PIAS1 inhibited the metastasis of gastric cancer cell by epithelial-mesenchymal transition regulation within the inflammatory microenvironment

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Abstract. Protein inhibitor of activated signal transducer and activator of transcription-1 (PIAS1) is an important regulator of the inflammatory signaling network, the expression of which was decreased in gastric cancer and implicated in the development of cancer. However, its mechanism has not been elucidated. The aim of the present study was to investigate the effect of PIAS1 on epithelial-mesenchymal transition (EMT) of gastric cancer cells within the inflammatory microenvironment. Recombinant adenovirus Ad5/F35-PIASl and Ad5/F35-null plasmids were constructed to transfect SGC7901 cells. Subsequently, these plasmids were confirmed by reverse transcription polymerase chain reaction and western blotting. The cells were treated with IL-6 or Ad5/F35-PIASl+IL-6, and the control cells were treated with Ad5/F35-null+IL-6. The morphological changes to the cells were observed using inverted microscopy. The effect of PIAS1 on cell migration and invasion was evaluated by scratch wound healing and Transwell chamber assays, and the protein expression of EMT markers and phosphatidylinositol 3-kinase (PI3K)/serine/threonine-protein kinase (Akt)/matrix metalloproteinase (MMP)-9 signaling pathway was examined by western blotting. Transfection with Ad5/F35-PIASl markedly increased the PIAS1 expression in SGC7901 cells. The cells acquired the more typical spindle-shape phenotype of mesenchymal cells following co-culture with IL-6; the cells co-cultured with IL-6 and Ad5/F35-PIASl acquired changes concordant with an epithelial phenotype. The overexpression of PIAS1 significantly decreased the migratory and invasive capacities of the SGC7901 cells (P<0.01). Western blotting indicated that the expression levels of E-cadherin protein in the cells treated with Ad5/F35-PIASl+IL-6 were increased significantly and the expression levels of zinc finger protein SNAI, Twist-related protein 1, vimentin and MMP-9, and the activation of PI3K/Akt proteins were decreased when compared with IL-6- or Ad5/F35-null+IL-6-treated cells (both P<0.01). PIAS1 may inhibit EMT in gastric cancer cells within the inflammatory microenvironment via the regulation of PI3K/Akt pathway activation, and may serve an important role in the inhibition of tumor invasion and metastasis with in this microenvironment.

Introduction

Gastric cancer is a common disease and a leading cause of cancer-associated mortality. According to a statistical study, ~70% of patients with gastric cancer in 2006 had lymph node metastasis at the time of diagnosis, leading to a median overall survival time of 16.7 months (1). Despite the standardization of surgical techniques and multimodal therapy, the postoperative survival of patients with advanced gastric cancer remains low in China due to the rates of invasion and metastasis (2).

Epithelial-mesenchymal transition (EMT) is a process by which cells lose epithelial characteristics and acquire mesenchymal properties, including the loss of all cell-cell contact, increased motility and invasion (3). Previous studies have indicated that the EMT phenotype is associated with advanced gastric cancer stages, that EMT is a key gastric cancer progression driver and that it serves a fundamental role during the early stages of gastric cancer invasion and metastasis (4). Investigating the potential mechanisms that modulate gastric cancer cell EMT and characterizing novel EMT regulators will increase the understanding of gastric cancer biology; it may also aid identification of novel biomarkers for the early detection of gastric cancer and of potentially efficient targets for preventative and curative anti-gastric cancer intervention approaches, preventing local and distant invasions (5).

Interleukin-6 (IL-6) is a cytokine that participates in acute inflammation (6). A previous study has indicated that IL-6

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led to EMT-associated changes via zinc finger protein SNAI (Snail) signaling pathway activation, and is involved in tumor progression, which is an important factor in tumor development (7). Elevated levels of IL-6 are associated with poor prognosis for a number of types of cancer (8).

Previous data have indicated that protein inhibitor of activated signal transducer and activator of transcription 1 (PIAS1), a downstream target protein of the signal transducer and activator of transcription (STAT) signaling pathway inhibitor, is associated with the anti-inflammatory response through the negative regulation of the STAT1 signaling pathway, which mediates inflammatory cell adhesion and inhibits inflammatory injury (9). A previous study demonstrated that the expression of PIAS1 in certain cancer cells was significantly downregulated or lost altogether (10). Therefore, PIAS1 functions as an inflammatory inhibitor and serves a role in the inhibition of cancer cell growth. A previous study indicated that the PIAS1 expression was primarily observed in the tissues of patients with gastric cancer, indicating that PIAS1 may be involved in the pathogenesis of cancer and verifying that PIAS1 may act as a marker for the preclinical detection and clinical assessment of patients with gastric cancer (11). Concurrently, the overexpression of PIAS1 protein was also demonstrated to inhibit the migration and invasion of gastric cancer cells (11). However, the mechanisms of its effect of gastric cancer were unclear.

On the basis of the aforementioned previous results, we hypothesized that the effects of PIAS1 may be due to the suppression of EMT by the phosphatidylinositol 3-kinase (PI3K)/serine/threonine kinase (Akt)/Snail signaling pathway and downregulated matrix metalloproteinase 9 (MMP-9) expression, which causes suppression of cell growth *in vitro*. To examine this hypothesis, the gastric cancer SGC7901 cell line was used, and the migration response of cells with upregulated PIAS1 expression was studied, indicating its potential effective mechanism for gastric cancer treatment.

Materials and methods

Reagents. A total of 10 rabbit or mouse monoclonal antibodies against epithelial (E)-cadherin (cat. no. SC71009), vimentin (cat. no. SC6260) were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA), Snail (cat. no. 3879), Twist-related protein 1 (Twist 1; cat. no. 46702), PIAS1 (cat. no. 3550), PI3K (cat. no. 4292), phosphorylated (P)-PI3K (cat. no. 4228), Akt (cat. no. 4685), P-Akt (cat. no. 4060) and MMP-9 (cat. no. 2270) were purchased from Cell Signaling Technology (CST; Beverly, MA, USA). Mouse monoclonal antibody against GAPDH (cat. no. AF1186) and horseradish peroxidase-conjugated goat anti-rabbit or mouse IgG were purchased from Shanghai Biyuntian Bio-Technology Co., Ltd. (Shanghai, China). Recombinant human IL-6 was produced by R&D Systems, Inc. (Minneapolis, MN, USA).

