SPANDIDOS PUBLICATIONS

# SiRNA-mediated PIAS1 silencing promotes inflammatory response and leads to injury of cerulein-stimulated pancreatic acinar cells via regulation of the P38MAPK signaling pathway

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**Abstract.** Our aim in this study was to investigate the changes of inflammatory response by protein inhibitor of activated signal transducer and activator of transcription 1 (PIAS1) gene silencing treatment in cerulein-stimulated AR42J cells, and relate them to changes in cell injury, thus providing evidence for developing clinical therapies. This study examined the effects of cerulein on the activity of P38 mitogen activated protein kinase (P38MAPK), c-jun NH2-terminal kinase/stress-activated protein kinase and the inflammatory mediators released by PIAS1 gene-silenced AR42J cells. Consequently, the markers including DNA ladder, cell apoptotic rat, cell cycles, levels of cell cycle and apoptotic related factors were used to determine the effects of PIAS1 gene silencing on the cerulein-induced cell injury. The results indicated that in the cerulein-stimulated PIASI silencing cells, the activity of P38MAPK was enhanced, while at the same time, the levels of inflammatory mediators such as the tumor necrosis factor-α, interleukin (IL)-1ß, IL-6 and matrix metallopeptidase-9, were markedly higher than those of other cerulein-stimulated cells. Thus, the cerulein-stimulated PIASI gene-silenced cells obviously increased cell arrest in the G1/M phase by increasing P21 and P27 expression, and also induced apoptosis by regulating the P53 signaling pathway. This study suggests that the down-regulation of PIAS1 is efficacious at enhancing the expression of inflammatory mediators and inducing cell injury in acute pancreatitis (AP), thus deteriorating the severity of disease. It provides evidence that PIAS1 is a potential therapeutic target for AP.

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*Key words:* protein inhibitor of activated signal transducer and activator of transcription 1, inflammation, apoptosis, pancreas, acinar cells

## Introduction

Acute pancreatitis (AP) is an inflammatory disease characterized by interstitial edema, vacuolization, acinar necrosis, and inflammatory infiltration in the pancreas. Approximately 25% of patients with AP develop a severe disease course that leads to systemic inflammatory response syndrome (SIRS), multi-organ dysfunction syndrome (MODS) and acute respiratory distress syndrome with mortality rates up to 50%. Its rapid clinical course, high mortality, and risk of life-threatening complications make AP a real challenge for clinical treatment. Although it has become increasingly clear that excessive SIRS is a determining factor in the process of AP (1), the exact mechanisms that regulate the severity of AP are still unknown.

The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway is an essential intracellular mechanism of cytokines and other stimuli that regulates gene expression and cellular activation, proliferation, and differentiation. The JAK/STAT pathway is controlled by different mechanisms, including receptor internalization, protein tyrosine phosphatases, and suppressors of cytokine signaling (2). Furthermore, the protein inhibitor of activated STAT (PIAS) is a family of critical negative regulators of the JAK/STAT signaling pathway, consisting of 4 members, PIAS1, PIAS3, PIASx, and PIASy (3). Gene activation analyses have shown that PIAS1 selectively regulates a subset of STAT-dependent genes, with a notable preference for inflammatory cytokines (4). It has also been shown that PIAS1 participates in anti-inflammatory responses through various regulated pathways in inflammatory disease (5). Subsequent studies found that PIAS1 null mice show hypersensitive response to LPS-induced septic shock (6). However, the role of PIAS1 in excessive SIRS in AP has not yet been fully determined. The aim of the current study was to explore the therapeutic potential target of PIAS1 in cell injury in AP.

AR42J is the currently available cell line that shows receptor expression and signal transduction mechanisms parallel to those of normal pancreatic acinar cells. Therefore, AR42J cells have been widely used as an *in vitro* model to study secretion, signal transduction, cytoskeleton function, apoptosis, and pancreatic inflammatory response of the exocrine pancreas (7). Cerulein over-stimulation is known to produce AP, and it is likely that under these conditions, the

inflammatory response production in the pancreatic acinar cells is increased leading to the damage of these cells (8). Therefore, in the present study, we used cerulein-stimulated AR42J cells as an *in vitro* model of AP. The PIAS1 gene silenced expression in the small interfering RNA (siRNA)-transfected AR42J cells, was used to investigate its effects and mechanisms for inflammatory response and cell injury in AP, subsequently providing therapeutic evidence for AP.

## Materials and methods

*Cell lines and reagents*. The pancreatic acinar cell line, AR42J (ATCC, Rockville, MD, USA), was cultured in DEME (Gibco BRL, Gaithersburg, MD, USA) plus 10% fetal bovine serum (Gibco BRL) and 1% penicillin/streptomycin (Sigma, St. Louis, MO, USA) in standard conditions (37˚C and 5% $CO_2$).

