Abstract. Human peritoneal mesothelial cells (HPMCs) in intact mesothelium have been demonstrated to protect against tumor peritoneal metastasis. We have previously reported that gastric cancer cells can induce peritoneal apoptosis, lead to damage of peritoneum integrity, and therefore promote peritoneal metastasis. In this study, we investigated the effects of TGF-ß1 on tumor-mesothelial interaction. Briefly, the levels of various soluble factors, in particular TGF-ß1, were measured. HMrSV5 cells, a human peritoneal mesothelial cell line, were co-incubated with TGF-ß1, gastric cancer cells, or gastric cancer cells and TGF-ß1 receptor inhibitor SB431542. The expressions of smad 2/3 and phosphorylated smad 2/3, indicator of TGF-ß/Smads pathway activation, were evaluated. Then the morphological changes of HPMCs were observed. The cell damage was quantitatively determined by fluorescent microscopy and flow cytometry. Tumor-mesothelial cell adhesion was also examined. Results showed a significant elevation of TGF-ß1 expression, which is accompanied by dramatically increased phosphorylated-smad 2/3 levels, after mesothelial cell co-culture with the gastric cancer cell line. In addition, mesothelial cells exposed to gastric cancer cells or TGF-ß1 became exfoliated and exhibited signs of injury, while blocking TGF-ß1 can partially inhibit these effects. These results indicate that soluble factors, such as TGF-ß1, produced in autocrine/paracrine manner in the peritoneal cavity, affect the morphology and function of mesothelial cells so that the resulting environment becomes favorable for peritoneal metastases.

Introduction

Peritoneal carcinomatosis remains a major obstacle that severely limits the further improvement of gastric cancer patients' prognosis after surgery (1). Peritoneal carcinomatosis is a peritoneal metastatic cascade that is composed of a series of events. It usually occurs at the late stage of tumor development and significantly contributes to gastric cancer-related mortality. The mechanisms of peritoneal metastasis of diffusely infiltrating carcinoma are not yet clearly understood.

Over 100 years ago, Paget et al proposed a 'seed and soil' theory: metastasis only occurs when tumor cells (seeds) survive and grow in a favorable organ/tissue micro-environment (soil) (2). It is conceivable that peritoneal carcinomatosis occurs as the peritoneal stroma environment promotes tumor cells proliferation by providing various growth factors and chemokines, thus promotes tumor metastasis. On the contrary, the healthy, intact mesothelial cells prevent cancer cells from infiltrating into the sub-mesothelial connective tissue by forming a layer of peritoneum barrier. Yashiro et al (1) have previously demonstrated that the layer of confluent, intact mesothelial cells hindered cancer cell invasion to the abdominal cavity. However, once the integrity of such barrier is disrupted, metastasis may occur because peritoneum provides a favorable environment for gastric cancer cells to grow. For example, Kiyasu et al (3) reported that mesothelial cells became hemispherical and exfoliated from the peritoneum prior to the peritoneal infiltration of cancer cells.

One of the most potent apoptotic and fibrotic stimuli for mesothelial cells is transforming growth factor ß1 (TGF-ß1), which belongs to the TGF-ß super-family. The TGF-ß super-family also includes other TGF factors, bone morphogenic proteins and activin families, all of which share similar structures, signaling pathways, and an overlap in biologic functions. TGF-ß is a 25 kD homodimeric polypeptide that can participate in a broad array of biologic activities such as

Transforming growth factor ß1 produced in autocrine/paracrine manner affects the morphology and function of mesothelial cells and promotes peritoneal carcinomatosis

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Abbreviations: HPMCs, human peritoneal mesothelial cells; AO/EB, acidine orange/ethidium bromide; SF-CM, serum-free conditional media

Key words: peritoneal carcinomatosis, stomach neoplasms, mesothelial cell, transforming growth factor ß1
normal development, wound healing and pathologic processes (4). It can regulate multiple cellular functions, including both inhibition and stimulation of proliferation, apoptosis and differentiation. TGF-β is also an inducer of extracellular matrix (ECM) protein synthesis and has been implicated as the key mediator of fibrogenesis in various tissues (5,6).

