PROTEINASE REQUIREMENTS OF EPIDERMAL GROWTH FACTOR–INDUCED OVARIAN CELL INVASION

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Ovarian cancer is a highly metastatic disease characterized by diffuse intraperitoneal metastases; however, the underlying mechanisms that contribute to the metastatic phenotype are not well understood. The epidermal growth factor (EGF) receptor family of tyrosine kinases has been associated with tumor progression and an invasive phenotype. In this study, we utilized 4 ovarian cancer cell lines, OVCA 432, DOV 13, OVEA6 and OVCA 429, to determine the effects of EGF on the regulation of proteolytic enzymes and their inhibitors, cellular migration and in vitro invasion. Induction of urinary-type plasminogen activator (u-PA) activity and tissue inhibitor of matrix metalloproteinase (TIMP)-1 was observed in all 4 cell lines. OVCA 432 cells showed strong PAI-1 induction; however, the other 3 lines displayed substantial baseline PAI-1 expression that was not induced by EGF. EGF-dependent stimulation of migration and induction of matrix metalloproteinase (MMP)-9 (gelatinase B) was observed in OVEA6 and OVCA 429 cells only. Upon EGF receptor activation, DOV 13, OVEA6 and OVCA 429 cells were induced to invade through an artificial basement membrane (Matrigel); however, no invasion was detected in OVCA 432 cells. Cell lines displaying induction of migration and MMP-9 (OVEA6 and OVCA 429) demonstrated robust EGF-induced invasion (5- to 20-fold), and cell invasion was substantially reduced in the presence of anti-catalytic MMP-9 antibody. Addition of anti-catalytic u-PA antibody inhibited the modest (<2-fold) EGF-induced invasion in a cell line that did not express MMP-9 (DOV 13) and in OVEA6 cells that displayed the highest baseline u-PA activity. Together, our findings indicate that multiple proteinases are important in ovarian cell invasion and implicate EGF induction of MMP-9 and migration as key components of more aggressive ligand-induced invasion. Int. J. Cancer 78:331–337, 1998.

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Ovarian carcinoma cell lines OVCA 432, DOV 13 and OVCA 429 were generously provided by Dr. R. Bast, Jr., M.D. Anderson Cancer Center (Houston, TX). OVCA6 cells were developed from ascites fluid obtained from ovarian cancer patients (Moser et al., 1996). Cells were grown under standard conditions in 75-cm2 cell culture flasks (Moser et al., 1994).
Cells were plated at a density of 1 x 10^5/well and then grown to near-confluence in 12-well plates at 37°C before switching to serum-free media. After 2 hr, cells were treated with fresh serum-free media and EGF was added at the concentrations indicated in the figure legends. After 24 hr, the conditioned media was collected and immediately used for analysis. Plasminogen activator levels were measured using a coupled assay that monitors plasmin generation (Stack et al., 1993). Absorbance at 405 nm was detected using a Molecular Devices (Menlo Park, CA) Microtiter Plate Reader. Assays were performed in quadruplicate, and the results shown are from 4 independent experiments (n = 4).

Baseline activity values for DOV 13 and OVEA6 cells were 1.3 ± 0.6 pmoles plasmin/min and 57.7 ± 5.5 pmoles plasmin/min, respectively. For zymographic analysis to detect MMP production, serum-free conditioned medium was collected from cells cultured as described above or from wells containing cells plated on Matrigel. Zymographic analysis was performed as described previously (Moser et al., 1994) following pre-activation of samples with 1.5 mM amino-phenylmercuric acetate (APMA) for 40 min at 37°C. MMP-9, TIMP-1 and PAI-1 ELISAs were performed according to the manufacturers’ specification.

Analysis of cell migration

The effect of EGF on collagen-driven cell migration was determined using phagokinetic assays, as previously described (Cha et al., 1996). Briefly, colloidal gold salts immobilized to coverslips were coated with 20 µg of type I collagen. Ovarian cancer cells (5,000) were plated on each coverslip, allowed to adhere for 2 hr and then treated with 100 nM EGF in the presence or absence of 20 µg/ml mouse IgG, anti-catalytic u-PA antibody or 10 µg/ml anti-catalytic MMP-9 antibody. Cells were allowed to migrate for an additional 16 hr before fixation in 1% formaldehyde/PBS. The relative area of phagokinetic tracks was analyzed through dark-field optics with an Olympus (Tokyo, Japan) inverted microscope using NIH Image v.1.58 to quantitate pixels, and migration was scored as [phagokinetic track area (pixels)/cell]. All experiments were performed in duplicate, and at least 70 cells were analyzed per coverslip.