The human gastric cancer SGC7901 cell line used in the present study was provided by Ruijin Hospital, Shanghai Jiaotong University School of Medicine (Shanghai, China) and maintained in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.), with addition of 100 U/ml penicillin and 100 μg/ml streptomycin

(Sigma-Aldrich; Merck, KGaA, Darmstadt, Germany) at 37°C and 5% CO_2.

Construction of expression plasmid. A replication-defective adenovirus serotype 5/F35 (Ad5/F35) was used as the vector (AGTC Gene Technology Co., Ltd., Beijing, China). Ad5/F35-PIAS1 was constructed by Shanghai Hongming Biotechnology Co. Ltd. (Shanghai, China) as follows: PIAS1 cDNA containing the full-length translated regions was obtained using the polymerase chain reaction with the following primers: Forward (EcoR V site is underlined); 5'-GCCGATATCATGGCGGACAGTGCGGAACTAAAG CAAATG-3' and 5'-ATTAAGCTTTCAGTCCAACGA GATAATGTCTGGTATAGT-3' (reverse, HindIII site is underlined). The sequence of PIAS1 was deposited in the GenBank database (cat no. AF167160.1) prior to being sub-cloned into 5 ul PDC316-MCMV-EGFP transfer plasmid (Microbix Biosystems Inc., Ontario, Canada) with green fluorescence by insertion of a fragment of green fluorescence protein (GFP) as previously described (12). This plasmid was co-transfected [multiplicity of infection (MOI), 10] into 293 cells (Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China) using lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), that were grown in Dulbecco modified Eagle medium plus 10% fetal calf serum (FBS; Gibco; Thermo Fisher Scientific, Inc.), penicillin, and streptomycin at 37°C in a 5% CO_2 incubator. Additionally, an Ad5/F35 plasmid containing an empty expression cassette was constructed for use as a control. All of the viral constructs were similar, with the exception of the PIAS1 gene, and the production and purification procedures were identical to our previous study (13).

Cell culture and gene transduction. When the SGC7901 cells reached 70% confluence, the process of transfection of Ad5/F35-PIAS1 (MOI, 10) was performed in Ad5/F35-PIAS1-treated cells. Concurrently, an additional group of SGC7901 cells were transfected with MOI 5 of the empty Ad5/F35-vector as Ad5/F35-null-treated cells. A third group of cells that underwent PBS treatment served as control cells. The samples were harvested for additional experiments at 24 h following transduction.

Fluorescence microscopy. The Ad5/F35-PIAS1-treated cells, Ad5/F35-null-treated cells and control cells were placed on glass slides and covered with a cover glass and the fluorescence of GFP was observed using an Olympus IX51 fluorescence microscope (Olympus Corporation, Tokyo, Japan).

Establishment of cell inflammatory microenvironment and groups. In the present study, the cells were divided into three groups: i) The IL-6-treated group: IL-6 (R&D Systems, Inc.). was added to FBS-free medium at a concentration of 50 ng/ml for 72 h in SGC7901 cells; ii) the Ad5/F35-PIAS1+IL-6-treated group: Ad5/F35-PIAS1 (MOI10) was added to SGC7901 cells following 48 h of IL-6 treatment; iii) the Ad5/F35-null+IL-6-treated group: Ad5/F35-null (MOI 5) was added to SGC7901 cells after 48 h of IL-6 treatment. The samples were harvested for additional experiments at 24 h following transduction.

Assessment of cell viability by MTT assay. A total of $2x10^3$ SGC7901 cells from each group were seeded onto a 96-well plate. After 24 h, 20 μl MTT (5 mg/ml) was added to each well. After 4 h, 100 μl dimethyl sulfoxide was added to each well subsequent to removal of the medium. Finally, the absorbance was detected with an enzyme calibrator at 570 nm and the cell viability (%) was calculated: (A_{570} of study group/A_{570} of control group) x100.

PIAS1 expression in cells by reverse transcription polymerase chain reaction (RT-PCR). Total RNA was isolated from cells of each group using a TRIzol® reagent kit (Life Technologies; Thermo Fisher Scientific, Inc.). Samples were analyzed with the Tiangen PCR purification kit (Tiangen Biotech Co., Ltd., Beijing, China), and the cDNA obtained from this reaction was mixed with a 50-μl reaction volume containing: 1X PCR buffer with 1.5 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphates (dNTPs), 0.05 U/μl Taq polymerase and 2 μM human PIAS1 gene-specific primers (PIAS1 forward primer: 5'-CCACGCCTTCCTGCTGTAGA-'3; PIAS1 reverse primer: 5'-TATCACACAGGCAGTCTTAGAT-'3) and amplified in an automated thermal cycler. The conditions of RT-PCR were as follows: 1 cycle for 5 min at 95°C; then 35 cycles for 45 sec at 94°C, 45 sec at 55°C, and 1 min at 72°C; then 1 cycle for 10 min at 72°C. The PCR products were separated by electrophoresis using 1.2% agarose gels and stained with ethidium bromide. The densities of cDNA bands were analyzed by scanning densitometry using the Image Analysis Gel Doc 2000 system and Quantity One software (version 4.4.0; Bio-Rad Laboratories, Inc., Hercules, CA, USA). The band densities were normalized to the GAPDH (the primer sequence of GAPDH, the forward primer: 5'GGCTGAGAACGGGAAGCTTGTC'3; the reverse primer: 5'CAGCCTTCTCCATGGTGGTGAAGA'3) band densities and the results were expressed as a ratio.

