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Regulation of Invasion of Epithelial Ovarian Cancer by Transforming Growth Factor-β[1]

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Objective. **The metastatic process in epithelial ovarian cancer is thought to involve surface shedding and subsequent dissemination of ovarian cancer cells, facilitated by localized proteolysis at the interface between ovarian cancer cells and peritoneal surfaces. The factors regulating the metastatic process, however, are not well understood. Transforming growth factor-β (TGF-β) is a multifunctional peptide that elicits numerous cellular effects pertinent to the metastatic process. The purpose of this study was to evaluate the regulatory role of TGF-β on metastasis in ovarian cancer.**

Methods. **We evaluated the effect of TGF-β on the metastatic characteristics (adhesion, invasion, motility, proteolysis) of five ovarian cancer cell lines (DOV-13 and OVCA 420, 429, 432, and 433), two short-term primary ovarian cancer cell cultures (OVCA 10 and OVCA 208), and five normal ovarian surface epithelial (NOSE) cell cultures (OSE 133, 185, 186, 188, and 189). The effect of TGF-β on invasion and proteolysis was quantified using a modified Boyden chamber invasion assay, zymography, a coupled colorimetric activity assay, and an HPLC-based quantitation of synthetic substrate cleavage.**

Results. **TGF-β significantly increased invasion in five of seven ovarian cancer cell lines in amounts ranging from 2- to 20-fold. In contrast, TGF-β significantly decreased invasion in two of five NOSE isolates by 50 to 80% and had no significant effect on invasion in three. TGF-β treatment increased matrix metalloproteinase (MMP) expression in OVCA 420 and 433 and DOV-13, resulting in MMP-dependent collagen cleavage and invasive activity. Addition of the MMP inhibitor GI12947 neutralized the enhancing effect of TGF-β on invasion. TGF-β had no effect on ovarian cancer cell motility and only increased adhesion in DOV-13.**

Conclusions. **These data suggest that TGF-β may enhance the invasiveness of ovarian cancers through induction of MMP activity.** **© 2001 Academic Press**

Key Words: **metastasis; ovarian carcinoma; metalloproteinase; invasion; TGF-β.**

INTRODUCTION

Epithelial ovarian cancer remains a highly lethal malignancy. It is the fourth leading cause of cancer deaths among women in the United States and causes 100,000 deaths annually in women worldwide [1]. Despite intensive research efforts over the past decade directed toward improved detection and treatment of ovarian cancer, the long-term survival of women with ovarian cancer has only improved modestly. Progress in the fight against ovarian cancer has been hampered by the lack of highly effective therapy to permanently eradicate disseminated intraperitoneal metastases, present in most patients at the time of diagnosis.

Little is known regarding the molecular mechanisms underlying the metastatic process in ovarian cancer. For other tumor types, Liotta and Rao's three-step model of invasion has described the biochemical events associated with tumor cell invasion and metastasis [2]. In this model, tumor cells first attach to the extracellular matrix through cell membrane receptors that mediate binding to matrix components such as laminin, collagen, and fibronectin. Subsequently, localized proteolysis initiated by tumor cells results in degradation of the matrix. This is followed by migration of tumor cells into the proteolytically modified matrix. Using repeated cycles of these three steps, tumor cells not only invade locally but also enter and exit the circulatory system and establish metastatic foci at distant sites.

Although it is commonly believed that ovarian cancers disseminate primarily through exfoliation occurring at the ovarian surface, ovarian cancers often demonstrate a capability to metastasize in a manner consistent with Liotta's model. Approximately 40% of women with advanced ovarian cancer have lymph node metastasis at presentation, and a similar fraction develop extra-abdominal metastasis late in the course of their disease. Furthermore, at a molecular level, ovarian cancers express cell surface receptors that facilitate adhesion to extracellular matrix components [3, 4], produce proteinases

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important in matrix degradation [5–7], and are capable of locomotion [4, 8]. We have previously shown that ovarian cancers have increased levels of matrix degrading proteinases when compared to normal ovarian epithelium and that ovarian cancer invasiveness correlates with expression of cellular proteinases [6–8]. In addition, recent studies suggest that ovarian cancer cells bind preferentially to type I collagen, which is abundant in the peritoneum, and that matrix metalloproteinase (MMP) expression or processing may be induced by type I collagen [9–11]. This suggests that localized proteolysis mediated by MMPs and directed at the interface between ovarian cancer cells and peritoneal tissues may play an important role in the localized invasion and dissemination of ovarian cancer cells in the peritoneal cavity. The diverse factors regulating proteinase secretion and the metastatic process overall, however, remain to be elucidated.

