βig-h3 supports gastric cancer cell adhesion, migration and proliferation in peritoneal carcinomatosis

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Abstract. βig-h3 is an extracellular matrix protein and its expression is highly induced by transforming growth factor (TGF-β). It has also been suggested to play an important role in the growth and invasion of colon and pancreatic cancers. In the present study, we demonstrated that βig-h3 is expressed in mesothelial cells, especially in patients with advanced gastric cancer. The positive rate of βig-h3 was significantly higher in cases with a more invasive and advanced serous-type, with visible peritoneal metastasis, and in peritoneal lavage cytological examination (PLC) (+) and peritoneal lavage fluid CEA mRNA(+) subgroups ($p<0.05$). Our study also showed that the expression of βig-h3 gradually increased with increasing TGF-β1 concentrations *in vitro* in a time-dependant manner. In addition, βig-h3 also induced human gastric carcinoma cell line (SGC-7901) cell adhesion in a dose-dependent manner and significantly increased cell migration and proliferation. The results suggest that βig-h3 expression in peritoneal mesothelial cells in gastric cancer patients is a marker of the biological behavior of gastric cancer and plays an important role in the process of peritoneal carcinomatosis.

Introduction

Although the therapeutic outcomes of patients with gastric cancer continue to improve, it remains the second leading cause of cancer-related mortality worldwide (1,2). Even when undergoing curative resection, almost half of patients with advanced cancers eventually die of peritoneal recurrence (3,4), a fact that insinuates the peritoneal metastatic cascade of gastric cancer and contributes significantly to gastric cancer-related mortality. To date, no mechanism has been specified by which gastric carcinoma undergoes peritoneal carcinomatosis.

Stephen Paget's 'seed and soil' theory of tumor metastasis may provide a clue useful for further research on peritoneal carcinomatosis of gastric cancer. This proposes, that the sites where metastasis occurs are defined not only by the tumor cells (seed) but also by the local microenvironment of the metastatic site (soil) (5). This is to say that the interactions between tumor cells and the local microenvironment at the secondary site are no less important than the biological activities of free cancer cells shed from primary gastric cancer in this process (6). Therefore, peritoneal carcinomatosis may occur as the peritoneal stroma environment promotes tumor cell attachment to the peritoneal mesothelium (7). This process requires the interactions between extracellular matrix proteins and signals produced by mesothelial cells and the corresponding adhesion molecules from tumor cells (8).

In our previous study, we demonstrated that transforming growth factor (TGF-β1) levels in peritoneal lavage fluid were correlated with the peritoneal metastasis of gastric cancer and TGF-β1 plays a key role in induction of peritoneal fibrosis, which in turn affected the adhesion and metastasis of gastric cancer cells.

βig-h3 is a TGF-β-induced extracellular matrix (ECM) protein, consisting of 4 fasciclin-1 (fas-1) homologous domains and an RGD motif at the C-terminus. The fas-1 domain is well-conserved in several proteins from different species, and has motifs interacting with the α3β1, α5β3 and α5β5 integins, through which it mediates adhesion and migration in several cell types (9,10). The βig-h3 transcript has been detected in a variety of human and mouse tissues including uterine tissue, heart, breast, prostate, skeletal muscle, testes, thyroid, kidney, liver and stomach (11). Some studies have revealed that βig-h3 expression is substantially elevated in colon and pancreatic cancers in comparison with corresponding normal tissues (12,13). The overexpression of βig-h3 in colon cancer cells promotes tumor metastasis, while the suppression of βig-h3 expression significantly decreases their metastatic potential *in vitro* (13).

In this study, we aimed to confirm whether TGFβ-1 induces peritoneal mesothelium cells to express βig-h3 and whether βig-h3 is involved in generating a suitable microenvironment for peritoneal dissemination and whether it induces gastric cancer cell migration to peritoneal tissue.