In vitro invasion

Analysis of in vitro invasion was performed as follows. Cells (1 x 10^5) were plated onto Matrigel-coated wells (11 µg/filter, 8 µm pore size) and incubated at 37°C for 72 hr. EGF (100 nM), in the presence or absence of 20 µg/ml mouse IgG, anti-catalytic u-PA antibody or 10 µg/ml anti-catalytic MMP-9 antibody, was added to invasion wells approximately 2 hr following plating of cells. Non-invading cells were removed from the top of the wells with a cotton swab, followed by fixation and staining with the Diff-Quick staining kit (Fisher). All experiments were completed in triplicate, and at least 15 fields/well were counted.

RESULTS

Characterization of EGF receptor and intermediate filament status in ovarian cancer cells

To evaluate the ability of ovarian cancer lines to respond to EGF, whole-cell lysates were prepared from OVCA 432, DOV 13,
OVEA6 and OVCA 429 cells treated with or without 10 nM EGF for 10 min prior to collection. EGF-treated extracts demonstrated ligand-dependent tyrosine phosphorylation of a band equivalent to the reported m.w. of the EGF receptor and of additional intracellular substrates, supporting the conclusion that these cells have a functioning EGF receptor (data not shown).

The mesodermally derived ovarian surface epithelium expresses both epithelial and mesenchymal characteristics in vivo and in cell culture. This relatively unique phenotypic plasticity is usually limited to immature or neoplastic epithelia (Czernobilsky et al., 1985; Auersperg et al., 1991). The intermediate filament composition of all 4 ovarian cancer cell lines was therefore assessed by immunofluorescence to determine whether there was a relationship between intermediate filament composition and an invasive phenotype. All ovarian epithelial carcinoma cells with a fibroblastic morphology (DOV 13, OVEA6 and OVCA 429) co-expressed vimentin and keratin, while the more epithelioid OVCA 432 cells expressed only keratin (data not shown).

**EGF regulation of u-PA activity and PAI-1 levels**

Plasminogen activators have been proposed to play a role in metastatic disease (Andreason et al., 1997) and are induced by EGF in other cell systems (Matrisian et al., 1990). Previous work has demonstrated that functional u-PA receptor is expressed on the surface of ovarian carcinoma cells, suggesting that the plasminogen activator pathway may be important for EGF-mediated invasion (Young et al., 1994). Conditioned media from all 4 cell lines exhibited increased u-PA production at concentrations of EGF greater than 1 nM (Table I). Rates of maximum induction of u-PA were 2.7±0.6 (OVCA 432), 2.8±0.5 (DOV13), 1.4±0.1 (OVEA6) and 4.7±2.0 (OVCA 429)-fold over control values. Uninduced uPA levels in OVEA6 cells were greater than 10-fold higher than those measured for the other 3 cell lines.

In addition to u-PA, higher levels of PAI-1 have been shown to correlate with a shorter patient survival in ovarian cancer (Pappot et al., 1995). EGF has previously been shown to affect PAI-1 expression in ovarian cells (Piquette et al., 1993). To evaluate the potential regulation of PAI-1 levels by EGF, PAI-1 antigen concentration in conditioned medium was measured by ELISA. An increase of PAI-1 antigen levels was observed in OVCA 432 conditioned media, with maximal stimulation at 1nM EGF (Table II). In the 3 vimentin-positive cell lines (DOV 13, OVEA6 and OVCA 429), uninduced PAI-1 levels were substantially higher than in OVCA 432 cells and PAI-1 activity was essentially unresponsive to EGF treatment (Table II).

