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TGFβ1 stimulates the secretion of matrix metalloproteinase 2 (MMP2) and the invasive behavior in human ovarian cancer cells, which is suppressed by MMP inhibitor BB3103

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Abstract

The present study investigated the modulatory role of transforming growth factor beta 1 (TGFβ1) on the secretion of matrix metalloproteinases (MMPs) and tested whether the altered secretion of MMPs could directly affect the invasive behavior of ovarian cancer cells. To this aim, human ovarian cancer SKOV3 cells were treated once with vehicle or various concentrations of TGFβ1 for 24 h. Gelatinase activities in conditioned media were analyzed by zymography and densitometry. TGFβ1 dose-dependently stimulated the secretion of a 68-kDa gelatinase, which was characterized as an MMP because its activity was inhibited by a metalloproteinase inhibitor 1,10-phenanthroline, and by a synthetic MMP inhibitor BB3103. In addition, we used aminophenylmercuric acetate (APMA) to activate latent gelatinases. APMA time-dependently decreased the activity of 68-kDa gelatinase, and increased the activities of 64- and 62-kDa gelatinolytic bands. The 68-kDa gelatinase was further characterized as MMP2 (gelatinase A) by immunoblotting analysis. We then tested TGFβ1 effect on the invasive potential of SKOV3 cells as assessed by the migration ability through reconstituted basement membrane, and further investigated whether TGFβ1 may act through modulating the MMP activity to affect ovarian cancer cell invasion. The results show that TGFβ1 stimulated the invasive behavior of SKOV3 cells, and that MMP inhibitor BB3103 abrogated this effect of TGFβ1. In conclusion, this study indicates that TGFβ1 may act partly through stimulating the secretion of MMP in promoting the invasive behavior of human ovarian cancer cells. Furthermore, this work supports the idea that specific MMP inhibitors of the hydroxamate class could be therapeutically useful in controlling cancer cell invasion/metastasis.

Abbreviations: APMA – aminophenylmercuric acetate; ECM – extracellular matrix; IgG – immunoglobulin G; MMP – matrix metalloproteinase; TGFβ1 – transforming growth factor beta 1

Introduction

Ovarian cancers of majority are adenocarcinomas arising from the ovarian epithelium, are the most common fatal gynecological malignancy [1, 2]. It has a high incidence of metastasis that generally remains localized within the peritoneal cavity [3]. The metastatic capability of cancer cells is considered to be the main cause for cancer death [4]. Therefore, understanding the mechanism of the metastatic process is of great importance in developing strategies for cancer prognosis and therapy. Metastasis is a complex multi-step process that involves changes in the interactions between the invasive cells and their microenvironment, the extracellular matrix (ECM). The role of matrix metalloproteinases (MMPs) in tumor cell-mediated ECM proteolysis is well established [5]. MMPs are zinc-dependent metalloproteinases which participate in the degradation of collagens and other extracellular matrix macromolecules [6]. Expression of MMPs, such as MMP-2 (gelatinase A) and MMP-9 (gelatinase B), has been linked to enhanced tumor invasion/metastasis in several *in vitro* and *in vivo* model systems [7–12]. Also, expression of MMP2 has been detected in ovarian tumors and carcinoma cell lines [9, 13–16]. Furthermore, down-regulation of MMPs or elevated levels of tissue inhibitors of metalloproteinases markedly reduces the cancer invasion/metastasis in human melanoma and fibrosarcoma [17–21].

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Studies have shown that growth factors can modulate MMP expression [22–25]. Among the intraovarian regulators of ovarian function, transforming growth factor β (TGFβ) is the most unique one for it inhibits proliferation of many cell types, and modulates the interaction between cells and the surrounding matrix [26, 27]. In normal cells, TGFβ generally enhances adhesion through increased matrix production and decreased ECM proteolysis [28]. However, TGFβ plays both positive and negative regulatory roles in tumorigenesis [29]. At early stages, TGFβ may act as a tumor suppressor when cells are still responsive to its antimitogenic effect. During malignant progression, TGFβ may function as a tumor promoter when cells become resistant to its growth inhibition by providing an appropriate microenvironment for tumor growth and metastasis. TGFβ thus receives considerable attention for its role in the progression of cancer formation. Enhanced expression of TGFβ is associated with various tumor types including breast, prostate, pancreas, liver, kidney, brain and some leukemias [30–36]. Also, several ovarian carcinomas overexpress TGFβ1 [37, 38]. TGFβ is further known to promote invasion/metastasis in carcinoma cells including gastric [39] and breast cancers [26, 40, 41].