Materials and methods

Reagents and instruments. βig-h3 antibodies and secondary antibodies were purchased from Santa Cruz Biotechnology

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(Santa Cruz, CA, USA). The human TGF-β1, epidermal growth factor (EGF), RT-PCR kit, dimethyl sulfoxide (DMSO), fibronectin (FN), bovine serum albumin (BSA) and trypsin were purchased from Sigma (St. Louis, MO, USA). DMEM, streptomycin and other cell culture supplies were from Gibco BRL (Grand Island, NY, USA). Fetal bovine serum (FBS) was obtained from HyClone (Logan, UT, USA). MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltrazolium bromide] was obtained from Fluka (Ronkonkoma, NY, USA). Human recombinant βig-h3 proteins and a βig-h3 ELISA kit were purchased from R&D (Minneapolis, MN, USA). A phase contrast microscope (Japan Nikon, Japan) and a spectrofluorometer (Japan Olympus, Japan) were used. Other laboratory reagents were obtained from Sigma.

Cell lines and culture. A human peritoneal mesothelial cell line HMrSV5 was kindly provided by Professor Youming Peng of the Second Hospital, Zhongnan University, Changsha, China and Professor Pierre Ronco, Hospital Tenon, Paris, France. This cell line was established after the infection of a fully characterized primary culture of human peritoneal mesothelial cells with an amphotropic recombinant retrovirus that encodes SV40 large-T Ag under the control of a Moloney virus long terminal repeat. A human gastric carcinoma cell line, SGC-7901, was obtained from the Cancer Research Institute of Beijing, China. These cell lines were cultivated in T75 tissue culture flasks in DMEM supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 20 mM hydroxyethyl-piperazineethanesulfonic acid (HEPES). Cultures were grown at 37˚C in a humidified 5% CO_2 and 95% air incubator.

Tissue samples. Human peritoneum tissue samples were obtained from 75 gastric cancer patients and 14 benign disease patients, who underwent surgery at the First Affiliated Hospital of the China Medical University from May to October 2011. The benign diseases included ileus, appendicitis and gastric adenomas. These tissue specimens were obtained from the lower anterior abdominal wall. No patients had received any form of radiation or chemotherapy prior to surgery. The local institutional review board approved our protocol for using patient samples; all patients provided written consent prior to participation in the study. All histology slides were staged and classified according to the UICC new TNM staging (7th edition) (14) and the serosa was classified according to Sun *et al* (15). The peritoneal tissues were directly obtained from the surgical site and immediately fixed in 10% buffered formalin and then embedded in paraffin. Sections (5 μm) were prepared for immunohistochemical staining.

Immunohistochemistry. To reveal the antigens, sections were placed in a 1-mM Tris solution (pH 9.0) supplemented with 0.5 mM EGTA [ethylenglycol-bis(β-amino-ethylether)-N,N,N',N'-tetraacetic acid] and heated using a microwave oven for 10 min. The non-specific binding of immunoglobulin was prevented by incubating the sections in 50 mM NH_4Cl for 30 min, followed by blocking in PBS supplemented with 1% BSA, 0.05% saponin and 0.2% gelatin. The sections were incubated overnight at 4˚C with an immune serum diluted in PBS supplemented with 0.1% BSA and 0.3% Triton X-100 (1:3,000), and labeling was visualized using a horseradish peroxidase-conjugated secondary antibody. Negative controls included omitting the primary antibody with normal rabbit IgG at an equivalent protein concentration.

Peritoneal lavage cytological examination and RT-PCR detection of CEA mRNA levels in the peritoneal lavage fluid. The peritoneal lavage fluid was collected from each patient. Briefly, during laparotomy, 100 ml of physiological saline was injected into the right upper quadrant or the Douglas pouch and approximately 60 ml was retrieved. The peritoneal lavage sample was immediately centrifuged at 2000 rpm for 10 min at room temperature. Approximately one half of each sample was used for cytopathological examination after conventional Papanicolaou staining; the remainder was dissolved in Isogen RNA extraction buffer and stored at -80˚C until use. Total RNA was isolated from these cells using the TRIzol reagent according to the manufacturer's instructions. A total amount of 1 mg of the total cellular RNA was then reverse-transcribed into cDNA for PCR amplification using a kit from Sigma. The sequences of the primers used were as follows: A: 5'-CATCATGATTGGAGTGCTGGTTG-3'; B: 5'-CACGATGTTGGCTAGGATGGTC-3'. Amplification consisted of an initial 5-min incubation at 95˚C and then 30 cycles of amplification using 30 sec of denaturation at 95˚C, 30 sec at 56˚C and 60 sec at 72˚C. The final extension was set for 10 min at 72˚C. Samples with visible 199-bp bands were designated as positive.