**FIGURE 1** – Effects of EGF on MMP-9 expression of OVEA6 and OVCA 429 cells. (a) OVEA6 cells (1×10^5) were grown to confluence in a 12-well dish and treated with various concentrations of EGF for 24 hr under serum-free conditions. The media were collected and APMA activated before being assayed by zymography on a 9% SDS-polyacrylamide gel. Lanes 1–6, 0, 0.1, 1.0, 10 and 100 nM EGF, respectively. Lane 7 is an MMP-2 standard lane. (b) OVCA 429 cells (1×10^5) were plated on Matrigel and treated with the indicated concentrations of EGF under serum-free conditions for 72 hr. The collected conditioned media were activated using APMA and assayed by zymography on a 9% gel. Lanes 1–4, 0, 0.1, 1.0 and 10 nM EGF, respectively; lane 5, 10 nM EGF+10 µg/ml of IM09L, an anti-catalytic MMP-9 MAb; lane 6, 100 nM EGF. Note that the IM09L antibody blocks propeptide processing of pro-MMP-9, as evidenced by the presence of the 92-kDa form in antibody-treated samples. Matrigel does not influence MMP production in any of the cell lines (data not shown). (c) Conditioned media from control and EGF-treated OVCA 429 cells, obtained as described above, were analyzed for MMP-9 antigen levels by ELISA. (d) Effect of EGF receptor-blocking antibody on MMP-9 production. OVCA 429 cells (1×10^5) were plated on plastic in the absence or presence of EGF (10 nM) and the EGF receptor-blocking antibody LA1 (5 µg/ml) as indicated. MMP-9 was measured in the conditioned media by ELISA.
EGF regulation of MMP-9 and TIMP-1 levels

MMPs are regulated by EGF in numerous cell types (Benbow and Brinckerhoff, 1997). As assessed by zymographic analysis, EGF had no effect on MMP-2 (gelatinase A) levels in conditioned media from any of the cells tested (data not shown but see Fig. 1b) and MMP-9 was not detected in OVCA 432 and DOV 13 conditioned media (data not shown). EGF induced MMP-9 production in OVCA 429 (Fig. 1b) culture media in a dose-dependent fashion. The same pattern of response was detected whether cells were plated onto Matrigel or tissue culture plastic, so there was no extracellular matrix requirement for the observed response. The absence of MMP-9 in OVCA 432 conditioned media (data not shown) and the increase in OVCA 429 MMP-9 secretion in response to EGF (Fig. 1c) were confirmed by ELISA. The ligand-dependent induction of MMP-9 is mediated by the EGF receptor as addition of a receptor-neutralizing antibody

TABLE III – EFFECT OF EGF ON TIMP-1 LEVELS IN CONDITIONED MEDIA

<table>
<thead>
<tr>
<th>Cell line</th>
<th>0</th>
<th>10 pM</th>
<th>100 pM</th>
<th>1 nM</th>
<th>10 nM</th>
<th>100 nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVCA 432</td>
<td>14.2 ± 0.5</td>
<td>17.4 ± 0.2</td>
<td>22.9 ± 0.2</td>
<td>50.5 ± 1.8</td>
<td>44.0 ± 0.2</td>
<td>43.4 ± 1.2</td>
</tr>
<tr>
<td>DOV 13</td>
<td>93.9 ± 0.5</td>
<td>88.4 ± 1.4</td>
<td>96.8 ± 1.4</td>
<td>93.6 ± 1.2</td>
<td>126.3 ± 1.7</td>
<td>124.6 ± 0.4</td>
</tr>
<tr>
<td>OVEA6</td>
<td>31.8 ± 1.1</td>
<td>26.5 ± 0.2</td>
<td>30.9 ± 0.8</td>
<td>35.3 ± 2.7</td>
<td>41.9 ± 1.8</td>
<td>39.4 ± 3.5</td>
</tr>
<tr>
<td>OVCA 429</td>
<td>24.4 ± 0.4</td>
<td>25.1 ± 0.5</td>
<td>30.3 ± 0.3</td>
<td>37.0 ± 3.2</td>
<td>32.0 ± 2.1</td>
<td>41.2 ± 1.8</td>
</tr>
</tbody>
</table>

1All 4 cell lines were grown to confluence in 12-well plates and then treated with various concentrations of EGF in serum-free media for 24 hr. Conditioned media were assayed in duplicate for TIMP-1 antigen levels by ELISA. Values are expressed as ng/ml ± SD between ELISA wells.

