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ORIGINAL ARTICLE

Effects of angiopoietin-1 on attachment and metastasis of human gastric cancer cell line BGC-823

Xi-Long Ou, Hui-Juan Chen, Wei-Hao Sun, Cheng Hang, Liu Yang, Yun-Yan Guan, Fang Yan, Bao-An Chen

Xi-Long Ou, Cheng Hang, Liu Yang, Bao-An Chen, Department of Gastroenterology, Zhongda Hospital, Southeast University, Nanjing 210009, Jiangsu Province, China
Hui-Juan Chen, Department of Gastroenterology, the Second Hospital of Nanjing, Southeast University, Nanjing 210003, Jiangsu Province, China
Wei-Hao Sun, Department of Geriatrics, First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China
Yun-Yan Guan, Department of Intensive Care Unit, Wuxi Hospital of Traditional Chinese Medicine, Wuxi 214001, Jiangsu Province, China
Fang Yan, Department of Oncology, Changhai Hospital, Shanghai 200433, China
Author contributions: Ou XL and Chen HJ contributed equally to this work; Ou XL, Chen HJ, Sun WH and Hang C designed the research; Ou XL, Chen HJ and Hang C performed the research; Yang L, Yan F and Guan YY contributed analytic tools; Ou XL, Chen HJ and Hang C analyzed the data; Ou XL and Chen HJ wrote the paper; Sun WH, Chen BA and Hang C wrote part of the paper.
Correspondence to: Xi-Long Ou, Associate Professor, Department of Gastroenterology, Zhongda Hospital, Southeast University, Nanjing 210009, Jiangsu Province, China. ouxilong@126.com
Telephone: +86-25-83272034 Fax: +86-25-83272011
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Abstract

AIM: To evaluate the effects of angiopoietin-1 (Ang-1) on adhesion of gastric cancer cell line BGC-823 and expression of integrin β1, CD44V6, urokinase-type plasminogen activator (uPA) and matrix metalloproteinase-2 (MMP-2).

METHODS: BGC-823 cells were transfected transiently with adenovirus-Ang-1 (Ad-Ang-1). Cells transfected transiently with adenovirus-green fluorescent protein (Ad-GFP) and untransfected cells were used as a negative and blank control group, respectively. The cell adhesion rate between cell and extracellular matrix (ECM) was determined by cell adhesion assay. To investigate whether Ang-1 could reinforce gastric carcinoma metastasis, we performed migration and invasion assays in BGC-823 cells. The mRNA and protein expression of integrin β1, CD44V6, uPA and MMP-2 were detected by reverse transcription polymerase chain reaction and Western blotting, respectively. The expression of integrin β1 and CD44V6 was measured by immunohistochemistry.

RESULTS: BGC-823 cells were transfected successfully. The adhesion rate increased significantly in the Ad-Ang-1 group ($P < 0.05$). The Ad-Ang-1-transfected group had a significant increase in migration and invasion compared with that of the mock-transfected and Ad-GFP groups. The mRNA and protein expression of integrin β1, CD44V6, uPA and MMP-2 in the Ad-Ang-1 group was higher than that in the Ad-GFP and blank control groups ($P < 0.05$). Compared with mock-transfected and Ad-GFP groups, integrin β1 and CD44V6 expression intensity greatly increased ($P < 0.05$).

CONCLUSION: Transfection of Ang-1 into human gastric cancer cell line BGC-823 can significantly increase expression of integrin β1 and CD44V6, by which cell adhesion and metastasis to the ECM are promoted.

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Key words: Angiopoietin-1; CD44V6; Cell adhesion; Gastric cancer; Integrin β1; Matrix metalloproteinase-2; Neoplasm metastasis; Urokinase-type plasminogen activator

Peer reviewer: Sharon DeMorrow, Assistant Professor, Division of Research and Education, Scott and White Hospital and The Texas A&M University System, Health Science Center College of Medicine, Temple, TX 76504, United States

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INTRODUCTION

Angiopoietin-1 (Ang-1) has been identified *via* secretion trap and homology cloning techniques as a family of structurally related proteins that bind with similar specificity and affinity to a common endothelial-cell-specific receptor tyrosine kinase, Tie2[1-3]. Ang-1 is

required for correct organization and maturation of newly formed vessels and promotes quiescence and structural integrity of adult vasculature. However, its functions and mechanisms in tumors are not clear[4,5]. In addition to Tie receptors, Ang-1 has been found to bind integrins. Experiments with blocking antibodies, as well as cells deficient in certain integrins, suggest Ang-1 can bind several different integrins, including $\alpha 2\beta 1$, $\alpha 5\beta 1$, $\alpha v\beta 3$ and $\alpha v\beta 5$[6-9]. Also, some researchers have confirmed that Ang-1 can support cell adhesion mediated by integrins[7]. Whether there is a relationship between Ang-1 and integrin β1 has not been established. In addition, Ang-1 can affect cell adhesion, and CD44V6 is a member of the cell adhesion family, therefore, we can infer that there is a relationship between Ang-1 and CD44V6. At the same time, in primary adenocarcinoma, cancer cell invasion is facilitated by interaction with the local stromal compartment, whereas metastatic growth requires the ability of the cancer cell to interact with the new host tissue before the cancer cells can invade and destroy the target organ. The local stromal compartment, which comprises inflammatory cells and fibroblasts, facilitates the process by secreting extracellular matrix (ECM)-degrading proteases, including matrix metalloproteinases (MMPs) and urokinase-type plasminogen activator (uPA). MMP-2 (also known as gelatinase A and the 72-kDa type Ⅳ collagenase) has the ability to degrade type Ⅳ collagen in the basement membrane, and it is one of the major stroma-derived MMPs. One proposed mechanism of MMP-2 activation is through the plasminogen activator/plasmin system, in which pro-uPA binds to uPA receptor (uPAR), through a specific amino-terminal sequence of its non-catalytic chain. This binding result in uPA activation, accelerates the conversion of plasminogen to plasmin on the cell surface, and localizes these enzymes to focal contact sites. Many studies have indicated that MMP-2 plays an important role in tumor invasion of the basement membrane. Therefore, we used adenovirus as a vector to transfect the cell line BGC-823, to explore the effects of Ang-1 on integrin β1, CD44V6, uPA and MMP-2 of human gastric cancer cell line BGC-823, and study the mechanism of adhesion to the ECM and metastasis of gastric cancer.