ELISA detection of βig-h3 levels in the supernatants. After the HMrSV5 cells were grown and treated with or without TGF-β1, the supernatants were collected and immediately centrifuged at 2000 rpm for 10 min at room temperature and stored at -80˚C until use. The βig-h3 levels were then assayed with a human βig-h3 ELISA kit according to the manufacturer's instructions. Data on the βig-h3 protein levels were expressed as the means ± SD.

Protein extraction and western blotting. After the HMrSV5 cells were grown and treated with or without TGF-β1, the total cellular protein was extracted using a lysis buffer and quantified by using protein quantification reagents from Bio-Rad. Next, 50 μg of the protein were suspended in a 5X reducing sample buffer, boiled for 5 min, electrophoresed on 10% SDS-PAGE gels and then transferred to a polyvinylidene difluoride membranes by electroblotting. The membrane was blocked in 1% BSA/0.05% Tween/PBS solution overnight at 4˚C, followed by incubation with the primary antibody for 24 h. A horseradish peroxidase-labelled goat anti-mouse IgG was used as the secondary antibody. The blots were then developed by incubation in a chemiluminescence substrate and exposed to X-ray films.

Immunofluorescence staining. The expression of βig-h3 in HMrSV5 cells was also analyzed by immunofluorescence microscopy. In brief, after the cells were grown and treated with or without TGF-β1, they were cultured on collagen-coated glass coverslips to confluency and then fixed in 4% paraformaldehyde in 20 mM HEPES (pH 7.4) and 150 mM NaCl for 20 min. The glas coverslips were rinsed 3 times and

Table I. Comparison of βig-h3 expression and various clinicopathologic features of the gastric cancer cases.

Clinicopathological features	Expression of βig-h3 in peritoneal mesothelial cells		p-value
	-	+	
Histologic grade			0.555
Differentiated	19	10	
Undifferentiated	27	19	
Lauren grade			0.362
Intestinal	22	17	
Diffuse	24	12	
Invasive depth			0.016
T1, T2, T3	32	12	
T4	14	17	
Lymph node metastasis			0.338
Negative	21	16	
Positive	25	12	
Types of serosa			
Normal and reactive type	13	3	0.037
Nodular type	19	9	
Tendonoid/color-diffused type	14	17	

A χ^2 test was used for this analysis.

Table II. Association between the expression of βig-h3 and various factors indicating peritoneal metastasis.

Factors indicating peritoneal metastasis	Expression of βig-h3 in peritoneal mesothelial cells		p-value
	-	+	
Visible peritoneal metastasis at surgery			0.002
Negative	43	19	
Positive	3	10	
PLC			0.005
Negative (-)	39	16	
Positive (+)	7	13	
CEA mRNA			0.027
Negative (-)	31	12	
Positive (+)	15	17	

PLC, peritoneal lavage fluid; CEA, carcinoembryonic antigen. χ^2 test was used for this analysis.

permeabilized with 1.2% Triton X-100 for 5 min, rinsed 3 times again and then incubated with 1% BSA/0.05% Tween/PBS for 1 h. Staining for the expression of βig-h3 was carried out with a primary rabbit antibody anti-βig-h3 (1:200) and then with a secondary antibody conjugated with FITC. The DNA dye To-PRO-3 (blue) was used for counterstaining. The stained cells were viewed under an immunofluorescence microscope.

Tumor cell adhesion assay. The cell adhesion assay was performed as described previously (16). Briefly, 96-well plates were coated with BSA, βig-h3 and FN (20 μg/ml) diluted in PBS at 4˚C overnight. Then the plates were rinsed with PBS and the uncoated surfaces were blocked with 2% BSA for 1 h. The SGC-7901 cells were suspended in medium at a density of $4x10^3$ cells/200 μl and added to each well of the coated plates. After incubation for 1 h at 37˚C, unattached cells were removed by rinsing with PBS, and the absorbance was measured at 570 nm in a Bio-Rad model 550 microplate reader. Experiments were repeated in triplicate. Data are reported as the mean ± SD.