Transforming growth factor-β (TGF-β) is a multifunctional peptide that elicits numerous cellular effects pertinent to the metastatic process including regulation of epithelial cell differentiation and proliferation, modulation of angiogenesis, synthesis of extracellular matrix proteins and integrin receptors, and alterations in production of matrix degrading proteinases and their inhibitors [12]. The purpose of this study was to evaluate the regulatory role of TGF-β on metastasis in ovarian cancer.

MATERIALS AND METHODS

Cell cultures. Five epithelial ovarian cancer cell lines (OVCA 420, OVCA 429, OVCA 432, OVCA 433, and DOV 13) were maintained as previously described in monolayer culture in tissue culture medium containing modified Eagle's medium (Hazelton Research Products Inc., Lenexa, KS) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco Laboratories, Grand Island, NY), sodium pyruvate, nonessential amino acids, glutamine, penicillin, and streptomycin [13].

Normal ovarian surface epithelial (NOSE) cells were obtained from the surface of human ovaries that had been removed at operation for treatment of benign gynecologic diseases and cultured as previously described [13]. The NOSE cells were maintained in monolayer culture in a 1:1 mixture of MCDB 105 and M199 medium (Sigma Chemical Co., St. Louis, MO) supplemented with 10% heat-inactivated FBS. The epithelial nature of the cells was verified both morphologically and by immunohistochemical staining with anti-cytokeratin antibodies AE1/AE3, which bind to ovarian epithelium but not to stroma. Purified nonimmune mouse immunoglobulin G (Coulter Immunology, Hialeah, FL) was used as a negative control. Staining with anti-factor-8 antibody was performed to confirm the absence of contaminating endothelial cells.

Primary short-term epithelial ovarian cancer cell cultures were established from the ascites of patients with Stage III and IV epithelial ovarian cancer as previously described [14]. Briefly, cells were removed from ascites by centrifugation and washed in MCDB105/M199 medium supplemented with 10% FBS, L-glutamine (2 mM), and penicillin (100 U/ml)/streptomycin (100 μg/ml). Purification of ovarian cancer cells was accomplished using both discontinuous gradient centrifugation with Percoll (Pharmacia, Piscataway, NJ) density gradients (1.070–1.023 g/cm^3) and selective removal of inflammatory cells with anti-CD45 antibodies and immunomagnetic beads. A cocktail of monoclonal antibodies known to be reactive with epithelial ovarian cancer cells, but not mesothelium or inflammatory cells, was used to confirm the purity of the primary ovarian cancer cell cultures [14].

Materials. Human recombinant TGF-β1 was obtained from R&D Systems (Minneapolis, MN). Plasminogen, plasminogen activator, and plasminogen activator inhibitor type 1 (PAI-1) were purchased from American Diagnostica (Greenwich, CT). GI 129471, a synthetic broad-spectrum MMP inhibitor, was provided as a generous gift from Glaxo, Inc. (Research Triangle Park, NC) [15]. The synthetic plasmin substrate D-Val-Leu-Lys-*p*-nitroanilide (VLKpNA) was a product of Sigma, whereas the synthetic MMP substrate Dnp-Pro-Leu-Gly-Leu-Trp-Ala-D-Arg-NH2 (DnpPLGLWAR-NH2) was purchased from Peptides International (Louisville, KY). A 48-well microchemotaxis and invasion chamber was purchased from Neuro Probe, Inc. (Cabin John, MD). Nucleopore 8-μm-pore filters were obtained from Costar (Cambridge, MA). Reconstituted basement membrane (Matrigel) was obtained from Collaborative Research (Lexington, MD). Cell culture reagents, fibronectin, and all other materials either were purchased from Sigma or were of the best commercial grade available.

Cell culture conditions and conditioned-medium collection. Cell cultures were seeded in serum-free medium at a density of 100,000 cells per well in 24-well plates (Costar) in the presence or absence (control) of TGF-β (10 ng/ml) for 24 h. The conditioned medium was then collected and analyzed for proteinase activity.

Metalloproteinase and plasminogen activator activity. Identification of MMP activity in ovarian cancer conditioned medium was performed as previously described [6]. Briefly, latent metalloproteinases were activated by incubation of conditioned medium with 1.5 mM aminophenylmercuric acetate (APMA) for 1 h at 37°C. Samples were then incubated for 30 min with nonreducing Laemmli sample dilution buffer and electrophoresed on 9% polyacrylamide gels containing 0.1% gelatin. Following electrophoresis, gels were washed with 2.5% Triton X-100 for 30 min and incubated overnight at 37°C in 0.1 M glycine, 10 mM CaCl2, 1 μM $ZnCl_2$, pH 8.3. After staining with Coomassie blue, regions of proteolytic activity were visualized as clear zones of lysis against a blue background. Samples of conditioned medium were normalized for differences in cell count before electrophoresis. All zymograms were repeated a minimum of three times.

