells. In some cases, these assays were carried out under sterile conditions to allow recovery of the invasive cells. The chambers were autoclaved and placed inside sterile Petri dishes (46). The uncoated filters were sterilized by UV. All solutions were sterile and the assembly of the chambers was carried out in a sterile hood. Two filters were used, including an 8-μm filter coated with matrigel and a second one with very small pores (1 μm) which was placed below it to retain the cells that passed the first filter. The second filter was coated with collagen IV to promote cell adhesion and the retention of cells on its surface. After 5 h, the matrigel and upper filter were removed. The lower filter, which contained the “invasive” cells, was rinsed and placed in a 13-mm tissue culture well containing complete culture medium. The cells were allowed to grow and were subsequently passed and reassayed for invasive behavior. Unselected control cells were assayed at the same passage number as the selected cells.

Morphology of Tumor Cells on Matrigel. The appearance of prostate cells cultured on the matrigel was assessed. Matrigel (0.5 ml/16-mm-diameter tissue culture dish well, i.e., 5 mg of matrigel/dish) was polymerized at 37°C for 30 min. A cell suspension (usually 5 × 10⁶ cells/well) in DMEM plus 10% fetal bovine serum was pipetted onto the top of the gel. Cells were photographed after 2-3 days.

In Vivo Assessment of Metastatic Activity. The metastatic capability of prostate-derived cells in vivo was assessed in nude mice by the intrasplenic assay (47). Cells (5 × 10⁶, viability over 95%) were injected into the medial tip of the spleen of mice anesthetized with methoxyflurane (Metofane). No significant bleeding or extravasation of cells was noted. The animals were killed by cervical dislocation 8 weeks after injection of the cells. Autopsies were performed and the organs (spleen, liver, foregut and hindgut mesentery, and lungs) were fixed, embedded in paraffin, and examined histologically.

RESULTS

To determine which conditions would allow a rapid but discriminating assay, we varied the amount of basement membrane matrigel placed on the filter and assessed the penetration of normal and malignant cells as a function of time. These studies showed that 50 μg of matrigel formed a continuous thin even barrier (Fig. 1) covering the surface of the filter but barely entering the pores. Few cells were found to penetrate through the matrigel during the first 3 h of the assay (Fig. 2A). After 5 and 6 h, many more cells from the malignant cell lines than from the nonmalignant controls were found to penetrate through the matrigel and filter. When lower levels of matrigel were used as a coating, more of the control cells penetrated and there was less of a difference between the malignant and control cells. Few cells were found to invade over a 5-h period when the amount of matrigel was increased to 100 μg/filter (Fig. 2B). However, a large number of malignant cells were able to invade through 100-μg matrigel in 24 h and through 200 μg after 48 h (data not shown). These observations indicate that the time for the invasive cells to pass through the barrier is proportional to the amount of matrix on the filter. For convenience, we selected a 5-h assay with 50 μg of matrigel on the filter for further investigation.

Various cells of known metastatic potential were examined in the invasion assay to determine if their ability to penetrate the reconstituted basement membrane matrigel correlated with their in vivo history. Indeed, those cell lines known to be malignant showed a higher rate of invasion than nonmetastatic tumor cells and the control cells showed little or no ability to penetrate the barrier (less than 2 cells/field) (Table 1). For example, various fibroblastic cell lines and a nonmetastatic clone (C110) from the K1735 mouse melanoma tumor did not invade the reconstituted matrix, whereas cells from several highly metastatic lines, including SW 620, a colon adenocarcinoma, and PA-1 teratocarcinoma and a metastatic clone (M2) from the K1735 tumor were highly invasive. In addition, the low metastatic line B16F1 of the B16 mouse melanoma demonstrated lower invasive activity than the more metastatic B16F10 line (22 cells/field versus 40 cells/field). B16BR₂ cells, obtained from a brain metastasis, were even more invasive (102 cells/field). We also studied B16BR₂ cells which had been collected after they had penetrated once through the reconstituted basement membrane and retested them in the invasion assay. These “selected” cells were more than twice as invasive in vitro as the parent line. Thus, this in vitro procedure not only characterizes the ability of cells to invade a basement membrane matrix but also allows the isolation of those cells which have successfully crossed the matrix barrier.

