Effects of αvβ6 Gene Silencing by RNA Interference in PANC-1 Pancreatic Carcinoma Cells

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Abstract. *Background: Integrin αvβ6 is up-regulated in a variety of human carcinomas and plays a crucial role in tumor invasion and metastasis. However, the function of αvβ6 in pancreatic carcinoma and its potential role in gemcitabine resistance remain unknown. Materials and Methods: Small interfering RNA (siRNA) targeting αvβ6 was constructed and transfected into PANC-1 cells. Effects of αvβ6 knockdown on cell proliferation, invasion, cell cycle progression, apoptosis and chemosensitivity to gemcitabine were investigated. Results: Expression of αvβ6 in PANC-1 cells was markedly suppressed by siRNA. Silencing of αvβ6 expression significantly inhibited cell proliferation and invasiveness, resulted in cell cycle arrest, and induced cell apoptosis. More importantly, αvβ6 knockdown enhanced chemosensitivity to gemcitabine and increased gemcitabine-induced caspase-mediated apoptosis. Conclusion: These findings suggest a novel mechanism by which αvβ6 contributes to pancreatic carcinoma progression. The combination of αvβ6 silencing and gemcitabine treatment may provide an effective therapeutic strategy for highly resistant pancreatic carcinoma.*

Pancreatic cancer is one of the malignancies with the worst prognosis because of aggressive invasion, early metastasis, and almost complete resistance to existing chemotherapeutic agents and radiation therapy (1). In the past few years, the use of gemcitabine (2',2'-difluorodeoxycytidine) has been shown to result in improved clinical symptoms and slightly longer overall survival in pancreatic cancer patients. Thus, gemcitabine has become the first-line treatment option for pancreatic cancer (2). However, chemoresistance to gemcitabine is increasing and has become a major cause of clinical treatment failure for pancreatic cancer. It is proposed that resistance to gemcitabine is mainly attributed to increased resistance to apoptosis (3). Consequently, new therapeutic strategies to induce apoptosis and enhance chemosensitivity to gemcitabine are urgently needed in this disease.

αvβ6 is a member of the integrin family, a group of cell adhesion molecules composed of two non-covalently bound transmembrane subunits (α and β). These proteins mediate cellular adhesion to extracellular matrix (ECM) and modulate cell proliferation, migration, invasion, and survival by triggering intracellular signaling pathways (4, 5). Inadequate or inappropriate cell-ECM interactions result in apoptosis (6). Overexpression of αvβ6 occurs in a number of epithelial tumors and has been shown to play a key role in tumor invasion and metastasis (7). Recently αvβ6 was defined as an independent unfavorable prognostic indicator in aggressive human colonic and gastric carcinomas (8, 9). Our previous study confirmed that αvβ6-mediated gelatinase B secretion is important in the progression of colonic cancer, and the transfection of antisense β6 gene can remarkably inhibit the growth of colon cancer cells *in vitro* and decrease their capability for tumor formation *in vivo* (10, 11). Our recent study also showed that cell apoptosis markedly increased after the function of αvβ6 in HT29 colon cancer cells was blocked by monoclonal antibody (12). However, the function of αvβ6 in pancreatic carcinoma cells and its potential role in gemcitabine resistance have not been elucidated.

RNA interference using small interfering RNA (siRNA) is a powerful technique that allows highly specific inhibition of individual gene expression (13). Previous reports have indicated that siRNA has advantages over antisense oligonucleotides when used to down-regulate gene expression, partly due to the greater resistance of siRNA to nuclease

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degradation (14). Here we show, for the first time, that inhibition of αvβ6 expression by siRNA increases the susceptibility of pancreatic carcinoma cells to gemcitabine *in vitro* and hinders pancreatic carcinoma progression.

Materials and Methods

Cell culture. Human pancreatic adenocarcinoma cell line PANC-1 was obtained from the American Type Culture Collection (Rockville, MD, USA) and maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; Sigma, St. Louis, MO, USA). Cells were cultured in a humidified (37˚C, 5% CO_2) incubator and passaged upon reaching 80% confluence.

siRNA synthesis. siRNAs were synthesized and purified by GenePharma, Inc. (Shanghai, China). We designed three siRNAs against the different positions of αvβ6 open reading frame (215-233, 221-239, and 737-755; GenBank accession no. NM_000888). These siRNAs were tested for the most effective targeting sequence. siRNA1, targeting the position 215-233, significantly reduced the expression of αvβ6 at mRNA and protein levels when compared with the other two siRNAs. Therefore, it was selected for subsequent experiments. The sequence of αvβ6 siRNA was sense: 5'-GCUAAAGGAUGUCAAUU AATT-3' and antisense: 5'-UUAAUUGACAUC CUUUAGCTA-3'. A scrambled version of αvβ6 siRNA was synthesized and used as a control siRNA (sense 5'-UUCUCCGAACGUGUCACGUTT-3', antisense 5'-ACG UGACACGUUCGGAGAATT-3'). All siRNAs were 21-nucleotides long and contained symmetric 3' overhangs of two deoxythymidines. The target specificity of these sequences was confirmed by BLAST search (http://www.ncbi.nih.gov/BLAST), bearing no homology to any relevant human genes. Homologous siRNAs were dissolved in buffer to a final concentration of 20 μM.