Scratch wound-healing assay. To measure cell motility, $4x10^5$ SGC7901 cells from each group were seeded in 6-well plates. A central linear wound was created by scraping the cell monolayer with a 200 μl sterile pipette tip. The media were carefully changed to remove any floating cells and cells were cultured at 5% CO_2 and 37°C for 24 h prior to fixation with 4% paraformaldehyde for 1 h at room temperature. The migration of cells into the empty areas in the scraped region was observed at 24 h and images of each plate were captured with a light inverted microscope (Olympus IX53; magnification, x200; Olympus Corporation, Tokyo, Japan) fitted with a digital camera (Olympus cellSens Standard; Olympus Corporation). The wound at 0 h was considered to be 100% of the average gap, and the percentage of the cleaned area at 24 h compared with time 0 was measured using Image Pro plus version 6.2 software (Media Cybernetics, Rockville, MD, USA).

Cell invasion assay. For the determination of cell invasion capabilities, Transwell chambers (Corning Incorporated, Corning, NY, USA) were used. The Transwell chamber membranes were covered with 75 μl Matrigel (2 mg/ml; BD Biosciences, Franklin Lakes, NJ, USA) and incubated for 2 h at 37°C. The SGC7901 cells from each group were grown in RPMI-1640 (10% FBS) medium were trypsinized and suspended at a density of $1x10^6$/ml in serum-free RPMI-1640 medium. A total of 200 μl cell suspension was placed into the upper compartment of the Transwell chambers. The lower compartment of the chambers was filled with 600 μl RPMI-1640 containing 10% FBS. The Transwell chamber systems were incubated in the humidified incubator at 37°C and 5% CO_2 for 24 h. Following incubation, non-invaded cells and Matrigel were removed from the top of the chamber with a cotton bud. At the bottom of the cell membrane, invaded cells were washed three times with PBS, fixed with 4% paraformaldehyde for 1 h at room temperature, prior to the paraformaldehyde being discarded. Subsequently, the membranes were air-dried and stained with 1% Giemsa solution for 15 min at room temperature. A total of 5 random fields were observed under an inverted microscope (magnification, x200) and the invasion rate was calculated to find the number of the invaded cells. The data representing the average cells of 5 fields were compared between the experimental and control groups. Each experiment was repeated three times.

EMT alterations of cells. The cell morphology in each group was observed in each of 5 randomly selected high-power fields using an inverted light microscope (Olympus IX53; magnification, x200; Olympus Corporation) fitted with a digital camera (Olympus cellSens Standard; Olympus Corporation).

Western blotting analysis. The SGC7901 cells from each group were washed twice with PBS and then homogenized in radio immunoprecipitation assay buffer. Following centrifugation at 12,000 x g at 4°C for 10 min, the supernatant was collected and stored at 80°C. The protein concentration of each sample was determined by BCA protein assay. Each sample was adjusted up to a desired protein content of 40 μg per lane, denatured in loading buffer and separated by electrophoresis on 9% SDS-PAGE at 100 V for 120 min. The separated proteins were transferred to polyvinylidene difluoride membrane using transfer buffer at 200 mA for 90 min. The membranes were blocked with 5% non-fat dry milk powder in TBS-0.1% Tween-20 for 1 h at room temperature, washed three times for 10 min each in TBS-0.1% Tween-20, and incubated with primary antibodies against E-cadherin, Snail, Twist 1, vimentin, PI3K, P-PI3K, Akt, P-Akt and MMP-9 with 1:1,000 dilution in TBS-0.1% Tween-20 overnight at 4°C. Membranes were washed three times for 10 min in TBS-0.1% Tween, the membranes were incubated with a second antibody, horseradish peroxidase-conjugated goat anti-rabbit IgG (cat. no. A0208; dilution, 1:500) or goat anti-mouse IgG (cat. no. A0216; dilution, 1:500) for 1 h at room temperature. Subsequent to washing three times with PBS, the membranes were detected using an Amersham enhanced chemiluminescence Western Blotting Detection kit (Amersham; GE Healthcare, Chicago, IL, USA), and then underwent densitometry using the Image Analysis Gel Doc 2000 system and Quantity One software version 4.4.0. GAPDH was determined in the similar manner with anti-GAPDH antibody (dilution, 1:500) as an endogenous control for other proteins.

Statistical analysis. All data were expressed as the mean ± standard deviation. Statistics were performed by SPSS version 13.0 (SPSS, Inc., Chicago, IL, USA). One-way analysis of variance with Dunnett's multiple comparison tests was used
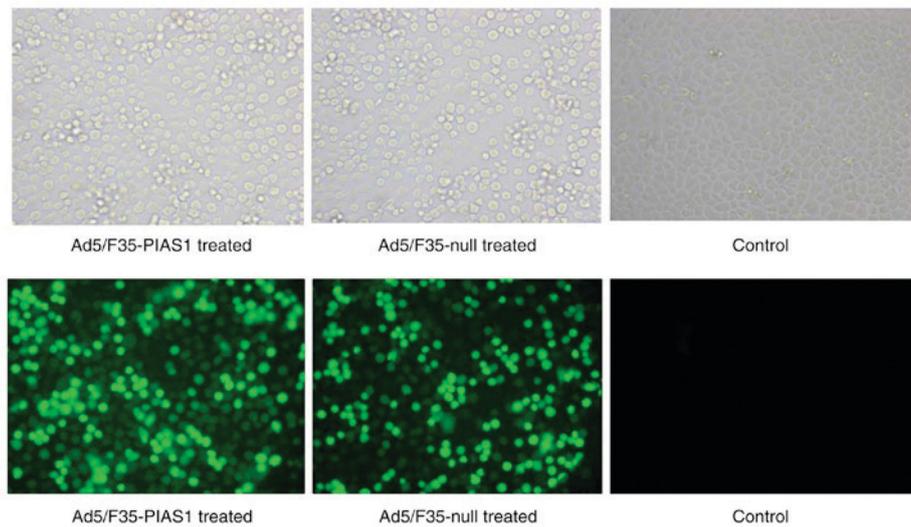
Figure 1. Levels of fluorescence intensity of Ad5/F35-PIAS1, Ad5/F35-null and PBS (control) treatments were investigated in SGC7901 cells by immunofluorescence microscopy (magnification, x200). PIAS1, protein inhibitor of activated signal transducer and activator of transcription 1.
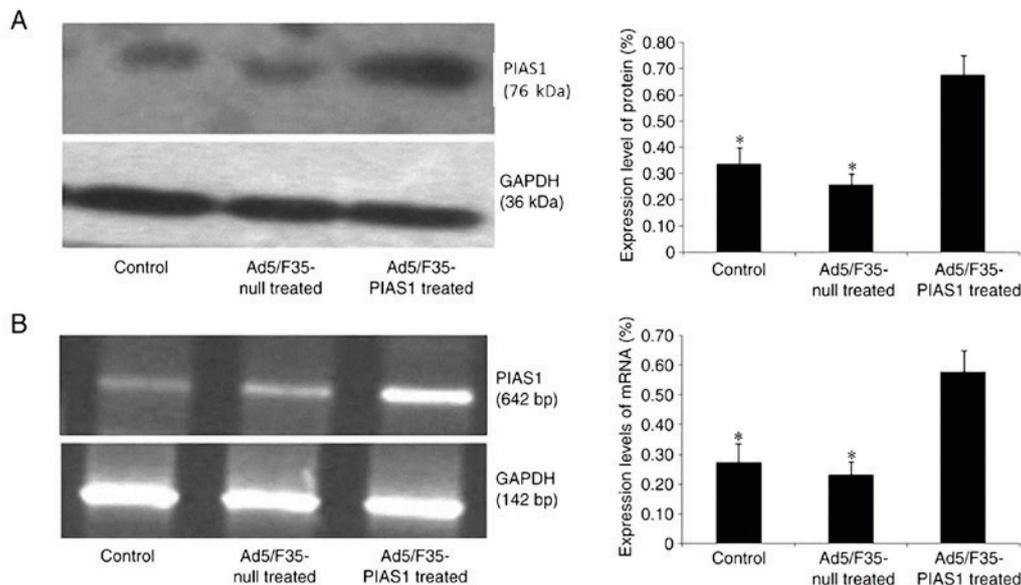