Our previous study demonstrated that the TGF-β1 level in peritoneal lavage fluid is significantly correlated with peritoneal metastasis and TNM stages of gastric cancer (7). Collectively, we hypothesize that disseminated gastric cancer cells infiltrate into abdominal cavity, where they secrete abundant inflammatory factors, such as TGF-β1, to induce apoptosis of peritoneal mesothelial cells. Thus, mesothelial cells become hemispherical and exfoliation occurs. Areas of the sub-mesothelial connective tissue are then exposed to peritoneal cavity and this injured peritoneum provides a favorable environment for peritoneal metastasis (8-14). In this study, we investigated the effects of TGF-β1 on the morphology and function of human peritoneal mesothelial cells to provide more information on the reciprocal interaction between tumor and mesothelial cells during peritoneal gastric cancer metastasis.

Materials and methods

Reagents. ELISA kits for TGF-β1, IL-8, uPA, VEGF, KGF, MMP-7, MMP-9, HGF, FGF, and EGF were obtained from R&D Systems, USA. Calcein-AM was obtained from Calbiochem, UK. Acridine orange/ethidium bromide (AO/EB) was obtained from Fluaka, USA. Smad2, Smad3, phosphorylated-Smad2, and phosphorylated-Smad3, actin antibodies, as well as second antibodies (goat anti-mouse IgG) were purchased from Santa Cruz Biotechnology Inc., USA. Dulbecco’s modified Eagle’s medium (DMEM) and fetal calf serum (FCS) were purchased from Gibco BRL, USA. Propidium iodide (PI) was from BioShop, Burlington, ON, Canada. Acridine orange/ethidium bromide (AO/EB) was obtained from Fluka, USA. Smad2, Smad3, phosphorylated-Smad2, and phosphorylated-Smad3, actin antibodies, as well as second antibodies (goat anti-mouse IgG) were purchased from Santa Cruz Biotechnology Inc., USA. Dulbecco’s modified Eagle’s medium (DMEM) and fetal calf serum (FCS) were purchased from Gibco BRL, USA. Propidium iodide (PI) was from BioShop, Burlington, ON, Canada. Human peritoneal mesothelial cells were isolated from human omentum. Briefly, omentum collected from consenting non-uraemic patients undergoing elective abdominal surgery was incubated in 0.05% (w/v) trypsin and 0.01% (w/v) EDTA for 20 min at 37°C. The harvested mesothelial cells were centrifuged at 150 x g for 5 min and then transferred into 75 cm² tissue culture flasks and cultured in humified 5% CO₂ incubator. DMEM was supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mmol/l L-glutamine and 20 mmol/l hydroxyethyl piperezine ethanesulfonic acid (HEPES, Gibco BRL). Medium was changed every two or three days.

The human gastric carcinoma cell lines, MKN-45, MKN-1, SGC-7901, BGC-823 and MGC-803 were obtained from the Department of Cell Biology, China Medical University. These cells were cultured in DMEM supplemented with 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mmol/l L-glutamine in humidified 5% CO₂ incubator at 37°C.

Preparation of serum-free conditional media (SF-CM). Conditional media from gastric cancer cells or HPMC cells was prepared as previously reported (1). Briefly, 5x10⁶ cells were seeded in a 100-mm tissue culture dish with regular medium for 3 days. Then the cells were washed twice with PBS and incubated with 3 ml of serum-free DMEM. Two days later, the SF-CM was collected and centrifuged at 1000 x g for 5 min, passed through filters (pore size, 0.45 μm) and stored at -20°C until use.

Enzyme-linked immunoassay (ELISA). The levels of TGF-β1, IL-8, uPA, VEGF, KGF, MMP-7, MMP-9, HGF, FGF and EGF in both the SF-CM and cell lysates of MKN-45, MKN-1, SGC-7901, BGC-823, MGC-803 and HMrSV5 cell lines were measured using human Quantikine ELISA kits (R&D Systems, Minneapolis, MN) following the manufacturer’s instructions.

To evaluate the effect of co-culture of both gastric tumor cells and mesothelial cells on TGF-β1 secretion, 8x10⁴ mesothelial cells/well were first cultured in flat-bottomed 96-well plates to sub-confluence. Then 4x10⁵ SGC-7901 cells were washed 3 times, added to the mesothelial cells and then co-cultured for additional 72 h. The supernatant was collected for ELISA test.