Type IV collagenase activity was demonstrated by monitor-

ing cleavage of type IV collagen (Sigma) as previously described [6]. Briefly, conditioned medium were incubated with type IV collagen for 24 h followed by electrophoretic separation of collagen degradation products on 7.5 SDS–polyacrylamide gels.

MMP activity was quantified as previously described [6] using the synthetic collagenase substrate Dnp-PLGLWAR-NH2. Conditioned medium samples (40 μl) were preactivated with APMA and incubated with 20 μM substrate in 0.05 M Tris, 0.2 M NaCl, 10 mM $CaCl_2$, pH 7.7, at 37°C for 18 h. Cleavage products were separated by reverse-phase HPLC using a C18 column. Substrate cleavage was quantified by monitoring column effluence at 370 nm.

Plasminogen activation was quantitated spectrophotometrically using a coupled assay that monitors the amidolytic activity of generated plasmin using the synthetic plasmin substrate VLKpNA. Plasminogen (0.3 μM) was incubated in 96-well microtiter plates in 20 mM Hepes, pH 7.4, in the presence of conditioned medium from control cells or cells treated with TGF-β (10 ng/ml). The plasmin substrate VLK-pNA (final concentration 0.3 mM) was added and plasminogen activation was quantitated by monitoring the absorbance at 405 nm with a Thermomax plate reader (Molecular Devices, Sunnyvale, CA). All samples were analyzed eight times.

In vitro invasion assay. Eight-micrometer-pore polycarbonate filters (Nucleopore) were coated on their upper surface with Matrigel and allowed to dry overnight. The Matrigel-coated filters were placed between the upper and lower plates of a 48-well microchemotaxis chamber (Neuro Probe). The lower portion of the invasion chamber was filled with NIH 3T3-conditioned medium to function as a chemoattractant. For experiments, 3.5×10^4 cells were placed into each of quadruplicate wells in the upper chamber in serum-free conditions (nutrient-supplemented minimal essential medium) in the presence or absence of TGF-β (10 ng/ml concentration), with or without the MMP inhibitor GI 129471 (100 nM) or PAI-1 (1 μg/ml). In addition, in separate experiments, ovarian cancer cell lines were grown in the presence or absence of TGF-β (10 ng/ml concentration) for 24 h prior to incubation in the invasion chamber to determine whether pretreatment with TGF-β influenced invasive behavior. The invasion chamber was incubated at 37°C under 5% carbon dioxide in 100% humidity for 9–18 h as indicated. The polycarbonate filter was then removed, fixed, and stained with hematoxylin and eosin. Noninvading cells adherent to the upper surface of the filter were wiped off gently with a cotton swab. The percentage of invasion (invading cells attached to under surface of the filter) was then determined for each well by calculating the percentage of the well filter surface area occupied by invading cells using computer-assisted image analysis. This was accomplished using the Optimas 4.0 image analysis software (Optimas Corporation, Edmonds, WA). Motility experiments were similarly performed with the exception that the polycarbonate filters were not coated with Matrigel. Motility assays were incubated for approximately 4 h prior to harvesting.

Adhesion assays. The effect of TGF-β on adhesion was quantitated using the Alamar blue indicator (Alamar, Sacramento, CA). Briefly, ovarian cancer cells (2.5×10^4 per well) were dispensed in 96-well plates in tissue culture medium (MEM) and MEM plus TGF-β (10 ng/ml, experimental wells) and incubated for 2 h. The plates were then washed three times with culture medium in order to remove nonplated, nonadherent cells. A total of 100 μl of culture medium, containing 10% alamar dye, was added to each well, and the wells were incubated for 4 h at 37°C in 5% CO_2. After being washed once, the optical density or absorbance of the medium in each well was measured at a wavelength of 630 nm using an ELISA reader. Reduction of Alamar dye by metabolically active cells results in a decrease in the optical density of the medium. The measured absorbance of the medium is thus inversely proportional to the number of cells present. Six replicates were used per condition.

Statistics. Student's *t* test was used to analyze experiments comparing two means. Adjustment for multiple comparisons was made where appropriate.

RESULTS

Normal ovarian surface epithelial cells were capable of invading through a reconstituted basement membrane. The invasiveness of NOSE was significantly less than that of OVCA 429, a moderately invasive ovarian cancer cell line. As can be seen in Fig. 1, OVCA 429 cells were 3–10 times more