MATERIALS AND METHODS

BGC-823 cell line and cell culture

Human gastric adenocarcinoma cell line BGC-823 (purchased by Chinese Academy of Sciences) was cultured in RPMI 1640 medium (Gibco) that contained 10% fetal bovine serum (FBS) and 10% penicillin/streptomycin, and maintained under an atmosphere of 5% CO_2 with humidity at 37℃.

Preparation of adenoviral vectors and the determination of multiplicity of infection (MOI)

The recombinant adenovirus vector that carried Ang-1 and control adenoviral vector that carried green fluorescent protein (GFP) were constructed and kindly provided by The First Affiliated Hospital of Nanjing Medical University[10]. The recombinant adenoviral vectors that expressed Ang-1 and GFP were amplified by infection of 293 cells. A suspension of 293 cells (5×10^8 cells/L) was added into the culture capsule. The linearized recombinant adenoviral vectors that expressed Ang-1 or GFP genes were co-transfected with Lipofectamine 2000 (Gibco) into 293 cells. The supernatant fluid that contained adenoviral vectors was transfected into more 293 cells after occurrence of a cytopathic effect. The viral extracts were propagated in 293 cells and were purified by CsCl (Sigma) density purification, and dialyzed and stored in dialysis buffer (Spectrum) with 10% glycerol at -70℃. Subsequently, the titer of each viral stock was measured by TCID50. 293 cells were seeded in 96-well plates (5×10^7 cells/L) for 24 h, and 100 μL dilute supernatant fluid that contained adenoviral vectors (10^{-1}-10^{-12}) was added respectively. Then, we calculated the titer of each viral stock after the cells were incubated with the complexes for 10 d. Subsequently, BGC-823 cells were seeded in 96-well plates (1×10^4cells/well). After 8 h exposure to control adenoviral vectors that contained medium at different MOI (10, 20, 30, 50 or 100) at 37℃, the medium was substituted with RPMI 1640 that contained 10% FBS. Forty-eight hours later, the cells that expressed GFP were counted by fluorescence microscopy to calculate efficiency of transfection. An MOI of 20 was the best.

Cell transfection

BGC-823 cells were seeded in 96-well plates (5×10^7 cells/L) 24 h before adenoviral transfection at 60%-70% confluence in medium that contained 10% FBS. The cells were infected at an MOI of 20 in serum-free medium with Ad-Ang-1 and Ad-GFP as a control adenoviral vector. Except for the above two groups, one additional control group was used, which consisted of uninfected cells.

Cell adhesion assay

Ninety-six-well plates were coated with 50 μL 10 mg/L fibronectin at 4℃. At the same time, 96-well plates were coated with 50 μL 10 mg/L BSA at 4℃ as a control ECM. A total of 1×10^5 cells in 100 μL of the medium were plated in 96-well plates at 37℃. After 1 h, 96-well plates were washed with PBS, and the medium was replaced with 50 μL 0.05% MTT solution and 150 μL RPMI 1640, and incubated for 4 h. After incubation, the MTT solution was removed, and the cells were suspended in 150 μL DMSO (Sigma). Absorbance was measured at 570 nm using a microplate reader (Bio-Rad Laboratories).

Invasion assays

The ability of BGC-823 cells to migrate through Matrigel-coated filters was measured using Transwell chambers with 8-μm pore polycarbonate filters coated with 300 μg Matrigel in the top side of the filter. BGC-823 cells were mock-transfected or transfected with Ad-GFP or Ad-Ang-1 (MOI = 20). After 36 h, the cells were trypsinized, resuspended in serum-free medium, seeded on the top

compartment of the chamber, and incubated for 24 h. At the end of incubation, the cells were stained, and the cells and Matrigel on the top surface of the filter were removed carefully with a cotton swab. The invasive cells that adhered to the bottom surface of the filter were quantified under a light microscope (× 200) (Nikon, Japan). The data were presented as the average number of cells attached to the bottom surface from randomly chosen fields. Each treatment condition was assayed using triplicate filters, and filters were counted at five areas.

Migration assay

The migration and invasiveness of BGC-823 cells were evaluated in six-well transwell chambers with upper and lower culture compartments that were separated by polycarbonate membranes with 8-μm sized pores. The three types of cells were detached, washed twice in PBS, and resuspended in serum-free RPMI 1640 medium. A total of 1×10^5 cells in 500 μL were placed in the upper chamber of a Millicell Insert (Millipore, USA), and the lower chamber was filled with 1.6 mL RPMI 1640/10% FBS. After a 4 h incubation period, the cells in the upper chamber that did not migrate were scraped away gently, and adherent cells on the lower surface of the insert were stained with hematoxylin and eosin and photographed. Triplicate assays were performed for each group of cells.

Reverse transcription polymerase chain reaction (RT-PCR) of integrin β1, CD44V6, uPA and MMP-2

Total RNA was isolated from BGC-823 cells using Trizol reagents (Invitrogen) according to the manufacturer's instructions, and utilized for RT-PCR. Primers designed for integrin β1 were as follows: forward, 5'-AATGAA GGGCGTGTTGGTAG-3', and reverse, 5'-AGACAC CACACTCGCAGATG-3'. Primers designed for CD44V6 were as follows: forward, 5'-GGCAACTCC TAGTAGTACAAC-3', and reverse, 5'-CAGCTGTCC CTGTTGTCGAAT-3'. Primers designed for uPA were as follows: forward, 5'-TCTGTGTGTGGGACTGAT GC-3', and reverse, 5'-GCCCTGACCTGAATCACA AT-3'. Primers designed for MMP-2 were as follows: forward, 5'-CTAGACAAGGGCCACAGACC-3', and reverse, 5'-GAGGAAGCAAACCTCGAACA-3'. Primers designed for β-actin, which was used as a loading control for the RT-PCR, were as follows: forward, 5'-CCACCC ATGGCAAATTCCATGGCA-3', and reverse, 5'-TCTAG ACGGCAGGTCAGGTCCAC-3'. After visualization of the PCR products by 20 g/L agarose gel electrophoresis with ethidium bromide staining gel, images were obtained and the densities of the products were quantified using a digital gel image analysis system (Pharmacia Biotech). The PCR fragments were identified according to their molecular mass using a DNA marker (DL 600 and 1200 marker, TaKaRa Biotech).