Western blotting. Human peritoneal mesothelial cells were cultured to sub-confluence in a 60-mm culture dish with 10% FCS-containing DMEM. The medium was then changed to either 1) SF-CM from gastric cancer cells SGC-7901 or 2) serum-free DMEM serving as control. Protein was extracted in a standard lysis buffer with protease inhibitors (sodium orthovanadate, phenylmethylsulfonyl fluoride, leupeptin, and aprotinin obtained from BioShop, Burlington, ON, Canada).

Protein lysate (20 μg) was electrophoresed with 12% SDS-PAGE gel, transferred to a nylon membrane, and probed with an antibody for Smad2, phosphorylated-Smad2, Smad3, and phosphorylated-Smad3. Following the incubation with secondary antibody, blots were developed by ECL Western blot substrate kit (Abcam, USA).

Morphological evaluation of mesothelial cells under various conditions by a phase contrast microscope. Human peritoneal mesothelial cells were cultured to sub-confluence in a 60-mm dish with 10% FCS containing DMEM. The medium was then changed to either 1) serum-free DMEM; 2) medium containing TGF-β1 (100 ng/ml); 3) SF-CM from gastric cancer cells or 4) SF-CM from gastric cancer cells supplemented with TGF-β1 receptor inhibitor SB431542. Twenty-four hours later, all groups were examined under a phase contrast microscope for alterations in size, shape, and integrity of cell membrane, cytoplasm and nucleus.

Detection of apoptosis. Human peritoneal mesothelial cells were cultured to sub-confluence in a 24-chamber plate with 10% FCS containing DMEM. The medium was then changed to either 1) serum-free DMEM; 2) medium containing TGF-β1 (100 ng/ml); 3) SF-CM from gastric cancer cells or 4) SF-CM...
from gastric cancer cells supplemented with TGF-ß1 receptor inhibitor SB431542. Forty-eight hours later, apoptosis was quantified by the following two methods: 1) Fluorescent methods by AO/EB staining to quantify alive, early apoptotic, late apoptotic, and necrotic cells. Briefly, both adherent and non-adherent cells were harvested and washed twice with PBS, and then immediately treated with acridine orange (100 μg/ml) for 5 min and ethidium bromide (100 μg/ml) for 5 min. Cells were then examined under fluorescence microscope. Cells containing normal nuclear chromatin will exhibit green nuclear staining. Cells containing fragmented nuclear chromatin (apoptotic cells) will exhibit orange to red nuclear staining. 2) Flow cytometry methods. Briefly, both adherent and non-adherent cells were harvested, re-suspended in PBS at a concentration of 1x10^6/ml and then fixed in 2-ml methanol for 30 min at 4˚C. After fixation, cells were re-suspended with PBS, treated with RNase (50 μg/ml), and re-suspended in PBS containing propidium iodide (PI) (0.05 mg/ml in 3.8 mol/l natrium citrate) at room temperature for 30 min. Then, the HPMCs were spun down and re-suspended in 1-ml PBS and analyzed using flow cytometry according to the manufacturer's instructions. The cells in the sub-diploid peak were considered as apoptotic cells.

Adhesion assay. Mesothelial cells/well (8x10^4) were incubated in flat-bottomed 96-well plates that were previously coated with 0.5% gelatin (Sigma Chemical Co., UK) and cultured to sub-confluence. Gastric carcinoma cells were detached with non-enzymatic cell dissociation solution (Sigma Chemical Co.), washed twice and incubated with DMEM containing 5 μM Calcein-AM (Calbiochem) at 37˚C for 30 min. Calcein-AM is a cell-permeable, non-fluorescent and hydrophobic compound, which is rapidly hydrolysed by cytoplasmic esterases, releasing the membrane-impermeable hydrophilic and highly fluorescent calcein (15,16). Then, 4x10^4 labeled tumor cells were washed 3 times with serum complete medium and added to the mesothelial cells for coculture at 37˚C for 3 h. Wells were then washed 3 times with 200 μl of serum complete medium to remove the non-adherent tumor cells. The remaining adherent tumor cells were measured for fluorescence intensity using a cytofluorometer (Titertek Fluoroskan II, Flow Laboratories, McLean, VA, USA). Another plate was seeded with labeled tumor cells for 3 h as positive control and its fluorescence intensity was considered as 100%.

