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RhTSG-6 inhibits IL-1β-induced extracellular matrix degradation and apoptosis by suppressing the p38, and JNK pathways in nucleus pulposus cells

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Abstract

Introduction. Intervertebral disc degeneration (IDD) is one of the major causes of low back pain (LBP) which seriously affects health and normal physical activity. Recombinant human tumor necrosis factor-α (TNF-α) induced protein 6 (rhTSG-6) has been reported to have therapeutic effects on a variety of inflammatory diseases, but the effect and mechanism of rhTSG-6 action in IDD are not fully understood. The present study was aimed to explore the functional role of rhTSG-6 in interleukin (IL)-1β-induced nucleus pulposus (NP) cell model.

Materials and methods. Experimental human NP cells were isolated from the patients with idiopathic scoliosis and treated with culture medium containing IL-1β (10 ng/mL) for 24 hours to induce extracellular matrix degradation and apoptosis, simulating an IDD model *in vitro.* The viability of NP cells was analyzed by the CCK-8 assay. The relevant mRNA and protein levels were measured by RT-qPCR and western blot. The apoptosis of NP cells was determined by flow cytometry analysis and western blot.

Results. Compared with the NP cells without IL-1β treatment, IL-1β caused approximately 70% reduction in the viability of NP cells, while RhTSG-6 partly increased the decrease of IL-1β on cell viabilities. Moreover, treatment with rhTSG-6 considerably attenuated the upregulation of extracellular matrix (ECM)-catabolic factors (MMP-3, MMP-13, ADAMTS-4, and ADAMTS-5), and increased the downregulation of ECM-anabolic factor (collagen II) in NP cells induced by IL-1β, indicating that ECM degradation was suppressed. Furthermore, rhTSG-6 also protected NP cells from IL-1β-induced apoptosis. Mechanically, rhTSG-6 inhibited the activation of members of mitogen-activated protein kinase (MAPK) pathway by blocking the phosphorylation of p38, c-Jun N-terminal kinase (JNK) and ERK in IL-1β-induced NP cells.

Conclusions. RhTSG-6 can attenuate ECM degradation and apoptosis in IL-1β-induced NP cells by inhibiting the p38, JNK and ERK pathways, which may contribute to its potential application in the therapy of IDD. (*Folia Histochemica et Cytobiologica 2020, Vol. 58, No. 3, 227–234)*

Key words: intervertebral disc degeneration; rhTSG-6; extracellular matrix degradation; apoptosis; IL-1β; nucleus pulposus cells

Introduction

Low back pain (LBP) is a common and multifactorial debilitating disease worldwide, leading to severe disability and a significant socio-economic burden [1]. Intervertebral disk degeneration (IDD) is believed to be one of the leading causes of LBP, which confuses 80% of the world's population [2]. Intervertebral disks (IVDs) are very important components of the human spine structure, maintaining the stability of the spine. IVDs are composed of the inner glycosaminoglycans (GAGs)-rich nucleus pulposus (NP) surrounded by the outer collagen-rich annulus fibrosus (AF) and cartilaginous endplate (CEPs) [3]. As

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the IDD progressed, the levels of pro-inflammatory cytokines (including TNF-α, IL-1β, IL-6 and etc.) and cell apoptosis increased. Moreover, during IDD the production of extracellular matrix (ECM)-catabolic proteinases [including disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS, which is a superfamily of 26 secreted molecules composed of ADAMTS proteases and ADAMTS-like proteins, and has the function of degrading ECM [4] and matrix metalloproteinases (MMPs)] were elevated, while the synthesis of type II collagen and aggrecan was decreased in NP tissue [5, 6]. Admittedly, up-regulated ADAMTS (including ADAMTS-4 and ADAMTS-5) and matrix MMPs (including MMP-1, MMP-3, MMP-7, MMP-9, and MMP-13) are responsible for the degradation of ECM components [4, 7, 8]. Therefore, inhibiting NP cell apoptosis and ECM degradation by NP cells may be therapeutic targets for delaying IDD.