Transfection of siRNA. PANC-1 cells were seeded into six-well plates at a density of 2.0×10^5 cells/well and allowed to adhere for 24 h. Cells were transfected using the Lipofectamine™ 2000 reagent (Invitrogen) according to the manufacturer's protocols. Briefly, 3 μl of siRNA and 6 μl of transfection reagent per well were diluted in 250 μl serum-free medium respectively. Diluted siRNA solution was then added to the diluted transfection reagent, mixed gently, and incubated at room temperature for 20 minutes. The transfection reagent/siRNA complex was added into each well containing cells and medium and incubated for 6 hours. The siRNA-containing medium was then replaced with normal DMEM containing 10% FBS. All assays were performed 48 h after transfection.

RNA extraction and RT-PCR analysis. Total cellular RNA was extracted from untreated and transfected cells (αvβ6 siRNA or control siRNA) using Trizol reagent (Sigma), and cDNA was synthesized according to the manufacturer's instructions (Promega, Madison, WI, USA). Equal amounts of cDNA were subjected to PCR analysis. The sequences of the primers were as followed: β6 integrin forward: 5'-AGGATAG TTCTGTTTCCTGC-3' and reverse: 5'-ATCATAGGAATATTTGGAGG-3', which generated a 141-bp amplicon; β-actin forward: 5'-GAGACCTTCAACACCCCAGCC-3', and reverse: 5'-AATGTCACGCACGATTTCCC-3', which generated a 264-bp amplicon and was used as an internal control. The amplification conditions were 33 cycles at 94˚C for 30 s, 51˚C for 30 s, and 72˚C for 30 s. The PCR products were electrophoresed in a 1.5% agarose gel and viewed by ethidium bromide staining. Data were analyzed with Alpha Imager software (Alpha Innotech Co., CA, USA) and the expression levels of αvβ6 were normalized to β-actin.

Western blotting analysis. Untreated and transfected cells were harvested and rinsed twice with PBS. Total cellular protein was extracted in 500 μl of lysis buffer (100 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1 mM $CaCl_2$, 1% Triton X-100, 0.1% SDS, 0.1% NP-40, 1 mM vanadate, 1 μg/ml pepstatin, 1 mM PMSF, 5 μg/ml aprotinin, and 1 μg/ml leupeptin) for 30 min on ice, and cleared by centrifugation at 12,000×g for 20 min at 4˚C. After the protein concentrations were measured using a BCA protein assay kit (Sigma), an equal amount of protein (30 μg) from each sample was subjected to 10% SDS-PAGE and then transferred onto nitrocellulose membranes (Amersham-Pharmacia Biotech, UK). After blocking with TBS-T solution containing 5% non-fat milk for 2 h at room temperature, membranes were incubated with the primary antibodies overnight at 4˚C. Anti-αvβ6 monoclonal antibody R6G9 was obtained from Chemicon International (Chemicon, Temecula, CA, USA) and was used at 1 μg/ml (1:1,000 dilution). Antibodies against cyclin B1, Bcl-2, Bax and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The membranes were then incubated for 1 h with horseradish peroxidase-conjugated anti-mouse/rabbit secondary antibodies (Sigma), and the immunoreactive bands were visualized using the ECL chemiluminescence method according to the manufacturer's instructions. The signals were analyzed with a ChemiImager 5500 imaging system (Alpha Innotech) and standardized with loading control.

MTT colorimetric assay. The effects of αvβ6 on cell proliferation and chemosensitivity to gemcitabine were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Results of the MTT assay have been shown to correlate well with [^{3}H]-thymidine incorporation in pancreatic cancer cell lines (15). Twenty-four hours following siRNA treatment, cells were seeded into 96-well plates at a density of 5×10^3 cells/well and allowed to adhere for 24 h, and cultured in the absence or presence of gemcitabine at concentrations ranging from 0.1 nM to 10 μM. Cellular proliferation was assessed at 48, 72, and 96 h after siRNA transfection. Gemcitabine-induced cytotoxicity was determined after 48 h of drug treatment. Plates were read using Microplate Reader (Bio-Tech Instruments, USA) at a wavelength of 570 nm, referenced to 650 nm. Each independent experiment was performed thrice. The 50% inhibitory concentration (IC_{50}) of gemcitabine was calculated from these results.