The following antibodies were purchased: Anti-PIAS1, anti-tumor necrosis factor (TNF)-α, anti-interleukin (IL)-1ß, anti-IL-6, anti-matrix metallopeptidase-9 (MMP-9), anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (all from Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-P38 mitogen activated protein kinase (P38MAPK), anti-phosphorylated P38MAPK (P-P38MAPK), anti-c-jun NH2-terminal kinase/stress-activated protein kinase (JNK/SAPK), anti-phosphorylated JNK/SAPK (P-JNK/SAPK) (all from Cell Signaling Technology, Inc., Danvers, MA, USA), anti-P21, anti-P27, anti-cyclin E, anti-Bcl-2, anti-Bax, anti-cleaved caspase 3 and anti-P53 (all from Santa Cruz).

The following reagents were purchased: TRIzol (Invitrogen, Groningen NL, USA), Lipofectamine 2000 (Invitrogen), RIPA buffer (Shanghai Biocolor BioScience & Technolgy Co., Shanghai, China), and the bicinchoninic acid (BCA) protein quantitative kit (Pierce, Rockford, IL, USA).

*SiRNA treatment*. The specific silencing of the rat PIAS1 gene expression was achieved by the siRNA technique. PIAS1-siRNA (5' CCG GAU CAU UCU AGA GCU UTT '3 and 5' AAG CUC UAG AAU GAU CCG GTT 3') and non-specific negative-siRNA (5' UUC UCC GAA CGU GUC ACG UTT 3' and 5' ACG UGA CAC GUU CGG AGA ATT 3') were chemically synthesized (Invitrogen) and effectively screened prior to this study (data no shown). The AR42J cells at 50-60% confluence were transfected with 20 nmol/ml siRNA using Lipofectamine 2000 (10 $\mu$l, Invitrogen) in a total volume of 500 ml/well growth medium. After 4-h incubation at 37˚C, 1.5 ml/well of growth medium were added and incubated for 24 h.

The AR42J cells were randomly allocated into 4 groups: The administration of phosphate buffered saline (25 $\mu$l, PBS), Lipofectamine 2000 (10 $\mu$l, liposome), negative-siRNA (20 nmol/ml) and PIAS1-siRNA (20 nmol/ml) was carried out 24 h prior to cerulein ($10^{-8}$ M) stimulation. One group of cells served as the control. The samples were harvested for 24 h after the onset of cerulein stimulation.

*The effect of PIAS1 gene silencing on the inflammatory response of AP*

*Western blot analysis*. The levels of P38MAPK, P-P38MAPK, JNK/SAPK, P-JNK/SAPK, TNF-α, IL-1ß, IL-6 and MMP-9 proteins, were investigated in each group using Western blot analysis. The cells were washed twice with PBS and then homogenized in RIPA 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 $\mu$g, and was thus denatured in loading buffer and separated by electrophoresis on 9% SDS polyacrylamide gel at 100 V for 120 min. The separated proteins were transferred onto polyvinylidene difluoride membranes by using transfer buffer at 200 mA for 90 min. The membranes were blocked with 5% non-fat dry milk in Tris-buffered saline (TBS)-0.1% Tween-20 for 1 h at room temperature, washed 3 times for 10 min each in TBS-0.1% Tween-20, and were each then incubated with a primary antibody including P38MAPK, P-P38MAPK, JNK/SAPK, P-JNK/SAPK, TNF-α, IL-1ß, IL-6 and MMP-9 with 1:1000 dilution in TBS-0.1% Tween-20 overnight at 4˚C. After washing 3 times for 10 min in TBS-0.1% Tween-20, the membranes were incubated with a second antibody, horseradish peroxidase-conjugated goat anti-rabbit or rabbit anti-mouse immunoglobulin G (Kangcheng Inc., Shanghai, China), for 1 h at room temperature. After washing, the membranes were detected by enhanced chemiluminescence methods (Amersham Biosciences, Piscataway, NJ, USA), and were then analyzed by scanning densitometry using the bio-image analysis system (Bio-Rad, Baltimore, MD, USA) for quantification. GAPDH was determined in a similar manner with anti-GAPDH antibody (diluted to 1:1000, Santa Cruz) as an endogenous control for other proteins.

*Reverse transcriptase-polymerase chain reaction (RT-PCR)*. The primers for rat TNF-α, IL-1ß, IL-6, MMP-9 and GAPDH were constructed based on the published rat TNF-α, IL-1ß, IL-6, MMP-9 and GAPDH nucleotide sequences and were synthesized by Sangon Technology Inc. (Shanghai, China). RT-PCR reactions were performed in the GeneAmp 9600 machine (Perkin-Elmer Inc., Wellesley, MA, USA). The primers were indicated as follows: TNF-α, 5' TCT CAT TCC TGC TCG TGG 3' and 5' CCA TTG GCC AGG AGG GCG TTG G 3'; IL-1ß, 5' TCT GTG ACT CGT GGG ATG 3' and 5' TCT TTG GGT ATT GTT TGG 3'; IL-6, 5' TGC CTT CTT GGG ACT GAT 3' and 5' CTG GCT TTG TCT TTC TTG TTA T 3'; MMP-9, 5' GAC GCA CAT CTC TCC TGC CG 3' and 5' GGC AAG GAT GGT CTA CTG GC 3'; and GAPDH, 5' GGC TGA GAA CGG GAA GCT TGT C 3' and 5' CAG CCT TCT CCA TGG TGG TGA AGA 3'.

