Original Article

TSG-6 Induces Apoptosis of Human Hypertrophic Scar Fibroblasts via Activation of the Fas/FasL Signalling Pathway

(TSG-6 / hypertrophic scar fibroblast / apoptosis / Fas/FasL)

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Abstract. Tumour necrosis factor-stimulated gene 6 (*TSG6*) is a protective inflammatory reaction gene which is upregulated by inflammatory processes. Recent studies suggest that TSG-6 exhibits anti-scarring effects. However, the mechanism of TSG-6 action in the scar formation remains poorly understood. We investigated whether TSG-6 affects growth of the human hypertrophic scar fibroblasts (HSFs) via Fas/FasL signalling pathway. Cultured HSFs were transfected with a vector carrying the *TSG6* gene (pLVX-Puro-TSG-6) or with a vector not containing the *TSG6* gene (pLVX-Puro). Untransfected HSFs served as a control group to both transfected HSFs. The expressions level of TSG-6 was up-regulated in the pLVX-Puro-TSG-6 group at the protein and mRNA level. MTT and flow cytometry were used to assess the effect of TSG-6 on the growth and apoptotic status of HSFs. Finally, qRT-PCR and western blot were used to measure the expression levels of Fas, FasL, FADD, caspase-3 and caspase-8 in each group. The apoptosis rate was significantly enhanced and the growth rate reduced in the HSFs transfected with the *TSG6* gene vector. The expression levels of Fas, FasL, FADD, caspase-3 and caspase-8 were significantly raised in the TSG-6 overexpressing HSFs. It is concluded that increased expression of TSG-6 may induce apoptosis of human hypertrophic scar fibroblasts via activation of the Fas/FasL signalling pathway.

Introduction

Wound healing is a very delicate and complicated process affected by many factors, and excessive healing can lead to the formation of a pathological scar (including hypertrophic scar and keloid) (Theoret, 2009). Dermal fibroblasts play an important regulatory role in wound healing (Wang et al., 2008). Studies have indicated that the formation of scar after tissue damage can be attributed to the inflammatory reaction, and the degree of pathological changes is closely related to the inflammatory reaction (Mak et al., 2009). Excessive scarring is a kind of dermal fibroproliferative disorder, which significantly affects the patient's quality of life, both physically and psychologically because of causing pain, pruritus and contractures. Therapy has become a difficult problem in the clinical work of plastic surgery due to the fact that pathogenesis of hypertrophic scar formation remains largely unknown. The possibility to inhibit proliferation of fibroblasts or induce their apoptosis becomes a very meaningful treatment strategy in the scar treatment research field (Gauglitz et al. 2011).

Tumour necrosis factor α-stimulated gene 6 (*TSG6*) was first reported in the early 1990s as a cDNA derived from TNF-treated cultures of normal human diploid FS-4 foreskin fibroblasts. The gene encodes a secreted 35 kDa polypeptide of 277 amino acids including a signal peptide of 17 amino acids, which is a member of the family of hyaluronate-binding proteins (Lee et al., 1990; Klampfer et al., 1994). Further research on the function of the *TSG6* gene showed that TSG-6 mRNA was not detectable in unstimulated cells, while they became readily expressed in the TNF-treated cells (Lee et al., 1992). TNF-α and IL-1 activate transcription of the *TSG6* gene in normal human fibroblasts through a promoter region (−165 to −58) that encompasses an AP-1 and a NF-IL6 site (Lee et al., 1993; Klampfer et al., 1994). TSG-6 can affect expression of various molecules that have important roles in the negative feedback

control of the tissue inflammatory response (Wisniewski and Vilcek, 1997; Choi et al., 2011). Tan et al. (2011) found that there was a significant reduction in TSG-6 levels within keloid scars compared with the dermis of unscarred skin, which might contribute to the pathological scar formation. In our previous research, we demonstrated that TSG-6 exhibited anti-inflammatory and anti-scarring effects in the rabbit ear model (Wang et al., 2015).

It has been found that the regulation of apoptotic phenomena during wound healing may be important in the scar establishment and in the development of pathological scars (Desmouliere et al., 1995). The cell-surface Fas receptor (Fas) is a member of the tumour necrosis factor (TNF) and nerve growth factor family of receptors (Nagata and Golstein, 1995; Schulze-Osthoff et al., 1998). Fas is widely distributed in skin cells (Oishi et al., 1994). Research has shown that stimulation of Fas receptors can induce apoptosis or proliferation in human dermal fibroblasts depending on the magnitude of Fas aggregation (Aggarwal et al. 1995; Freiberg et al., 1997; Jelaska and Korn, 1998).

In our current study, it was hypothesized that TSG-6 may induce apoptosis of human hypertrophic scar fibroblasts via activation of the Fas/FasL signalling pathway. To validate this hypothesis, expression vector pLVX-Puro-TSG-6 was constructed and stably transfected to human hypertrophic scar fibroblasts (HSFs). The effects of overexpressed TSG-6 on the growth and apoptosis of HSFs were then evaluated. Further, the expression levels of Fas, FasL, FADD, caspase-3 and caspase-8 in HSFs with various expression levels of TSG-6 were determined.

Material and Methods

Patient selection and tissue collection

Ten participants (Table 1) with untreated hypertrophic scar were recruited from patients undergoing scar revision surgery at the First Affiliated Hospital of Anhui Medical University. This study received approval from the Research Ethics Committee of the First Affiliated Hospital of Anhui Medical University.