Cell invasion assay. The invasive capability of PANC-1 cells was determined using Matrigel-coated transwell invasion chambers (8 μm pore size; BD Biosciences, Bedford, MA, USA). Untreated and transfected cells at 48 h after siRNA treatment were collected respectively, and 5×10^4 cells from each group were added to the upper chamber in 350 μl of culture medium containing 1% FBS. The lower chamber was filled with 750 μl DMEM containing 10% FBS. After incubation at 37˚C for 24 h, cells that invaded through the membrane were fixed with methanol, stained with Trypan blue, and counted under a light microscope.

Cell cycle and apoptosis analysis by flow cytometry. The effects of αvβ6 siRNA treatment on cell cycle progression and cellular

apoptosis were determined by flow cytometry. In brief, cells were harvested following trypsinization, washed once with cold PBS, and fixed in 70% ethanol. DNA staining was carried out by resuspending cells in a solution of PBS containing 20 μg/ml RNase A, 50 μg/ml propidium iodide (PI) and incubation at 37˚C for 30 min. The stained cells were analyzed using a Coulter Epics XL flow cytometer (Beckman Coulter, USA). The cells included in the sub-G_1 population were considered to be apoptotic cells.

Apoptosis assay by Annexin V staining. To assess apoptosis, cells were stained with an Annexin V-FITC apoptosis assay kit (Zymed, USA) following the protocol provided by the manufacturer. Briefly, pre-treated (siRNA, gemcitabine, or combination of both) and untreated cells were harvested, washed twice with cold PBS and resuspended in 100 μl 1×Annexin-binding buffer. Subsequently, 5 μl of Annexin V/FITC and 10 μl of PI solution (20 μg/ml) were added to each cell suspension. After 15 min incubation in the dark at room temperature, stained cells were analyzed by flow cytometry (FACS Calibur; Becton-Dickinson, USA) using CellQuest software. All the samples were assayed in triplicate, and apoptotic fraction was calculated as follows: apoptotic cell number/total cell number ×100%.

Caspase-3 activity assay. Caspase-3 activity was assayed using a fluorometric protease assay kit (Biovision, USA) following the manufacturer's instructions. Cells were homogenized on ice with kit-provided lysis buffer. An aliquot of 50 μl of supernatant was incubated with an equal volume of the reaction buffer containing fluorogenic peptide substrate for 2 h at 37˚C. The fluorescence at 400 nm excitation and 505 nm emission was read using a fluorescent plate reader. At least three determinations were made for each sample.

Statistical analysis. Data were expressed as the mean±SD and were representative of at least three independent experiments. The statistical comparisons were made by Student's *t*-test and one-way ANOVA. A value of $p<0.05$ was considered statistically significant.

Results

αvβ6 siRNA suppresses the expression of αvβ6 at both protein and mRNA levels. Our preliminary experiment confirmed that human pancreatic carcinoma cell line PANC-1 inherently expresses high levels of αvβ6 and has relatively high gemcitabine chemoresistance. This cell line was therefore chosen to evaluate the knockdown effect of αvβ6-specific siRNA. We simultaneously used a FAM-labeled control siRNA in our transfection experiments, which allowed us to easily track oligomer uptake and transfection efficiency by fluorescence microscopy. The results showed that transfection efficiency exceeded 80% at 48 h after transfection. The ability of αvβ6 siRNA to inhibit αvβ6 expression was quantified by Western blotting analysis. Up to 87% inhibition of αvβ6 protein expression was observed in cells transfected with αvβ6 siRNA but not with control siRNA, and this persisted 96 h after transfection. The β-actin expression was unaffected by either control or αvβ6 siRNA, indicating that non-specific down-regulation of protein expression did not occur (Figure 1A). Inhibition of αvβ6 mRNA expression by αvβ6 siRNA, but not control siRNA, was confirmed at the transcription level using RT-PCR analysis at the same time point (Figure 1B).

Silencing of αvβ6 expression inhibits cell proliferation. We tested the effect of inhibition of αvβ6 expression on the proliferation of PANC-1 cells. As shown in Figure 2A, αvβ6 siRNA reduced the cell proliferation in a time-dependent manner. At 96 h after transfection, the proliferation rate of αvβ6 siRNA-transfected cells decreased to 60.1±4.7% compared to the untreated cells, whereas those transfected with control siRNA showed no apparent antiproliferative effect at any indicated time point.

Silencing of αvβ6 expression inhibits cell invasion. To evaluate the effect of αvβ6 on cell invasion, transwell invasion assays were carried out. The results showed that transfection with αvβ6 siRNA significantly reduced the number of PANC-1 cells that invaded through the Matrigel-coated membrane compared with untreated cells. There were approximately 76±11 cells in each field from three independent experiments in the untreated group, while this number decreased to 15±3 cells in the αvβ6 siRNA group. On the other hand, control siRNA had no significant effect on the invasive capability of PANC-1 cells (Figure 2B).