The RT-PCR conditions were as follows: One cycle for 5 min at 95˚C, 35 cycles for 45 sec at 94˚C, 45 sec at 60˚C (TNF-α), 45 sec at 56˚C (IL-1ß, MMP-9), 45 sec at 55˚C (IL-6, GAPDH), 45 sec at 72˚C, and 1 cycle for 10 min at 72˚C. The PCR products were stained with ethidium bromide and separated by electrophoresis on 1.2% agarose gels. The densities of the cDNA bands were analyzed by scanning densitometry using Gel Doc 2000 software (Bio-Rad, Baltimore, MD, USA). The band densities were normalized to the GAPDH band densities and the results were expressed as the ratio.

*The effect of PIAS1 gene silencing on cell injury of AP*

*The detection of cell cycle*. The cell concentration was adjusted to $1x10^5$/ml, and then the cells were washed with PBS and

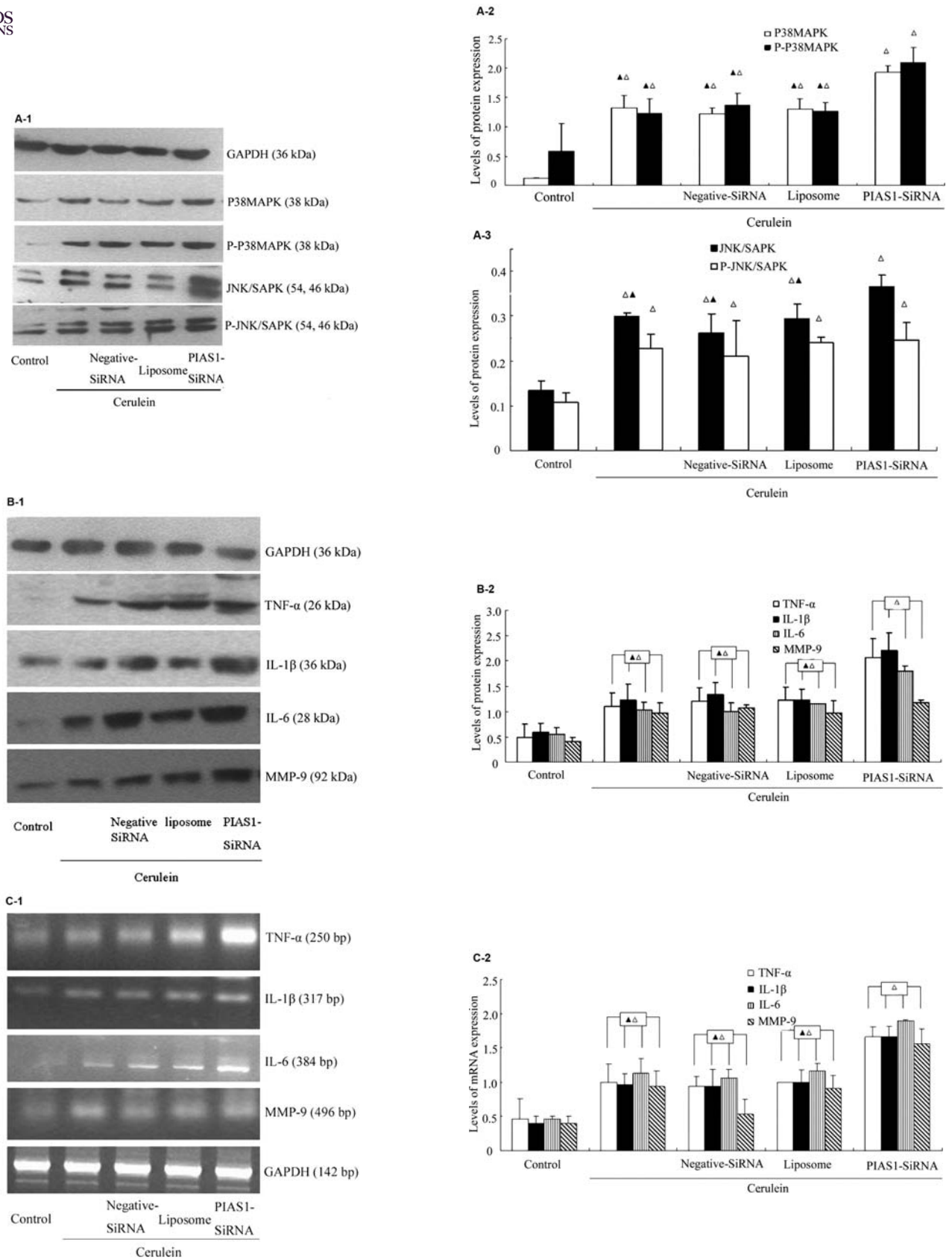



Figure 1. Data are representative of the results of the effect of PIAS1 gene silencing on the inflammatory response of cerulein-stimulation AR42J cells. (A) Representative Western blot analysis of proteins in the AR42J cells detected with P38MAPK, P-P38MAPK, JNK/SAPK, P-JNK/SAPK and GAPDH antibodies in each group. The graphs show the protein levels of P38MAPK, P-P38MAPK, JNK/SAPK, and P-JNK/SAPK (measured as the ratio of P38MAPK, P-P38MAPK, JNK/SAPK, or P-JNK/SAPK to GAPDH by band density) in each group. (B) Representative Western blot analysis of proteins in AR42J cells detected with TNF-α, IL-1ß, IL-6, MMP-9 and GAPDH antibodies in each group. The graph shows the protein levels of TNF-α, IL-1ß, IL-6, and MMP-9 (measured as the ratio of TNFα, IL-1ß, IL-6, or MMP-9 to GAPDH by band density) in each group. (C) Detection by RT-PCR of specific mRNA for TNF-α, IL-1ß, IL-6, and MMP-9 in each group. The amplification of GAPDH mRNA was used as the internal control, and the mRNA levels of TNFα, IL-1ß, IL-6 and MMP-9 in the respective cells, were analyzed (measured as the ratio of TNFα, IL-1ß, IL-6, or MMP-9 to GAPDH band density). △ vs. control group, P<0.05; ▲ vs. PIAS1-siRNA + cerulein group, P<0.05.