Hypertrophic scar fibroblast cultures

Specimens from the excised tissue were dissected free of fat and cut into pieces smaller than 1 mm in diameter. Tissues were explanted in 25 cm² tissue culture flasks (Corning Coster Corpration, Corning, NY) in a growth medium that consisted of Dulbecco's Modified Eagle's Medium (DMEM), L-glutamine, 100 µg/ml streptomycin, 100 U/ml penicillin (all Gibco, Fisher Scientific, NY) and foetal calf serum (HyClone, Fisher Scientific) and incubated at 37 °C in an atmosphere with 5% carbon dioxide and relative humidity of 100 percent. The medium was changed every 2–3 days. Seven days after the primary culture, cells were passaged every 3–4 days.

Construction of recombinant lentiviruses carrying the TSG6 gene

Total RNA was extracted using the Trizol reagent (Invitrogen, Carlsbad, CA) from the above isolated fibroblasts. cDNA was synthesized using PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time: Takara Bio Inc., Otsu, Shiga, Japan). The *TSG6* gene (GenBank: AJ421518) was amplified by PCR using the following primer sequences: forward 5′-GGAATTCA TGATCATCTTAATTTACT-3′ and reverse 5′-CGGGAT CC TAAGTGGCTAAATCTTCC-3′. The amplification product and lentiviral plasmid pLVX-Puro (Clontech Inc., Mountain View, CA) were ligated with T4 DNA ligase after double digestion by restriction enzymes *Eco*RI and *Bam*HI. The product of the ligation was transformed into competent *E. coli* DH5α cells. Recombinant plasmids were identified by PCR and DNA sequencing. The target plasmid was named pLVX-Puro-TSG-6.

Packaging of recombinant lentiviruses

The target plasmid with pHelper 1.0 and pHelper 2.0, which was extracted and purified using an endotoxin-free plasmid extracting kit, was used to transfect 293T cells (Clontech Inc.) by Lipofectamine 2000 (Thermo Fisher, Waltham, MA) according to the manufacturer's instructions. After 8 h of transfection, the medium was replaced by complete medium, and after 48 h of culture,

Table 1. Patient information

Patient Number	Gender	Age	Position	Cause of Scar
01	Male	25	Upper Arm	Scald
02	Female	16	Back	Surgery
03	Male	7	Upper Arm	Scald
04	Male	21	Chest	Scald
05	Female	19	Chest	Burn
06	Female	9	Upper Arm	Burn
07	Female	23	Shoulder	Surgery
08	Male	18	Back	Surgery
09	Female	17	Abdomen	Surgery
10	Male	15	Shoulder	Surgery

the supernatant containing lentivirus particles was collected and centrifuged at 250 g for 5 min, meanwhile the cell debris was removed. Cells and debris were further removed by filtering the supernatant and its volume was condensed to obtain a high concentration of lentivirus. The virus titre in 293T cells was measured by using the end-point dilution assay.

Transfection of HSFs with recombinant lentiviruses

HSFs in the exponential phase of growth were divided into three groups: future pLVX-Puro-TSG-6 HSFs, pLVX-Puro HSFs, and untransfected HSFs. The cells were cultured in a 6-well plate at a concentration of 8×10^4 cells/ml for 24 h at 37 °C in an atmosphere with 5% CO_2. The supernatant was discarded and 2 ml of the growth medium consisting of DMEM and 10% foetal calf serum was added. When cells were fused to 30 %, the medium was removed, and two wells in the pLVX-Puro-TSG-6 group and two wells in the pLVX-Puro group were transfected with pLVX-Puro-TSG-6 or pLVX-Puro with a multiplicity of infection (MOI) = 10. Following addition of 50 μl of Polybrene, 2 ml of growth medium was added. In two wells of the untransfected HSF control group, 2 ml of growth medium only was added. Stable transfectants of pLVX-Puro-TSG-6 and pLVX-Puro were obtained by puromycin continuous selection at a concentration of 2.5 μg/ml. The stably transfected cells were verified by quantitative real-time PCR and western blot analysis.

Flow cytometry assay of cell apoptosis

Cells were collected after digestion with trypsin without EDTA and washed twice with ice-cold PBS. Cells in the amount of 1×10^6 were centrifuged and resuspended in 400 μl of 1× Binding Buffer. Five μl of Annexin V-FITC (Bestbio, Shanghai, China) was added according to the manufacturer's protocol. After 15 min of incubation at 4 °C in the dark, propidium iodide was added and the incubation extended for another 5 min. The apoptosis rate was detected by flow cytometry (BD FACSVerse, Piscataway, NJ). Each experiment was repeated three times.

MTT assay of cell viability

Cells were incubated at 37 °C and 5% CO_2 in 96-well plates at a concentration of 1×10^5 cells/ml for 24 h. MTT (5 mg/ml, Sigma-Aldrich, St. Louis, MO) was dissolved in RPMI medium, filtered through a 0.2 μm filter and stored at –20 °C. Twenty-four hours after cell adherence, 20 μl of the MTT solution was added to each well and the plates were incubated at 37 °C for 4 h. Subsequently, the plates were centrifuged at 800 g for 20 min and 100 μl of DMSO was added to each well and mixed thoroughly to dissolve the crystals. The OD values were determined at 490 nm wavelength using an Infinite M200 microplate reader (Tecan, Salzburg, Austria) after rocking the cells for 10 min. Each group comprised six replicate wells, and the experiment was repeated three times.