The conventional therapy for ovarian cancers includes surgery and chemotherapy using cytotoxic drugs [42]. More recently, a new therapeutic strategy, the combined treatment with cytotoxic drugs and synthetic MMP inhibitors, has been employed in clinical trials. The first MMP inhibitor used clinically is batimastat (also named BB-94) [42, 43]. A recent study has shown that batimastat inhibits metastasis of a rat mammary carcinoma [44]. Moreover, batimastat alone or in combination with a cytotoxic drug reduces tumor growth and increases the survival rate of mice bearing human ovarian or pancreatic cancer xenografts [45–47]. Among the hydroxamate derivatives of MMP inhibitors, batimastat has a disadvantage of low solubility in aqueous solution; to overcome this limitation more soluble derivatives have been developed, including BB-3103 [48].

The effects of TGFβ1 on ECM proteolysis and cell invasion, and their inter-relationship in ovarian carcinoma remain unclear. Therefore, the objectives of this study were to investigate the effects of TGFβ1 on MMP production and invasive behavior in human ovarian epithelial cancer cells using the SKOV3 cell line. To further investigate whether TGFβ1 may act through modulating the MMP activity to affect ovarian cancer cell invasion, a synthetic MMP inhibitor BB-3103 was used.

Materials and methods

Materials

The SKOV3 human ovarian carcinoma cell line was obtained from the American Type Culture Collection (Rockville, Maryland). Human TGFβ1 was purchased from Upstate Biotechnology Co. (Lake Placid, New York). Engelbreth-Holm-Swarm sarcoma tumor extract was prepared as previously described [49]. BB-3103 was provided by British Biotech Pharmaceuticals Ltd (Oxford, UK). Most chemicals were purchased from Sigma Chemical Co (St. Louis, Missouri); sources for others are indicated individually below.

Cell culture and treatment

SKOV3 cells were plated in 24-well plates (Becton Dickinson Labware, Franklin Lakes, New Jersey) at approximately 5×10^5 viable cells per well in 800 μl of DMEM medium containing 5% fetal bovine serum, and incubated at 37 °C, 5% CO_2-95% air. Cells were allowed to attach for 24 h, and then incubated in serum-free medium (DMEM containing 0.1% lactalbumin hydrolysate) for 16 to 18 h before the beginning of treatment. Cells were treated with various concentrations of TGFβ1 once alone in 800 μl of serum-free medium. After 24-h incubation, conditioned media were collected, cleared by centrifugation, and stored at -70 °C until the performance of gelatin zymography and immunoblotting analysis. The cell number was determined using hemacytometer.

Gelatin zymography analysis

Gelatin zymography was performed as previously described [50]. In brief, medium samples were electrophoresed on a 10% SDS-polyacrylamide gel containing 0.1% gelatin from porcine skin. There were no significant differences in the mean cell number among treatment groups under our culture condition as determined using hemacytometer. The volume of each medium sample analyzed was the same. Electrophoresis was run in 25 mM Tris-HCl, pH 8.0 containing 192 mM glycine and 0.1% SDS at 15 mA/gel during stacking and at 20 mA/gel during separation. After electrophoresis, gels were washed twice in 2.5% Triton X-100 for 40 min each, and in reaction buffer (50 mM Tris-HCl, pH 8.0 containing 5 mM $CaCl_2$, 0.02% NaN_3) for 15 min. Gels were incubated in reaction buffer at 37 °C for approximately 40 h, then stained with 0.25% Coomassie brilliant blue R-250 in 10% acetic acid–30% ethanol, and destained in the same solution without dye. Quantification of gelatinases was achieved by computerized image analysis using a two-dimensional laser scanning densitometer (Molecular Dynamics, Sunnyvale, California). The relative quantitative analysis was performed on the same gelatinase activity band of different treatment groups in reference to the value of control group that is defined as 100%. There is a linear relationship between the loading volume of a sample and the density of the same gelatinase band under our study conditions.

Characterization of gelatinases

To further characterize the gelatinases secreted by SKOV3 cells, gels after electrophoresis and Triton-wash were incubated in reaction buffer containing a proteinase inhibitor. The inhibitors tested included 5 mM 1,10-phenanthroline (a general metalloproteinase inhibitor), 1 mM phenylmethylsulfonyl fluoride (a serine/cysteine proteinase inhibitor) [51]