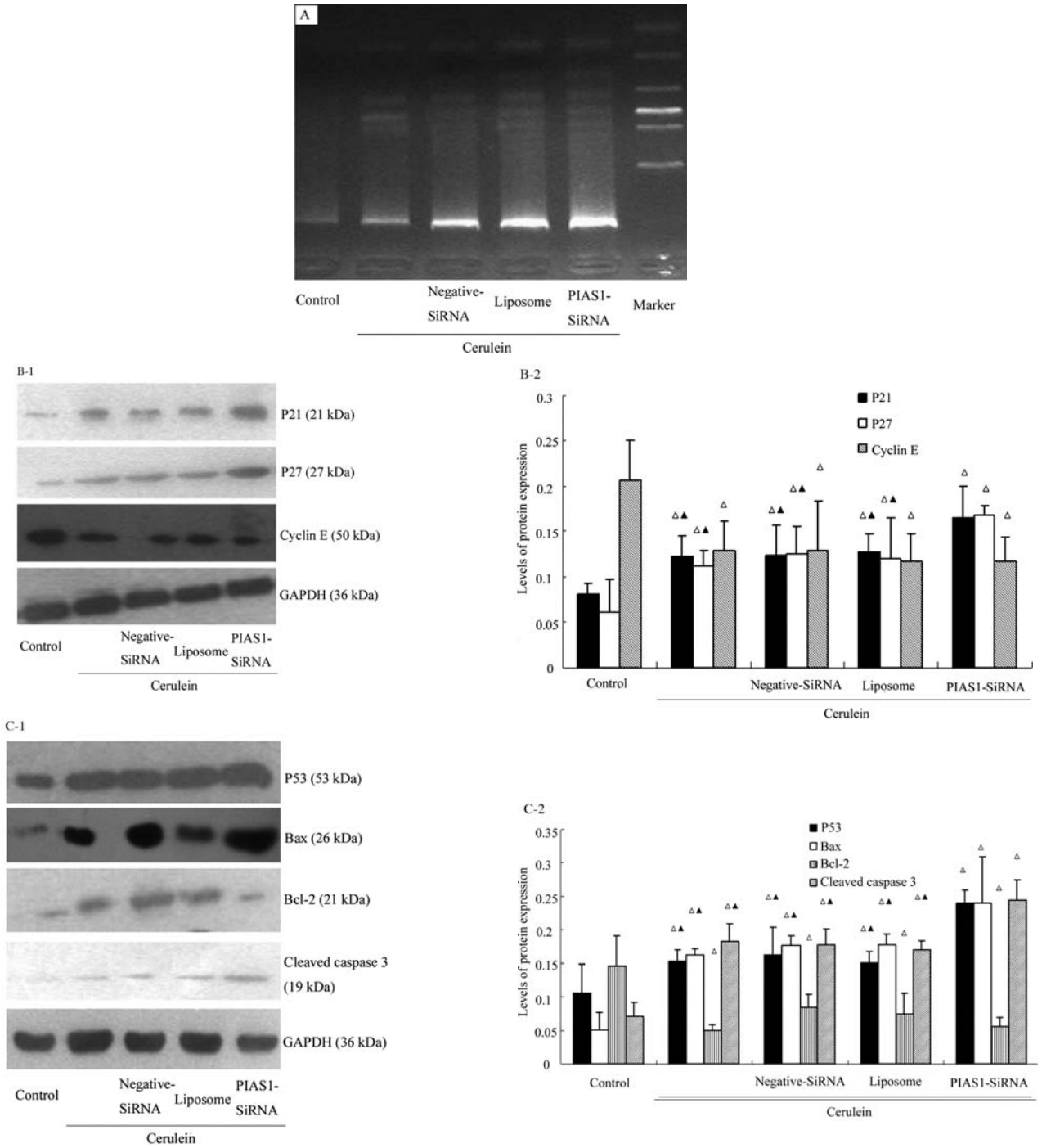


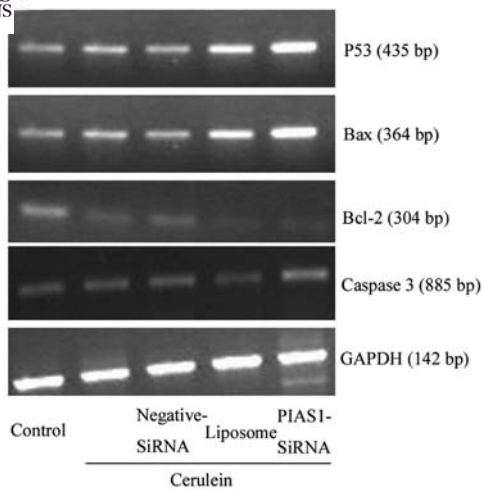

Figure 2. Data are representative of the results of the effective mechanism of PIAS1 gene silencing on the apoptosis AR42J cells induced by cerulein stimulation. (A) Genomic DNA fragments were extracted and resolved on 1.2% agarose gels. Apoptotic DNA fragmentation was visualized by ethidium bromide staining in each group. A typical ladder pattern of inter-nucleosomal DNA fragmentation was detected in all of the cerulein-stimulated cells. (B) Representative Western blot analysis of proteins in the AR42J cells detected with P21, P27, cyclin E and GAPDH antibodies in each group. The graph shows the protein levels of P21, P27 and cyclin E (measured as the ratio of P21, P27, or cyclin E to GAPDH by band density) in each group. (C) Representative Western blot analysis of proteins in the AR42J cells detected with P53, Bcl-2, Bax, cleaved caspase 3 and GAPDH antibodies in each group. The graph shows the protein levels of P53, Bcl-2, Bax and cleaved caspase 3 (measured as the ratio of P53, Bcl-2, Bax, or cleaved caspase 3 to GAPDH by band density) in each group.

suspended in 500 $\mu$l propidium iodide/Triton X-100/RNase staining solution. After 15-min incubation in the dark, the cell cycle was analyzed using a FACScan flow cytometer (Becton-Dickinson, Mountain View, CA, USA).