Western blot analysis

Western blot was performed to detect the protein levels of TSG-6, Fas, FasL, FADD, caspase-3 and caspase-8. Twenty μg of each protein sample was resolved using 10% SDS-PAGE. After electrophoresis, the separated proteins were transferred to PVDF membranes (Millipore, Billerica, MA). The membranes were blocked with 5% bovine serum albumin for 2 h at room temperature and incubated with primary polyclonal antibodies against TSG-6 (Abcam, ab132329), Fas (Abcam, ab133619), FasL (Abcam, ab68338), FADD (Abcam, ab108601), caspase-3 (Abcam, ab32042), caspase-8 (Abcam, ab32125) and GAPDH (Abcam, ab37168) at 1/2,000 dilution overnight at 4 °C. The blots were developed with goat anti-rabbit IgG secondary antibodies (Abcam, Cambridge, MA) at 1/10,000 dilution for 1 h at room temperature. Signals were then visualized by enhanced chemiluminescence detection reagents (Millipore) and photographed by the Bio-Spectrum Gel Imaging System (UVP, Upland, CA). The band intensity was measured using the Image-Pro Plus software (Meyer instruments, Inc., Houston, TX).

Quantitative real-time PCR

Total RNA was extracted using the TRIzol method (Invitrogen, Carlsbad, CA). Purified RNA (1 μg) was reverse transcribed using the PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (Takara Bio Inc., Otsu, Shiga, Japan). The resulting cDNA samples were amplified by real-time PCR with SYBR® Premix Ex TaqTM II (Tli RNaseH Plus: Takara Bio Inc.) and the 7500 Real Time PCR System (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. PCR primer sequences used are listed in Table 2. The thermocycler parameters were as follows: 95 °C for 10 min, 40 cycles of 95 °C for 30 s, 58 °C for 1 min, and 72 °C for 1 min, and 1 cycle of 95 °C for 1 min, 60 °C for 30 s, and 95 °C for 30 s. The levels of mRNA expression were calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Statistical analysis was carried out by conducting one-way ANOVA using the GraphPad Prism 5.0 software (GraphPad Software, La Jolla, CA) and the data were expressed as mean ± SD. Tukey's post-hoc multiple comparison test was used to examine the statistical significance between the groups and a P value < 0.05 was considered to be significant.

Results

Expression of TSG-6

The specific band of the TSG-6 protein was observed at about 30 kD of the marker in western blots. The absorbance density ratio of TSG-6 to GAPDH was signifi-

Table 2. Primer sequences used in qRT-PCR analysis

Gene	Forward (5'–3')	Reverse (5'–3')
FasL	TGGGGATGTTTCAGCTCTTC	GTGGCCTATTTGCTTCTCCA
Fas	CAAGGGATTGGAATTGAGGA	ACCTGGAGGACAGGGCTTAT
FADD	CTGGGGAAGAAGACCTGTGT	GCTGTCGATCTTGGTGTCTG
Caspase-8	GGAGGAGTTGTGTGGGGTAA	AGTATCCCCGAGGTTTGCTT
Caspase-3	GGAGGCCGACTTCTTGTATG	ACTGTTTCAGCATGGCACAA
GAPDH	CAGGAGGCATTGCTGATGAT	GAAGGCTGGGGCTCATTT

cantly increased in pLVX-Puro-TSG-6 cells compared with pLVX-Puro cells and untransfected HSF cells (P < 0.05). There was no significant difference between pLVX-Puro cells and untransfected HSF cells (Fig. 1).

Quantitative reverse transcription PCR (qRT-PCR) showed that the expression of TSG-6 mRNA was significantly higher in pLVX-Puro-TSG-6 cells compared with pLVX-Puro cells and untransfected HSF cells (P < 0.05), but there was no significant difference between pLVX-Puro and HSF cells (P >0.05) (Fig. 2). This indicated that the TSG-6 mRNA and protein were markedly expressed in the HSFs containing the pLVX-Puro-TSG-6 gene construct.

Effects of TSG-6 on HSF apoptosis

The apoptotic rate in pLVX-Puro-TSG-6 cells was 37.2 %, significantly higher than in pLVX-Puro cells (12.7 %) and untransfected HSFs cells (15.7 %) (P < 0.05). However, there was no significant difference between pLVX-Puro and untransfected HSFs cells (P > 0.05). This suggested that the overexpression of TSG-6 significantly promotes apoptosis in hypertrophic scar fibroblasts (Fig. 3).

Effects of TSG-6 on cell viability

The MTT cell viability assay revealed a reduced growth rate in pLVX-Puro-TSG-6 cells in comparison to pLVX-Puro and untransfected cells at the 24 h and later intervals of incubation (Fig. 4). Conversely, there was no significant difference between the growth kinet-
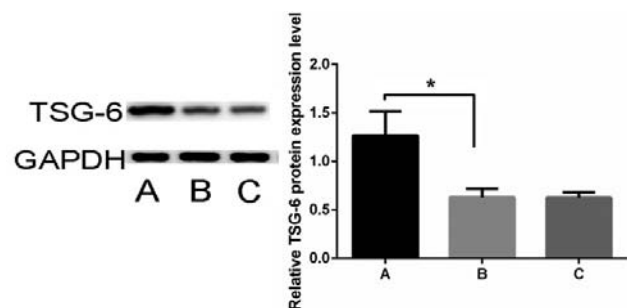
ics in pLVX-Puro and untransfected HSF cells. This demonstrates that the overexpression of TSG-6 can inhibit HSF growth.