Tumor cell migration assay. A cell migration assay was performed using Transwell plates. The undersurface of the membrane was coated with BSA, βig-h3 and FN (20 μg/ml) diluted in PBS, at 4˚C overnight. Then the plates were rinsed with PBS and uncoated surfaces were blocked with 2% BSA for 1 h. The SGC-7901 cells ($4x10^4$) per well in 200 μl complete medium were seeded in the upper compartment of the plates. After 6 h of migration, cells in the upper chamber of the filter were removed and non-migrating cells on the top of the filters were removed with a cotton swab. SGC-7901 cells on the lower side of the filter were fixed with 8% glutaraldehyde and then stained with 0.25% crystal violet in 20% methanol. Each cell experiment was repeated in triple-wells and for each well the numbered cells were counted in 9 randomly selected microscopic high-power fields.

Proliferation assay. A total of 24-well culture plates were coated with BSA, βig-h3 and FN (20 μg/ml) diluted in PBS at 4˚C overnight. Then the plates were rinsed three times in PBS and uncoated surfaces were blocked with PBS containing 2% BSA for 1 h at 37˚C. The plates were rinsed again and $5x10^3$ SGC-7901 cells were added to each well in 1 ml culture medium. Although the initial cell adhesion efficiency was different depending on the substrates, most of the cells became adherent within a few hours, thus giving the same cell numbers. Then, we subjected SGC-7901 cells to serum starvation for 24 h, which should have brought most of the cells into the G0 phase of the cell cycle. After incubation for 24 h, SGC-7901 cell proliferation was assessed by counting cells after trypsinization using a hematocytometer at 24 h intervals. Cell numbers at 0 h indicate the numbers at 24 h after the initial cell seeding, showing that there was no difference in the initial cell numbers at the 0 h point under different conditions. Experiments were repeated in triplicates. Data are reported as the mean ± SD.

Statistical analysis. All the statistical analyses were carried out with the SPSS 16.0 statistical package (SPSS Inc., Chicago, IL, USA). All data were summarized as the mean ± SD, where appropriate. The two-tailed χ^2 test or Student's t-test was performed in order to compare the different groups.

Differences were considered statistically significant at a p-value ≤0.05.

Results

βig-h3 expression of peritoneal mesothelial cells in gastric cancer patients and its relation to pathological factors. Histological sections were examined to localize βig-h3 expression in peritoneum tissue. Immunohistochemical staining showed that there was a positive staining in the peritoneal mesothelial cells (Fig. 1). βig-h3 was confirmed positive in the peritoneal tissue in 29 patients with gastric cancer and in 1 with benign lesions, the difference being significant (p=0.03). In the gastric cancer group, the positive rate of βig-h3 was significantly higher in the more invasive and advanced serous type subgroups (Table I).

Association of βig-h3 expression and various factors indicating peritoneal metastasis. In the gastric cancer group, there were 13 patients with visible peritoneal metastasis, 20 with PLC(+) and 32 with CEA mRNA(+) (Fig. 2). The positive rate of βig-h3 was significantly higher in the subgroups with visible peritoneal metastasis, PLC(+) or CEA mRNA(+) (p<0.05) (Table II).

Expression of βig-h3 is induced by TGF-β1 in HMrSV5 cells. βig-h3 is known as one of the target genes of TGF-β. To determine whether TGF-β1 induces βig-h3 production in HMrSV5 cells, the βig-h3 protein levels were measured by ELISA in the culture supernatants of HMrSV5 cells incubated with various concentrations of TGF-β1 for different period of time. TGF-β1 increased the βig-h3 protein levels in the culture supernatants in dose- and time-dependent manners (Fig. 3A and B). The maximum increase was observed at 50 ng/ml and 24 h. A similar result was observed in the western blot analysis of HMrSV5 cells (Fig. 3C and D). We assessed whether EGF affected βig-h3 expression in HMrSV5 cells. Different concentrations of recombinant human EGF (Sigma) ranging from 1 to 50 ng/ml were added and incubation was carried out for 48 h, resulting in no effect on the βig-h3 production by HMrSV5 cells at any concentration (data not shown). In addition, we used an immunofluorescence microscopy to confirm the protein data; the TGF-β1-treated HMrSV5 cells exhibited a higher level of βig-h3 than the control (Fig. 3E and F).