The adhesion percentage was calculated as: 100x fluorescence intensity of the experimental group/positive control. The experimental groups are: 1) mesothelial cells incubated with gastric cancer cells; 2) mesothelial cells pretreated with TGF-ß1 (100, 50, 10 ng) for 2 h, and then incubated with gastric cancer cells; 3) mesothelial cells pretreated with TGF-ß1 receptor inhibitor SB431542 for 2 h, and then incubated with gastric cancer cells; 4) gastric cancer cells incubated without mesothelial cells were prepared as a
positive control group. All the experiments were repeated 3 times.

**Statistical analysis.** All data are expressed as mean ± SD. Statistical analysis was performed using the Student’s t-test. P<0.05 was considered as significant.

**Results**

**Enzyme-linked immunoassay (ELISA).** We examined the levels of various soluble factors, including TGF-ß1, IL-8, uPA, VEGF, KGF, MMP-7, MMP-9, HGF, FGF, and EGF from multiple cancer cell lines including MKN-45, MKN-1, SGC-7901, BGC-823, MGC-803 and a human peritoneal mesothelial cell line HM-1SV5. Interestingly, all five gastric cancer cell lines showed abundant levels of TGF-ß1 (Fig. 1). In addition, we also observed a reasonable level of TGF-ß1 from HPMCs cells. This result indicated that the TGF-ß1 pathway might be active in the normal HPMC biological functions (Fig. 1).

It is widely accepted that TGF-ß1 plays a critical role in tumor invasion and metastasis. We then further investigated the role of TGF-ß1 in the reciprocal interaction between gastric tumor cells and HPMCs. We co-cultured both gastric tumor cells and HPMCs cells for 72 h and found that TGF-ß1 expression was greatly increased in the co-culture system compared to individual culture condition (Fig. 2). The TGF-ß1 level in co-culture was 4 times higher when compared to HPMCs cell culture alone. This indicates that TGF-ß1 may be actively involved in the reciprocal communication between gastric tumor and mesothelial cells, and both

<table>
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<th>HPMC</th>
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<td>TGF-ß1 (pg/ml/10^6 cells)</td>
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<td>478.71±24.59</td>
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Figure 2. The level of TGF-ß1 expression before and after tumor-mesothelial co-culture. Mesothelial cells were incubated in flat-bottomed 96-well plates and cultured to sub-confluence. SGC-7901 cells were added to the mesothelial cells and then co-incubated for 72 h. TGF-ß1 level was then measured by ELISA. The image shows the level of TGF-ß1 expression in supernatants from HPMC (open bars) or SGC-7901 (open bars) or co-culture (closed bars). Bars represent mean ± SD of three independent experiments (four replicates per experiment), P<0.05 as compared to each group.

Figure 3. Western blot analyses of TGF-ß1/smads2, 3 proteins. Mesothelial cells were treated for 24 h with either gastric cancer cells SGC-7901 and serum-free DMEM.

Figure 4. Morphological changes in human peritoneal mesothelial cells under phase contrast microscopy (x40). (A) Morphology of mesothelial cells cultured in serum-free DMEM; (B) Exfoliation and naked areas of mesothelial cells after treatment with 100 ng/ml TGF-ß1; (C) Morphological changes in mesothelial cells after treatment with gastric cancer cells; (D) Morphological changes were partly suppressed in mesothelial cells after treatment with gastric cancer cells + TGF-ß1 receptor inhibitor SB431542 (IC_{50} 94 nmol/l).
autocrine and paracrine TGF-ß1 may contribute to the disrupted mesothelial integrity, therefore facilitating peritoneal invasion/metastasis.

Western blotting. To further investigate the role of abundant TGF-ß1 in gastric cancer cell infiltration through mesothelial cells, we next examined the activation of TGF-ß/Smad pathway, which is known to be involved in promoting cell invasion. Interestingly, we observed a dramatic upregulation of both phosphorylated-smad2 and phosphorylated-smad3 levels after HPMCs co-culture with gastric cancer cells SGC-7901, while the total smad2 and smad3 levels remained similar (Fig. 3). These results clearly demonstrated that the TGF/Smad pathway is highly activated when mesothelial cells were co-cultured with gastric tumor cells, and which may contribute to the damaging effect on mesothelial cell function and integrity in preventing gastric tumor invasion/metastasis.