Silencing of αvβ6 expression causes G_2/M cell cycle arrest and induces cell apoptosis. To examine the mechanism responsible for αvβ6 siRNA-mediated cell growth inhibition, cell cycle distribution was assessed by flow cytometry. The results showed that treatment with αvβ6 siRNA caused a significant inhibition of cell cycle progression in PANC-1 cells (Figure 3A). Compared to control siRNA-treated cells, a 53% increase in the G_2/M population was observed at 48 h after αvβ6 siRNA transfection. At 96 h post-transfection, 41% of αvβ6 siRNA-transfected cells were arrested at the G_2/M phase, suggesting an inability of these cells to complete mitosis without sufficient αvβ6 activity (Figure 3B). The accumulation of cells at the G_2/M phase was confirmed by the elevated protein levels of cyclin B1, a known G_2/M phase marker (Figure 4A). Concomitantly, the percentage of cells forming the sub-G_1 population notably increased in αvβ6 siRNA-transfected cells when compared to control siRNA-treated cells (Figure 3A).

To confirm the increase of αvβ6 siRNA-induced apoptosis in PANC-1 cells, Annexin V-FITC staining assays were performed. As shown in Figure 5C, the apoptotic rate of αvβ6 siRNA-treated cells was increased to 13.5±2.3% at 96 h after transfection, whereas control siRNA had no significant effect on cell apoptosis. The expression of apoptosis-related proteins was also analyzed. Western blotting showed that after the cells were treated with αvβ6

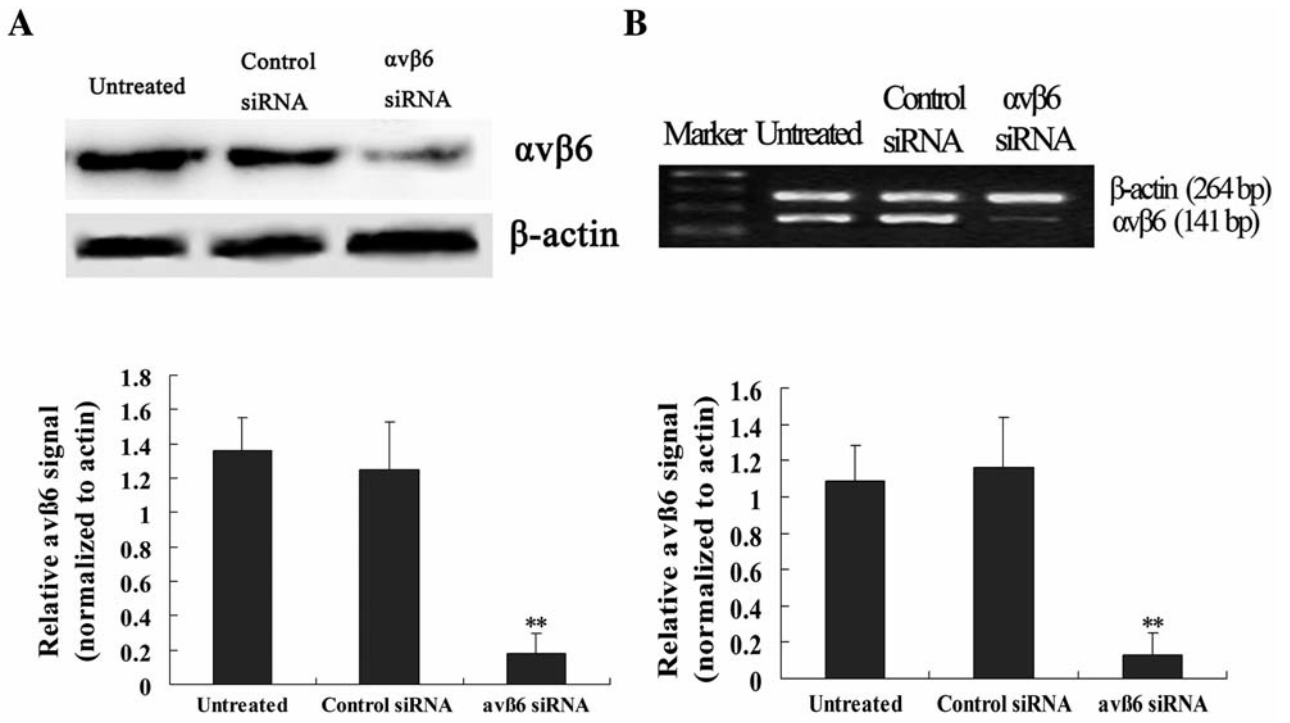

Figure 1. *Inhibition of αvβ6 expression by siRNA in PANC-1 cells. A, Representative Western blotting analysis of αvβ6 expression 96 h after siRNA transfection. The αvβ6 expression was not affected in control siRNA-treated cells, compared with untreated cells, whereas significant inhibition of αvβ6 expression was found in αvβ6 siRNA-treated cells. The expression of β-actin was not affected by either control or αvβ6 siRNA treatment. Densitometric values are shown as the mean±SD of three independent determinations. **p<0.01 versus control siRNA. B, RT-PCR analysis of αvβ6 mRNA expression 96 h after siRNA transfection. Inhibition of αvβ6 mRNA expression by αvβ6 siRNA, but not control siRNA, was confirmed. **p<0.01 versus control siRNA.*