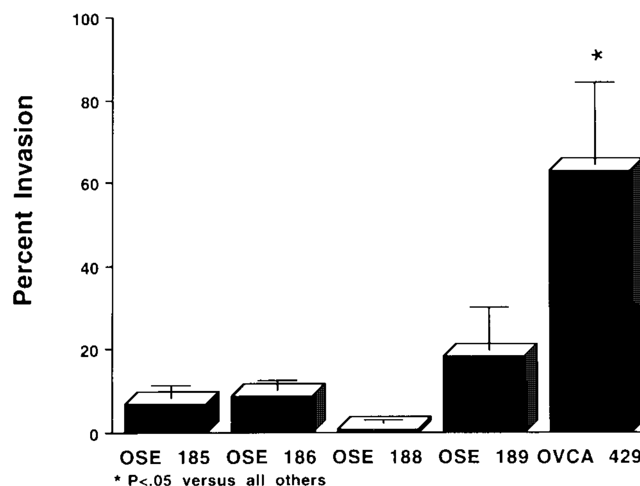

FIG. 1. Comparison of the relative invasiveness of four different normal ovarian surface epithelial (NOSE) cell cultures and OVCA 429. Cells (3.5×10^4) were seeded onto Matrigel-coated porous filters in the presence or absence of TGF-β (10 ng/ml) as indicated. Following incubation for 18 h, filters were stained and invading cells were quantified using computer-assisted image analysis. Although NOSE invade Matrigel, the percentage of invasion is significantly less than that of moderately invasive OVCA 429 ($P < 0.05$).

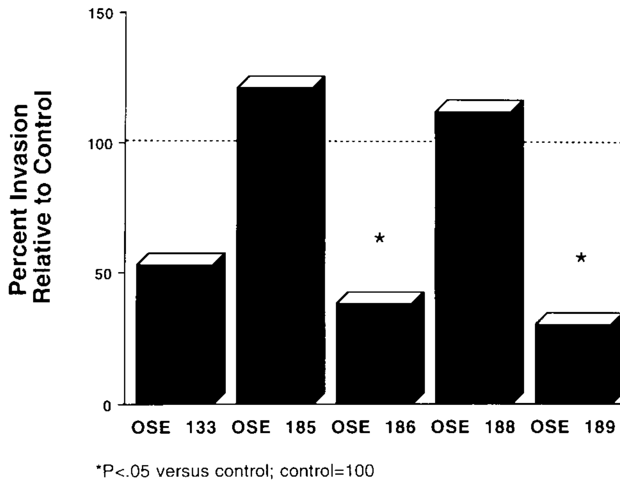

FIG. 2. Effect of TGF-β on NOSE invasion. Cells were cultured in the presence or absence of TGF-β (10 ng/ml) as indicated and invasion was evaluated as described in Fig. 1. Invasion of untreated controls is designated as 100%. *$P < 0.05$.

invasive than the NOSE cell cultures OSE 185, 186, 188, and 189. In control motility assays, normal ovarian epithelial cells were highly motile (data not shown).

In the presence of TGF-β, a significant decrease in invasion (50–80%) was observed in two of five NOSE cell cultures (OSE 186 and 189, Fig. 2), and no significant effect was noted in three (OSE 133, 185, and 188). In sharp contrast to the effect observed with NOSE, TGF-β significantly increased invasion in four of five ovarian cancer cell lines (Fig. 3: OVCA 420, 429, and 433 and DOV-13) and in one of two primary ovarian cancer cell cultures (OVCA-10). Overall, a 2- to 20-fold increase in invasive activity was induced by TGF-β. The invasion enhancing effects of TGF-β were identical, regardless of whether ovarian cancer cells were incubated in invasion assays with TGF-β or pretreated with TGF-β for 24 h prior to placement in invasion chambers.

To determine the mechanism by which TGF-β enhanced invasion in the ovarian cancer cell lines, experiments were performed to study the effect of this peptide growth factor on adhesion, motility, and protease production. TGF-β had no significant effect on adhesion in any of the cancer cell lines except DOV-13, in which adhesion was increased by 25%, $P < 0.05$ (Fig. 4). TGF-β had no significant effect on motility in any of the cancer cell lines (Fig. 5). The effect of TGF-β on plasminogen activator varied between cell lines (Table 1), with increased (OVCA 432), decreased (OVCA 433), or unaltered (OVCA 420, OVCA 429, DOV 13) activity observed. Evaluation of matrix metalloproteinase expression expression by gelatin zymography indicated an increase in expression of a 72-kDa MMP that comigrated with MMP-2 in DOV 13 cells (Fig. 6) as well as in OVCA 420 and OVCA 433 cells (not shown). A dose-dependent stimulation in MMP-2 expression was observed with increasing concentrations of TGF-B (Fig. 6A). Increased expression correlated with enhanced collagenase activity, as evidenced by degradation of type IV collagen by conditioned medium derived from TGF-β-stimulated cells (Fig. 6B). Type IV collagen cleavage was MMP-