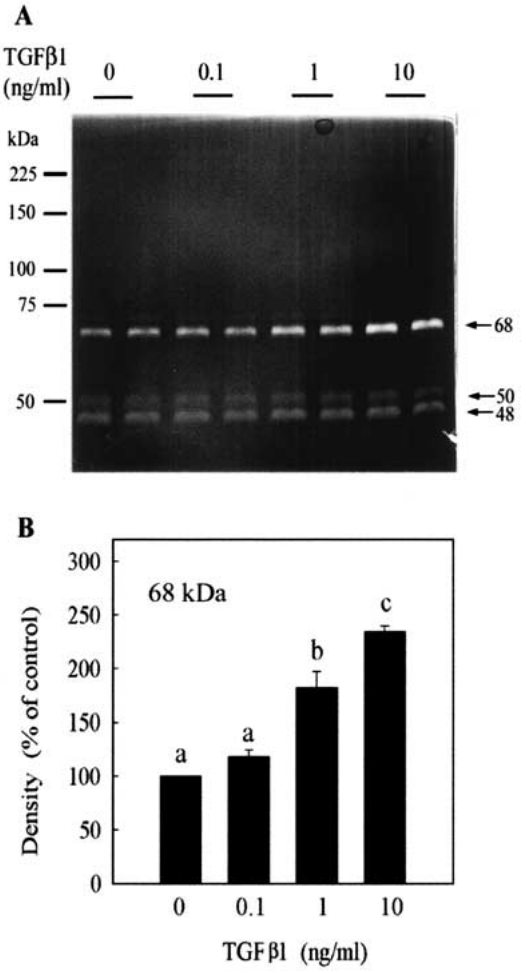

Figure 1. Effect of TGFβ1 on the secretion of gelatinase from cultured SKOV3 ovarian cancer cells. Cells were treated with various concentrations of TGFβ1 (0.1 to 10 ng/ml) for 24 h. Conditioned media were collected and analyzed by gelatin zymography. (A) A representative gelatin zymogram of conditioned media. Duplicate samples for each treatment were loaded on the same gel for analysis. (B) Quantitative analysis of 68-kDa gelatinase was performed using scanning densitometry. Each point represents mean ($\pm$SE) of mean percentage density of duplicate samples from three separate experiments. Percentage of density was calculated using the mean density of control value as 100%. Data were analyzed using analysis of variance and multiple range test. Different lower-case letters (a, b, c) indicate significant differences among groups ($P < 0.05$).

and various concentrations of BB-3103 (a specific inhibitor of matrix metalloproteinases). In addition, latent gelatinases were activated by incubation of the medium sample with 1.5 mM aminophenylmercuric acetate (APMA) for 15, 30 and 60 min at 37 °C prior to gelatin zymography analysis [52].

Immunoblotting analysis of gelatinases

Each conditioned medium of 1.5 ml was concentrated and desalted using microconcentrator (mol wt cut-off, 10 kDa; Millipore Corporation, Bedford, Massachusetts), lyophilized and resuspended in Laemmli SDS sample buffer. Samples were then electrophoresed on a 12% SDS polyacrylamide gel and electrotransferred to a PVDF membrane (Micron Separations Inc, Westborough, Massachusetts) in Towbin buffer (25 mM Tris-HCl, pH 8.3 containing 192 mM glycine, 1.3 mM SDS and 20% methanol) using a Hoefer semidry transfer unit (San Francisco, California). The membrane blot was blocked for 1 h in Tris-buffered saline containing 0.05% Tween 20 (TBST) and 5% nonfat dry milk. The membrane was then incubated with mouse monoclonal antibody against human MMP2 (Oncologix Inc, Gaithersburg, Maryland) or a control normal mouse immunoglobulin G (IgG) for 1 h. The membrane was washed three times with TBST for 10 min each, and then incubated with peroxidase-conjugated sheep anti-mouse IgG (Amersham Co, Little Chalfont, Buckinghamshire, UK) for 40 min. The membrane was washed three times with TBST, and subsequently subjected to enhanced chemiluminescence detection system (Amersham ECL Plus) conducted according to the manufacturer's protocol.

Cell invasion assay

The invasive activity of the tumor cells was determined using an *in vitro* assay as previously described [53] with slight modifications. Briefly, 8-μm pore size polycarbonate filters (Millipore, Bedford, Massachusetts) were coated with approximately 12.5 μg protein of reconstituted basement membrane (Engelbreth-Holm-Swarm sarcoma tumor extract). Approximately 5×10^4 cells were inoculated into each inner well, and incubated for 1 h at 37 °C, 5% CO_2-95% air before the beginning of treatment. Cells were then given the following treatments: vehicle control, 10 ng/ml TGF-β1, or TGF-β1 in combination with various concentrations of the MMP inhibitor BB-3103. The final volumes of the inner and outer wells were 400 μl and 600 μl, respectively. After an incubation period of 72 h, the filters were fixed in 3% glutaraldehyde in PBS for 30 min, permeablized with 0.1% Triton X-100 for 5 min, and then stained with hematoxylin. Cells remaining on the inner surface of the filter were removed with a cotton swab. Invasive cells adhering to the under surface of the filter were counted using light microscope.