*The detection of cell cycle-related protein expression*

*Western blot analysis*. The cells were treated as described above for the indicated time-period, and then the levels of P21, P27 and cyclin E proteins were detected with P21, P27 and cyclin E antibodies, respectively (diluted to 1:500) in each group by Western blot analysis as described above.

*DNA ladder detection*. The cells were washed twice with PBS and DNA was extracted with an apoptotic DNA ladder detection kit according to the manufacturer's instructions (Biyuntian Co., Shanghai, China). Then 8.5 $\mu$l of the DNA



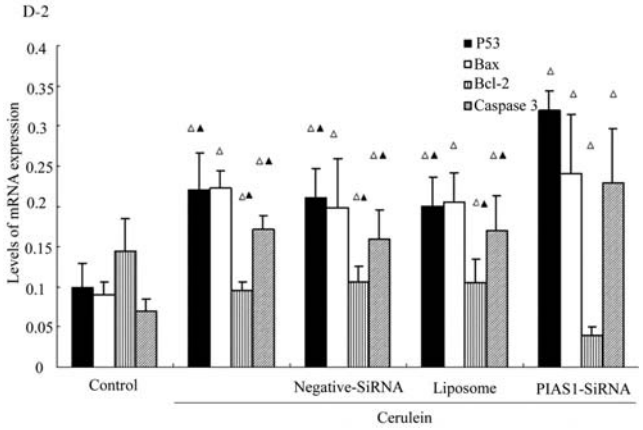




Figure 2. (D) Detection by RT-PCR of specific mRNA for P53, Bcl-2, Bax and caspase 3 in each group. The amplification of GAPDH mRNA was used as the internal control, and the mRNA levels of P53, Bcl-2, Bax and caspase 3 in the respective cells, were analyzed (measured as the ratio of P53, Bcl-2, Bax, or caspase 3 to GAPDH band density). △ vs. control group, P<0.05; ▲ vs. PIAS1-siRNA + cerulein group, P<0.05.

sample were mixed with 1.5 $\mu$l of 6X loading buffer, electrophoresed on 1.2% agarose gels containing ethidium bromide at 60 V, and observed using Bio-Rad gel image analysis system.