Expression of genes and proteins involved in the Fas/FasL signalling pathway

The qRT-PCR results showed that the mRNA expression levels of Fas, FasL, FADD, caspase-3 and caspase-8 were significantly increased in pLVX-Puro-TSG-6 HSF cells compared with pLVX-Puro and untransfected HSF cells (P < 0.05), but that there was no significant difference between pLVX-Puro and untransfected HSFs (Fig. 5).

The western blotting results showed significantly increased protein expression levels of Fas, FasL, FADD, caspase-3 and caspase-8 in the TSG-6-overexpressing HSFs (P < 0.05) (Fig. 6). These results suggest that the Fas/FasL signalling pathway is activated by TSG-6.

Discussion

The abnormal hypertrophic scarring process arises not only from overproduction of collagen in the extracellular matrix (ECM) (Fujiwara et al., 2005), but also
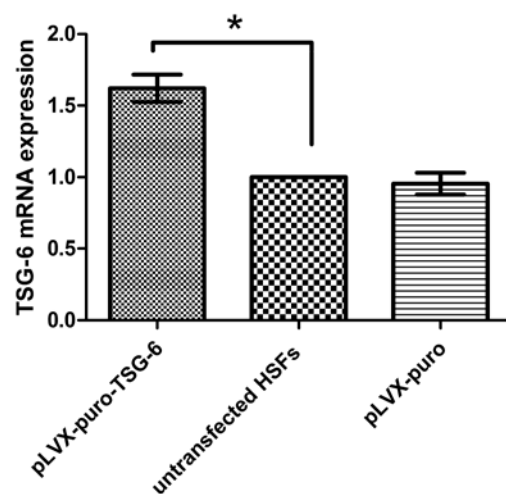

Fig. 1. TSG-6 protein expression. **A**: pLVX-Puro-TSG-6 HSFs. **B:** untransfected HSFs. **C**: pLVX-Puro HSFs. The band intensity ratio of TSG-6 to GAPDH was significantly increased in pLVX-Puro-TSG-6 HSFs compared with pLVX-Puro and untransfected HSFs (P < 0.05).

Fig. 2. TSG-6 mRNA expression (N = 4, *P < 0.05). The expression of TSG-6 mRNA in pLVX-Puro-TSG-6 HSFs was significantly increased compared with pLVX-Puro and untransfected HSFs (P < 0.05).
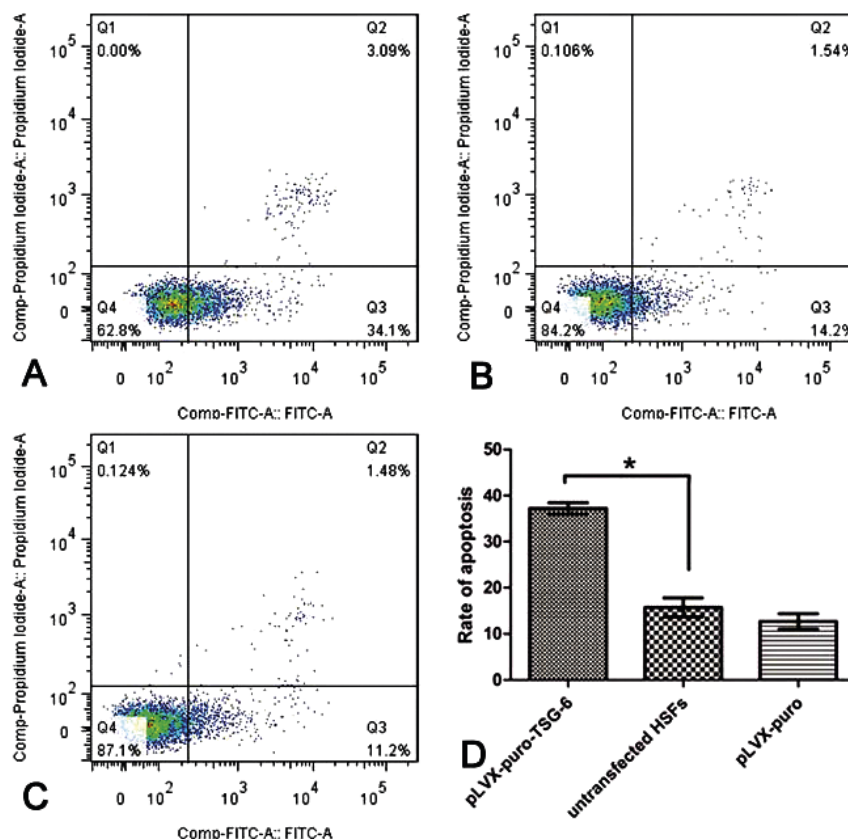
Fig. 3. Effect of TSG-6 on apoptosis in HSFs (N = 4, *P < 0.05). **A**: pLVX-Puro-TSG-6 HSFs. **B**: untransfected HSFs. **C**: pLVX-Puro HSFs (examples). **D**: The apoptosis rate in pLVX-Puro-TSG-6 HSFs was significantly higher than the apoptosis rate in pLVX-Puro and untransfected HSFs (P < 0.05).

from high numbers of fibroblasts in hypertrophic scars resulting from their excessive proliferation (Nakaoka et al., 1995; Bellemare et al., 2005) and/or reduced apoptosis (Messadi et al., 1998; Chen et al., 2012). The expression of the TSG-6 protein was negative in normal skin tissues but increased after stimulation with various inflammatory agents (Milner and Day, 2003). Tan et al. (2011) found that TSG-6 levels were significantly reduced in the keloid scars compared with the dermis of unscarred skin. It can thus be hypothesized that insufficient TSG-6 expression may be one of the factors that lead to the formation of pathologic hypertrophied scars. In our previous study, we injected the recombinant human TSG-6 protein into hypertrophic scars using a rabbit ear model, and the obtained results indicated that TSG-6 exhibited anti-inflammatory and anti-scarring effects (Wang et al., 2015). Therefore, in the present study, we overexpressed TSG-6 in cultures of fibroblasts derived from the skin of patients suffering from hypertrophied scars.