Morphological alterations of HPMCs after co-culture with gastric tumor cells. In addition, we observed dramatically different morphology of HPMCs after co-culture with gastric tumor cells. In particular, the non-treated HPMCs exhibited the typical polygonal and cobblestone-like morphology (Fig. 4A). In contrast, cells treated with gastric cancer cells or TGF-ß1 for 24 h underwent significant morphological alterations (Fig. 4B and C). Cells were spindle-like with reduced cytoplasm volume, and with a scattered distribution indicating reduced cell-cell adhesion. Moreover, some cells rounded up and detached from the culture dish, leading to exposed surface area (Fig. 4B and C). Interestingly, the above morphological alterations were effectively inhibited by the TGF-ß1 inhibitor treatment (Fig. 4D).

Detection of apoptosis. We then characterized the effect on apoptosis of HPMC cells after co-culture with gastric tumor cells. In control group, serum-free DMEM was not able to
induce apoptosis of mesothelial cells (Fig. 5A, a). After
HPMC line HMrSV5 cells were treated with gastric cancer
cell conditional medium or TGF-ß1 for 48 h, we observed
significant morphological indications of cell apoptosis, such
as condensation of chromatin, nuclear fragmentation and
apoptotic bodies, by AO/EB staining (Fig. 5A, b and c).
Interestingly, TGF-ß1 receptor inhibitor SB431542 could
partly suppress the apoptotic morphological changes
(Fig. 5A, d). The above apoptotic effect of co-culture and
TGF-ß1 treatment was also confirmed by flow cytometric
analysis. As is shown in Fig. 5B, the apoptotic rate of
mesothelial cells incubated with gastric cancer conditional
medium and TGF-ß1 was 60.98±3.07% and 11.11±0.71%,
respectively. In contrast, apoptosis in control group was
significantly lower at 0.70±0.16%. Most importantly, TGF-
ß1 receptor inhibitor SB431542 treatment dramatically
suppressed apoptosis induced by gastric tumor cell
co-culture. This clearly indicated that the apoptotic
effect on mesothelial cells by gastric tumor cells are
contributed by TGF-ß1 upregulation and the TGF/Smad
pathway activation.

Adhesion assay. To determine the effect of TGF-ß1 on tumor-
mesothelial cell adhesion, a monolayer of mesothelial cells
were incubated with TGF-ß1 (100, 50, and 10 ng/ml), gastric
tumor cell conditional medium, or both gastric tumor cell
conditional medium and TGF-ß1 receptor inhibitor
SB431542 for 2 h. Then fluorescence labeled tumor cells
were added to the monolayer of mesothelial cells for another
3 h. After rinsing away the non-adherent tumor cells from
the co-culture, the fluorescence intensity was measured to
represent the remaining adherent tumor cells on the
mesothelial layer. We found that TGF-ß1 can promote the
adhesion of gastric tumor cells to mesothelial cells in a
dose-dependent manner (Fig. 6A). We also found that less
gastric tumor cells were able to adhere to mesothelial cell
monolayer when compared to the control non-coated plate
(42.19±2.10% compared to 100% in the control). The
presence of TGF-ß1 receptor inhibitor SB431542
significantly inhibited the adhesion of tumor cells to
mesothelial cells, indicating that the TGF-ß1 produced by
autocrine/paracrine pathways during co-culture plays a
critical role in promoting the tumor cell adhesion to
mesothelial cells (Fig. 6A and B). There is a positive
correlation between dose of TGF-ß1 and adhesion.

Discussion

According to the ‘seed and soil’ theory; metastases only
occur when tumor cells encounter a favorable micro-
environment where they can survive and proliferate rapidly.
It has been previously reported that healthy mesothelial cells
can prevent tumor cell invasion and metastasis by providing
an intact barrier. Interestingly, tumor cells are also known to
secrete various factors to induce damage or apoptosis in
mesothelial cells and the disrupted mesothelial cells will then
promote tumor cells invasion (17-19). We hypothesize that
the damaged peritoneum may provide such a micro-
environment for scirrhoid gastric cancer cell metastasis. We
have previously demonstrated that cancer cells can secrete
soluble factors into abdominal cavity to induce damage and apoptosis of peritoneal mesothelial cells (20,21). Then the mesothelial cells became hemispherical, and detached from the mesothelial layer, leading to the exposure of sub-mesothelial connective tissue. This injured peritoneum may function as a favorable environment for peritoneal metastasis (22,23). However, what these soluble factors secreted by cancer cells are that trigger the damaging cascade remain unclear.