Statistics

Data were analyzed using analysis of variance and Duncan's multiple range test.

Results

Effect of TGFβ1 on gelatinase secretion in SKOV3 ovarian cancer cells

Gelatinase activity in conditioned media of SKOV3 cells treated with various concentrations of TGFβ1 for 24 h was determined using gelatin zymography and densitometric analysis. A representative gelatin zymogram is shown in Figure 1A, which indicates that SKOV3 cells secrete three major gelatinases with estimated molecular sizes of 68, 50 and 48 kDa. TGFβ1 (0.1 to 10 ng/ml) dose-dependently increased the secreted activity of 68-kDa gelatinase (Figure 1B). Densitometric analysis showed that stimulation

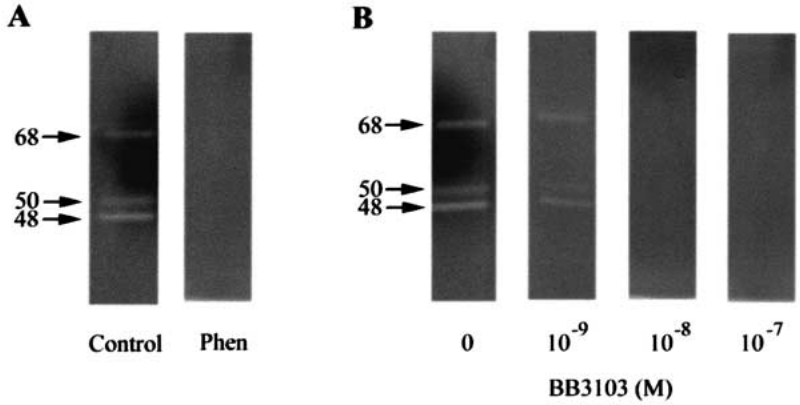

Figure 2. Effect of proteinase inhibitors on the activity of gelatinases secreted from TGFβ1-treated SKOV3 cell cultures. Gelatinase activity was analyzed by gelatin zymography. (A) Representative zymograms of a control gel and a gel treated with 5 mM 1,10-phenanthroline (phen), a metalloproteinase inhibitor. Treatment with 1 mM phenylmethylsulfonyl fluoride, a serine/cysteine proteinase inhibitor, did not inhibit gelatinase activities (data not shown). (B) Representative zymograms of gels incubated with various concentrations of BB-3103, a specific inhibitor of matrix metalloproteinases.

by TGFβ1 at the concentration of 10 ng/ml caused a 2.3-fold increase in the 68-kDa gelatinolytic band as compared to control (Figure 1B). Also, TGFβ1 did not appear to influence the cell number under our culture condition as determined by hemacytometer (data not shown).

Characterization of gelatinases secreted by SKOV3 cells

All three gelatinases of 68-, 50- and 48-kDa were characterized as metalloproteinases, but not serine/cysteine proteinases, because their activities were inhibited by 1,10-phenanthroline but not by phenylmethylsulfonyl fluoride (Figure 2A). In order to determine whether these proteinases were MMPs, a synthetic MMP inhibitor, BB-3103, was used. BB-3103 dose-dependently (10^{-9} to 10^{-7} M) inhibited the activity of all three major gelatinases (Figure 2B). In addition, we used aminophenylmercuric acetate (APMA) to activate latent gelatinases. When SKOV3 conditioned medium was treated with 1.5 mM APMA, there were time-dependent decreases in the activities of 68-, 50- and 48-kDa gelatinases and increases in the activities of 64- and 62-kDa as well as 43-, 40- and 35-kDa gelatinolytic bands (Figure 3). Finally, this study demonstrates that the 68-kDa gelatinase, whose secretion from SKOV3 cells was stimulated by TGFβ1, was an MMP2-like proteinase as determined by immunoblotting analysis using a monoclonal antibody against human MMP2 (Figure 4).

Effect of TGFβ1 on the invasive behavior of SKOV3 ovarian cancer cells

An *in vitro* assay was used to determine the effect of TGFβ1 on the invasive behavior of SKOV3 cells as previously described [53]. TGFβ1 at 10 ng/ml promoted an approximately 1.8 fold increase in the number of invasive SKOV3 cells compared with control (Figure 5). To further understand whether MMPs may act as potential mediators in TGFβ1-stimulated SKOV3 cell invasion, a synthetic MMP inhibitor BB-3103 was used. BB-3103 at concentrations of 10 to 50 μM completely suppressed the stimulatory effect of TGFβ1 on SKOV3 cell invasion (Figure 5).