*The detection of cell apoptotic rate*. The cells were washed twice with PBS at 4˚C, re-suspended in 250 ml of a combination buffer solution, and the cell concentration was adjusted to $1x10^5$/ml. Annexin V/FITC (5 $\mu$l) and 5 $\mu$l propidium iodide were added. After incubation in the dark for 15 min, the cells were analyzed using a FACScan flow cytometer (Becton-Dickinson).

*The detection of apoptosis-related mRNAs and protein expression*

*Western blot analysis*. The cells were treated as described above for the indicated time-period, and then the levels of P53, Bax, Bcl-2 and cleaved caspase 3 proteins were detected with P53, Bax, Bcl-2 and cleaved caspase 3 antibodies, respectively (diluted to 1:500) in each group by Western blot analysis as described above.

*RT-PCR*. The primers for rat P53, Bax, Bcl-2, caspase 3 and GAPDH were constructed based on the published rat P53, Bax, Bcl-2, caspase 3 and GAPDH nucleotide sequences and were synthesized by Sangon Technology Inc. RT-PCR reactions were performed in the GeneAmp 9600 machine. The primers were indicated as follows: P53, 5' TCT GGG ACA GCC AAG TCT GT 3' and 5' GGA GTC TTC CAG TGT GAT GA 3'; Bax, 5' ACA AAG ATG GTC ACG GTC TG 3' and 5' ACC AAG AAG CTG AGC GAG TGT C 3'; Bcl-2, 5' AGA CAG CCA GGA GAA ATC AAA CAG 3' and 5' TGC ACC TGA CGC CCT TCA C 3'; caspase 3, 5' GCT AGC TAG CAT GGA GAA CAC TGA AAA CTC AGT G 3' and 5' GCT ACT CGA GGT GA T AAA AAT AGA GTT CTT TTG TG 3'; and GAPDH, 5' TGA CGG GGT CAC CCA CAC TGT GCC CAT CTA 3' and 5' CTA GAA GCA TTT GCG GTG GAC GAT GGA GGG 3'.

The RT-PCR conditions were as follows: One cycle for 5 min at 95˚C, 35 cycles for 45 sec at 94˚C, 45 sec at 56˚C (P53), 45 sec at 55˚C (Bcl-2, GAPDH), 45 sec at 54˚C (Bax), 45 sec at 57˚C (caspase 3), 1 min at 72˚C, and 1 cycle for 10 min at 72˚C. The PCR products were stained with ethidium bromide and separated by electrophoresis on 1.2% agarose gels. The densities of the cDNA bands were analyzed by scanning densitometry using Gel Doc 2000 software (Bio-Rad). The band densities were normalized to the GAPDH band densities and the results were expressed as the ratio.

*Statistical analysis*. All data were expressed as the means ± standard deviation (SD). Statistics were calculated using the SPSS program, version 10.5. The one-way Analysis of Variance (ANOVA) with Dunnett's multiple comparison tests was used for comparisons. A P-value of <0.05 was considered statistically significant.

## Results

*The effect of PIAS1 gene silencing on the inflammatory response*. The levels of P38MAPK, P-P38MAPK, JNK/SAPK and P-JNK/SAPK protein expressions were investigated in each group, as shown in Fig. 1A. The results show that the above-mentioned markers were increased in all of the cerulein-stimulated compared to the control cells. These markers were also higher in the cerulein-stimulated PIAS1 gene-silenced cells compared to the cerulein-stimulated cells administered with negative-siRNA, liposome, or PBS (P<0.05, respectively). The exception was the P-JNK/SAPK protein. The expression of this protein showed no differences between the cerulein-stimulated cells administered with negative-siRNA, liposome, or PBS (P>0.05, respectively).

Furthermore, the effects of PIAS1-siRNA on P38MAPK down-stream regulating inflammatory mediators such as TNF-α, IL-1ß, IL-6, and MMP-9 genes and protein expressions were determined by Western blot analysis or RT-PCR, as

shown in Fig. 1B and C. The results showed that cerulein caused an increase in the protein expressions and mRNA levels of TNF-α, IL-1ß, IL-6 and MMP-9 (P<0.05, all of the cerulein-stimulated cells vs. the control cells, respectively). The PIAS1 gene silencing treatment enhanced the effects of cerulein on TNF-α, IL-1ß, IL-6 and MMP-9 expressions (P<0.05, vs. all of the other cerulein-stimulated cells and the control cells, respectively).