Fas-mediated apoptosis can occur through two distinct signalling pathways. One is the extrinsic pathway, induced by FasL or Fas agonistic antibodies. The intracellular domain of Fas, called the death domain (DD), binds adaptor protein Fas-associated protein with death domain (FADD), which in turn recruits procaspase-8, resulting in the formation of the death-inducing signalling complex (DISC). Within the DISC, the initiator procaspase-8 molecules are activated by dimerization and subsequent auto-processing. The active caspase-8 can directly cleave effector caspases such as caspase-3, which is the effector caspase responsible for most of the cellular changes characteristic of apoptosis. The intrinsic pathway is initiated in response to cellular stress. The amount of caspase-8 that is activated within the DISC is very low and insufficient to directly activate the downstream executioner procaspases. Triggering of apoptosis depends on the activation of pro-apoptotic Bcl-2 family members such as BH3 interacting domain death agonist (Bid) and Bax/Bak. Ultimately, cytochrome c is released from the intermembrane space of the mitochondrion into the cytosol, where it binds to Apaf-1 to form the apoptosome. The apoptosome recruits and activates caspase-9, which in turn activates caspase-3 and -7 (Barnhart et al. 2003; Legembre et al., 2005; Shawgo et al., 2009; Hao and Mak, 2010).

MTT and flow cytometry assays revealed that the upregulated TSG-6 expression could significantly inhibit HSF growth and significantly induce apoptosis. Therefore, qRT-PCR and western blot analyses were used to determine the expression level of the genes and proteins involved in the Fas/FasL signalling pathway. The results showed that the expression levels of Fas/FasL signalling pathway-related genes and proteins were significantly
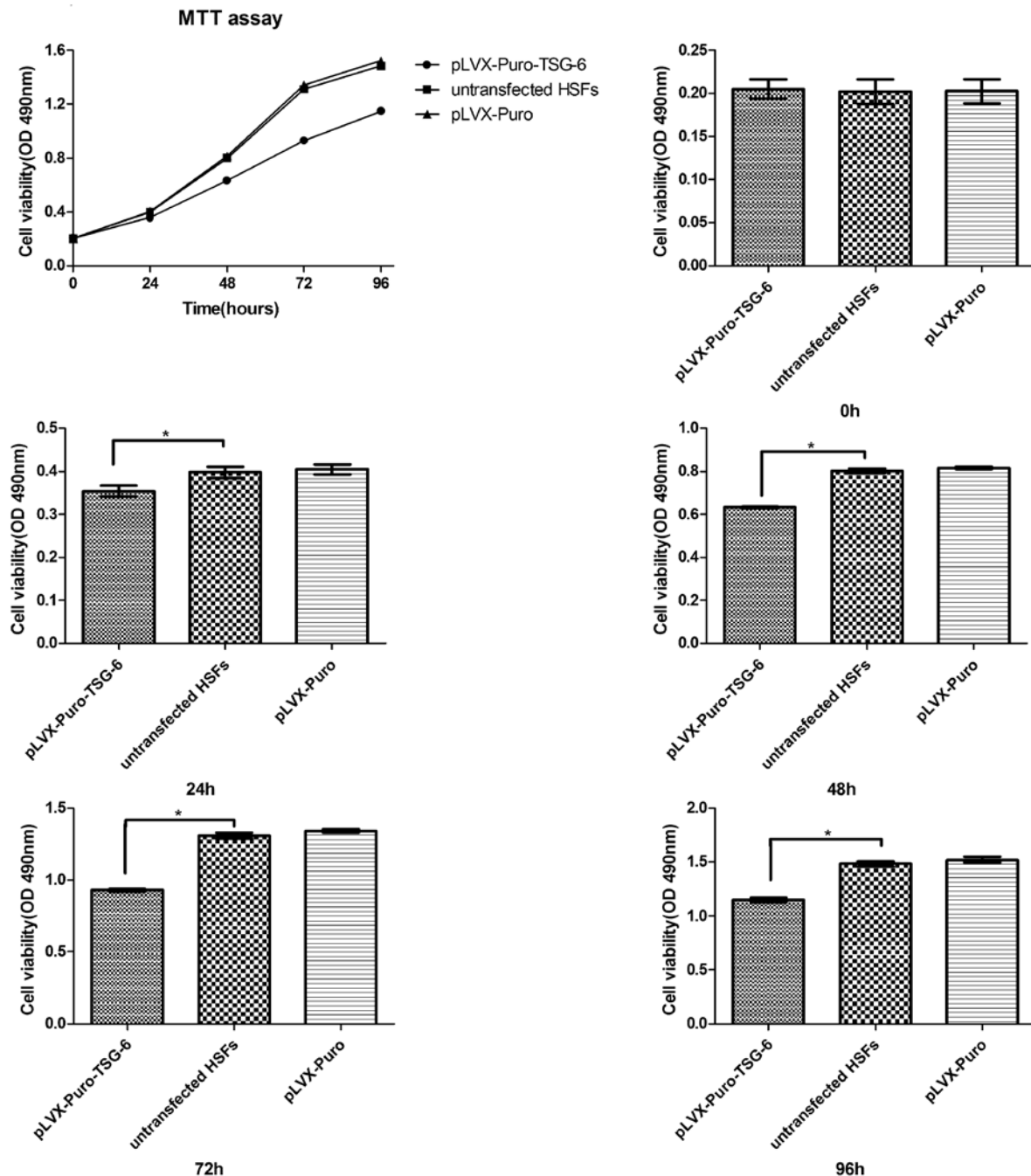
Fig. 4. Effect of TSG-6 on viability of HSFs (N = 3, *P < 0.05). pLVX-Puro-TSG-6 HSFs had a reduced growth rate compared with pLVX-Puro and untransfected HSFs at 24 h and later intervals (P < 0.05).