Discussion

This study demonstrates for the first time that TGFβ1 stimulates the secretion of 68-kDa gelatinase, identified as MMP2, in human ovarian epithelial cancer cells SKOV3, and that contributes at least partly to TGFβ1 promotion of the invasive behavior of SKOV3 cells. The secretion of a 68-kDa gelatinase was reported earlier in another ovarian epithelial carcinoma cell line DOV13 [9]. Also, MMP2 was detected in ovarian cancer cells, SKOV3 and OV432 by immunocytochemistry [13]. In addition, the expression of MMP2 was more clearly detected in malignant ovarian tumors than in benign ones [13, 14]. Previous studies also showed that TGFβ1 up-regulated 72-kDa and 92-kDa gelatinolytic activities in colon carcinoma [27] and mammary adenocarcinoma [54]. Our finding that TGFβ1 promoted the invasive behavior of ovarian cancer SKOV3 cells is consistent with earlier studies showing that TGFβ1 stimulated invasion/metastasis of carcinoma cells including gastric [39] and breast cancers [26, 40, 41]. The expression of MMPs is positively correlated with the invasion/metastatic potential of tumor cells in several *in vitro* and *in vivo* model systems [7–12]. Such correlation is also clearly seen in our study on ovarian epithelial cancer SKOV3 cells.

The present study further investigated whether TGFβ1 may act through modulating the MMP activity to affect ovarian cancer cell invasion. To target that, a synthetic MMP inhibitor BB-3103 was used. The current available synthetic MMP inhibitors can be categorized into two classes, peptidyl hydroxamate and prodomain peptides of MMP zymogens. The mechanisms for inhibition of MMPs are different between the two classes of inhibitors. The former acts through competition with the zinc binding domain of MMPs, which is essential for catalytic activity, while the latter acts through maintaining MMPs as latent proenzyme forms [42, 55]. This study shows that BB3103, an MMP inhibitor of the hydroxamate class, dose-dependently inhibits the activities of major gelatinases, including an MMP2-like proteinase, secreted in ovarian cancer SKOV3 cells. Furthermore, we have made the novel observation that the promoting effect

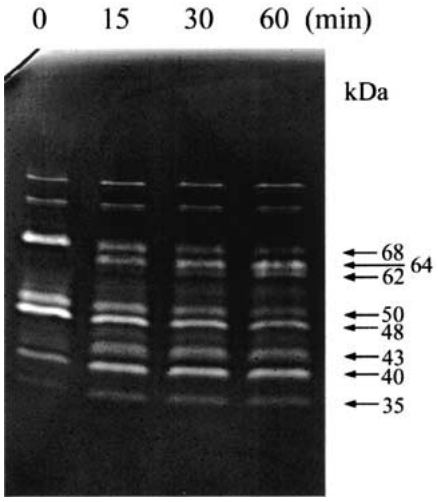

Figure 3. Effect of aminophenylmercuric acetate (APMA) on the activation of gelatinases secreted in SKOV3 cell culture. Conditioned medium was concentrated approximately five folds using a microconcentrator (mol wt cut-off, 10 kDa) prior to APMA treatment. Conditioned medium was treated with 1.5 mM APMA, and then analyzed by gelatin zymography. Aliquots (20 μl) of conditioned medium were removed before and at 15, 30 and 60 min after APMA treatment to activate latent gelatinases.

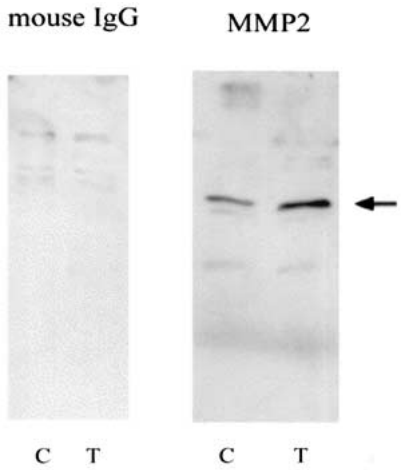

Figure 4. Immunoblotting analysis of the TGFβ1-stimulated MMP2-like proteinase secreted by SKOV3 cells. Cells were treated with vehicle control (C) or 10 ng/ml TGFβ1 (T) for 24 h. Conditioned media of 1.5 ml each were concentrated prior to immunoblotting analysis. Left panel, control normal mouse IgG; right panel, monoclonal antibody against human MMP2.

of TGFβ1 on the invasive capacity of SKOV3 cancer cells is suppressed by concomitant treatment with BB3103. Also, as noted earlier, TGFβ1 stimulates the secreted activity of a BB3103-inhibitable MMP2-like proteinase in SKOV3 cells. Thus, the results of the current study suggest that TGFβ1 may act at least partly through up-regulation of MMP release to facilitate ovarian cancer cell invasion.