Figure 2. Evaluation of PIAS1 expression was investigated in SGC7901 cells in each group. (A) Western blot analysis demonstrated the increased expression of PIAS1 in SGC7901 cells following transfection with Ad5/F35-PIAS1 at 24 h. The control cells and the cells transfected with Ad5/F35-null indicated low expression levels of PIAS1 at 24 h. These results indicated similar changes to the results of the (B) reverse transcription-polymerase chain reaction analysis. *P<0.01 vs. Ad5/F35-PIAS1 treated group. PIAS1, protein inhibitor of activated signal transducer and activator of transcription 1.

to perform comparisons between groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Transfection capability of Ad5/F35-PIAS1. According to the results of immunofluorescence microscopy, the cells of Ad5/F35-PIAS1 and Ad5/F35-null transfection exhibited high fluorescent intensity, but the control cells demonstrated a fluorescent intensity (Fig. 1).

PIAS1 gene mRNA and protein expression in gastric cancer cell. According to western blotting and RT-PCR analyses, following 24 h of Ad5/F35-PIAS1 transfection, the PIAS1

protein (Fig. 2A) and mRNA (Fig. 2B) expression levels in SGC7901 cells were significantly increased when compared with control and Ad5/F35-null-transfected cells (P<0.01).

Assessment of cell viability. The cell viability rate of Ad5/F35-PIAS1+IL-6 cells (42.2±12.3%) was significantly decreased compared with that of IL-6 (72.4±11.2%) or Ad5/F35-null+IL-6 (69.7±9.8%)-treated cells (P<0.01) (Data not shown).

Cell invasion and migration assay. IL-6 treatment increased the migratory activity of the SGC7901 cells, and treatment with Ad5/F35-PIAS1 inhibited IL-6-induced migration. The scratch wound healing assay indicated that
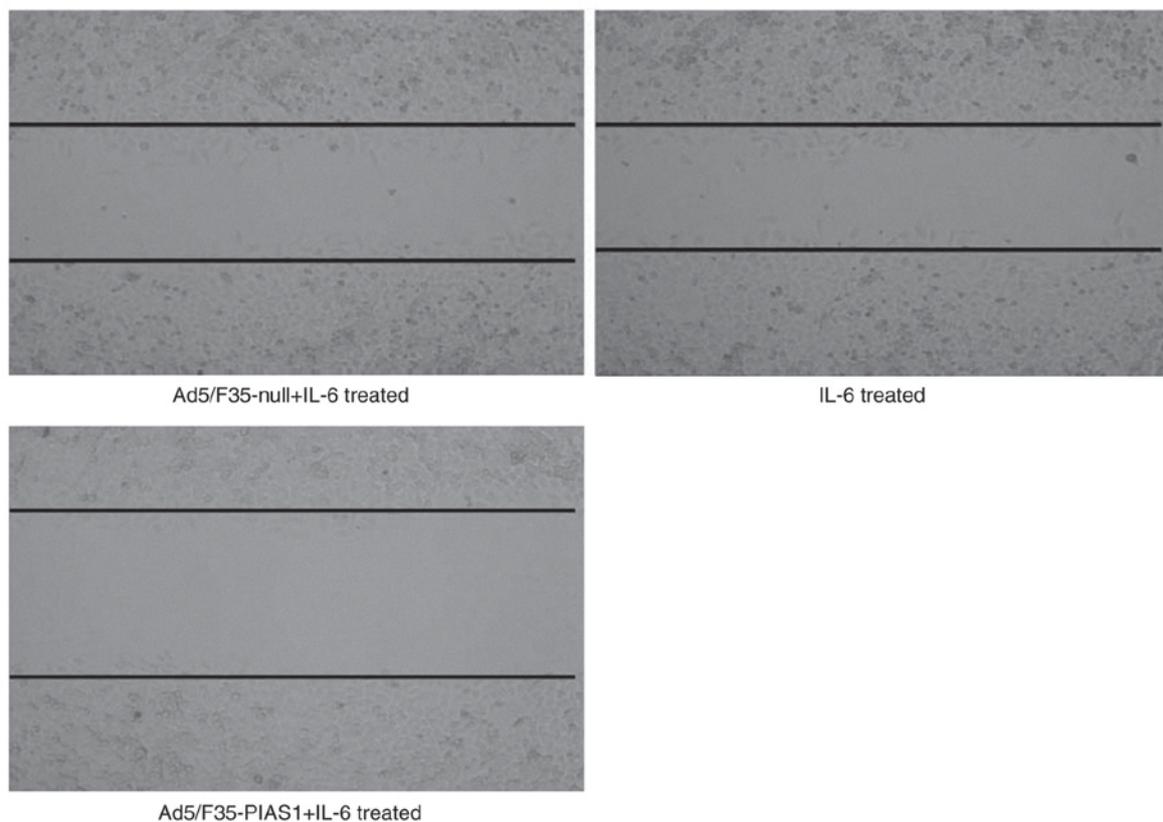
Figure 3. Migration of SGC7901 cells investigated by scratch wound healing assay in each group at 24 h following transduction. (magnification, x200). IL-6, interleukin-6.