Western blotting

Western blotting was utilized for the detection of integrin β1 and CD44V6. Cell lysates were prepared and separated by 15% SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked for 1 h at room temperature in 5% milk solution. PAGE was detected by incubating the transferred membrane overnight at 4℃ with anti-human rabbit monoclonal antibody (Cell Signaling Technology, USA) at 1:100 dilution in 5% milk solution. Secondary antibody, anti-rabbit IgG/horseradish peroxidase, was added and cells were incubated for 2 h at room temperature. The signal was detected by the ECL detection system (Amersham), according to the manufacturer's protocol, and imaged digitally by Kodak Formatter (Kodak, Japan).

Immunocytochemistry for integrin β1 and CD44V6

BGC-823 cells were prepared as a suspension that contained 1×10^8 cells/L and were added to a six-well plate that contained many small glass slides. The cells were infected with recombinant adenovirus vector as before at 70% confluence on the glass slides. These small glass slides were taken out after 36 h. Expression of integrin β1 and CD44V6 was determined immunohistochemically with an anti-integrin β1 (Beijing Zhongshan Jinqiao Biotech), anti-CD44V6 mouse monoclonal antibody (Beijing Zhongshan Jinqiao Biotech), respectively, at a dilution of 1:100, and using the streptavidin-peroxidase technique with the SPtm kit (SP-9000; Maixin). Negative controls were prepared by substituting PBS for the primary antibodies, and known positive controls were included in each staining run. Finally, positive expression was measured *in situ* by Image-Proplus 4.5.

Statistical analysis

Data are presented as mean ± SE. Means among multiple groups were detected by one way ANOVA. Statistically significant differences were determined by Q test and were defined as $P < 0.05$.

RESULTS

Transfection efficiency of the adenovirus vector in BGC-823 cells

As MOI increased, the number of BGC-823 cells expressing GFP protein increased. None of cells could be transfected when MOI was 0 (Data was not shown). Nearly all the cells could be transfected when MOI was 20 (Figure 1A). However, became detached and died when MOI was 30 or 50 (Figure 1B and C).

Cell adhesion assay

We analyzed the effects of BGC-823 cells on cell adhesion after transfection with Ang-1. Compared to control group, the rate of adhesion of integrin β1 and CD44V6 in the Ad-Ang-1 group greatly increased ($P < 0.05$) (Table 1).

Invasion assay

Overexpression of Ang-1 was induced by Ad-Ang-1. There was no difference between the control and Ad-GFP groups (Figure 2A and B). The Ad-Ang-1-transfected cells showed significantly greater invasion than the mock-transfected and Ad-GFP groups (Figure 2C). These results

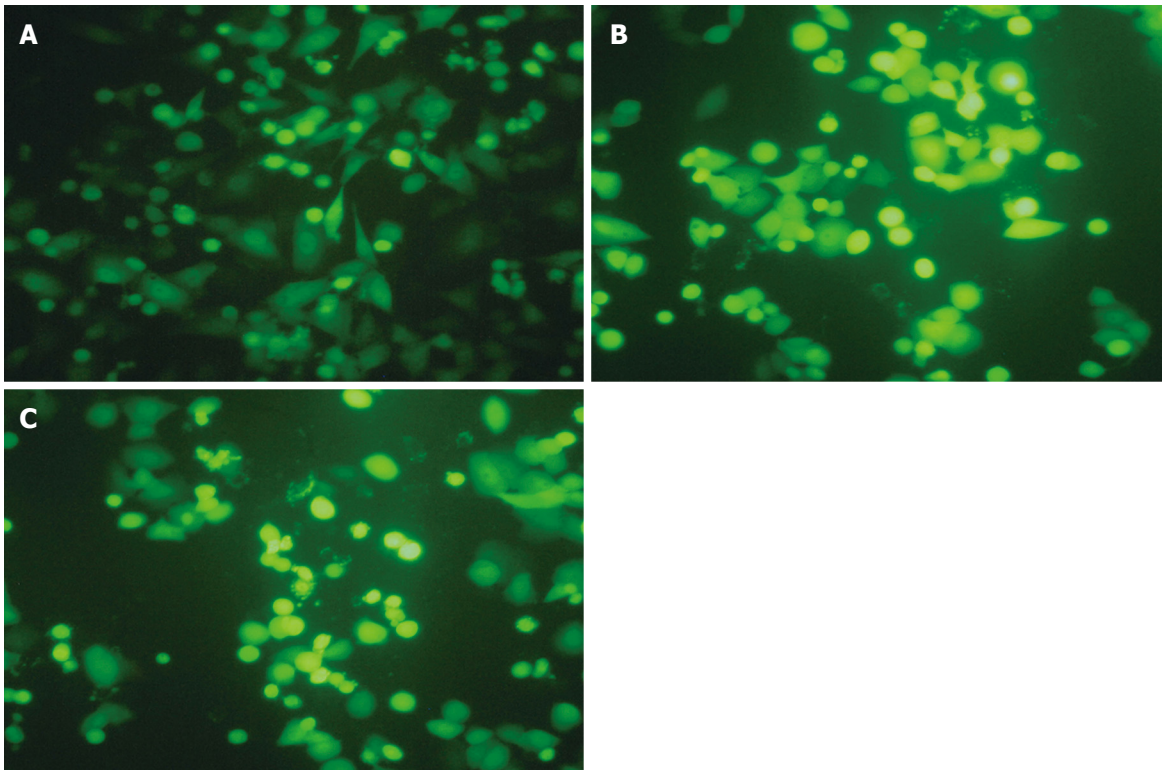

Figure 1 Expression of fluorescence in BGC-823 cells (× 200). As MOI increased, so did the number of BGC-823 cells that expressed GFP protein. A: Nearly all the cells could be transfected when MOI was 20; B: Most cells became detached and died at MOI = 30; C: Most cells became detached and died at MOI = 50. Diagrams are representive of at least three independent experiments.