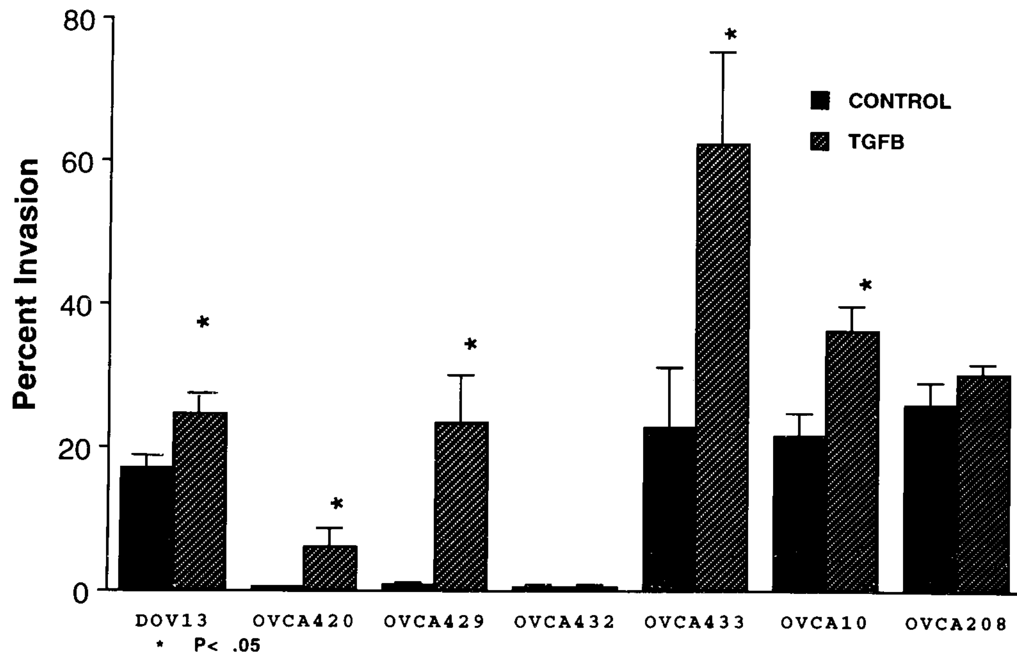

FIG. 3. Effect of TGF-β on ovarian cancer cell invasion. Cells (3.5×10^4) were seeded onto Matrigel-coated porous filters in the presence or absence of TGF-β (10 ng/ml) as indicated. Following incubation for 12 h, filters were stained and invading cells were quantified using computer-assisted image analysis ($P < 0.05$ relative to untreated control).

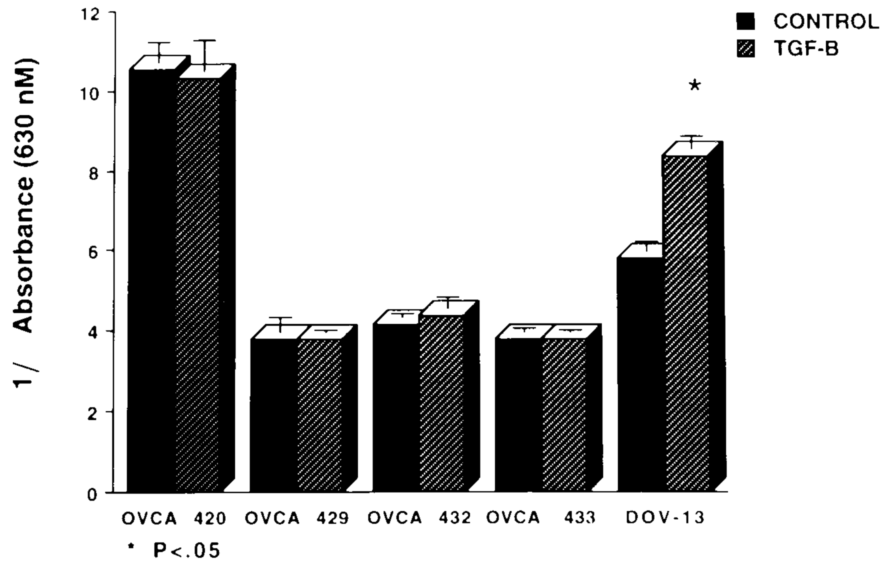

FIG. 4. Effect of TGF-β on adhesion of ovarian cancer cells. Cells (2.5×10^4) were plated in 96-well plates in the presence or absence of TGF-β (10 ng/ml) as indicated and incubated for 2 h. After being washed, adherent cells were stained with Alamar blue and adherent cells detected by monitoring absorbance at 630 nm (*$P < .05$ relative to untreated controls).

dependent, as coincubation with the zinc-specific chelator *o*-phenanthrolene abrogated collagenase activity (Fig. 6B, lane 4). This result was confirmed by quantitation of MMP-2 activity in DOV-13 conditioned medium by HPLC [6, 16], demonstrating a 3.5-fold increase in synthetic substrate cleavage activity in response to TGF-β (Fig. 6C). Further, the invasion enhancing effect of TGF-β on DOV-13 was completely neutralized by the matrix metalloproteinase inhibitor GI 129471, suggesting that MMP-dependent proteolysis contributes to the invasive behavior of ovarian cancer cells (Fig. 7). In control experiments, addition of the plasminogen activator inhibitor PAI-1 failed to neutralize the invasion-enhancing effect of TGF-β on any of the ovarian cancer cell lines (not shown). These data suggest that TGF-β enhances invasion of ovarian cancer cells via a mechanism involving induction of MMP activity.