Metastasis is the most life-threatening event for cancer patients [4]. To escape the primary tumor site and colonize a new body site, cancer cells acquire the capability of breaking through the restriction of their physical coupling to the extracellular microenvironment [56]. It has been reported that up-regulation of ECM-degrading proteinases is a common process in several types of tumors linking to their invasive capabilities [7–12]. Thus, development of a therapeutic strategy by blocking the up-regulated matrix metalloproteinases may prove to be an effective way to prevent metastasis of cancer cells. BB-3103 belongs to the hydroxamate class of MMP inhibitors including the well known batimastat [48, 42]. Batimastat has been shown to inhibit the growth of cancer xenografts and increase the survival incidence of nude mice bearing human ovarian and pancreatic cancers [45–47]. Further, the therapeutic effect of combined treatment with batimastat and cytotoxic drugs was even better than treatment with either drug alone [46, 47]. Batimastat is currently under phase III clinical trial; the treatment outcome will be expected in the near future. Also, a specific prodomain peptide was shown to inhibit gelatinase activity and invasive behavior of fibrosarcoma and melanoma cell lines [55]. This study also supports the idea that synthetic MMP inhibitors of the hydroxamate class could prove therapeutically useful in controlling cancer cell invasion/metastasis, the main cause of cancer deaths.

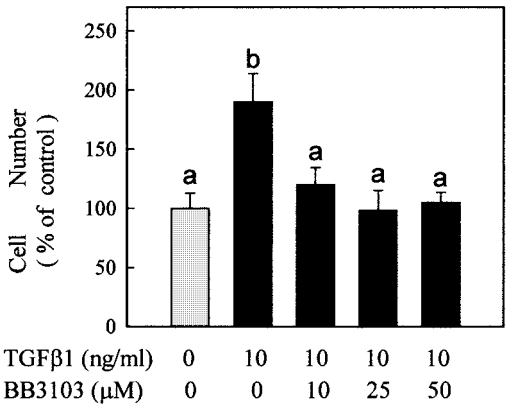

Figure 5. Effect of TGFβ1 on the invasive behavior of SKOV3 ovarian cancer cells. Cells were incubated with vehicle control, 10 ng/ml TGFβ1 alone or in combination with various concentrations of BB-3103 for 72 h. At the end of culture, cells were fixed, stained and counted under a light microscope for cells which had penetrated through the reconstituted basement membrane. Each bar represents mean ($\pm$SE) percentage of cell number using control value as 100%. $n = 13$–17 per treatment group. Data were analyzed using analysis of variance and multiple range test. Different lower-case letters (a, b) indicate significant differences among groups ($P < 0.05$).

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References

1. Richardson GS, Scully RE, Nikrui N et al. Common epithelial cancer of the ovary. N Engl J Med 1985; 321: 474–83.
2. Silverberg E, Boring CC, Squires TS. Cancer statistics. CA- Cancer J Clin 1990; 40: 9–19.