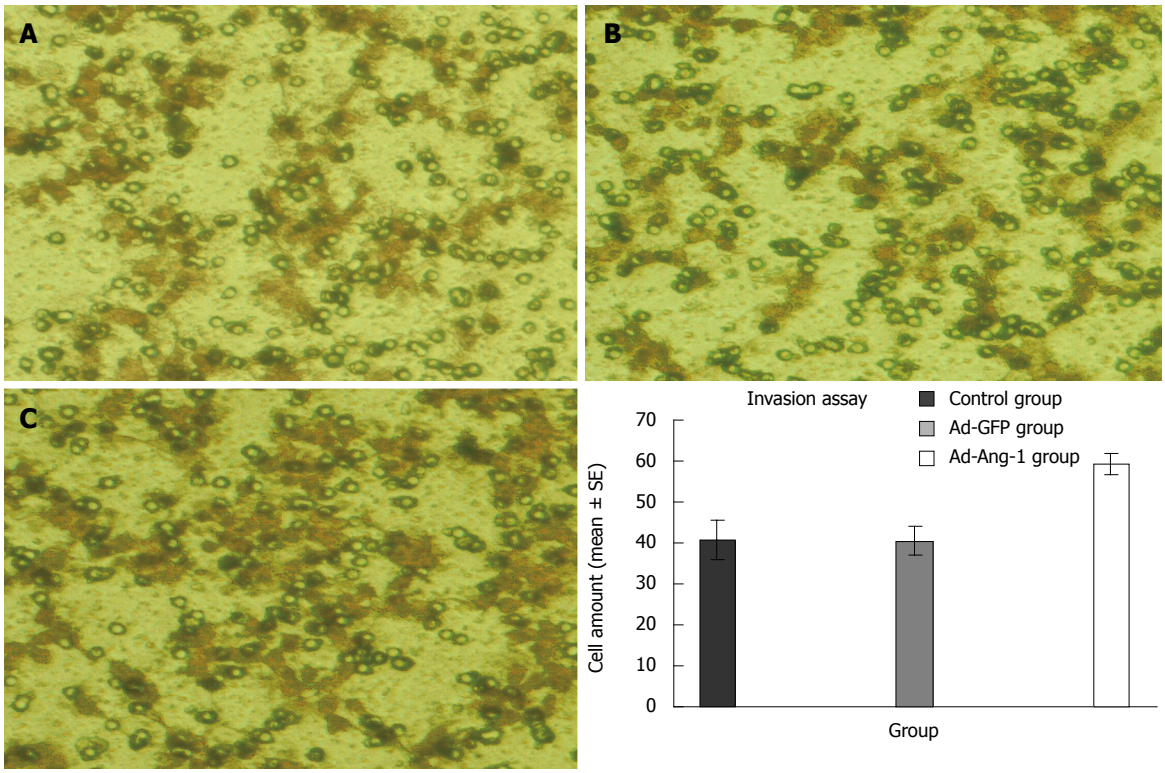

Figure 2 Invasion assay in BGC-823 cells (× 200). A: Control group; B: Ad-GFP-transfected group; C: Ad-Ang-1-transfected group. There was no difference between the control and Ad-GFP groups. Ad-Ang-1-transfected cells had a significant increase in invasion compared with the mock-transfected and Ad-GFP groups ($P < 0.05$). Diagrams are representive of at least three independent experiments.

provide evidence that Ang-1 enhances gastric carcinoma invasion ($P < 0.05$).