Ad5/F35-PIAS1+IL-6-treated cells (9.80±2.22%) exhibited a slower migration rate compared with IL-6 (19.03±2.70%) and Ad5/F35-null+IL-6-treated cells (18.42±4.42%; Fig. 3). Similarly, the Transwell migration assay demonstrated that the Ad5/F35-PIAS1+IL-6-treated cells (28.44±3.57) were associated with significantly lower migration than IL-6- (54.44±7.74) and Ad5/F35-null+IL-6-treated cells (53.22±11.50; P<0.01; Fig. 4).

EMT alterations in gastric cancer cells. The spacing of IL-6- and Ad5/F35-null+IL-6-treated SGC7901 cells was more spread out and had lost their cell-cell contacts, whereas the Ad5/F35-PIAS1+IL-6-treated cells remained tightly attached with typical epithelial cell characteristics (Fig. 5).

Effect of PIAS1 on EMT in gastric cancer. To confirm the effect of PIAS1 on the change in EMT of gastric cancer cells, western blotting was performed for EMT markers; the results indicated that the protein expression of Snail, Twist 1 and vimentin were decreased in Ad5/F35-PIAS1+IL-6-treated cells; however, the E-cadherin protein expression level was increased when compared with those of IL-6 and Ad5/F35-null+IL-6-treated cells (P<0.01, respectively; Fig. 6).

PIAS1 suppresses EMT by inhibiting the PI3K/Akt-MMP-9 signaling pathway. As demonstrated by western blotting, IL-6 promoted the activation of PI3K-Akt and increased MMP-9 protein expression. However, a decrease in the expression levels of total PI3K and Akt protein was observed in the

Ad5/F35-PIAS1+IL-6-treated cells when compared with those of IL-6 and Ad5/F35-null+IL-6-treated cells (P<0.01, respectively). Densitometry results also suggested that the expression levels of MMP-9 protein in the Ad5/F35-PIAS1+IL-6-treated cells were significantly decreased compared with that of the IL-6 and Ad5/F35-null+IL-6-treated cells (P<0.01, respectively; Fig. 7).

Discussion

Gastric cancer was the fifth most common cancer world wide, comprising 6.8% of the total number of new cases diagnosed in 2012 (14). Despite previous advances in surgical techniques and the development of chemotherapy and radiotherapy, the mortality of gastric cancer remains high, with a 5-year survival rate of <30% (15). To provide data that will enable the development of novel therapeutic strategies, it is crucial to elucidate the molecular mechanisms that promote the migration and invasion properties of gastric cancer. Metastasis is defined as the process of dissemination of cancer cells from their origin to a distant organ, a complex process involving several stages, which are as follows: i) The activation of EMT; ii) local invasion; iii) intravasation; iv) the ability to survive in the bloodstream; v) extravasation, whereby tumor cells exit the bloodstream; and vi) establishment of tumor cells in the tissues of a distant organ (16).