increased in the pLVX-Puro-TSG-6 cells overexpressing TSG-6. This indicates that the high expression of *TSG6* gene could increase the apoptosis rate of HSFs and markedly reduce their growth rate via activation of the Fas/FasL signalling pathway.

To sum up, we suggest that a high expression level of TSG-6 can induce apoptosis in hypertrophic scar fibroblasts via activation of the Fas/FasL signalling pathway. This may provide a mechanism how TSG-6 could prevent development of hypertrophic scars, without excluding some other mechanism through which TSG-6 may modulate skin scar formation.

Conclusions

The levels of genes and proteins involved in the Fas/FasL signalling pathway were significantly increased in the fibroblasts obtained from hypertrophic scars when the expression level of TSG-6 was up-regulated. The present study showed that up-regulation of TSG-6 expression could reduce growth of hypertrophic scar fibroblasts through activation of the Fas/FasL signalling.
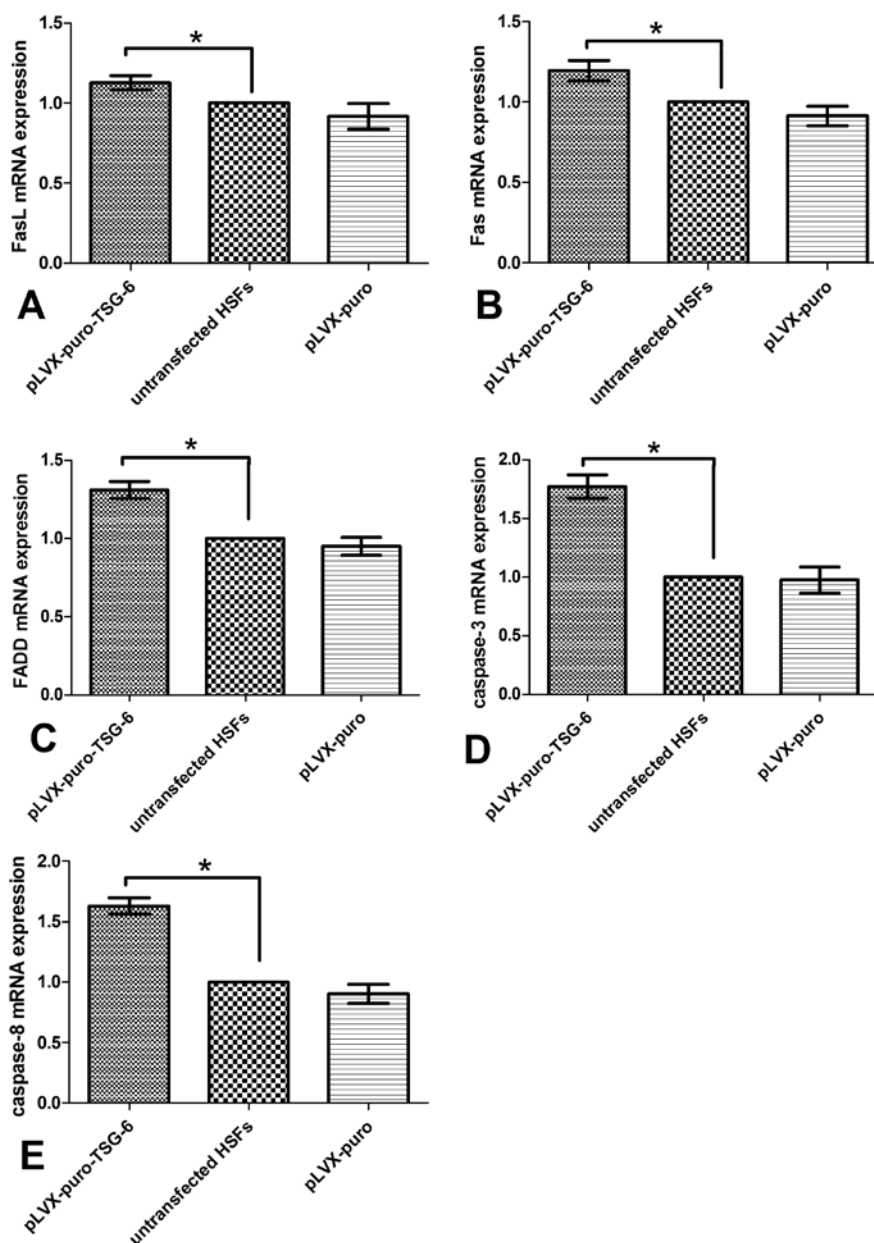
Acknowledgements

Fig. 5. Relative mRNA expression levels of FasL, Fas, FADD, caspase-3 and caspase-8 (N = 4, *P < 0.05). **A**: FasL, **B**: Fas, **C**: FADD, **D**: caspase-3, **E**: caspase-8 mRNA expression level in HSFs with various TSG-6 expression levels.