Migration assay

Cell migration was greater in the Ad-Ang-1 group than

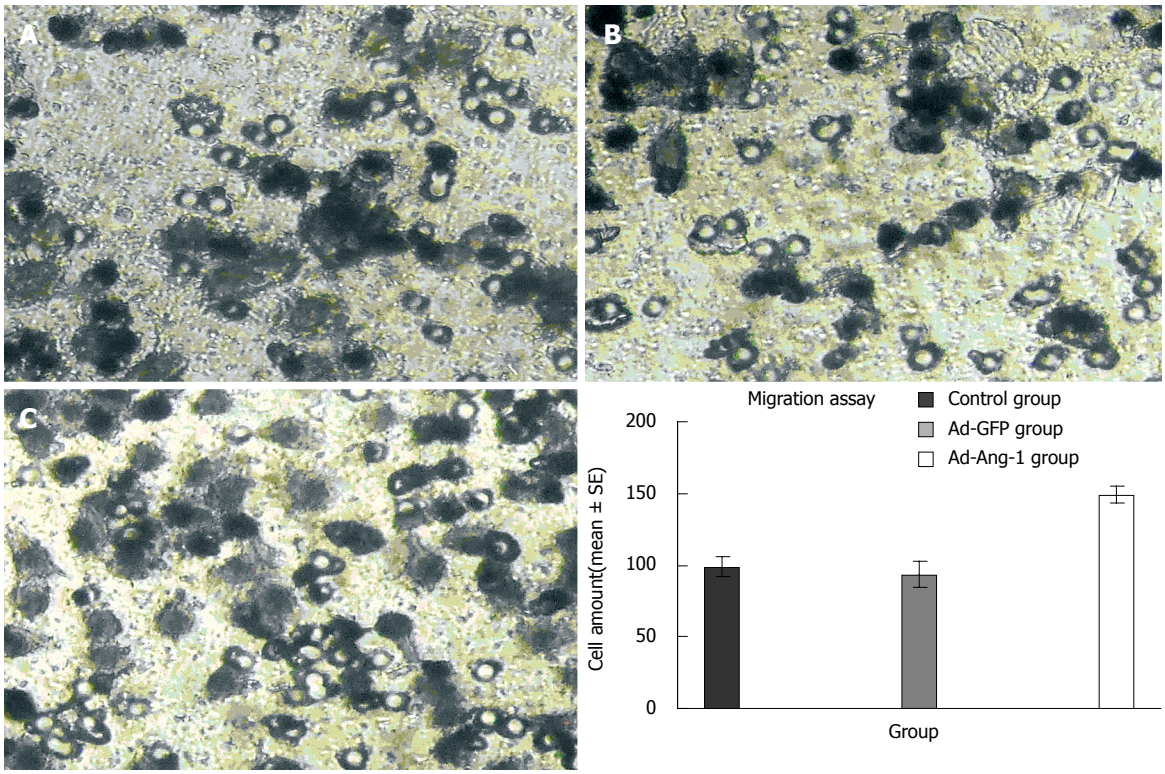

Figure 3 Migration assay in BGC-823 cells (× 200). A: Control group; B: Ad-GFP-transfected group; C: Ad-Ang-1-transfected group. There was no difference between the control and Ad-GFP groups. In the Ad-Ang-1 group, there were more cells than in the control and Ad-GFP groups ($P < 0.05$). Diagrams are representive of at least three independent experiments.

Table 1 Cell adhesion assay (mean ± SE)

Group	Experiment group cell A_{570}/BSA group cell A_{570}				Cell adhesion rate (%)
	1	2	3	4	
Control group	1.23 ± 0.071	1.28 ± 0.025	1.29 ± 0.032	1.28 ± 0.010	27.20
Ad-GFP group	1.172 ± 0.006	1.342 ± 0.036	1.264 ± 0.006	1.294 ± 0.016	26.80
Ad-Ang-1 group	1.756 ± 0.020	1.589 ± 0.007	1.502 ± 0.011	1.957 ± 0.005[a]	70.10

[a]Compared to control group and Ad-GFP group $P < 0.05$. Cell adhesion rate = (experiment group cell A_{570}/BSA group cell A_{570}-1) × 100%. There was no difference between the control and Ad-GFP groups. Compared to the control and Ad-GFP groups, the rate of adhesion of integrin β1 and CD44V6 in the Ad-Ang-1 group greatly increased ([a]$P < 0.05$).

in the control and Ad-GFP groups (Figure 3C), but there was no difference between the latter two groups (Figure 3A and B). These results demonstrate that Ang-1 enhances gastric carcinoma migration ($P < 0.05$).

RT-PCR of integrin β1, CD44V6, uPA and MMP-2

RT-PCR revealed that the mRNA expression of integrin β1, CD44V6, uPA and MMP-2 was increased in the Ad-Ang-1-transfected group ($P < 0.05$) (Figure 4).

Western blotting of integrin β1, CD44V6, uPA and MMP-2

Western blotting revealed that protein expression of integrin β1, CD44V6, uPA and MMP-2 was increased in the Ad-Ang-1-transfected group ($P < 0.05$) (Figure 5).

Expression of integrin β1 and CD44V6 protein

Integrin β1 and CD44V6 protein were both detected in BGC-823 cells in all three groups by using their respective antibodies. The staining intensity of integrin β1 in the Ad-Ang-1-transfected group was 0.183 ± 0.014, which was significantly higher than that in the control (0.114 ± 0.023, $P < 0.05$) and Ad-GFP-transfected (0.149 ± 0.013, $P < 0.05$) groups (Figure 6A). Compared with the other groups, expression of CD44V6 was also up-regulated (Figure 6B) ($P < 0.05$). The staining intensity of this protein in the Ad-Ang-1-transfected group (0.147 ± 0.011) was higher than that in the control (0.089 ± 0.007) and Ad-GFP-transfected (0.065 ± 0.021) groups.

DISCUSSION

The angiopoietin family of morphogens has an essential function in vascular and lymphatic growth and remodeling[11]. The role of Ang-1 is controversial. Ang-1 is sequestered by peri-endothelial and vascular smooth muscle cells, and acts specifically on endothelial cells