*The effect of PIAS1 gene silencing on pancreatic acinar cell apoptosis*. In order to further investigate the role of PIAS1 on cerulein-induced apoptosis, the cell cycle was measured by flow cytometry. The amount of cells in the G1 population increased from 21.05±5.88% in the control cells to 34.97±8.5, 38.24±10.74, 37.7±9.87 and 52.01±9.52% in the cerulein-stimulated cells treated with PBS, negative-siRNA, liposome, or PIAS1-siRNA (P<0.05, respectively). The amount of cell significantly arrested in the G1 phase in the cerulein-stimulated PIAS1 gene-silenced cells was higher compared to that of the other cerulein-stimulated cells (P<0.05, respectively). At the same time, the increasing cell population at the S phase was accompanied by a decrease in the cell population at the G1 phase of the cell cycle, and the amount of cells in the S population decreased from 71.453±8.19% in the control to 49.49±7.64, 44.52±7.88, 49.97±13.64 and 30.07±9.91% in the cerulein-stimulated cells treated with PBS, negative-siRNA, liposome, or PIAS1-siRNA (P<0.05, respectively). The amount of cells also decreased in the cerulein-stimulated PIAS1 gene-silenced cells compared to the other cerulein-stimulated cells (P<0.05, respectively).

As shown in Fig. 2A, after cerulein stimulation in the cells for 24 h, a typical ladder pattern of inter-nucleosomal DNA fragmentation was detected using agarose gel electrophoresis, which was enhanced in the cerulein-stimulated PIAS1 gene-silenced cells. After cerulein stimulation for 24 h, the apoptotic rates of the cells were markedly increased (PBS, negative-siRNA, liposome, and PIAS1-siRNA-treated cerulein-stimulated cells, 22.12±7.23, 19.03±4.13, 23.04±6.51 and 33.04± 8.19%, respectively) compared to those of the control cells (7.01±6.66%, P<0.05). At the same time, in the cerulein-stimulated PIAS1 gene-silenced cells, the apoptotic rates of the cells were greatly increased compared to those of the other cerulein-stimulated cells (P<0.05, respectively).

Furthermore, in order to identify the mechanisms involved in PIAS1-SiRNA-induced apoptosis and cell cycle arrest in cerulein-stimulated pancreatic acinar cells, the G1 phase cycle-related proteins including P21, P27 and cyclin E, were examined as shown in Fig. 2B. The expression of the cyclin E protein was found to be markedly reduced in all of the cerulein-stimulated cells, and the protein levels of P21 and P27 were increased compared to the control cells (P<0.05, respectively). Furthermore, the expressions of the P21 and P27 proteins, but not cyclin E, were increased in the cerulein-stimulated PIAS1 gene-silenced cells with compared to the other cerulein-stimulated cells (P<0.05, respectively). In addition, the expressions of apoptosis-regulatory factors including P53, Bax, Bcl-2 and caspase 3 were investigated by Western blot analysis and RT-PCR (Fig. 2C and D). The results revealed that in all of the cerulein-stimulated cells, the P53, Bax, and cleaved caspase 3 proteins levels were greatly increased and the Bcl-2 protein levels were down-regulated, compared to those of the control cells (P<0.05, respectively). Simultaneously, the levels of P53, Bax and cleaved caspase 3 proteins were significantly enhanced in the PIAS1 gene-silenced cells compared to the other cerulein-stimulated cells (P<0.05), respectively. As expected, the mRNA levels of the apoptosis-promoting factors, P53, Bax and caspase 3, were highly expressed and the levels of the apoptosis inhibitor, Bcl-2, were slightly expressed in all of the cerulein-stimulated compared to the control cells (P<0.05, respectively). However, the mRNA levels of the apoptosis-promoting factors, P53 and caspase 3, were increased in the cerulein-stimulated PIAS1 gene-silenced cells compared to the other cerulein-stimulated cells (P<0.05, respectively). The mRNA levels of the apoptosis inhibitor, Bcl-2, were lower in the cerulein-stimulated PIAS1 gene-silenced cells compared to those the other cerulein-stimulated cells (P<0.05, respectively).

## Discussion

AP is a disease of variable severity and certain patients experience mild, self-limited attacks, whereas others manifest a severe, highly morbid and frequently lethal attack. It is currently believed that, during the early phase of AP, pancreatic acinar cells produce and release excessive inflammatory cytokines, thus causing the inflammatory cascade to run out of control, and consequently inducing or exacerbating SIRS, and finally resulting in MODS (9). In the progression of the disease, the generation of inflammatory mediators is believed to occur subsequent to pancreatic acinar cell injury, and these 'downstream' events are believed to influence the severity of the disease (10). Apoptosis is involved in cell injury in AP which is often induced by inflammatory mediators such as TNF-α, reactive oxygen species, and so on (11). The observations of previous studies have led to the hypothesis that apoptosis could be a favorable response to pancreatic acinar cells in the early phase of AP, although the excessive apoptotic response of pancreatic acinar cells could induce the severity of AP in the later phase of the disease (12). Therefore, the apoptosis of pancreatic acinar cells plays an important role of self-protection. However, it has also been found to play an important role in the emergence and development of AP.