References

Aggarwal, B. B., Singh, S., LaPushin, R., Totpal, K. (1995) Fas antigen signals proliferation of normal human diploid fibroblast and its mechanism is different from tumor necrosis factor receptor. *FEBS Lett.* **364**, 5-8.

Barnhart, B. C., Alappat, E. C., Peter, M. E. (2003) The CD95 type I/type II model. *Semin. Immunol.* **15**, 185-193.

Bellemare, J., Roberge, C. J., Bergeron, D., Lopez-Valle, C. A., Roy, M., V. J. Moulin, V. J. (2005) Epidermis promotes dermal fibrosis: role in the pathogenesis of hypertrophic scars. *J. Pathol.* **206**, 1-8.

Chen, L., Wang, Z., Li, S., Zhao, G., Tian, M., Sun, Z. (2012) SFRP2 and slug contribute to cellular resistance to apoptosis in hypertrophic scars. *PLoS One* **7**, e50229.

Choi, H., Lee, R. H., Bazhanov, Y. Oh, J. Y. Prockop, D. J. (2011) Anti-inflammatory protein TSG-6 secreted by activated MSCs attenuates zymosan-induced mouse peritonitis by decreasing TLR2/NF-κB signaling in resident macrophages. *Blood* **118**, 330-338.

Desmouliere, A., Redard, M., Darby, I., Gabbiani, G. (1995) Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar. *Am. J. Pathol.* **146**, 56-66.

Freiberg, R. A., Spencer, D. M., Choate, K. A., Duh, H. J., Schreiber, S. L., Crabtree, G. R., Khavari, P. A. (1997) Fas signal transduction triggers either proliferation or apoptosis in human fibroblasts. *J. Invest. Dermatol.* **108**, 215-219.

Fujiwara, M.,Muragaki, M. Y., Ooshima, A. (2005) Keloid-derived fibroblasts show increased secretion of factors involved in collagen turnover and depend on matrix metalloproteinase for migration. *Br. J. Dermatol.* **153**, 295-300.
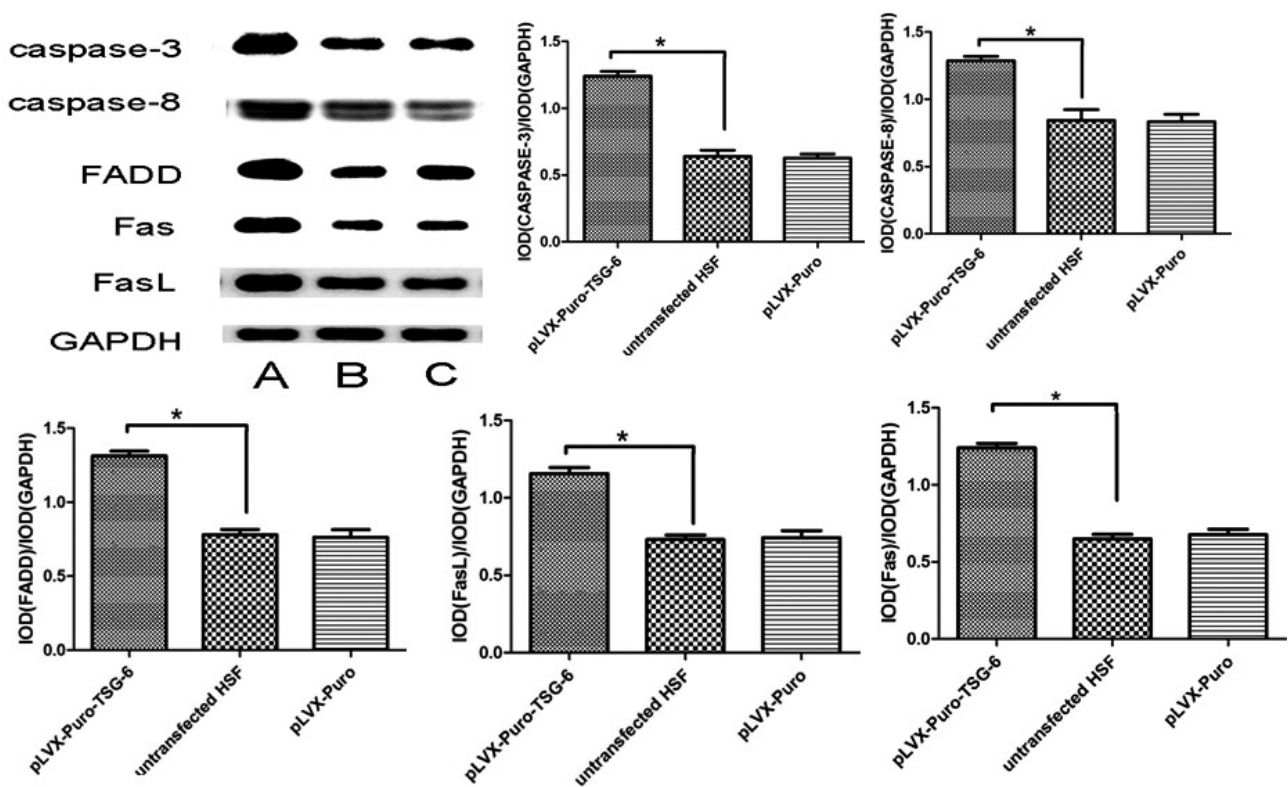
Fig. 6. Protein expression levels of caspase-3, caspase-8, FADD, Fas, and FasL (*P < 0.05). **A**: pLVX-Puro-TSG-6 HSFs. **B**: untransfected HSFs. **C**: pLVX-Puro HSFs. The protein expression levels of Fas, FasL, FADD, caspase-3 and caspase-8 were significantly higher in pLVX-Puro-TSG-6 HSFs.