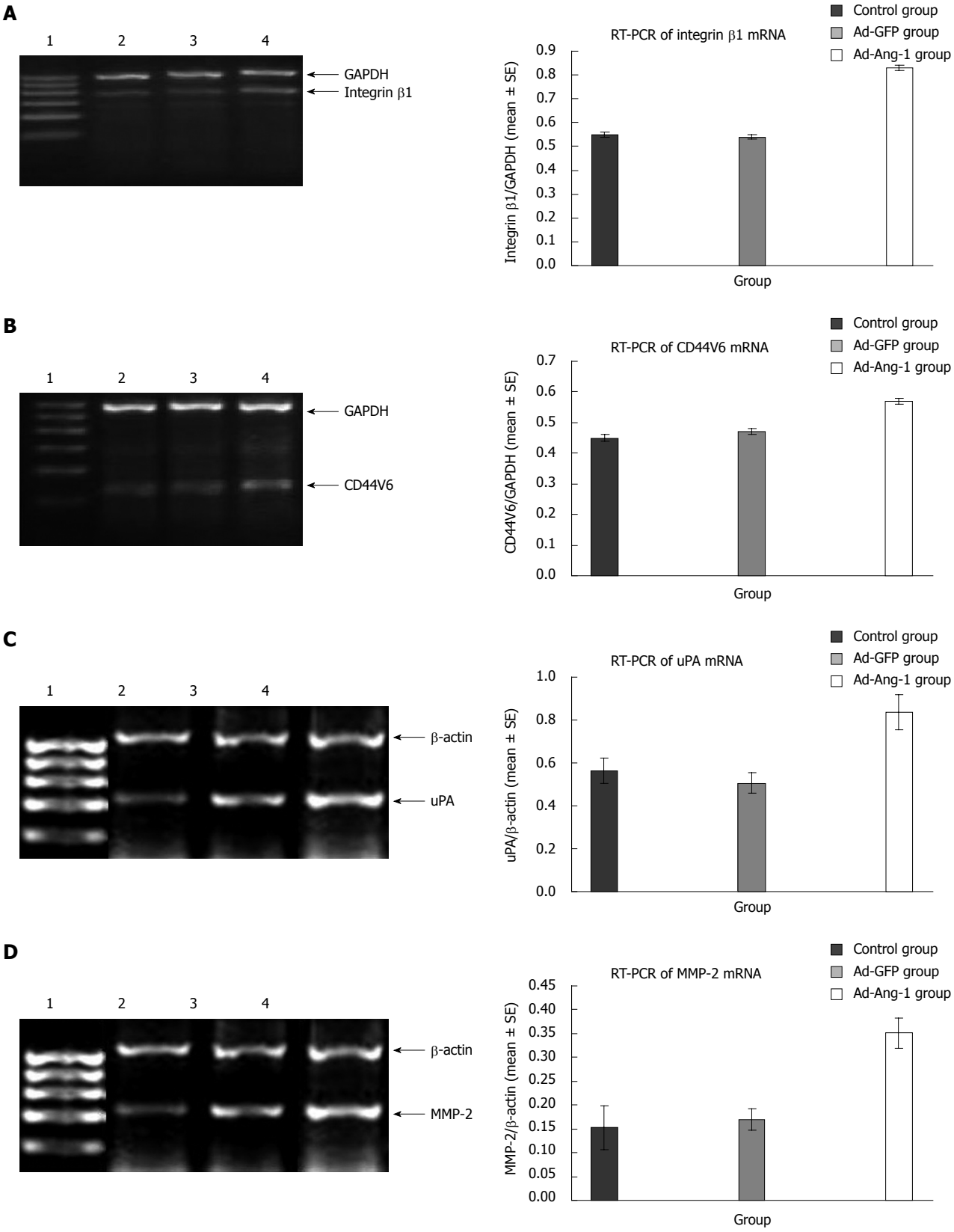

Figure 4 Expression of integrin β1 (A), CD44V6 (B), uPA (C) and MMP-2 (D) mRNA. 1: Markers: 100, 200, 300, 400, 500 and 600 bp; 2: Control group; 3: Ad-GFP-transfected group; 4: Ad-Ang-1-transfected group. A: Ang-1 group: 0.830 ± 0.010; control group and Ad-GFP group: 0.551 ± 0.009 and 0.543 ± 0.010, respectively; B: Ang-1 group: 0.570 ± 0.012; control group and Ad-GFP group: 0.451 ± 0.014 and 0.472 ± 0.013, respectively; C: Ang-1 group: 0.837 ± 0.081; control group and Ad-GFP group: 0.563 ± 0.059 and 0.506 ± 0.048, respectively; D: Ang-1 group: 0.351 ± 0.032; control group and Ad-GFP group: 0.153 ± 0.046 and 0.170 ± 0.023, respectively. These results show that the expression of integrin β1, CD44V6, uPA and MMP-2 mRNA was increased in the Ang-1 group ($P < 0.05$). Diagrams are representive of at least three independent experiments.

(ECs) through binding and activation of the cell surface tyrosine kinase receptor Tie2[12]. The angiopoietin family