βig-h3 supports the adhesion, migration, and proliferation of HMrSV5 cells. Since βig-h3 mediates the adhesion of several cell types (10,11,16), its ability to mediate the adhesion of HMrSV5 cells was examined by using a recombinant βig-h3 protein. In addition, the effects of βig-h3 on the migration and proliferation of the SGC-7901 cells were also examined. For the cell adhesion assay, a cell culture plate with recombinant βig-h3 protein was used. As shown in Fig. 4A, βig-h3 significantly increased SGC-7901 cell adhesion compared to BSA, and there was no significant difference between βig-h3 and FN. Subsequently, the ability of βig-h3 to mediate the SGC-7901 cells proliferation was tested. The βig-h3 had the strongest ability to induce proliferation, followed by FN and BSA in descending order (Fig. 4B) and the difference was significant. In the migration assay, βig-h3 and FN significantly increased the SGC-7901 cell migration compared to BSA (Fig. 4C-E). Cells seeded on βig-h3-coated culture plates showed a marked increase in number compared to those seeded on BSA-coated plates.

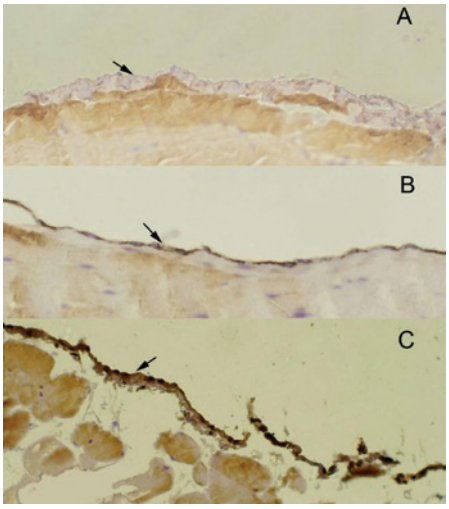

Figure 1. (A) βig-h3 was not detected in the peritoneal mesothelial cells. (B and C) βig-h3 was detected in the peritoneal mesothelial cells (x40 magnification). Peritoneal mesothelial cells were immunostained with the anti-βig-h3 antibody as described in 'Materials and methods'. Arrowheads indicate the peritoneal mesothelial lining.

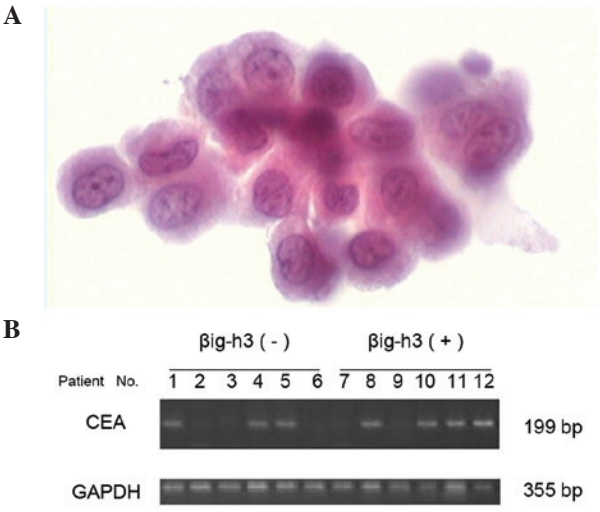

Figure 2. (A) Peritoneal lavage fluid (PLC) (+). (B) Representative RT-PCR results for peritoneal washes from gastric carcinoma patients. Specific 199-bp bands for CEA are evident in 15 of 46 washes from βig-h3-negative patients (lanes 1, 4, 5) and 17 of 29 washes from βig-h3-positive patients (lanes 8, 10, 11, 12). The integrity of extracted RNA was confirmed by RT-PCR amplification of GAPDH mRNA.