EMT is a multistage reprogramming process that is also important in the physiological process of embryogenesis. During EMT, epithelial cells are closely arranged and
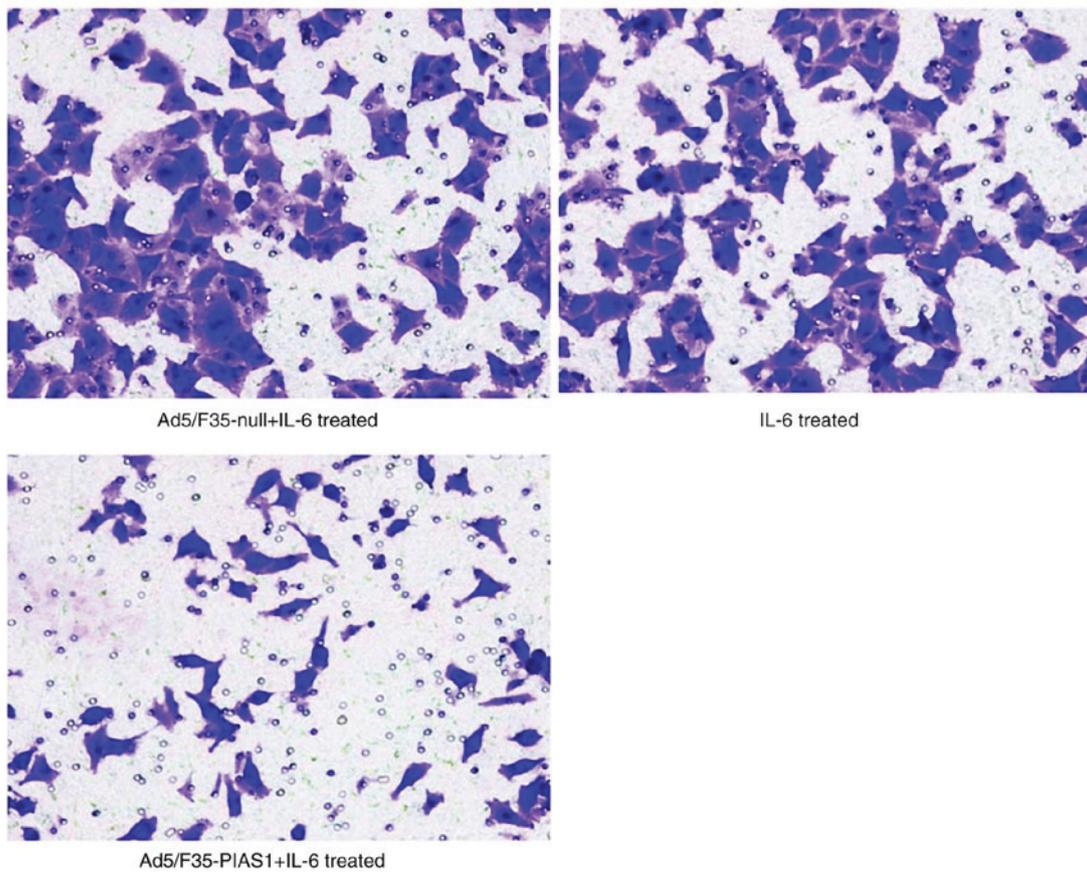
Figure 4. Invasion of SGC7901 cells were detected using Transwell chambers in each group at 24 h following transduction. (magnification, x200). IL-6, interleukin-6.
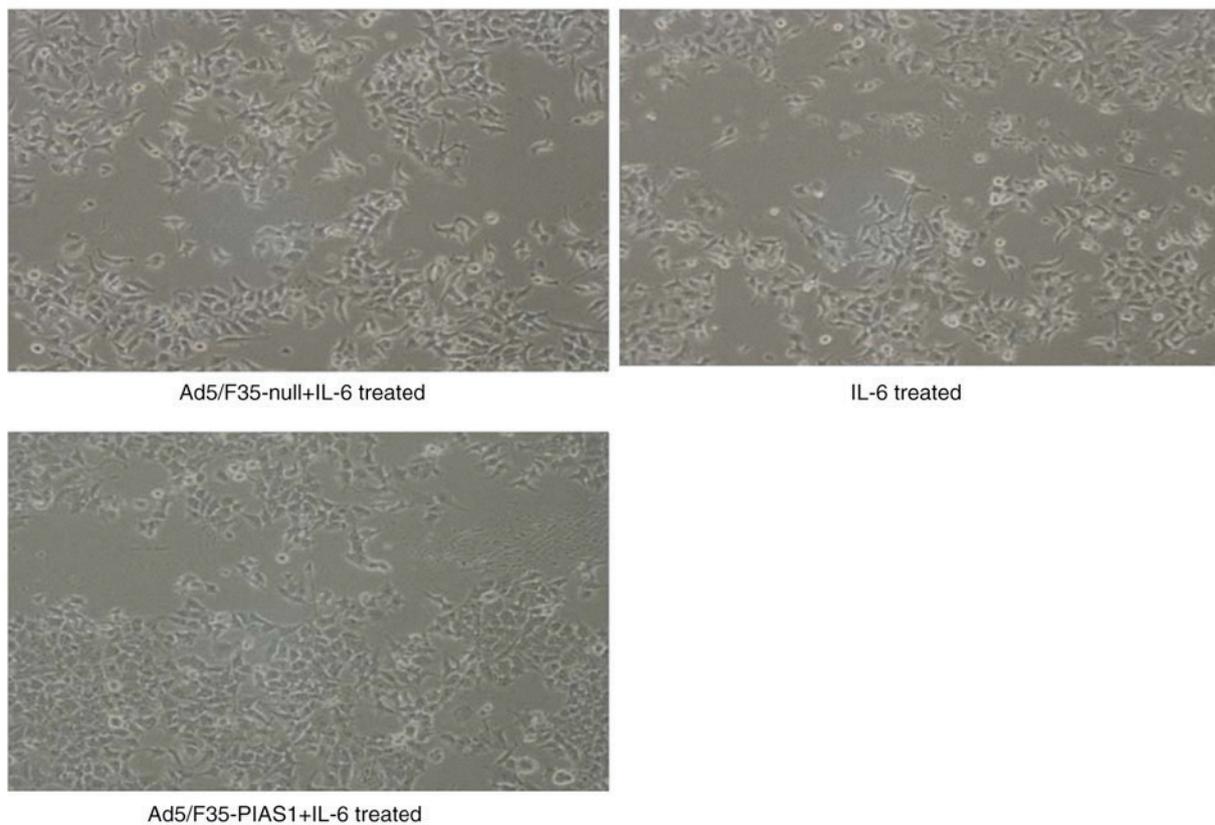