RNA interference mediated by siRNA is an important defense mechanism that binds to complementary target mRNA, while specifically targeting these sequences for degradation, resulting in the inhibition of protein expression to prevent and/or treat disease. In addition, siRNA have been extensively tested against inflammatory disease in animal models (13). In our previous study, chemically synthesized siRNA were shown to successfully inhibit the replication of PIAS1 in AR42J cells. The aim of this study was to use the PIAS1 gene-silenced AR42J cells to investigate the potential role of PIAS1 in the regulation of inflammatory mediators and cell injury of cells in an *in vitro* model of AP.

Related data have provided evidence that the MAPKs are widely represented in the family of serine/threonine protein kinases in mammalian cells. The MAPKs amplify and integrate signals from a diverse range of stimuli to elicit

te physiological responses, which include cellular tion, differentiation, inflammatory response, and apoptosis (14). The P38 MAPK and JNK/SAPK are both called stress protein kinases, based upon their activation in response to stress conditions such as heat and osmotic shock, cytokines, protein synthesis inhibitors, antioxidants, and DNA damaging agents (15). Their activation in response to stress has contributed greatly to the expression or production of inflammatory mediators such as TNF-α, IL-1, IL-6 and MMP-9 in cells. These modules are generally believed to be part of the cellular stress response machinery in the onset of inflammation in the pancreas (16). Other evidence has also confirmed that the activation of MAPKs, including P38MAPK and JNK/SAPK, was induced rapidly and transiently during AP (17). This study found that the incubation of AR42J cells with cerulein strongly enhanced the phosphorylation of P38MAPK and JNK/SAPK. The results of this study showed the promoting effect of the PIAS gene-silenced cells on P38MAPK activation that significantly abrogated cerulein-induced inflammatory mediator production, thus confirming the role of P38MAPK in the process of AP and the correlation with PIAS1 expression. However, the PIAS1 gene silencing of the AR42J cells did not affect the cerulein-induced JNK/SAPK activation.

In the development of AP, it has been proposed that the expression levels of the inflammatory mediators, such as TNF-α, IL-1ß and IL-6, were elevated, thus mediating the systemic complications of the disease (18). MMP-9 is a structurally related endopeptidase and $Zn^{2+}$-containing enzyme that degrades a wide range of extra-cellular matrix components. The increased expression of MMP-9 has been observed in response to the TNF-α stimulated inflammatory response. Our previous study showed that the increased expression of MMP-9 had a close correlation with the clinical deterioration of AP (19,20). In this study, the expression levels of these inflammatory mediators were increased in the cerulein-stimulated AR42J cells. At the same time, in the PIAS1 gene-silenced AR42J cells, the above-mentioned markers were also significantly higher compared to the cerulein only-stimulated cells. The results indicate that PIAS1 could be involved in the negative regulation of inflammatory mediator production in an *in vitro* model of AP via regulating the P38MAPK signaling pathway.

The cell cycle inhibitors, P21 and P27, which block cell cycle progression by inhibiting cyclin E, have been extensively studied, and their increased levels have been documented during the lesion formation of cells induced by the TNF-α signaling pathway (21). In addition, the P53 signaling pathway plays a very important role in cell cycle arrest and apoptosis induced by the up-regulation of the P21 expression (22,23). In order to examine cell injury in an *in vitro* model of AP, cell apoptosis, cycle, and the expression of apoptosis-regulatory factors, were explored. The results showed that in the cerulein-stimulated PIAS1 gene-silenced AR42J cells, there was a typical DNA ladder pattern of inter-nucleosomal fragmentation, an increase in apoptotic rate, increase in cell cycle arrest in the G1 phase, and a decrease in arrest in the S phase. The PIAS1 gene silencing of the cerulein-stimulated AR42J cells induced an increase in P53 levels, and the expressions of the downstream targets of p53 including P21, P27, Bax capsase 3, were increased following the cerulein treatment of the PIAS1 gene-silenced cells. However, the expression levels of the apoptotic inhibitor, Bcl-2, were decreased in the cerulein-stimulated PIAS1 gene-silenced AR42J cells compared the cerulein only-stimulated AR42J cells. The results indicate that PIAS1 could be involved in the P53 signaling pathway activation of apoptosis induced by cerulein in pancreatic acinar cells by regulating inflammatory mediators.

In conclusion, our results provide evidence that cerulein rapidly triggers the P38MAPK signaling pathway activity to mediate the transcription of inflammatory mediators in pancreatic acinar cells. Our study also documents a novel effect of PIAS1 gene silencing in an *in vitro* model of AP, resulting in the enhanced activity of P38MAPK, the increased inflammatory mediator production and in the induction of apoptosis. These mechanisms could promote the progress of inflammatory response and subsequent cell injury in AP.

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