The chemotactic response of the cells was also tested to determine if the failure of the nonmetastatic tumor cells and normal cells to invade was due to their lacking this response. Indeed, all the normal cells tested here plus the nonmetastatic tumor lines showed a good chemotactic response (data not
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Fig. 1. Invasion assay: Boyden chamber assembly used for the invasion assay is shown in the upper left; section of a matrigel-coated (50 µg protein) filter is shown in the upper right; lower surfaces of an invasion membrane when NIH 3T3 (lower left) and T24/3T3 (lower right) are assayed for invasion.

shown). When matched cell lines were examined, such as 3T3 and 3T3 transfected with ras (Fig. 3) and the invasive and noninvasive lines from the 1735 melanoma clones C110 and M2 (data not shown), we found that the chemotactic response of the malignant cell was somewhat greater than its control line (1.5 to 2 times), but that invasiveness was some 15-fold greater. These results indicate that both the noninvasive and invasive cells are able to migrate to chemoattractants and that the basement membrane represents the critical barrier in our assay.

Studies on Prostate Cells. We also tested several human prostatic epithelial cell lines for their invasive activity in vitro and for their metastatic capability in vivo. These cells were studied because they represent a graded series differing in their malignancy in the human and in their metastatic abilities in nude mice. Normal prostatic cells did not invade the basement membrane matrigel nor form metastases in the nude mouse (data not shown). Primary benign hyperplastic prostatic cells, which did not form tumors in the animal after intrasplenic assay, were not invasive (Table 2). The prostatic carcinoma line (Du 145) gave rise to local tumors but showed base-line levels of invasiveness in vitro and very little metastatic activity in vivo. In contrast, cells from the rare liver metastasis of the Du 145 line, designated Du LM, and a highly malignant prostate tumor line, PC 3, invaded the matrix and also formed metastases in vivo. The organs involved were liver, parietal peritoneum, mesenteric and mediastinal lymph nodes, and lung parenchyma. This suggests that the in vitro invasion assay may supplement the intrasplenic assay for detecting metastatic variants and for quantitating metastatic potential.

No morphological differences were noted in the appearance of the various lines of human prostate cells whether normal or malignant when cultured on plastic substrates. However, when plated onto a matrigel substrate, the cells showed striking differences. Under these conditions, the normal cells formed secretory domes and the low-grade carcinoma cells aggregated into tight colonies. In contrast, the metastatic lines extended, spread, and formed branching, invasive colonies (Fig. 4, right). This distinctive behavior was apparent after 24 to 48 h in
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Fig. 2. A, time dependence of the invasion of the matrigel-coated filters for nonmalignant (NIH 3T3 and Du 145) and malignant lines (T24/3T3 and PC 3). B, dependence of varying amounts of matrigel coating on the invasiveness of nonmalignant (NIH-3T3 and MRC-5) and malignant (T24/3T3 and Hs 242/3T3) lines.

Table 1 Invasiveness of various cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Origin</th>
<th>Malignancy in vivo</th>
<th>Invasiveness in vitro (cells/field)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td></td>
<td>No</td>
<td>0° (15)*</td>
</tr>
<tr>
<td>MRC-5</td>
<td>Embryonic fibroblast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HT1080</td>
<td>Fibrosarcoma</td>
<td>Yes</td>
<td>8 (9)</td>
</tr>
<tr>
<td>ME-180</td>
<td>Cervical carcinoma (metastasis)</td>
<td>Yes</td>
<td>14 (6)</td>
</tr>
<tr>
<td>Malme 3M</td>
<td>Melanoma (metastasis)</td>
<td>Yes</td>
<td>14 (3)</td>
</tr>
<tr>
<td>A 204</td>
<td>Rhabdomyosarcoma</td>
<td>Yes</td>
<td>28 (3)</td>
</tr>
<tr>
<td>SW 620</td>
<td>Colon adenocarcinoma (metastasis)</td>
<td>Yes</td>
<td>30 (6)</td>
</tr>
<tr>
<td>PA-1</td>
<td>Ovary teratocarcinoma (ascites)</td>
<td>Yes</td>
<td>38 (24)</td>
</tr>
<tr>
<td>Mouse</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3H/10T½</td>
<td>Fibroblastoid</td>
<td>No</td>
<td>2 (15)</td>
</tr>
<tr>
<td>NIH 3T3</td>
<td>Fibroblastoid</td>
<td>No</td>
<td>2 (24)</td>
</tr>
<tr>
<td>T24/3T3</td>
<td>Ha-ras-transfected 3T3</td>
<td>Yes</td>
<td>30 (18)</td>
</tr>
<tr>
<td>HS242/3T3</td>
<td>Ha-ras-transfected 3T3</td>
<td>Yes</td>
<td>44 (12)</td>
</tr>
<tr>
<td>K 1735 C110</td>
<td>Melanoma</td>
<td>No</td>
<td>4 (30)</td>
</tr>
<tr>
<td>K 1735 M2</td>
<td>Melanoma</td>
<td>Yes (high)</td>
<td>44 (30)</td>
</tr>
<tr>
<td>B16F1</td>
<td>Melanoma</td>
<td>Yes (low)</td>
<td>22 (9)</td>
</tr>
<tr>
<td>B16F10</td>
<td>Melanoma</td>
<td>Yes (high)</td>
<td>40 (9)</td>
</tr>
<tr>
<td>B16Br2</td>
<td>Melanoma</td>
<td>Yes (high)</td>
<td>102 (24)</td>
</tr>
<tr>
<td>Br1 MG</td>
<td>B16Br2, matrigel selected</td>
<td>ND</td>
<td>&gt;200 (6)</td>
</tr>
</tbody>
</table>