Figure 5. Epithelial-mesenchymal transition in SGC7901 cells was investigated in each group at 24 h following transduction. (magnification, x200). IL-6, interleukin-6.
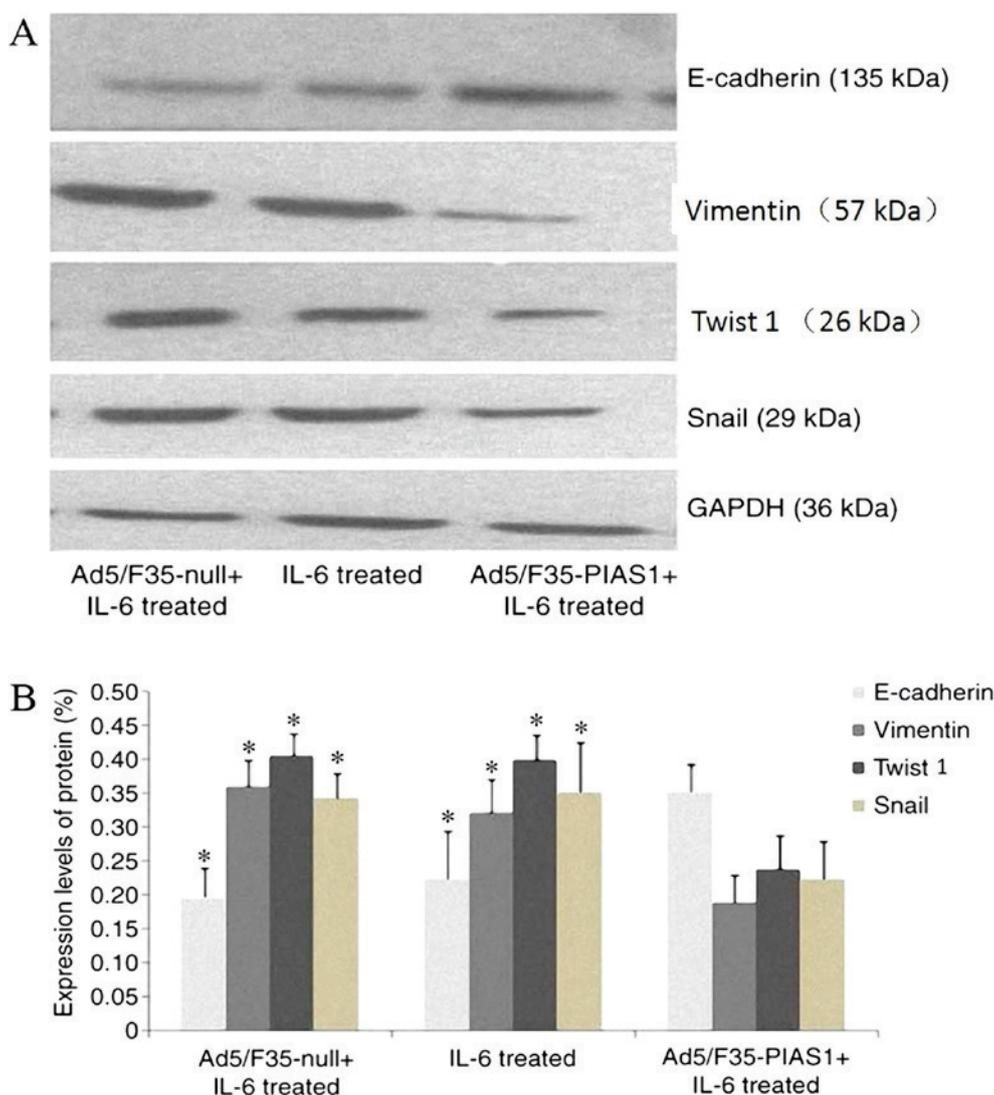
Figure 6. Expression levels of proteins involved in epithelial-mesenchymal transition. (A) Levels of E-cadherin, Snail, Twist 1 and vimentin in SGC7901 cells incubated with Ad5/F35-null+IL-6, IL-6, Ad5/F35-PIAS1+IL-6 were investigated using western blotting. (B) Densitometric analysis of western blotting data. *P<0.01 vs. Ad5/F35-PIAS1+IL-6 treated group. PIAS1, protein inhibitor of activated signal transducer and activator of transcription 1; IL-6, interleukin-6; E-cadherin, epithelial cadherin; Twist 1, Twist-related protein 1; Snail, zinc finger protein SNAIL.

undergo a phenotypic alteration to acquire a mesenchymal phenotype, which involves a loss of polarity and intercellular adhesion, cytoskeletal disorganization that promotes motility and remodeling of the surrounding microenvironment, which is also commonly identified in various physiological processes, including wound healing, inflammation and fibrosis (17). A previous study has also demonstrated that EMT is associated with the acquisition of malignant characteristics in gastric cancer cells, suggesting that EMT is a vital step in tumor progression and metastasis (18). An additional previous study has indicated that the mechanism of EMT expression is regulated by various factors and signaling pathways, including E-cadherin, Snail, Twist 1 and vimentin (19). The levels of Twist 1, Snail and vimentin expression are all upregulated in patients with gastric cancer, whereas the level of E-cadherin is decreased in these patients (20). A study has indicated that the reduction of E-cadherin is considered to be an important EMT feature, which serves critical roles in EMT by changing the components of intercellular adhesion

and regulating diverse signaling pathways activated by the PI3K/Akt signaling pathway (21).