IL-1β and other pro-inflammatory cytokines are expressed at high levels in degenerative IDD tissues and cells and have been demonstrated to play essential roles in development of IDD [9–11]. IL-1β has also been reported to regulate pathological processes of NP cells in the disk, including inflammatory response, cell apoptosis, and MMP production and ECM homeostasis, further leading to destruction of the physiological structure and function of IVD and the instability of the spine, which ultimately causing LBP [12, 13]. Induction of NP cells with IL-1β has been widely reported as an *in vitro* model for simulating the process of IVD [14–16]. Mechanically, reversing the effect of IL-1β on the cell apoptosis and ECM degradation of NP cells may delay the progression of IDD.

Tumor necrosis factor-α (TNF-α)-induced protein 6 (TSG-6) is a 35 kDa protective inflammatory response protein that mediates inflammatory cell migration, adhesion, involvement in immune regulation and extracellular matrix remodeling by binding to hyaluronic acid, chondroitin sulfate or proteoglycans, and thus plays an inflammatory regulatory role [17–21]. Numerous evidences have demonstrated that administration of recombinant human TSG-6 (rhTSG-6) has been used in a variety of disease models and has demonstrated a broad and strong anti-inflammatory effect. Tuo *et al.* demonstrated that rhTSG-6 could stabilize retinopathy in Ccl2 -/- /Cx3cr1 -/- mice [22]. Li *et al.* found that rhTSG-6 could regulate microglia polarization in rats with subarachnoid hemorrhage and reduce inflammatory brain injury [23]. The protective effects of rhTSG-6 have also been well studied in osteoarthritis. TSG-6 is reported to be up-regulated in rheumatoid arthritis and osteoarthritis and as a biomarker for the progression of knee osteoarthritis [24, 25]. Mindrescu *et al.* indicated that rhTSG-6 improved collagen-induced arthritis in DBA/1J mice [26]. Tellier *et al.* suggested that TSG-6 could attenuate cartilage damage in a rat model of osteoarthritis [27]. A recent study showed that TSG-6 secreted by bone marrow mesenchymal stem cells (BMSCs) attenuated IL-1β-induced NP cell degeneration by inhibiting the activation of the TLR2/ /NF-κB signaling pathway [28]. However, the effects of rhTSG-6 on apoptosis and ECM degradation in IL-1β-induced NP cells still remain unclear.

In this study, we aimed to investigate the influence of rhTSG-6 on ECM degradation and apoptosis in IL-1β-induced NP cells and to explore its potential molecular mechanism.

Materials and methods

Isolation and culture of NP cells. NP cells were isolated from 10 patients with idiopathic scoliosis (average age 22.8 years, range 18–45) as described in previous studies [15, 28]. Thereafter, NP cells were cultured in DMEM/F12 (Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin mix at 37°C in a humidified atmosphere with 5% CO_2.

Cell viability analysis. Cell viability was evaluated by Cell Counting Kit-8 (CCK-8; Dojindo Co, Kumamoto, Japan). The NP cells were seeded in a 96-well plate at a density of 5×10^5 cells/well until reaching 80–90% confluence. The cells were treated with different concentrations of rhTSG-6 (0, 0.5, 1, 1.5, 2 μg/mL) to determine its effects on viability of NP cells. The cells were pre-treated with different concentrations of rhTSG-6 (0, 0.5, 1, 1.5, 2 μg/mL) for 2 h in NP cells then treated with or without 10 ng/mL IL-1β for 24 h at 37°C. Subsequently, 10 μL of CCK-8 solution was added into each well and the cells were incubated at 37°C for 2 h. The optical density at 450 nm was measured using a microplate reader (BioTek Instruments, Inc., Winooski, VT, USA).