* Data are expressed as migrated cells/field (x160).
* Numbers in parentheses, number of assays run for each line. SD was less than 10%.
* ND, not determined.

culture. The normal prostate and low-grade prostate carcinoma cells did not proliferate readily on the matrix, whereas the metastatic lines reached high density and totally lysed the matrix in the dish over the course of a few weeks (data not shown).

Studies on H-ras Oncogene-transfected Cells. ras oncogenes have been shown to be involved in a wide variety of human and animal malignancies (48, 49). The most frequent activating regions in these ras oncogenes have been localized to single point mutations at either position 12 or 61 in their coding sequences. NIH 3T3 cells transfected with ras oncogenes acquire a malignant phenotype and have been reported to show metastatic properties as well (50, 51). NIH 3T3 transfectants containing H-ras oncogenes with position 12 (T24/3T3) or 61 (Hs242/3T3) mutations were assessed for their invasive activity.

Table 2 Comparison of in vitro invasiveness and in vivo metastatic ability of primary and established prostate cell lines

<table>
<thead>
<tr>
<th>No. of animals developing visceral metastasis after intrasplenic injection/total animals</th>
<th>No. of cells which have invaded/field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign prostate hyperplasia</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>DU 145 (prostate carcinoma line)</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Du LM (Du 145, liver metastasis)</td>
<td>22 ± 6</td>
</tr>
<tr>
<td>PC 3</td>
<td>17 ± 3</td>
</tr>
<tr>
<td></td>
<td></td>
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Fig. 4. Appearance of prostate cells on plastic (left) and on matrix (right). A and B, benign prostate hyperplasia; C and D, low metastatic carcinoma Du 145; E and F, high metastatic carcinoma (PC 3); G and H, high metastatic carcinoma line (Du LM).
in vitro. Both cell lines readily penetrated the matrix in the assay with little difference in their invasiveness (Fig. 2, left; Table 1). Both cell lines also formed branching colonies on matrigel-coated dishes (not shown).

**DISCUSSION**

The invasiveness of tumor cells represents one of several important properties necessary for the formation of metastases (6). Many test systems have been used to obtain an assessment of the ability of cells to cross tissue barriers. Often whole organs or complex tissues such as the amnion, lens capsule, and bladder have been used (20), as well as in vivo determinations (51). It is assumed that it is the basement membrane in these tissues that creates a critical barrier to the passage of cells, although this has not yet been shown directly. Recently Terranova et al. (25) compressed collagen IV and laminin into a disc along with collagen I and interposed this barrier between two chambers, one of which contained tumor cells. Over the course of 1-2 days, cells from metastatic cell lines were found to migrate through the barrier, particularly in response to certain factors present in the tissue to which the cells appear to be targeted (19). Benign tumor cells and normal cells, with the exception of neutrophils, would not penetrate the disc. The barrier to the passage of cells was shown to be collagen type IV and laminin.