Discussion

In the present study, we demonstrated, that βig-h3 was expressed in mesothelial cells, especially in patients with advanced gastric cancer. The positive rate of βig-h3 was significantly higher in the more invasive and advanced serous-type, with visible peritoneal metastasis, in PLC(+) and CEA mRNA(+) subgroups. All the data indicated the expression of βig-h3 in mesothelial cells were closely related to peritoneal

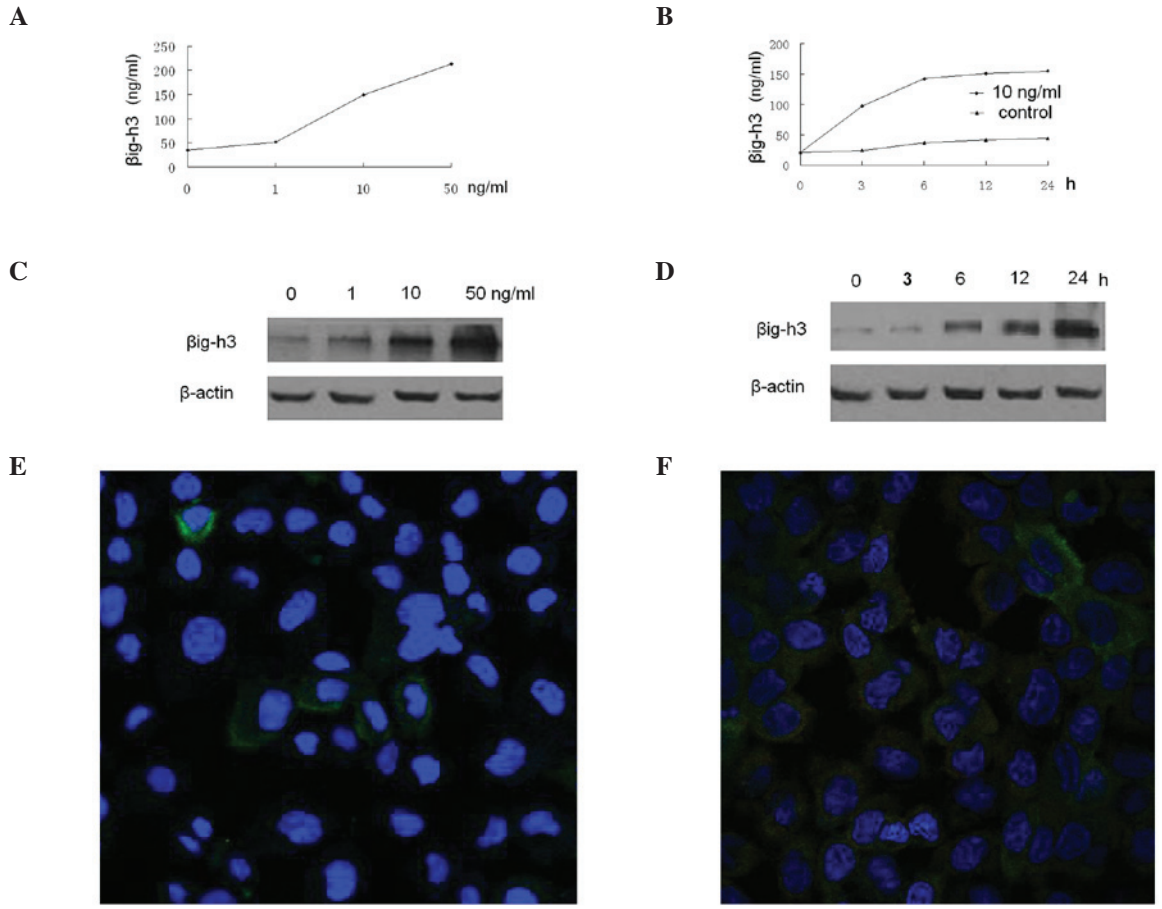

Figure 3. Effects of TGF-β1 on expression of βig-h3. The control groups were treated with RPMI1640 cell culture with 10% FBS. Serum-starved HMrSV5 cells were incubated with increasing concentrations (0, 1, 10, 50 ng/ml) of TGF-β1 for 24 h and 10 ng/ml of TGF-β1 for 3, 6, 12, 24 h. (A and B) βig-h3 protein levels in cell supernatant were estimated by ELISA analysis. (C and D) βig-h3 protein levels in the cells were estimated by western blot analysis. The cells were fixed for immunostaining with a polyclonal antibody against βig-h3. βig-h3 was visualized by FITC (green), and nuclei were visualized by To-PRO-3 (blue) under immunofluorescence confocal microscopy. (E) Control. (F) TGF-β1-treated for 24 h (50 ng/ml). Data represent the means ± SD of at least three determinations. All images were obtained at x100 magnification.