siRNA for 96 h, the expression of anti-apoptotic protein Bcl-2 was down-regulated remarkably, while the expression of pro-apoptotic protein Bax was up-regulated compared to that of control siRNA-transfected cells (Figure 4B). Taken together, these results suggest that siRNA-mediated silencing of αvβ6 could cause G_2/M cell cycle arrest and induce cell apoptosis, and that the mitochondrial pathway may play a major role in the apoptosis of PANC-1 cells induced by αvβ6 gene silencing.

αvβ6 siRNA enhances chemosensitivity to gemcitabine. Given that αvβ6 siRNA has inhibitive effects on cell proliferation and cell cycle progression, we next tested the potential application of αvβ6 siRNA in sensitizing pancreatic carcinoma cells to gemcitabine. Treatment with control siRNA had no apparent effect on the IC_{50} of PANC-1 cells. In contrast, αvβ6 siRNA resulted in a 52% decrease in the gemcitabine IC_{50} relative to that of control siRNA transfectants (Figure 5A).

αvβ6 siRNA increases gemcitabine-induced caspase-mediated apoptosis. Finally, the combined effect of αvβ6 siRNA and gemcitabine treatment on the induction of cell apoptosis was determined. Forty-eight hours following siRNA transfection, cells were treated with gemcitabine at a concentration of 0.1 nM. Cell apoptosis was quantified after 48 hours of drug treatment. The results showed that the combination of αvβ6 siRNA and gemcitabine induced significant increases in cell apoptosis over that of each agent alone in PANC-1 cells (Figure 5B). Both the apoptotic fraction and caspase-3 activity induced by exposure to gemcitabine significantly increased following transfection of αvβ6 siRNA but not control siRNA (Figure 5C and 5D). In addition, when the transfected cells were exposed to gemcitabine in the presence of caspase inhibitor z-VAD-fmk (Calbiochem, USA), the increase in the apoptotic fraction induced by αvβ6 knockdown was significantly reduced, indicating that gemcitabine-induced apoptosis is caspase dependent (Figure 5C).

Discussion

Pancreatic carcinoma continues to be a formidable disease with a poor prognosis. Drug resistance to gemcitabine remains a significant problem in the clinical treatment of pancreatic cancer. A better understanding of the molecular

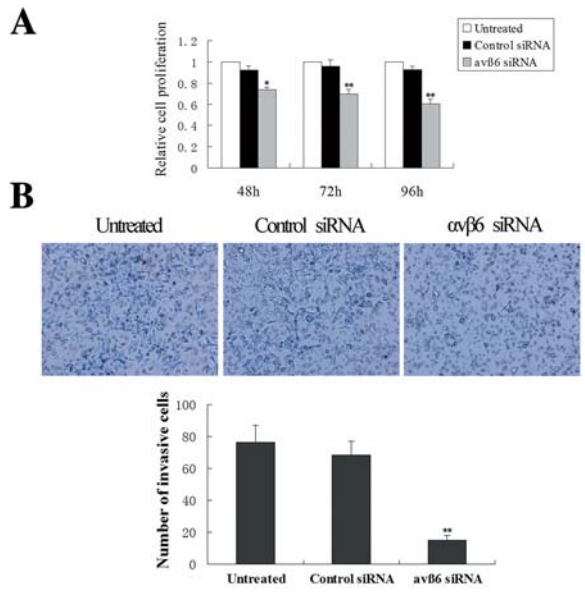

Figure 2. *Effects of αvβ6 gene silencing on cell proliferation and invasiveness. A, The αvβ6 siRNA notably reduced the cell proliferation in a time-dependent manner, while control siRNA had no significant effect on cell proliferation. *p<0.05, **p<0.01 versus control siRNA. B, The αvβ6 siRNA significantly reduced the number of PANC-1 cells which invaded through the Matrigel-coated membrane compared with untreated cells, while control siRNA had no significant effect on the invasive capability of PANC-1 cells. Results are shown as the mean±SD of invaded cells in three independent experiments. **p<0.01 versus control siRNA.*