It was the purpose of the present study to develop a simple, more rapid method to assay the invasiveness of tumor cells through basement membrane. The 1 to 2 days required for the cells to migrate through the compressed layer of collagen IV, laminin, and collagen I necessitates sterility during the assay. Here we found that we could adjust the amount of matrigel on the filter and in this way vary the stringency of the barrier. Few cells from any source migrated through the reconstituted basement membrane at >100 µg/disc over the course of 6 h and all cells showed the ability to cross the barrier at lower levels of protein (12.5 µg/ml). An excellent degree of discrimination was observed at 50 µg/ml matrigel. At this concentration, the invasiveness of the cells was correlated with their malignant potential. In this assay, the cells cross the basement membrane matrix more rapidly than has been observed in other in vitro test systems using tissue matrices (1-3 days) (20) and in vivo systems (3-5 days) (52). These data are not inconsistent with our findings since the amount of time required for the cells to invade in this assay is directly proportional to the amount of matrix present. In addition, the in vitro assay described here does not have a continuous layer of endothelial cells on top of the basement membrane. It is estimated that the endothelial cell layer delays tumor cells from contacting the basement membrane in vivo for up to 2 days (52). The introduction of chemoattractants to the bottom chamber in the form of fibroblast-conditioned media speeded the movement of the invasive cells and was found to be necessary for maximal penetration over a 5- to 6-h period. This is in accordance with the suggestion (19, 20) that the migration and invasion of malignant cells into a target organ may be partially dependent on local chemotactic factors. Recently, autocrine motility factors have been described in tumor cell-conditioned media (53). The kinetics of the invasion process suggests that the numbers of cells crossing the barrier may increase with time as cells penetrate through the tunnels created by the first wave of migrating cells, as suggested previously by Kramer et al. (26), who also used matrigel as a substrate.

This rapid chemoinvasion assay measures the ability of the cells to (a) attach to the matrix; (b) degrade the matrix; and (c) migrate toward a chemoattractant. These events are considered to be important steps in the metastatic spread of tumor cells through basement membrane in vivo (20). Evidence for the degradation of the matrigel during the migration of the cells has been observed at the morphological level (26). Further, the addition of inhibitors of collagenase to the invasion chambers has been found to reduce the degradation of the matrix and to block cell invasion, and collagenase IV was found to be produced by the malignant cells and was detected in the lower compartment of the Boyden chamber. The degradation of labeled collagen IV added to the matrigel was also observed. These results indicate that invasion requires proteolysis of matrix components. While cell migration is a necessary activity for tumor cell invasion (13, 14), many nonmetastatic and highly metastatic cell lines give comparable chemotactic responses. Since in the chemoinvasion assay only the metastatic cells invade, it appears that the ability to degrade the basement membrane barrier is essential for the passage of these cells.

All cells characterized as invasive and metastatic in vivo which we tested were able to invade matrigel in vitro. NIH 3T3 cells were not invasive but NIH 3T3 cells transfected with activated ras oncogenes were able to cross the matrigel. In addition, MCF-7 breast carcinoma cells transfected with the ras oncogene were found to be highly invasive in this in vitro assay (54). These data are in accordance with other reports describing ras-transfected cells as being metastatic and invasive (50, 51). It is possible that this assay could be adapted and used to select for cells acquiring invasive properties after transformation with oncogenes or with DNA from metastatic cells.

Cells invading the reconstituted basement membrane can be isolated and studied. These cells appear to be a more invasive subpopulation of the parent line as determined on reassy. Such behavior is consistent with reports of Fidler and Kripke (55, 56) who showed that tumor cells are heterogeneous and that the invasive cells form a subpopulation in the parent tumor. We have preliminary evidence that some matrigel-selected cells are not only more invasive in vitro but are also more metastatic in vivo. However, we do not expect that this will be true with all cells, since a variety of other factors including antigenicity could be limiting their spread.

Perhaps the most striking results were obtained with the prostate cells. Again invasiveness in vitro was correlated with the biological behavior in vivo. While the malignant and benign cells show little difference in morphology on plastic substrates, they are readily distinguished when grown on matrigel with the malignant cells forming many processes. Such differences in appearance and in activity as well as in the ability to isolate the more invasive cells in the population suggest that this material will be of general use in studying the interaction of cells with basement membrane. Such an assay also has the potential for being used as a rapid in vitro method for screening agents that inhibit invasive behavior, but are not necessarily cytotoxic.

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