3. Berek JS, Martinez-Maza O. Molecular and biologic factors in the pathogenesis of ovarian cancer. J Reprod Med 1994; 39: 241–8.
4. Sporn MB. The war on cancer. Lancet 1996; 347: 1377–81.
5. Stetler-Stevenson WG, Liotta LA, Kleiner DE Jr. Extracellular matrix 6: Role of matrix metalloproteinases in tumor invasion and metastasis. FASEB J 1993; 7: 1434–41.
6. Woessner JF Jr. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. FASEB J 1991; 5: 2145–54.
7. Bonfil RD, Reddel RR, Ura H et al. Invasive and metastatic potenial of a v-Ha-ras-transformed human bronchial epithelial cell line. J Natl Cancer Inst 1989; 81: 587–94.
8. Mignatti P, Rifkin DB. Biology and biochemistry of proteinases in tumor invasion. Physiol Rev 1993; 73: 161–95.
9. Moser T L, Young TN, Rodriguez GC et al. Secretion of extracellular matrix-degrading proteinases is increased in epithelial ovarian carcinoma. Int J Cancer 1994; 56: 552–9.
10. Powell WC, Knox JD, Navre M et al. Expression of the metalloproteinase matrilysin in DU-145 cells increases their invasive potential in severe combined immunodeficient mice. Cancer Res 1993; 53: 417–22.
11. Sreenath T, Martrisian LM, Stetler-Stevenson W et al. Expression of matrix metalloproteinase genes in transformed rat cell lines of high and low metastatic potential. Cancer Res 1992; 52: 4942–7.
12. Thompson EW, Yu M, Bueno J et al. Collagen induced MMP-2 activation in human breast cancer. Breast Cancer Res Treat 1994; 31: 357–70.
13. Afzal S, Lalani EI-N, Foulkes WD et al. Matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-2 expression and synthetic matrix metalloproteinase-2 inhibitor binding in ovarian carcinomas and tumor cell lines. Lab Invest 1996; 74: 406–21.
14. Autio-Harmainen H, Karttunen T, Hurskainen T et al. Expression of 72 kilodalton type IV collagenase (gelatinase A) in benign and malignant ovarian tumors. Lab Invest 1993; 69: 312–21.
15. Campo E, Merino MJ, Tavassoli FA et al. Evaluation of basement membrane components and the 72 kDa type IV collagenase in serous tumors of the ovary. Am J Surg Pathol 1992; 16: 500–7.
16. Naylor MS, Stamp GW H, Davies B et al. Expression and activity of MMPs and their regulators in ovarian cancer. Int J Cancer 1994; 58: 50–6.
17. Albini A, Melchiori A, Santi L et al. Tumor cell invasion inhibited by TIMP-2. J Natl Cancer Inst 1991; 83: 775–9.
18. Hendrix MJ, Wood WR, Seftor EA et al. Retinoic acid inhibition of human melanoma cell invasion through a reconstituted basement membrane and its relation to decreases in the expression of proteolytic enzymes and motility factor receptor. Cancer Res 1990; 50: 4121–30.
19. Khokha R, Zimmer MJ, Graham CH et al. Suppression of invasion by inducible expression of tissue inhibitor of metalloproteinase-1 (TIMP-1) in B16-F10 melanoma cells. J Natl Cancer Inst 1992; 84: 1017–22.
20. Khokha R. Suppression of the tumorigenic and metastatic abilities of murine B16-F10 melanoma cells *in vivo* by the overexpression of the tissue inhibitor of metalloproteinase-1. J Natl Cancer Inst 1994; 86: 299-304.
21. Koop S, Khokha R, Schmidt EE et al. Overexpression of metalloproteinase inhibitor in B16F10 cells does not affect extravasation but reduces tumor growth. Cancer Res 1994; 54: 4791–7.
22. Brown PD, Levy AT, Margulies IM et al. Independent expression and cellular processing of Mr 72,000 type IV collagenase and interstitial collagenase in human tumorigenic cell lines. Cancer Res 1990; 50: 6184–91.
23. Matrisian JM. The matrix-degrading metalloproteinases. BioEssays 1992; 14: 455–62.
24. Ogata Y, Pratta MA, Nagase H et al. Matrix metalloproteinase 9 (92-kDa gelatinase/type IV collagenase) is induced in rabbit articular chondrocytes by cotreatmemt with interleukin 1 beta and a protein kinase C activator. Exp Cell Res 1992; 210: 245–9.
25. Weinberg WC, Brown PD, Stetler-Stevenson WG et al. Growth factors specifically alter hair follicle cell proliferation and collagenolytic activity alone or in combination. Differentiation 1990; 45: 168–78.
26. Gorsch SM, Memoli VA, Stukel TA et al. Immunohistochemical staining for transforming growth factor β1 associates with disease progression in human breast cancer. Cancer Res 1992; 52: 6949–52.
27. Shimizu S, Nishikawa Y, Kuroda K et al. Involvement of transforming growth factor β1 in autocrine enhancement of gelatinase B secretion by murine metastatic colon carcinoma cells. Cancer Res 1996; 56: 3366–70.
28. Massague J. The transforming growth factor-β family. Annu Rev Cell Biol 1990; 6: 597–641.
29. Akhurst RJ, Balmain A. Genetic events and the role of TGFβ in epithelial tumor progression. J Pathol 1999; 187: 82–90.
30. Travers MT, Barrett-Lee PJ, Berger U et al. Growth factor expression in normal, benign, and malignant breast tissue. BMJ 1988; 296: 1621–4.
31. Steiner MS, Barrack ER. Transforming growth factor-β1 overproduction in prostate cancer: effects on growth *in vivo* and *in vitro*. Mol Endocrinol 1992; 6: 15–25.
32. Friess H, Yamanaka Y, Buchler M et al. Enhanced expression of transforming growth factor-β isoforms in pancreatic cancer correlates with decreased survival. Gastroenterology 1993; 105: 1846–56.
33. Ito N, Kawata S, Tamura S et al. Elevated levels of transforming growth factor β messenger RNA and its polypeptide in human hepatocellular carcinoma. Cancer Res 1989; 51: 4080–3.
34. Gomella LG, Sargent ER, Wade TP et al. Expression of transforming growth factor α in normal human adult kidney and enhanced expression of transforming growth factors α and β1 in renal cell carcinoma. Cancer Res 1989; 49: 6972–5.
35. Jennings MT, Maciunas RJ, Carver R et al. TGF-β1 and TGF-β2 are potent growth regulators for low-grade and malignant gliomas *in vitro*: evidence in support of an autocrine hypothesis. Int J Cancer 1991; 49: 129–39.
36. Niitsu Y, Urushizaki Y, Koshida Y et al. Expression of TGF-beta gene in adult T cell leukemia. Blood 1988; 71: 263–6.
37. Bristow RE, Baldwin RL, Yamada SD et al. Altered expression of transforming growth factor-β ligands and receptors in primary and recurrent ovarian carcinoma. Cancer 1999; 85: 58–668.
38. Gordinier ME, Zhang HZ, Patenia R et al. Quantitative analysis of transforming growth factor β1 and 2 in ovarian carcinoma. Clin Cancer Res 1999; 5: 2498–2505.
39. Maehara Y, Kakeji Y, Kabashima A et al. Role of transforming growth factor-beta 1 in invasion and metastasis in gastric carcinoma. J Clin Oncol 1999; 17: 607–14.
40. Albo D, Berger DH, Wang T N et al. Thrombospondin-1 and transforming growth factor-beta1 promote breast tumor cell invasion through up-regulation of the plasminogen/plasmin system. Surgery 1997; 122: 493–9.
41. Walker RA, Dearing SJ, Gallacher B. Relationship of transforming growth factor β1 to extracellular matrix and stromal infiltrates in invasive breast carcinoma. Br J Cancer 1994; 69: 1160–5.
42. Beckett RP, Davidson AH, Drummond AH et al. Recent advances in matrix metalloproteinase inhibitor research. Drug Discov. Today 1996; 1: 16–26.
43. Talbot DC, Brown PD. Experimental and clinical studies on the use of matrix metalloproteinase inhibitors for the treatment of cancer. Eur J Cancer 1996; 32A: 2528–33.
44. Eccles SA, Box GM, Court WJ et al. Control of lymphatic and hematogenous metastasis of a rat mammary carcinoma by the matrix metalloproteinase inhibitor batimastat (BB94). Cancer Res 1996; 56: 2815–22.
45. Davies B, Brown PD, East N et al. Synthetic matrix metalloproteinase inhibitor decreases tumor burden and prolongs survival of mice bearing human ovarian carcinoma xenografts. Cancer Res 1993; 53: 2087–91.
46. Giavazzi R, Garofalo A, Ferri C et al. Batimastat, a synthetic inhibitor of matrix metalloproteinase, potentiates the antitumor activity of cisplatin in ovarian carcinoma xenografts. Clin Cancer Res 1998; 4: 985–92.
47. Haq M, Shafii A, Zervos EE et al. Addition of matrix metalloproteinase inhibition to conventional cytotoxic therapy reduces tumor implantation and prolongs survival in a murine model of human pancreatic cancer. Cancer Res 2000; 60: 3207–11.
48. Ancuta P, Fahmi H, Pons J-F et al. Involvement of the membrane form of tumor necrosis factor-α in lipopolysaccharide-induced priming of mouse peritoneal macrophages for enhanced nitric oxide response to lipopolysaccharide. Immunology 1997; 92: 259–66.
49. Hahm HA, Ip MM. Primary culture of normal rat mammary epithelial cells within a basement membrane matrix, I. Regulation of prolifer-