Gauglitz, G. G., Korting, H. C., Pavicic, T., Ruzicka, T., Jeschke, M. G. (2011) Hypertrophic scarring and keloids: pathomechanisms and current and emerging treatment strategies. *Mol. Med.* **17**, 113-125.

Hao, Z., T. Mak, T. W. (2010) Type I and type II pathways of Fas-mediated apoptosis are differentially controlled by XIAP. *J. Mol. Cell Biol.* **2**, 63-64.

Jelaska, A., Korn, H. J. (1998) Anti-Fas induces apoptosis and proliferation in human dermal fibroblasts: differences between foreskin and adult fibroblasts. *J. Cell. Physiol.* **175**, 19-29.

Klampfer, L., Lee, T. H., Hsu, H., Vilcek, J., Chen-Kiang, S. (1994) NF-IL6 and AP-1 cooperatively modulate the activation of the TSG-6 gene by tumor necrosis factor α and interleukin-1. *Mol. Cell. Biol.* **14**, 6561-6569.

Lee, T. H., Lee, G. W., Ziff, E. B., Vilcek, J. (1990) Isolation and characterization of eight tumor necrosis factor-induced gene sequences from human fibroblasts. *Mol. Cell. Biol.* **10**, 1982-1988.

Lee, T. H., Wisniewski, H. G., Vilcek, J. (1992) A novel secretory tumor necrosis factor-inducible protein (TSG-6) is a member of the family of hyaluronate binding proteins, closely related to the adhesion receptor CD44. *J. Cell. Biol.* **116**, 545-557.

Lee, T. H., Klampfer, L., Shows, T. B., Vilcek, J. (1993) Transcriptional regulation of TSG6, a tumor necrosis factor- and interleukin-1-inducible primary response gene coding for a secreted hyaluronan-binding protein. *J. Biol. Chem.* **268**, 6154-6160.

Legembre, P., Daburon, S., Moreau, P., Ichas, F., de Giorgi, F., Moreau, J. F., Taupin, J. L. (2005) Amplification of Fas-mediated apoptosis in type II cells via microdomain recruitment. *Mol. Cell. Biol.* **25**, 6811-6820.

Mak, K., Manji,A., Gallant-Behm, C., Wiebe, C., Hart, D. A., Larjava, H., Hakkinen, L. (2009) Scarless healing of oral mucosa is characterized by faster resolution of inflammation and control of myofibroblast action compared to skin wounds in the red Duroc pig model. *J. Dermatol. Sci.* **56**, 168-180.

Messadi, D. V., Le, A., Berg, S., Huang, G., Zhuang, W., Bertolami, C. N. (1998) Effect of TGF-β 1 on PDGF receptors expression in human scar fibroblasts. *Front. Biosci.* **3**, a16-22.

Milner, C. M., Day, A. J. (2003) TSG-6: a multifunctional protein associated with inflammation. *J. Cell Sci,* **116(Pt 10)**, 1863-1873.

Nagata, S., Golstein, P. (1995) The Fas death factor. *Science* **267**, 1449-1456.

Nakaoka, H., Miyauchi, S., Miki , Y. (1995) Proliferating activity of dermal fibroblasts in keloids and hypertrophic scars. *Acta Derm. Venereol.* **75**, 102-104.

Oishi, M., Maeda, K., Sugiyama, S. (1994) Distribution of apoptosis-mediating Fas antigen in human skin and effects of anti-Fas monoclonal antibody on human epidermal keratinocyte and squamous cell carcinoma cell lines. *Arch. Dermatol. Res.* **286**, 396-407.

Schulze-Osthoff, K., Ferrari, D., Los, M., Wesselborg, S., Peter, M. E. (1998) Apoptosis signaling by death receptors. *Eur. J. Biochem.* **254**, 439-459.

Shawgo, M. E., Shelton, S. N., Robertson, J. D. (2009) Caspase-9 activation by the apoptosome is not required for Fas-mediated apoptosis in type II Jurkat cells. *J. Biol. Chem.* **284**, 33447-33455.

Tan, K. T., McGrouther, D. A., Day, A. J., Milner, C. M., A. Bayat, A. (2011) Characterization of hyaluronan and TSG-6 in skin scarring: differential distribution in keloid scars, normal scars and unscarred skin. *J. Eur. Acad. Dermatol. Venereol.* **25**, 317-327.

Theoret, C. (2009) Tissue engineering in wound repair: the three "R"s - repair, replace, regenerate. *Vet. Surg.* **38**, 905-913.

Wang, H., Chen, Z., Li, X. J., Ma, L., Tang, Y. L. (2015) Anti-inflammatory cytokine TSG-6 inhibits hypertrophic scar formation in a rabbit ear model. *Eur. J. Pharmacol.* **751**, 42-49.

Wang, J., Dodd, C., Shankowsky, H. A., Scott, P. G., Tredget, E. E. (2008) Deep dermal fibroblasts contribute to hypertrophic scarring. *Lab. Invest.* **88**, 1278-1290.

Wisniewski, H. G., Vilcek, J. (1997) TSG-6: an IL-1/TNF-inducible protein with anti-inflammatory activity. *Cytokine Growth Factor Rev.* **8**, 143-156.