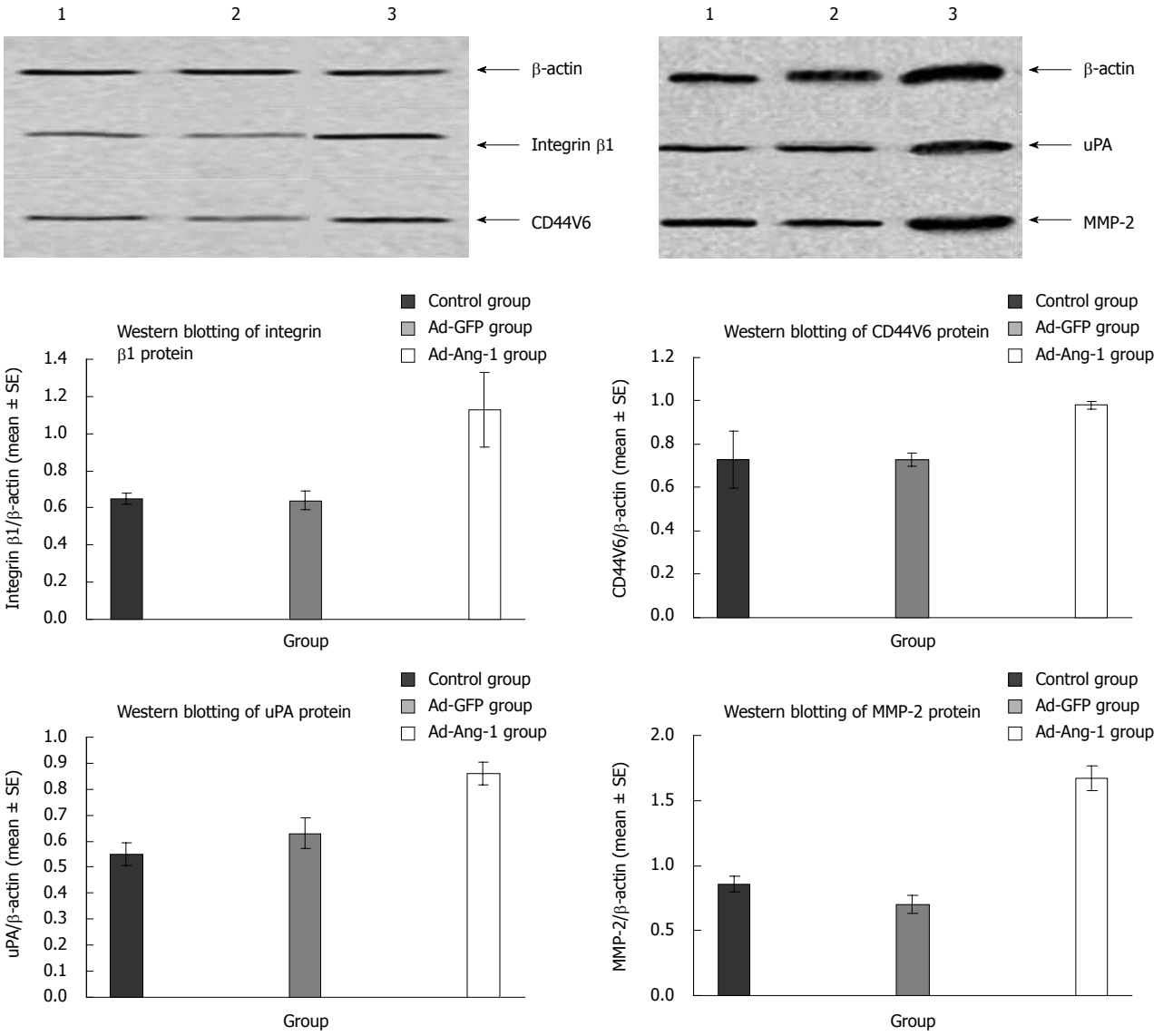

Figure 5 Expression of integrin β1 and CD44V6, uPA and MMP-2 protein. 1: Control group; 2: Ad-GFP-transfected group; 3: Ad-Ang-1-transfected group. After transfection with Ang-1, the protein level was greater than that in the control and Ad-GFP groups, but there was no difference between the control and Ad-GFP group. Diagrams are representive of at least three independent experiments.

of proteins contain an N-terminal coiled-coil domain, as well as a C-terminal fibrinogen-like domain that shares a high degree of homology to the analogous domains in the ECM proteins tenascin C and fibrinogen γ and β[1,2,13]. In a study of a pro-B cell line that stably expressed Tie2, it has been found that addition of Ang-1 to the culture medium enhanced cell adhesion to fibronectin[14]. In another study, fluorescence-activated cell-sorter Tie2-positive hematopoietic cells also responded to Ang-1 treatment with increased adherence to a fibronectin-coated substrate[15]. It has been shown recently that Ang-1 promotes cell adhesion[16], and that this process is mediated by α5-integrin in ECs[7]. Moreover, the finding that Ang-1 can bind ECM extracts from carcinoma cells[17] has offered new insights into understanding the role of Ang-1 in modulating the angiogenic microenvironment.

Cancer progression depends on an accumulation of metastasis-supporting genetic modifications and physiological alterations. Often, these physiological changes are regulated by cell signaling molecules, which target signal transduction pathways and ultimately, gene expression. Cell adhesion is the main step in cancer metastasis, and it is mediated by integrin heterodimers[18]. Cross-talk between integrins and growth factor receptors has been shown to coordinate biological processes through the regulation of downstream and inside-out signaling pathways[19-23]. Tyrosine kinase receptors and integrins share many downstream effectors.

Integrins have crucial roles in angiogenesis[24] and allow vascular cells to adapt their adhesive machinery to the so-called "provisional" ECM components, such as fibronectin, collagen and vitronectin, that are exposed by basement degradation around sprouting vessels[25]. Integrins αvβ3, αvβ5, α2β1 and α5β1 are upregulated in newly formed blood vessels[26,27], and αvβ3 and αvβ5 antagonists inhibit angiogenesis *in vitro* and *in vivo*[28,29]. Integrins can exist in different functional states that regulate their biological functions[30]. Additionally,