The cascade reaction of PI3K/Akt is one of the vital intracellular signal transduction systems, participating in numerous physiological progressions, such as cell growth, proliferation, differentiation and apoptosis. The constitutive activation of the PI3K/Akt signaling pathway has been noted during the malignant transformation of various cell lines and implicated in carcinogenesis and metastatic potential of human cancer (22). A previous study identified that PI3K/Akt modulated cancer metastasis via the regulation of EMT (23). IL-6 is a potent inflammatory cytokine that is released by inflammatory cells and cancer cells. IL-6 mediates several important physiological functions, including the control of the acute-phase inflammatory response and the support of cell growth and survival (24). Several studies have suggested that IL-6 is a key mediator of the development of metastatic potential in cancer cells (25,26). Previous studies have indicated that IL-6 promotes the initial steps
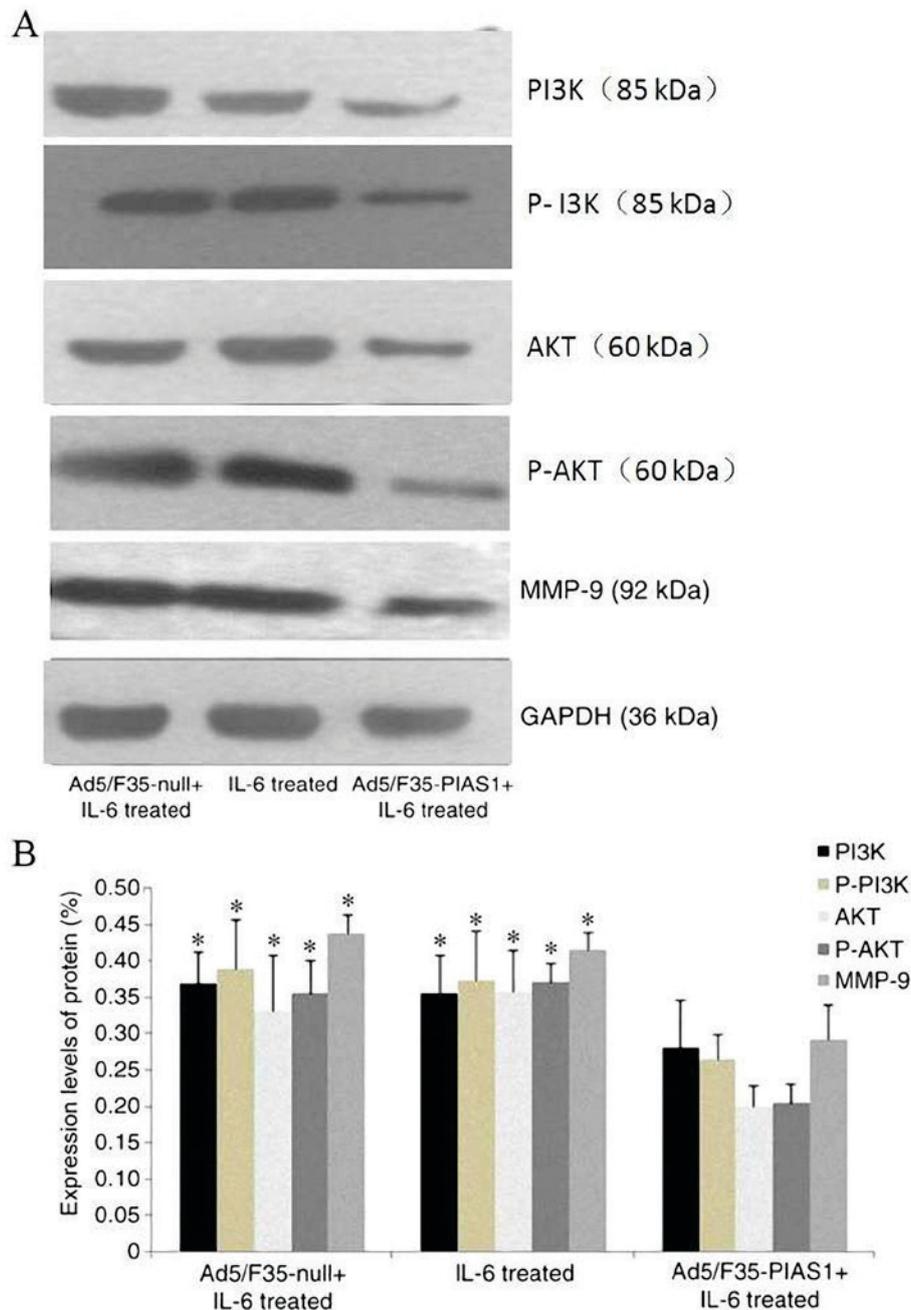
Figure 7. Expression levels of proteins involved in P-PI3K, PI3K, P-Akt, Akt and MMP-9 in SGC7901 cells incubated with Ad5/F35-null+IL-6, IL-6, Ad5/F35-PIAS1+IL-6 were investigated using (A) western blotting and (B) densitometric analysis of the western blotting data. *P<0.01 vs. Ad5/F35-PIAS1+IL-6 treated group. PI3K, phosphatidylinositol 3-kinase; P-PI3K, phosphorylated PI3K; Akt, serine/threonine kinase; P-Akt, phosphorylated Akt; MMP-9, matrix metallopeptidase 9; PIAS1, protein inhibitor of activated signal transducer and activator of transcription 1; IL-6, interleukin-6.

of cancer metastasis, potentially by upregulating MMP-9 through the PI3K/Akt signal pathway (27,28), and it has been demonstrated that IL-6 induces EMT through the expression of molecular markers (29,30). Therefore, the present study evaluated the expression of the primary biomarkers of EMT using western blotting and RT-PCR in SGC7901 cells treated with IL-6. Expression of the epithelial marker E-cadherin was downregulated, whereas the associated transcription factors involved in EMT, namely vimentin and Twist 1, were upregulated. Upon stimulation with IL-6, SGC7901 cells secreted MMP-9 and induced invasiveness. These data indicate that during cancer pathogenesis, elevated levels of

IL-6 may promote metastasis by acting on the inflammatory microenvironment. This finding is consistent with previous data, which indicate that IL-6 promotes tumor growth and malignant progression in gastric cancer, and that the induction of EMT is associated with tumor progression and the poor prognosis of patients with gastric cancer (31). This indicates that activating the IL-6 pathway also serves an important role in tumor microenvironment.

PIAS1 was originally identified as an inhibitor of STAT1 (32). It is well-known that activated STAT factors may regulate gene expression and thereby affect cell differentiation, proliferation, angiogenesis and apoptosis. PIAS1 already

has been identified as a negative regulator of tumor suppressors, including p53 and p73 (33). To address whether PIAS1 targeting could be used to improve gastric cancer therapy, PIAS1 expression in primary tumors of all stages in metastatic lesions from patient tissues was analyzed. The data from the present study were complemented by functional experiments following PIAS1 upregulation expression *in vitro*. A previous study demonstrated that a loss of PIAS1 led to the enhanced proliferation of tumor cells, while another study found an association between the reduced expression of PIAS1 and gastric cancer development (11,34). Similar to these data, the elevated PIAS1 expression in gastric cancer was observed in the present study, and a pro-proliferative role for the protein in this malignancy was proposed. On the basis of the data of the present study, we hypothesized that the elevated expression of PIAS1 in gastric cells impairs the transcriptional activity of the PI3K/Akt signaling pathway. The present study also confirms the effect of elevated PIAS1 expression on EMT in gastric cancer.

The results of present study indicated that IL-6-treated cells exhibited significantly increased migratory and invasive capabilities, as assessed by scratch wound healing and invasion assays. Transfection with the Ad5/F35-PIAS1 plasmid inhibited IL-6-induced migration, and the scratch wound healing assay indicated that Ad5/F35-PIAS1+IL-6-treated cells exhibited a notably slower viability recovery rate compared with the IL-6 and Ad5/F35-null+IL-6-treated cells. Similarly, the results of the Transwell invasion assay indicated that the Ad5/F35-PIAS1+IL-6-treated cells were significantly associated with decreased rates of migration compared with the other groups. These results indicated that PIAS1 was important for gastric cancer cell invasion and cell migration. Consequently, PIAS1 led to a reduction in the activation of the PI3K/Akt signaling pathway and MMP-9 protein expression in SGC7901 cells. Therefore, these cells may inhibit the initiation of EMT, leading to the increased expression of E-cadherin, and consequently a downregulation of Snail, Twist 1 and vimentin protein expression.

Taken together, these data confirm that PIAS1 upregulation causes the inhibition of EMT in an inflammatory microenvironment, which consequently results in decreased cell migration. On the basis of the present data, it was concluded that PIAS1 may be a promising novel target for treatment of gastric cancer.

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