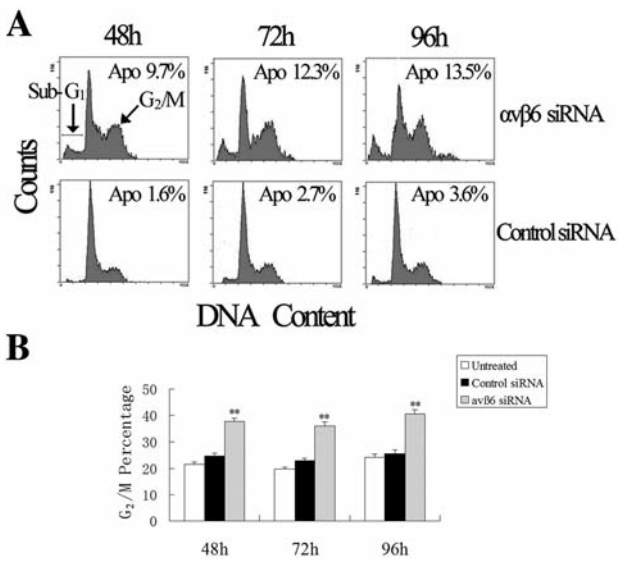

Figure 3. *Effects of αvβ6 gene silencing on cell cycle progression and cell apoptosis. A, Representative histograms showing the cell cycle profiles of αvβ6 siRNA- and control siRNA-transfected cells. The peaks corresponding to sub-G_1 and G_2/M phases are indicated by arrows. The percentage of the sub-G_1 proportion markedly increased in αvβ6 siRNA-transfected cells. B, Silencing of αvβ6 expression caused G_2/M cell cycle arrest. Data represent the mean±SD of three independent experiments. **p<0.01 versus control siRNA.*

biology of pancreatic cancer progression might potentially lead to more effective clinical management and provide novel candidates for targeting therapy. One major aim of our study was to investigate the role of αvβ6 in pancreatic cancer progression and its potential implication for gemcitabine resistance.

We found that αvβ6 siRNA was able to effectively down-regulate αvβ6 expression at both mRNA and protein levels with high specificity. Silencing αvβ6 expression significantly inhibited cell proliferation and invasion, caused G_2/M cell cycle arrest, and induced apoptosis in pancreatic carcinoma cells. More importantly, αvβ6 gene silencing significantly enhanced gemcitabine sensitivity and increased gemcitabine-induced caspase-mediated apoptosis, suggesting that the combination of αvβ6 depletion and gemcitabine treatment has synergistic antitumor effect and may represent a novel therapeutic strategy for highly resistant pancreatic carcinoma.

The epithelial-restricted integrin αvβ6 is usually not detectable in normal epithelium tissues but is up-regulated in a variety of malignant tumors, including colon, breast, stomach, ovary, squamous cell and pancreatic carcinomas (7-9, 16-18). Integrin αvβ6 can regulate the expression of proteinase, such as urokinase plasminogen activator and matrix metalloproteinase-9 (MMP-9), which results in increased matrix degradation and facilitation of tumor cell invasion and metastasis (17, 19). Our previous study indicated that αvβ6 promotes MMP-9 secretion and mediates the potential of colon cancer cells to colonize in and metastasize to the liver (20). There is a direct binding between αvβ6 and extracellular signal-regulated kinase 2, and this novel binding defines a direct signal pathway for αvβ6 to regulate the progression of malignant tumors (21). Therefore, αvβ6 has been proposed as a new therapeutic target for aggressive carcinomas. Inhibition of αvβ6 activity by antisense oligonucleotides, monoclonal antibodies, and small molecule inhibitors has been developed in recent years. Although limited data exist, there is experimental support for the idea of αvβ6-directed therapy (7).

RNA interference is a gene-silencing method with sequence-specificity at post-transcriptional level, by which double-stranded RNA inhibits gene expression by degradation of the corresponding mRNA. This technique was successfully used to down-regulate the gene expression, thus leading to many attempts to explore its potential therapeutic values (22). Cao *et al*. (23) reported that knockdown of av integrin with siRNA promoted apoptosis and enhanced G_2/M arrest in breast cancer cells. However,