DISCUSSION

A more detailed understanding of the molecular events that regulate the metastatic process in epithelial ovarian cancer is

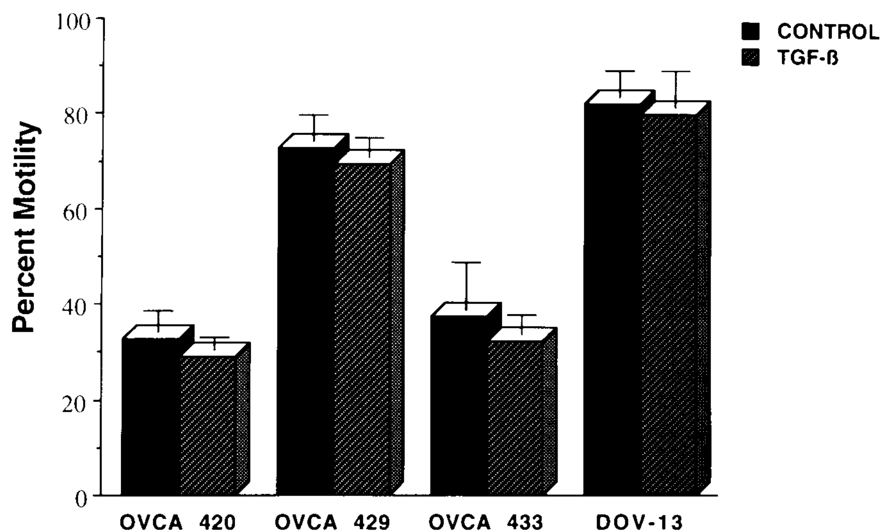

FIG. 5. Effect of TGF-β on motility of the ovarian cancer cell lines. Cells (3.5×10^4) were seeded onto uncoated porous filters in the presence or absence of TGF-β (10 ng/ml) as indicated. Following incubation for 4 h, filters were stained and migrating cells were quantified using computer-assisted image analysis ($P < 0.05$ relative to untreated control).

TABLE 1
Effect of TGF-β on Plasminogen Activator Activity

Cell line	TGF-β (10 ng/ml)	Plasminogen activator activity (mol plasmin/min/cell) × 10^{13}
OVCA420	−	2.8 ± 0.31
OVCA420	+	3.0 ± 0.02
OVCA429	−	0.5 ± 0.1
OVCA429	+	0.45 ± 0.08
OVCA432	−	0.45 ± 0.1
OVCA432	+	1.34 ± 0.12*
OVCA433	−	4.35 ± 0.45
OVCA433	+	2.56 ± 0.53*
DOV13	−	4.50 ± 0.65
DOV13	+	3.82 ± 0.34

Note. Cells were cultured as described in the presence or absence of TGF-β (10 ng/ml) as indicated and plasminogen activator activity was measured using a coupled colorimetric assay. Results are expressed as moles of plasmin generated per minute per cell.

* $P < 0.05$.

currently emerging. For other tumor types, the successful establishment of metastatic colonies requires that tumor cells escape normal growth regulatory controls, acquire the ability to invade and spread throughout host tissues, survive the rigors of the circulation, attach and thrive in tissues at distant sites, and develop a blood circulation to supply essential nutrients. Each one of these steps involves a complex interaction between tumor cells and host tissues and can be further regulated by components of the extracellular matrix, as well as by cytokines and growth factors released by both tumor cells and host cells. In contrast to other tumors, ovarian cancers may represent a unique model for metastasis, in that ovarian cancer cell dissemination throughout the abdominal cavity may occur preferentially using detachment, attachment, and localized invasion steps, without the requirement for hematogenous or lymphatic dissemination or angiogenesis. Factors which promote localized attachment and detachment, such as adhesion and proteolysis, may therefore be more important determinants of ovarian cancer metastasis than is the case for other tumors.

The search for potent regulators of ovarian cancer metastasis led us to evaluate the effect of TGF-β on the metastatic phenotype in ovarian cancer cells. Previously, we have shown that the normal ovarian epithelium is markedly susceptible to the growth inhibitory effects of TGF-β [17]. In addition, we reported that TGF-β had potent growth inhibitory effects on 19 of 20 primary ovarian cancer cell cultures derived from the malignant ascites of women with ovarian cancer [14]. Given that TGF-β induces a number of cellular effects pertinent to the metastatic process, including expression of adhesion molecules and proteinases, and the strong susceptibility of ovarian epithelial cells to the effects of TGF-β, we sought to determine whether TGF-β could regulate the metastatic process in ovarian carcinoma.