ation by hormones and growth factors. In Vitro Cell Dev Biol 1990; 26: 791–802.
50. Hwang J-J, Lin S-W, Teng C-H et al. Relaxin modulates the ovulatory process and increases secretion of different gelatinases from granulosa and theca-interstitial cells in rats. Biol Reprod 1996; 55: 1276–83.
51. Hibbs MS, Hasty KA, Seyer JM et al. Biochemical and immunological characterization of the secreted forms of human neutrophil gelatinase. J Biol Chem 1985; 260: 2493–500.
52. Salamonsen LA, Nagase H, Suzuki R et al. Production of matrix metalloproteinase 1 (interstitial collagenase) and matrix metalloproteinase 2 (gelatinase A; 72kDa gelatinase) by ovine endometrial cells *in vitro*: Different regulation and preferential expression by stromal fibroblasts. J Reprod Fertil 1993; 98: 583–9.
53. Albini A, Iwamoto Y, Kleinman K et al. A rapid *in vitro* assay for quantitating the invasive potential of tumor cells. Cancer Res 1987; 47: 3239–45.
54. Welch DR, Fabra A, Nakajima M. Transforming growth factor β stimulates mammary adenocarcinoma cell invasion and metastatic potential. Proc Natl Acad Sci USA 1990; 87: 7678–82.
55. Melchiori A, Albini A, Ray JM et al. Inhibition of tumor cell invasion by a conserved peptide sequence from the matrix metalloproteinase enzyme prosegment. Cancer Res 1992; 52: 2353–6.
56. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100: 57–70.