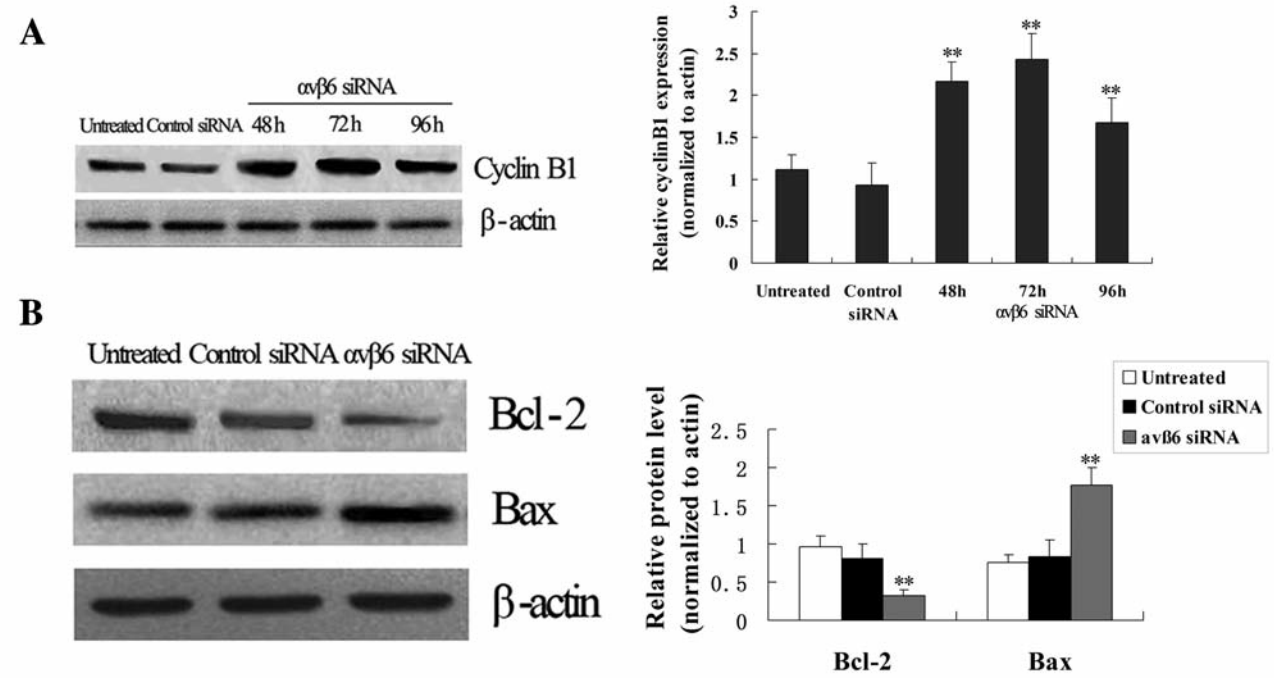

Figure 4. *Effects of αvβ6 gene silencing on the expression of cyclin B1, Bcl-2 and Bax in PANC-1 cells. A, Western blotting analysis showed the elevated protein levels of cyclin B1 in αvβ6 siRNA-transfected cells. B, After the cells were treated with αvβ6 siRNA for 96 h, the expression of anti-apoptotic protein Bcl-2 was remarkably down-regulated, while the expression of pro-apoptotic protein Bax was up-regulated. Densitometric values are shown as the mean±SD of three independent determinations. **p<0.01 versus control siRNA.*

the av integrin family includes five members (αvβ1, αvβ3, αvβ5, αvβ6, and αvβ8), so this method has a broad-spectrum anti-integrin effect. In this study, αvβ6 siRNA was chosen to knock down αvβ6 expression because of it having more advantages than oligonucleotide-based techniques (14). Here we show that integrin αvβ6 plays an important role in promoting cell cycle progression and inhibiting apoptosis in PANC-1 pancreatic carcinoma cells, and that a mitochondrial mechanism is involved in the process of αvβ6 silencing-induced apoptosis. These findings are in agreement with our recent observation in colon cancer (12) and represent a novel way by which αvβ6 contributes to cancer progression.

More importantly, our results demonstrate that αvβ6 is an important determinant for chemoresistance to gemcitabine. Silencing of αvβ6 expression by siRNA enhances gemcitabine sensitivity and increases gemcitabine-induced cytotoxicity. We have shown that silencing αvβ6 expression may potentially enhance the efficacy of gemcitabine in pancreatic carcinoma cells. Based on the findings that αvβ6 is frequently overexpressed in carcinomas but not in normal epithelial tissues, gene therapy targeting αvβ6 would afford high specificity with low toxicity.

However, RNA interference mediated by siRNA is generally transient, largely due to the decrease of siRNA intensity during cell division. Our recent study reported that plasmid vector-based shRNA effectively down-regulated αvβ6 expression in colon cancer cells (24). Strategies using plasmid- and virus-based siRNA delivery systems need to be established and additional studies on animal models should be performed in order to improve the potency of this approach and further confirm the importance of targeting therapy against αvβ6.

In summary, we firstly explore the role of αvβ6 in cell proliferation, invasion, cell cycle progression, apoptosis and chemoresistance to gemcitabine in pancreatic carcinoma cells. Silencing of αvβ6 expression inhibited the progression of pancreatic carcinoma cells and increased chemosensitivity to gemcitabine, suggesting that αvβ6 is a potential therapeutic target. The combination of αvβ6 silencing and gemcitabine treatment may provide an effective therapy strategy for highly resistant pancreatic cancer, which might ultimately improve patients' clinical outcome.