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNAs were extracted from cells and using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. First-strand cDNA was reverse transcribed from RNAs using reverse transcriptase kit (Takara, Dalian, China). qRT-PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, CA, USA). Relative expression levels were calculated with the $2^{-\Delta\Delta Ct}$ method and normalized to GAPDH. The sequence of primers used for RT-qPCR analysis were as follows: MMP-3, forward: 5'-AAAATCAAGCAG-CGGCGAAG-3', and reverse: 5'-CTCGCGCATAAAAG-CGTCTG-3'; MMP-13, forward: 5'-GATGCCTACTGGGT

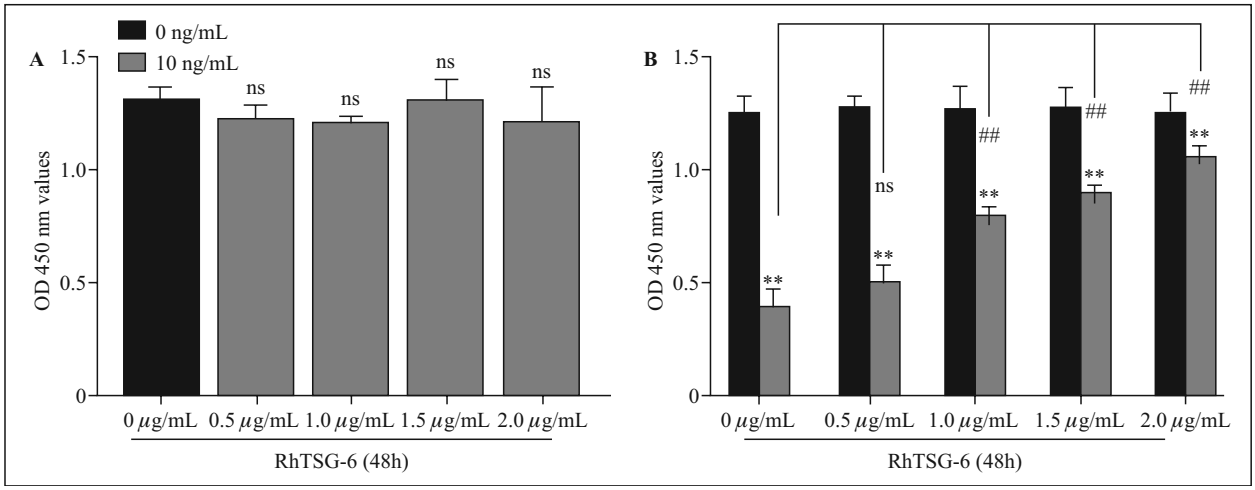

Figure 1. Cell viability of human NP cells following treatment with rhTSG-6 and IL-1β. **A.** Nucleus pulpous (NP) cells were incubated in DMEM/F12 medium containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin mix for 24 h and then indicated concentrations of rhTSG-6 were added for 48 h. Thereafter, viability of NP cells was measured by a CCK-8 assay as described in Methods. **B.** Effect of rhTSG-6 on the viability of NP cells stimulated by IL-1β as detected by a CCK-8 assay. Data are expressed as mean ± SD, $^{**}P < 0.01$, *vs.* 0 ng/mL IL-1β group; $^{\#\#}P < 0.01$, *v*s. 10 ng /mL IL-1β + 0 μg/mL rhTSG-6 group.

GGAG-3' and reverse: 5'-AAAGACGGAAATGGGAGA-3'; ADAMTS-4, forward: 5'-ACCCAAGCATCCGCAATC-3' and reverse: 5'-TGCCCACATCAGCCATAC-3'; ADAMTS-5, forward: 5'-GACAGTTCAAAGCCAAAGACC-3' and reverse: 5'-TTTCCTTCGTGGCAGAGT-3'; Collagen II, forward: 5'-CTCCATGTTGCAGAAG ACTTTCA-3' and reverse: 5'-TTCATGCATCCGCTAGTCCCTTCT-3'; GAPDH, forward: 5′-CGAGATCCCTCCAAAATCAA-3′ and reverse: 5′-TTCACACCCATG ACGAACAT-3′.