Over the past decade, significant progress has been made to better understand TGF-ß signaling in both physiological and pathological scenarios (24). In particular, TGF-ß1 has been demonstrated to be a key mediator of fibrosis in both experimental and human peritoneal dialysis-induced mesothelial injury (25,26). Whether the introduction of TGF-ß1 can promote gastric tumor-dysregulation and infiltration into peritoneum, and if so whether TGF-ß1 inhibitor can prevent such an event remain poorly understand.

Our study demonstrated a significant level of TGF-ß1 expression in all gastric cancer cell lines we examined. We also observed a decent level of TGF-ß1 in HPMC, which indicates that TGF-ß1 pathway and its related pathways may be involved in normal HPMC biological functions. We observed a dramatic increase of TGF-ß1 from HPMC cells when HPMC cells were co-cultured with gastric tumor cells than the individual HPMC cell culture alone. This indicates that TGF-ß1 pathway may play a role in the reciprocal communication of gastric tumor cells and mesothelial cells, and it potentially contributes to gastric tumor cell invasion through mesothelial cell layer. More importantly, the higher TGF-ß1 expression after co-culture also leads to increased p-smad2 and p-smad3 levels, which clearly indicate the TGF/Smad pathway activation.

It is known that after TGF-ß1 ligand binding with TGF-ß receptors on the cell membrane, the receptor kinase is activated and then leads to receptor Smads (both smad2 and smad3) phosphorylation. The p-smad2/3 will then be translocated into nucleus where they form heteromeric complex with smad4, and functions as transcription factors to regulate various downstream genes expression (27). The TGF/Smad pathway can regulate multiple cellular functions including inhibition and stimulation of cell growth, cell death or apoptosis, and cellular differentiation. In this study, we found that the p-smad2 and p-smad3 levels in HPMCs are significantly elevated, while the total level of smad 2, 3 remain similar, after co-culture with gastric cancer cells SGC-7901. The results indicate that elevated TGF-ß1 expression can lead to TGF/Smads pathway activation in HPMCs.

Considering the significant function of TGF/Smads in regulating cell proliferation and apoptosis, we hypothesized that the TGF-ß1, produced by both autocrine and paracrine via gastric tumor cells and mesothelial cells respectively, can affect mesothelial cell viability. We observed under phase contrast microscope that mesothelial cells became hemispherical and exfoliation occurred when gastric cancer cell or TGF-ß1 were added into mesothelial cell culture. Furthermore, both fluorescent microscopy and flow cytometric analysis confirmed that apoptosis of HPMCs was dramatically increased in response to TGF-ß1 treatment and gastric cancer cell co-culture. This indicated that factors, in particular TGF-ß1, secreted in abdominal cavity by invading cancer cells, induce damage and apoptosis of mesothelial cells, lead to exfoliation, and result in eventual metastasis after the barrier of metastasis is severely disrupted. TGF-ß1 receptor inhibitor SB431542 can partially suppress the above effects, supporting the involvement of TGF pathway.

To investigate whether the introduction of TGF-ß1 could enhance tumor-mesothelial adhesion and if TGF-ß1 receptor inhibitor SB431542 could reduce this adhesion, we incubated the mesothelial cells with TGF-ß1, or with cancer cell conditional medium with and without TGF-ß1 receptor inhibitor for 2 h. Then, we added tumor cells for another 3 h to evaluate the adhesion ability to mesothelial cells under various conditions. Interestingly, TGF-ß1 increases tumor-mesothelial adhesion in a dose-dependent manner. Consistently, the inhibition of TGF/Smad pathway by SB431542 led to a significant decrease of tumor-mesothelial adhesion. The findings are consistent with previous studies that TGF-ß1 may enhance tumor-mesothelial cell adhesion (28,29).

It should be noted that the effects of gastric cancer were stronger than TGF-ß1 alone; this indicates that some other soluble factors secreted by gastric cancer cells may also contribute to the effect. However, blocking TGF-ß1 pathway activation can partially inhibit these effects. This indicated that while various soluble factors contributed to tumor-mesothelial interaction, TGF-ß1 might be a key regulator. Further studies are required to elucidate the detailed mechanisms of the TGF-ß/Smad signaling activation induced by gastric cancer cells.

In conclusion, soluble factors, such as TGF-ß1, produced by autocrine/paracrine in peritoneal cavity can regulate the morphology and behavior of mesothelial cells so that the altered mesothelial cells may provide a favorable environment for the peritoneal dissemination of cancer cells (30-33).

Acknowledgements

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