TABLE IV – EFFECTS OF EGF ON OVARIAN CANCER CELL MIGRATION

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Matrix</th>
<th>[EGF] (nM)</th>
<th>Antibody</th>
<th>MU/cell ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVCA 432</td>
<td>Collagen I</td>
<td>0</td>
<td>Mouse IgG</td>
<td>1,940 ± 350</td>
</tr>
<tr>
<td>DOV 13</td>
<td>Collagen I</td>
<td>100</td>
<td>Mouse IgG</td>
<td>1,960 ± 290</td>
</tr>
<tr>
<td>OVEA6</td>
<td>Collagen I</td>
<td>0</td>
<td>MAb 394</td>
<td>1,700 ± 320</td>
</tr>
<tr>
<td>OVCA 429</td>
<td>Collagen I</td>
<td>0</td>
<td>Mouse IgG</td>
<td>7,390 ± 3,020</td>
</tr>
<tr>
<td>OVEA6</td>
<td>Collagen I</td>
<td>100</td>
<td>Mouse IgG</td>
<td>7,500 ± 2,480</td>
</tr>
<tr>
<td>DOV 13</td>
<td>Collagen I</td>
<td>0</td>
<td>MAb 394</td>
<td>7,310 ± 2,310</td>
</tr>
<tr>
<td>OVEA6</td>
<td>Collagen I</td>
<td>100</td>
<td>MAb 394</td>
<td>7,300 ± 2,310</td>
</tr>
<tr>
<td>OVCA 429</td>
<td>Collagen I</td>
<td>0</td>
<td>Mouse IgG</td>
<td>2,670 ± 730</td>
</tr>
<tr>
<td>OVEA6</td>
<td>Collagen I</td>
<td>100</td>
<td>MAb 394</td>
<td>2,750 ± 410</td>
</tr>
<tr>
<td>DOV 13</td>
<td>Collagen I</td>
<td>0</td>
<td>Mouse IgG</td>
<td>2,520 ± 460</td>
</tr>
<tr>
<td>OVEA6</td>
<td>Collagen I</td>
<td>100</td>
<td>MAb 394</td>
<td>4,660 ± 1,120</td>
</tr>
<tr>
<td>OVCA 429</td>
<td>Collagen I</td>
<td>0</td>
<td>MAb 394</td>
<td>2,250 ± 460</td>
</tr>
<tr>
<td>DOV 13</td>
<td>Collagen I</td>
<td>100</td>
<td>MAb 394</td>
<td>4,220 ± 1,000</td>
</tr>
<tr>
<td>OVEA6</td>
<td>Collagen I</td>
<td>100</td>
<td>IM09L</td>
<td>4,380 ± 990</td>
</tr>
</tbody>
</table>

1Cells were plated on colloidal gold-coated coverslips overlaid with type I collagen in the absence or presence of 100 nM EGF as indicated. In addition, either 20 µg/ml of mouse IgG or anti-catalytic u-PA antibody (394) or 10 µg/ml anti-catalytic MMP-9 antibody (IM09L) was added 2 hr after plating. Cells were allowed to migrate for an additional 16 hr prior to fixation in 0.1% formaldehyde. DOV 13 cells demonstrated an inflated migration value due to multiple fibroblastic processes on collagen type I. The relative area of phagokinetic tracks was measured using NIH Image, and values are expressed as migration units (MU, pixels of migration) ± SD per cell.

FIGURE 2 – EGF induction of migration in OVEA6 and OVCA 429 cells on a collagen type I matrix. Cell migration analyses were performed as described in “Material and Methods”. EGF stimulated migration in OVEA6 and OVCA 429 cells. The observed ligand-dependent migratory response was diminished when OVEA6 cells were treated with an anti-catalytic u-PA antibody (394). EGF-induced OVCA 429 cellular migration was consistently 2-fold higher than control, and addition of the u-PA antibody had little effect on migration (Table IV).
(LA1) blocked the EGF-dependent induction of MMP-9 in OVCA 429 cells (Fig. 1d).

TIMP-1 inhibits the activity of MMP-9, and expression of TIMP-1 can be regulated by growth factor-signaling pathways (Hosono et al., 1996). Modest induction (<2-fold) of TIMP-1 by EGF was observed in DOV 13, OVEA6, and OVCA 429 conditioned media (Table III). As with PAI-1 (Table II), OVCA 432 cells displayed the greatest induction of TIMP-1 (3-fold, Table III).