Western blot. Total proteins were extracted by using RIPA lysis buffer (Beyotime Biotechnology Co., Ltd., Shanghai, China) and separated with 10% SDS-PAGE and transferred onto a PVDF membrane. After blocking with 5% non-fat milk in TBST for 1 h, the membranes were incubated overnight at 4°C with the following primary antibodies: anti-MMP3 (ab52915, 1:1000, Abcam), anti-MMP13 (ab39012, 1:3000, Abcam), anti-ADAMTS4 (ab185722, 1:500, Abcam), anti-ADAMTS5 (ab41037, 1:250, Abcam), anti-Collagen II (ab34712, 1:1000, Abcam), anti-Bcl-2 (ab196495, 1:1000, Abcam), anti-cleaved caspase-3 (ab49822, 1:500, Abcam), anti-p38 (ab170099, 1:1000, Abcam), phosphor-p38 (1:1000, ab195049, Abcam), anti-JNK (1:1000, #9258, Cell Signaling), anti-phospho-JNK (1:1000, #4668, Cell Signaling), anti-ERK (1:500, ab17942, Abcam), anti-phospho-ERK (1:500, ab214362, Abcam), followed by incubation with the HRP-labeled secondary antibody at room temperature for 1h. The bands were then visualized by electrogenerated chemiluminescence reagent (Pierce, Rockford, IL, USA), and analyzed by using Image J software. GAPDH was used as an internal control.

Flow cytometry analysis. Cell apoptosis was determined using the Annexin V-FITC Apoptosis Detection kit (Beyotime Biotechnology Co., Ltd., Shanghai, China) according to the manufacturer's instructions. Briefly, cells were collected, washed, and stained with 10 μL of Annexin V-FITC buffer and 10 μL of propidium iodide (PI) in dark for 30 minutes at room temperature. Then the stained cells were analyzed using a FACS flow cytometer (BD Biosciences, San Jose, CA, USA).

Statistical analysis. All data were expressed as mean ± SD of three independent experiments. Statistical analysis was performed by using SPSS20.0 (SPSS, Inc., Chicago, IL, USA). Significant differences were evaluated using student's t test or one-way ANOVA. $P < 0.05$ was considered as statistically significant.

Results

Cell viability of human NP cells following treatment with rhTSG-6

Firstly, NP cells were treated with different concentrations (0, 0.5, 1, 1.5, 2 μg/mL) of rhTSG-6, and cell viabilities were determined by using CCK-8 assay. As revealed in Figure 1A, the results suggested that rhTSG-6 was not cytotoxic and had no effect on the proliferation of NP cells. Subsequently, NP cells were stimulated by 10 ng/mL IL-1β for 24 h to evaluate whether rhTSG-6 can alleviate the cytotoxic effects of IL-1β on NP cells. Our findings showed that IL-1β caused approximately 70% reduction in the viability of NP cells, while rhTSG-6 co-treatment could grad-