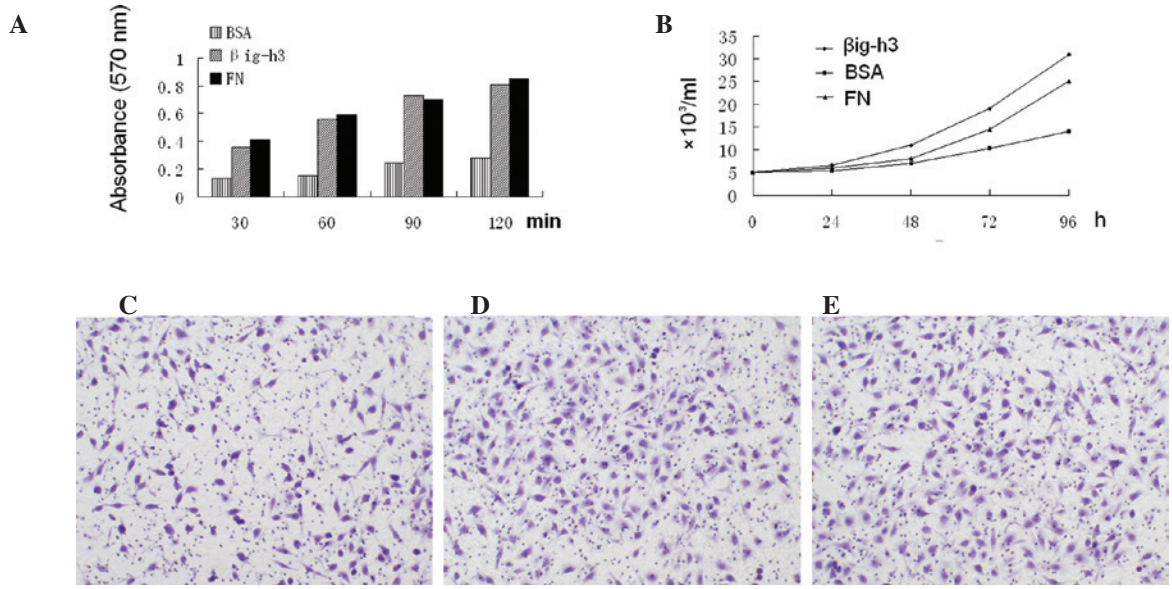

Figure 4. (A) βig-h3 and FN significantly increased SGC-7901 cell adhesion compared to BSA in a time-dependent manner; the maximal absorbance was observed at 120 min. (B) Ability of bovine serum albumin (BSA), FN and βig-h3 to induce SGC-7901 cell proliferation was asserted and the inducing effect was increased in turn. (C-E) A cell migration assay was performed with transwell plates. The cell membrane surface was coated with BSA, FN and βig-h3, and cells were seeded in the upper compartment of the filter. Cells that migrated to the lower side of the filter were fixed, stained and counted. A total of (C) 84±9 (BSA), (D) 159±25 (FN) and (E) 171±38 (βig-h3) cells migrated to the lower side. Difference between BSA and βig-h3, or BSA and FN was significant ($p<0.05$). There was no significant difference between βig-h3 and FN.

metastasis. Our study subsequently showed, that the expression of βig-h3 increased gradually with elevated TGF-β1 concentrations and in a time- and dose-dependent manner. βig-h3 induced HMrSV5 cell adhesion and significantly increased its migration and proliferation.