**EGF regulation of cell migration**

Both proteolytic activity and cell motility can contribute to an invasive response, and EGF can modulate epithelial cell migration. In this regard, we measured ligand-dependent migration for each of the cell lines (Table IV). As we have previously reported that ovarian carcinoma cells preferentially adhere to collagen type I (Moser et al., 1996), cells were plated on a thin layer of collagen type I and migration was measured with or without 100 nM EGF. EGF did not promote migration of OVCA 432 and DOV 13 cells on collagen type I matrix (Table IV). In contrast, OVEA6 and OVCA 429 cellular migration was stimulated by 100 nM EGF (Fig. 2, Table IV). A relationship may exist between u-PA/u-PA receptor and cellular migration, possibly through plasmin generation or a u-PA receptor-signaling mechanism (Andreasen et al., 1997). Furthermore, antibodies against u-PA have been shown to inhibit migration of some cell types, including epithelial cells (Andreasen et al., 1997). Because EGF induced u-PA activity in each cell line (Table I), we wanted to address the role of u-PA activity in EGF-stimulated migration. An anti-catalytic u-PA antibody (394) did not inhibit basal (OVCA 432, DOV 13) or EGF-stimulated (OVCA 429) cell migration on collagen type I; however, EGF-dependent OVEA6 cellular migration on collagen type I was eliminated upon addition of the anti-catalytic u-PA antibody (Table IV, Fig. 2). In contrast, an anti-catalytic MMP-9 antibody did not inhibit EGF-stimulated migration of the 2 cell lines (OVEA6 and OVCA 429) that displayed EGF-dependent MMP-9 induction (Table IV).

**EGF regulation of cell invasion**

In the preceding experiments, we showed that EGF co-regulates uPA, MMP-9 and migration in OVEA6 and OVCA 429 cells. Matrigel-coated invasion chambers were utilized to determine whether regulation of these activities had an impact on ligand-induced ovarian cancer cell invasion. EGF markedly stimulated invasion of OVEA6 and OVCA 429 cells (Fig. 3b,c). In contrast, OVCA 432 cells did not invade Matrigel basement membrane (data not shown) and the addition of exogenous plasminogen (300 nM) did not promote a response either in the absence or the presence of EGF (data not shown), though plasmin is generated under these conditions (Table I). The ligand-stimulated invasion of DOV 13 cells was of a lesser magnitude (approx. 2-fold) than that of OVEA6 or OVCA 429 cells (Fig. 3a).

Neutralizing antibodies to u-PA and MMP-9 were used to determine the contribution of each protease to the invasive response. Inclusion of the MMP-9-neutralizing antibody had no effect on DOV 13 cell invasion, as would be expected by the lack of MMP-9 production in the presence or absence of EGF (data not shown). However, the anti-uPA antibody fully inhibited EGF-dependent invasion of DOV 13 cells (Fig. 3a), indicating that uPA activity is crucial to the invasive capacity of this cell line. EGF-stimulated invasion of OVEA6 cells was blocked 65–85% by addition of 20 µg/ml of the u-PA antibody and 50–70% by co-incubation with 10 µg/ml of the anti-catalytic MMP-9 antibody. Simultaneous addition of both antibodies completely eliminated EGF-induced invasion, suggesting that uPA and MMP-9 activities contribute to OVEA6 cell invasion. In contrast, EGF-dependent invasion of OVCA 429 cells was inhibited by the anti-catalytic MMP-9 antibody but not by the u-PA-blocking antibody (Fig. 3c). In each case, the concentrations of anti-catalytic uPA antibody used blocked all PA activity (data not shown) and the anti-catalytic MMP-9 antibody effectively blocked pro-MMP-9 processing, as

![Figure 3](image-url) – EGF stimulates in vitro invasion of DOV 13, OVEA6 and OVCA 429 cancer cells. (a) DOV 13 (1.6 × 10⁵), (b) OVEA6 (1.6 × 10⁵) or (c) OVCA 429 (1.0 × 10⁵) cells were plated onto polycarbonate filters (8-µm pores) coated with Matrigel (11 µg/ml) in the presence or absence of EGF (100 nM), anti-catalytic u-PA antibody 394 (20 µg/ml), anti-catalytic MMP-9 antibody IM09L (10µg/ml) or control IgG (20 µg/ml) as indicated. All invasion assays were conducted in triplicate with at least 15 fields counted per well. Values are expressed as number of invasive cells found within a set grid using a 20x objective (bars: SD).
shown in Figure 1b, lane 5. Our findings suggest that MMP-9 induction is a primary contributor to EGF-stimulated OVCA 429 cell invasion.