The results of our study suggest that TGF-β enhances the invasiveness of ovarian cancer cells and that this may be at least partly mediated by induction of MMP activity. This hypothesis is supported by the observation that TGF-β increased the MMP expression in three of five ovarian cancer cell lines shown to undergo enhanced invasiveness in response to TGF-β. In addition, we found that the enhancing effect of TGF-β on invasion of DOV-13 cells was neutralized by a broad-spectrum MMP inhibitor. Although TGF-β also increased u-PA activity in OVCA 432, this was not associated with increased invasiveness. We did not note any effect of TGF-β on ovarian cancer cell motility and found that TGF-β increased adhesion only in the cancer cell line DOV-13.

Previously, we have reported that ovarian cancers secrete TGF-β [17]. In addition, others have reported that primary and recurrent ovarian carcinomas have significant overexpression of TGF-β mRNA transcripts relative to normal ovarian tissue [18]. The finding that ovarian cancer cell invasion is enhanced

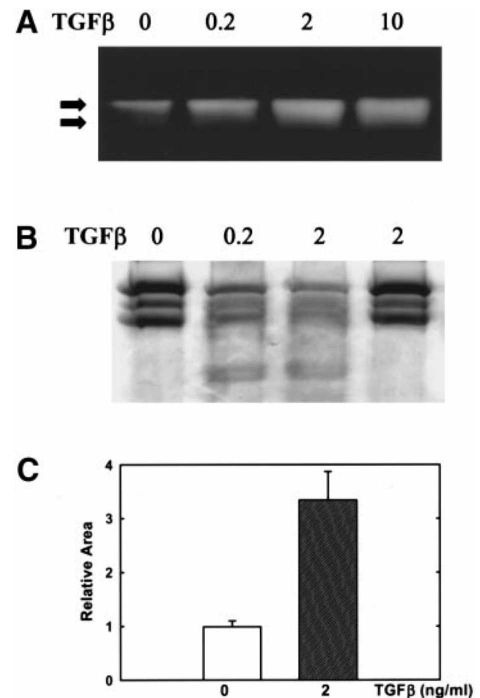

FIG. 6. Effect of TGF-β on MMP expression and activity. (A) Dose-dependence of TGF-β MMP induction. DOV13 cells were treated with increasing concentrations of TGF-β as indicated, and conditioned medium were evaluated for MMP expression by gelatin zymography. Arrows indicate the migration position of pro- and active MMP-2. (B) TGF-β induces type IV collagenase activity. DOV13 cells were cultured in the absence or presence of TGF-β as indicated and conditioned medium were incubated with type IV collagen (20 μg) at 25°C for 18 h. Collagen IV degradation was monitored by electrophoretic analysis on 7.5% polyacrylamide gels. Control experiments contained the MMP inhibitor *o*-phenanthrolene (lane 4). (C) Quantitation of MMP-2 activity in DOV13 conditioned media. DOV13 cells were treated with TGF-β (10 ng/ml) as indicated, and conditioned medium were incubated with the synthetic MMP substrate Dnp-PLGLWAR-NH2 (20 μM) for 18 h. Substrate cleavage was quantified by reverse-phase HPLC and results are quantified as the relative area of product/substrate peaks.