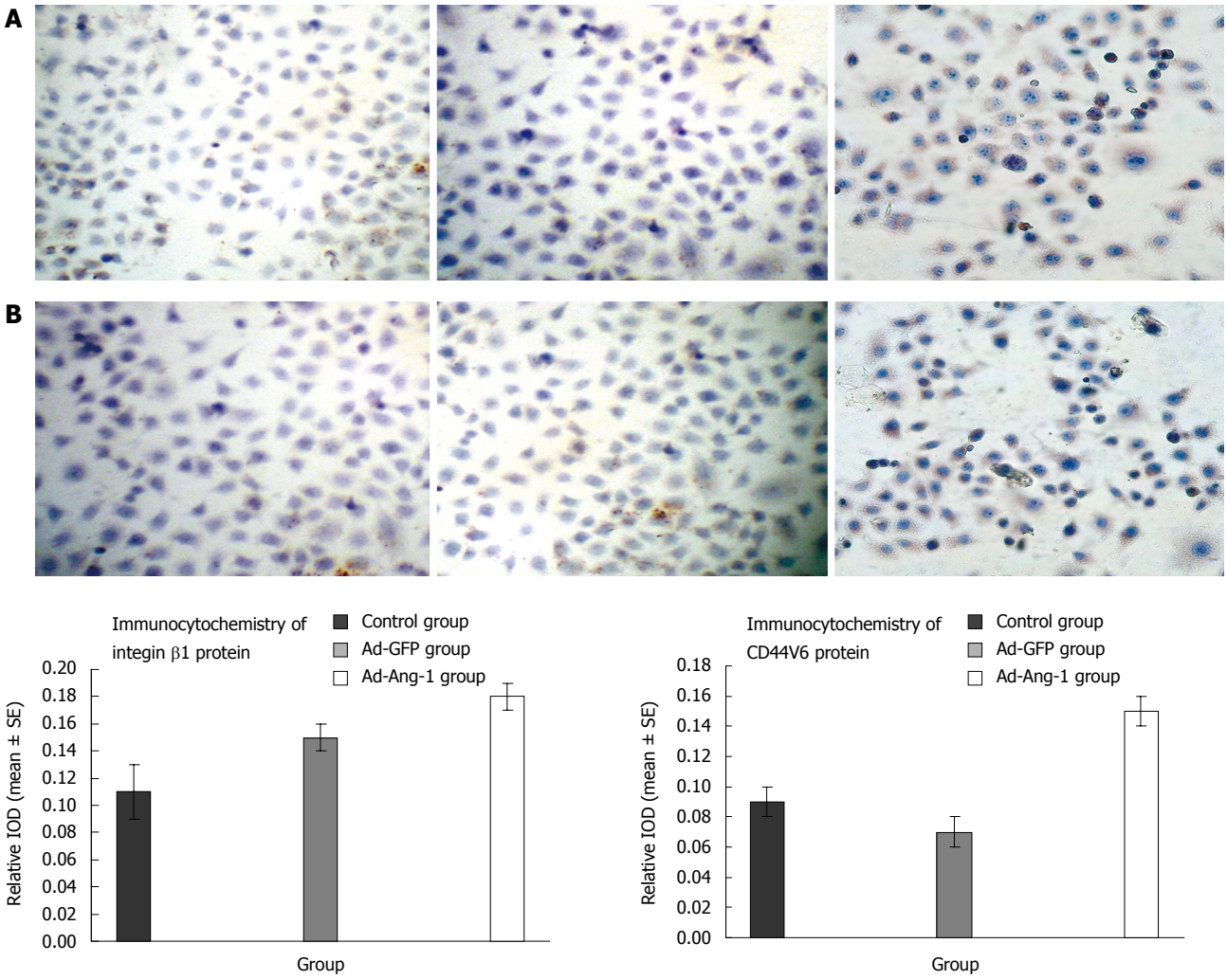

Figure 6 Expression of integrin β1 (A) and CD44V6 (B) protein (× 200). A: Expression of integrin β1 in the Ad-Ang-1-transfected group was 0.183 ± 0.014, which was significantly higher than that in the control group (0.114 ± 0.023, $P < 0.05$) and Ad-GFP-transfected group (0.149 ± 0.013, $P < 0.05$); B: Compared with the other groups, expression of CD44V6 was also up-regulated. The staining intensity for this protein in the Ad-Ang-1-transfected group (0.147 ± 0.011) was higher than that in the control group (0.089 ± 0.007, $P < 0.05$) and Ad-GFP-transfected group (0.065 ± 0.021, $P < 0.05$). Diagrams are representive of at least three independent experiments.

integrin β1 plays a key role in gastric cancer, and it can mediate cell adhesion.

Integrin β1 is a receptor of Ang-1, therefore, we speculate whether there is a relationship between integrin β1 and Ang-1. In addition, CD44 is a member of the immunoglobulin superfamily that is expressed on most epithelial and non-epithelial cells. Functionally, CD44 binds hyaluronate in the ECM to maintain tissue/organ structure, promote cell aggregation and mediate cell movement, and CD44 variant isoforms, especially CD44v6, have been identified as protein markers for metastasis in hepatocellular, breast, colorectal and gastric cancer[31-34]. Therefore, we speculate whether Ang-1 can affect the expression of CD44v6. Moreover, the expression of MMP-2 was stronger in metastatic tissues than in primary tumors. Up regulation of MMP-2 in neoplastic foci might be helpful to gastric carcinogenesis and metastasis[35]. In this model, the development of angiogenesis was highly dependent upon MMP-2 expression[36]. It has been reported that Ang-1 contributes to increased secretion of MMP-2 and decreased secretion of tissue inhibitors of metalloproteinase-2. Vascular endothelial growth factor (VEGF) and Ang-1 have an immunomodulatory role in airway remodeling[37]. uPA is a serine protease that functions in the conversion of the circulating zymogen plasminogen to the active, broad-spectrum serine protease plasmin. Plasmin, in turn, mediates the pericellular proteolysis of ECM components, and activates other proteases such as MMPs and collagenases, which leads to further degradation and remodeling of the ECM. It has been found that uPA levels are elevated in various malignancies, including breast, pancreatic, gastric, lung and colorectal carcinoma[38]. uPA expression is correlated with enhanced VEGF-induced tumor angiogenesis and may play a role in invasion and nodal metastasis of gastric carcinoma, thereby serving as a prognostic marker of gastric cancer[39]. Human gastric cancer cell lines express uPA mRNA and activity, which correlates with their peritoneal seeding potential[40]. Therefore, we also detected the expression of uPA and MMP-2. We explored the effects of Ang-1 on integrin β1, CD44V6, uPA and MMP-2 in the human gastric cancer cell line BGC-823, and studied the mechanism of adhesion and metastasis in gastric cancer.

We found that cell adhesion increased clearly in the Ad-Ang-1-transfected group. Therefore, we considered whether Ang-1 can affect the expression of integrin β1 and CD44V6 through cell adhesion. We found that mRNA and protein expression of integrin β1 and CD44V6 in BGC823 cells was enhanced, using RT-PCR, Western blotting and immunocytochemistry. We also found that Ang-1 affected the expression of uPA and MMP-2 to increase the invasiveness of gastric cancer. We suggest that there is a signaling pathway between Ang-1, integrin β1, CD44V6, uPA and MMP-2, and through this pathway, Ang-1 can affect the attachment and metastasis of carcinoma.

COMMENTS

Background

Gastric carcinoma is one of the most common cancers worldwide. The mortality of gastric carcinoma is currently rising faster than for any other cancer in China. About 60% of patients die from metastasis of gastric cancer. Progression of this disease may be associated with overexpression of angiopoietin-1 (Ang-1).

Innovations and breakthroughs

Recent reports have highlighted that Ang-1 has been found to bind integrin β1 and matrix metallopro-teinase-2 (MMP-2). Ang-1 may influence cell adhesion, invasion and migration. This is believed to be the first study to show that over-expression of Ang-1 can affect adhesion, invasion and migration of BGC823 cells through the expression of integrin β1, CD44V6, MMP-2 and urokinase-type plasminogen activator (uPA).

Applications

By understanding how Ang-1 affects attachment and metastasis, this study may represent a future strategy for therapeutic intervention in the treatment of patients with gastric carcinoma.

Terminology

Ang-1 is a member of the angiopoietin family. It has essential functions in vascular and lymphatic growth and remodeling. Integrin β1 and CD44V6 are members of the cell adhesion family. They mediated cell and extracellular matrix adhesion. MMP-2 and uPA are associated with tumor invasion and migration.

Peer review

The present study demonstrates a role for Ang-1 in adhesion, migration and invasion of human gastric cancer cells. Specifically, transient transfection of Ang-1 increases attachment and migration of this cell line. The experimental design is well thought out and the results are intriguing.

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