Stephen Paget's 'seed and soil' theory of tumor metastasis has been adopted by most scholars. To date, however, most of the relevant studies focus more on the 'seed' rather than the 'soil'. It is generally believed, that gastric cancer cells first acquire some particular ability to readily physically invade the peritoneal cavity occupying a unique position to eventually metastasize to the peritoneum. However, a more complicated process may be involved. For example, peritoneal mesothelial cells may also change in favor of implantation of gastric cancer cells to peritoneal tissue (7). In this study, we observed the expression of βig-h3 in peritoneal mesothelial cells and the positive rate of βig-h3 was significantly higher in the more invasive and advanced serous-type subgroups. Previously, the depth of invasion and the serosal changes were reported to be significant risk factors for the prediction of peritoneal recurrence (15,17). Thus, we proposed the hypothesis that the βig-h3 expression in peritoneal mesothelial cells in gastric cancer patients may be a marker of the biological behavior of gastric cancer and could predict peritoneal metastasis. To confirm our hypothesis, we further evaluated the relationship between the expression of βig-h3 and the other factors indicating peritoneal metastasis. The cytologic examination of the lavage fluid obtained during surgery is a conventional method to detect free cancer cells in the peritoneal space and is considered a gold standard for predicting peritoneal metastasis (18). In recent years, however, some investigations have demonstrated that the CEA RT-PCR analysis of peritoneal lavage fluids was more sensitive than conventional cytology (17,19). Thus, both examinations were performed. Our study demonstrated, that the positive rate of βig-h3 was significantly higher in the visible peritoneal metastasis PLC(+) and CEA mRNA(+) subgroups. The results further support our hypothesis.

βig-h3 is an extracellular matrix protein, which was first identified as a gene induced in A549 cells, after treatment with TGF-β1, and was subsequently reported to be present in several cell types including skin fibroblasts (6), corneal epithelial cells, and chondrocytes (9-11). We first proved it was present in human peritoneal mesothelial cells by immunohistochemical staining. To further confirm our conclusion, an *in vitro* experiment was performed. The result showed that TGF-β1 increased the βig-h3 protein levels in the culture supernatants of HMrSV5 cells in a dose- and time-dependent manner. A similar result was observed in western blot analysis and immunofluorescence staining in evaluating the βig-h3 protein levels in HMrSV5 cells. Our previous study showed, that TGF-β1 levels in the peritoneal wash-fluid were significantly higher in patients with gastric cancer than in those with benign disease and increased along with the development of the disease (20-22). Our current study indicated that the TGF-β1 levels in the peritoneal wash fluid might play a key role in promoting peritoneal mesothelial cells to express βig-h3. We have also noted that the concentration of TGF-β1 in the peritoneal wash-fluid was lower than the one used *in vitro* to treat mesothelial cells. This may be attributed to the natural differences between *in vivo* and *in vitro* experiments. In addition, another substance in the peritoneal wash-fluid, secreted by gastric cancer cells, may have also contribute to this effect.

It is understood that the attachment of malignant cells to the peritoneal mesothelium is a critical step in the peritoneal dissemination of a disease (5,7). Previous studies have suggested that this process is mediated by the interaction between the extracellular matrix and the corresponding adhesion molecules from the gastric cancer cells (8). Moreover, the extracellular matrix may serve to anchor the cancer cells (7,23,24). Although the biological roles of the βig-h3 are largely unknown, the most extensive literature regarding βig-h3 to date suggests that it acts as a cell adhesion substrate, regulates cell growth, interconnects other matrix components and transduces TGF-β-mediated signaling. Several recent studies have revealed, that βig-h3 regulates cell growth and migration in colorectal and pancreatic cancer cells (12,13). However, little is known about the effect of βig-h3 on gastric cancer cells. Our results showed that βig-h3 induces gastric cancer cell adhesion, migration and proliferation. βig-h3 activities on HMrSV5 cell adhesion and migration were comparable with those of FN, although the activity of βig-h3 was somewhat lower than that of FN. Notably, the βig-h3 exhibited a stronger ability to induce SGC-7901 cell proliferation than FN. All the above findings indicate a possible role for βig-h3 in the development of gastric cancer, promoting a suitable environment for gastric cancer cell adhesion, migration, proliferation and finally peritoneal metastasis.

In conclusion, peritoneal mesothelial cells do express βig-h3. TGF-β1 increased the βig-h3 protein levels both in the culture supernatants and in the HMrSV5 cells in a dose- and time-dependent manner *in vitro*. βig-h3 induced gastric cell adhesion, migration and proliferation. These data suggest, that βig-h3 expression in peritoneal mesothelial cells in gastric cancer patients may be a marker of biological behavior of gastric cancer and play an important role in the process of peritoneal carcinomatosis. These data provide a sound scientific rationale for further investigation into the use of βig-h3 as a therapeutic target for peritoneal metastasis of gastric cancer.

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