Acknowledgements

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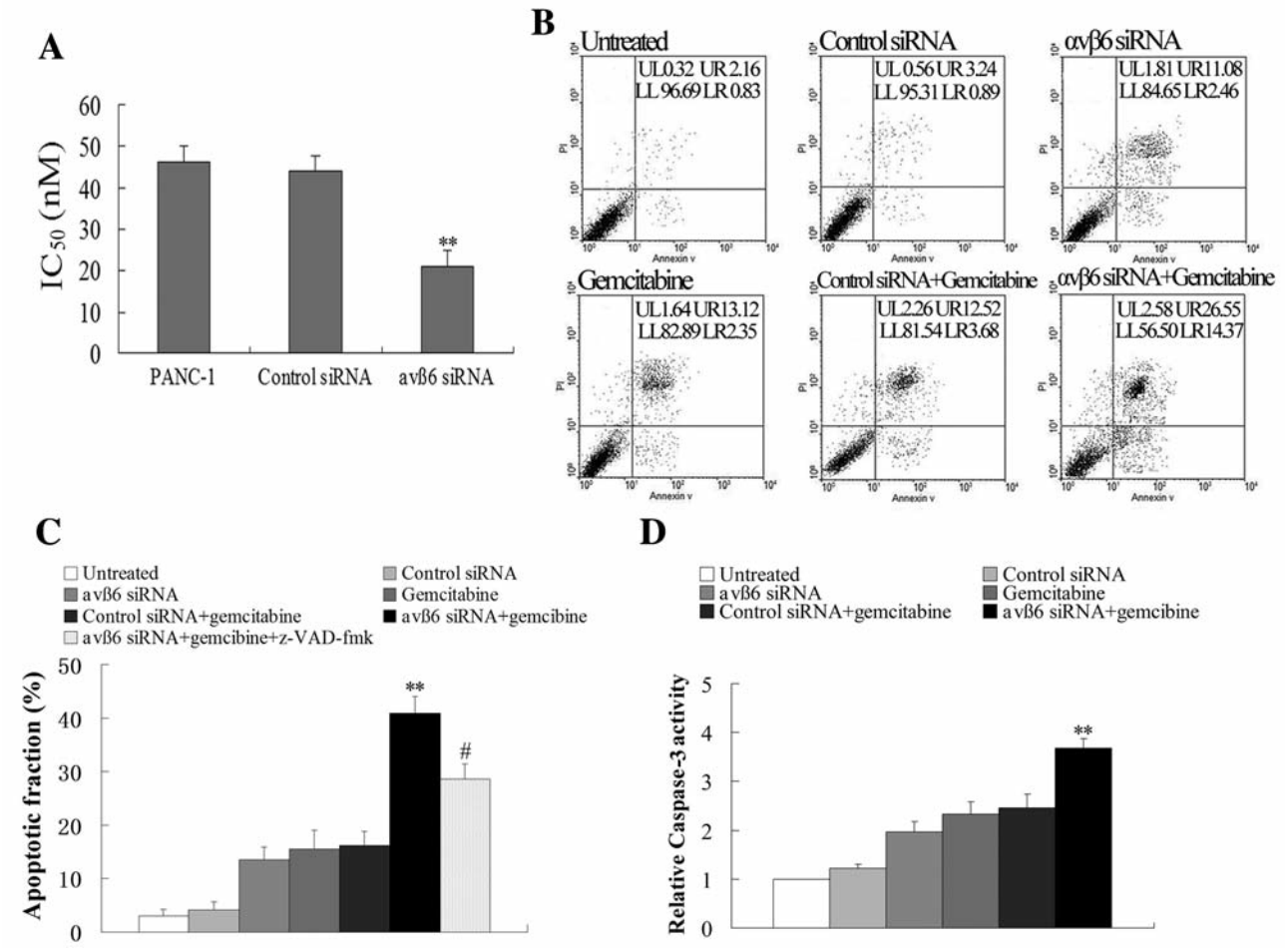

Figure 5. *The silencing of αvβ6 gene enhances chemosensitivity of PANC-1 cells and increases gemcitabine-induced caspase-mediated apoptosis. A, The IC_{50} value of gemcitabine decreased in αvβ6 siRNA-transfected cells. B, Enhancement of gemcitabine-induced apoptosis by αvβ6 siRNA was confirmed by Annexin V staining. C, The apoptotic fraction following gemcitabine exposure increased in αvβ6 siRNA-transfected cells. D, The activity of caspase-3 following gemcitabine exposure increased in αvβ6 siRNA-transfected cells. Data represent the mean±SD from triplicate independent experiments. **p<0.01 versus control siRNA. #p<0.01 versus absence of caspase inhibitor.*

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