DISCUSSION

Tumor progression to the metastatic phenotype is a complex process involving changes in cell adhesion, migration and degradation of the extracellular matrix (Mignatti and Rifkin, 1993). The factors that contribute to metastatic progression are not well understood, but aberrant expression or activity of the erbB family of receptor tyrosine kinases may play a role (Eccles et al., 1995). The observation that over-expression of the EGF receptor and erbB-2 is associated with poor prognosis for ovarian cancer patients (Bartlett et al., 1996; Meden et al., 1994) suggests a relationship between these receptors and cellular responses associated with metastasis. Receptor-mediated induction of proteinases represents one possible mechanism linking the receptors to tumor progression and metastasis.

Over-production of proteinases of the PA and MMP families has previously been reported in ovarian cancer cells and tissues (Stack et al., 1998); however, the individual contribution of each proteinase family remains unclear. Studies have shown, on the one hand, that u-PA levels increase with tumor progression and that anti-sense to u-PA in ovarian cells reduced invasion in vitro and in vivo (Andreason et al., 1997). On the other hand, previous work on macrophage colony-stimulating factor (CSF-1) induction of ovarian cancer cell invasion (Chambers et al., 1995) concluded that saturable levels of anti-uPA antibodies only partially inhibited CSF-1-induced invasion, suggesting that uPA is not an exclusive mediator of ovarian cancer cell invasion.

MMPs represent another family of proteinases that are candidate mediators of ovarian cancer cell invasion. Although many studies have reported a correlation between increased MMP-9 expression and tumor metastasis, it has been difficult to establish whether gelatinase activity in general or MMP-9 in particular is the key determinant of increased invasiveness (Himmelstein et al., 1995). There is some evidence that the MMP-9:MMP-2 ratio is greater in ovarian cancer tissues when compared with normal ovarian tissues (Stack et al., 1998), and constitutive over-expression of MMP-9 in rat embryo cells transfected with the MMP-9 gene resulted in a metastatic phenotype in vitro and in vivo (Bernhard et al., 1994), thereby strengthening the relationship between MMP-9 and invasion. Similarly, our studies support a link between growth factor-induced MMP-9 expression and subsequent cell invasion in an ovarian tumor model.

In the cell lines examined, co-induction of u-PA and MMP-9 and migration by EGF corresponded to the greatest invasive potential through a Matrigel matrix. As in the studies of Chambers et al. (1995), we did not find a strict correlation between growth factor-induced u-PA activity and invasion. Our data indicate that although u-PA activity was increased by EGF in all 4 cell lines, there was minimal EGF-induced invasion in cell lines that do not additionally produce MMP-9 (Table V). Furthermore, whereas anti-catalytic u-PA antibody inhibited EGF-induced invasion of DOV 13 and OVEA6 cells, it had little effect on OVCA 429 cells, which displayed the greatest magnitude of EGF-induced invasion. However, anti-catalytic MMP-9 antibody was an effective inhibitor of EGF-induced invasion of both OVEA6 and OVCA 429 cells. These findings suggest that although u-PA may play an important role in ovarian tumor invasion, induction of u-PA is not sufficient for a maximal response and may require co-regulation of other enzymatic activities, such as MMP-9.

In addition to MMP-9 induction, we observed that stimulation of migration on collagen type I matrix correlated with EGF-induced invasion. This suggests that ligand-induced motility, in conjunction with induction of specific proteinases, is an important component of EGF-induced invasion in ovarian cancer cells. It has been proposed that u-PA may modulate cell migration (Andreason et al., 1997), and a defect in migration of an ovarian cancer cell line has been associated with an impaired urokinase-induced tyrosine phosphorylation-signaling event (Mirshahi et al., 1997). Interestingly, we found that addition of anti-catalytic u-PA antibodies blocked EGF-stimulated migration and reduced EGF-mediated invasion in OVEA6 cells. It is not known whether u-PA activation of plasminogen or a u-PA-mediated signaling event is important for migration in these cells.

In summary, we find a relationship between EGF-induced MMP-9 activity, cell migration and subsequent cell invasiveness. Activation of the EGF receptor increased u-PA activity in each cell line examined, but co-regulation of uPA and MMP-9 corresponded to the greatest invasive capacity. Our results illustrate that induction of MMP-9 by EGF is a key component of EGF-induced ovarian cell invasion. These findings outline a potential mechanistic relationship between over-expression or aberrant activity of the erbB receptor tyrosine kinase family in ovarian cancer and the metastatic nature of this disease.

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REFERENCES