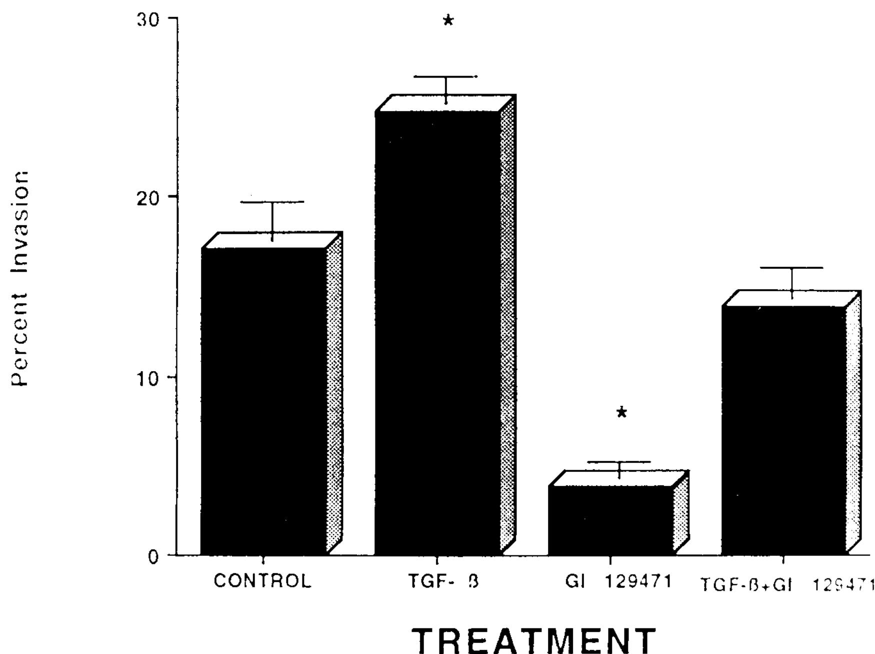

FIG. 7. TGF-β-induced invasion of DOV13 cells is MMP dependent. DOV13 cells were treated in the presence or absence of TGF-β or the broad spectrum MMP inhibitor GI129471 (100 nM) and invasion was analyzed as described in Fig. 4 (* $P < 0.05$ relative to control).

by TGF-β raises the possibility that ovarian cancers may enhance their own invasiveness via an autocrine loop, through release of TGF-β followed by increased proteolysis in response to TGF-β. In this fashion, ovarian cancer cells may direct localized proteolysis, facilitating both localized invasion in the peritoneal cavity and detachment from the primary tumor or metastatic deposits.

The findings from this study suggest that normal ovarian epithelial cells are capable of penetrating extracellular matrix. This is not surprising, given the dynamic nature of the ovarian epithelium, which regularly undergoes repair associated with ovulation. Ovulation, with its associated disruption and subsequent repair of the ovarian surface epithelium, can be likened to the tissue remodeling, turnover, and repair that occur with wound healing. During this process, normal epithelial cells must attach to basement membrane, migrate, and degrade matrix. It is interesting to speculate that migration and invasion of ovarian epithelial cells associated with wound healing may lead to entrapment of some cells within the stroma and to the development of epithelial inclusion cysts commonly seen in the ovary. It is likely, however, that the ovarian epithelium is restrained from unbridled proliferation and invasion by normal growth regulatory controls. In this regard, it is possible that TGF-β may normally play the dual role of inhibiting both proliferation and invasion of normal ovarian epithelial cells and that disregulation of TGF-β—mediated pathways may be one of the mechanisms associated with malignant transformation of the ovarian epithelium.

A common property of malignant cells is an aberrant response to normal growth regulatory controls. Similar to our findings in ovarian cancer, TGF-β has been shown to enhance the invasive phenotype in other cell types such as mammary adenocarcinoma [19–21], pulmonary carcinoma [22], choriocarcinoma [23], glioma [24], hepatoma [25], colon carcinoma [26, 27], pancreatic cancer [28, 29], prostate cancer [21, 30, 31], and melanoma [32]. The enhancing effect of TGF-β on invasion has been primarily related to induction of tumor cell protease secretion, although TGF-β has been shown to enhance both adhesion and motility both in glioma cells and in the A549 lung cancer cell line [22, 24]. It is important to note, however, that TGF-β does not uniformly enhance invasion in all malignant cells. TGF-β has been shown to inhibit invasion in both fibrosarcoma [33] and renal carcinoma [34] cells. In these cell types, TGF-β effects decreased proteolysis either by decreasing cellular release of proteinases or by increasing the release of protease inhibitors. Thus, the ultimate effect of TGF-β on tumor cell invasion appears to be tissue specific as well as dependent upon the net effect of TGF-β on the balance of proteinases and their respective inhibitors.

One prior study by Yamada *et al.* did not find that MMP-2 production was regulated by TGF-β in ovarian cancer cells [35]. Interestingly, Yamada *et al.* also noted that most primary ovarian cancer cells cultures established in their laboratory were resistant to the growth inhibitory effects of TGF-β, which is contrary to the experience that we have had in our laboratory. Yamada's study differed from ours in that their ovarian cancer cell cultures were derived from solid ovarian tumors, whereas all of the ovarian cancer cell lines that we studied have been derived from ascites. It is possible that ovarian cancer

cells present in ascites have a different metastatic phenotype than those in solid tumor explants.

The available experimental evidence suggests that TGF-β may play opposite roles with respect to the process of carcinogenesis and metastasis: early on, TGF-β functions as a potent tumor suppressor, whereas in advanced tumors, TGF-β enhances tumor growth and metastatic potential [36–41]. The switch from a tumor suppressor to a tumor enhancer is probably associated with the acquisition of aberrations in the TGF-β signaling pathway, ranging from defects in TGF-β receptors to mutations in the genes that encode for the Smad proteins that transfer the TGF-β signal from the cell membrane to the nucleus. In the absence of growth inhibitory effects of TGF-β, the stimulatory effects of TGF-β on matrix deposition, proteolysis, and angiogenesis become prominent, thereby conferring a growth advantage to tumor cells.

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