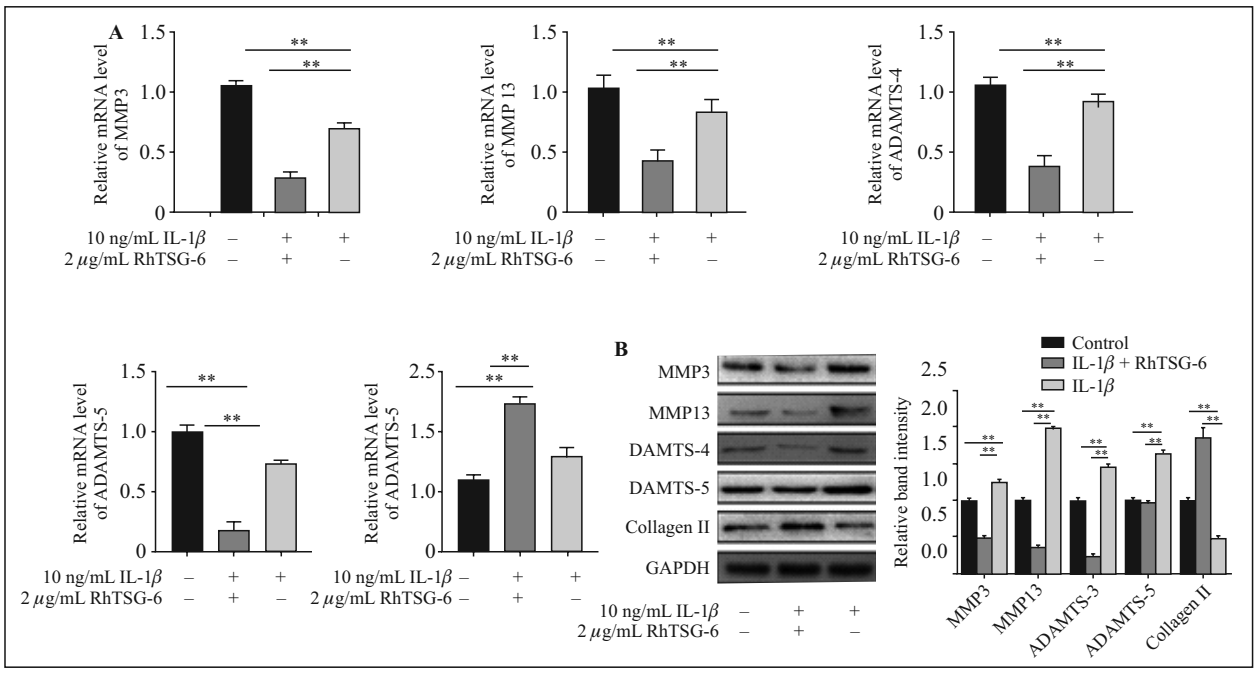

Figure 2. Inhibitory effects of rhTSG-6 on IL-1β-induced expression of ECM components in human NP cells. **(A)** RT-qPCR and **(B)** Western blot analyses were used to evaluate the mRNA and protein expression of MMP3, MMP13, ADAMTS-4, ADAMTS-5 and collagen II. GAPDH served as an internal control. Data are expressed as mean ± SD, $^{**}P < 0.01$.

ually reverse the inhibitory effect of IL-1β on cellular viability in a dose-dependent manner, especially at the concentration of 2 μg/mL (Fig. 1B). Therefore, the concentration of rhTSG-6 used in our subsequent experiments was 2 μg/mL.

Inhibitory effects of rhTSG-6 on IL-1β-induced ECM degradation in human NP cells

To investigate whether rhTSG-6 affects IL-1β-induced ECM degradation in human NP cells, the expression levels of the mRNA and protein associated with ECM progression of NP cells were detected by western blot and RT-qPCR (Fig. 2A and B). The results showed that IL-1β significantly increased the mRNA and protein expression levels of MMP3, MMP13, ADAMTS-4 and ADAMTS-5, but decreased the mRNA and protein expression level of collagen II, whereas co-treatment with rhTSG-6 partly reversed the effects induced by IL-1β.

RhTSG-6 protects NP cells against IL-1β-induced apoptosis

Since cell apoptosis is closely related to the development of IDD, our study further explored the effect of rhTSG-6 on apoptosis of IL-1β-induced NP cells. Flow cytometry analysis demonstrated that IL-1β increased the apoptosis rate of NP cells, whereas rhTSG-6 prevented the apoptosis of NP cells induced by IL-1β. Consistently, the protein level of Bcl-2 (anti-apoptotic protein) was decreased, and the expression level of cleaved caspase-3 (pro-apoptotic protein) was increased in the IL-1β-stimulated NP cells, whereas the effects induced by IL-1β stimulation were alleviated by rhTSG-6 treatment (Fig. 3C).

Effects of rhTSG-6 on the IL-1β-induced activation of p38, JNK and ERK signaling pathways in NP cells

To elucidate the potential mechanisms responsible for rhTFG-6 protective effect on IL-1β-induced NP cells, we evaluated the role of rhTFG-6 in regulating p38 and JNK pathways. As displayed in Figure 4A, the levels of phosphorylated p38, phosphorylated JNK and phosphorylated ERK were significantly increased by IL-1β treatment, indicating activation of the p38, JNK and ERK pathways in the IL-1β-treated human NP cells. However, co-treatment with rhTFG-6 markedly inhibited IL-1β-induced activation of the p38, JNK and ERK pathways.

Discussion

IDD is a major cause of LBP which seriously endangers public health and has become a serious public health problem with high disease rate, disability rate and high medical costs [29, 30]. IDD is characterized by

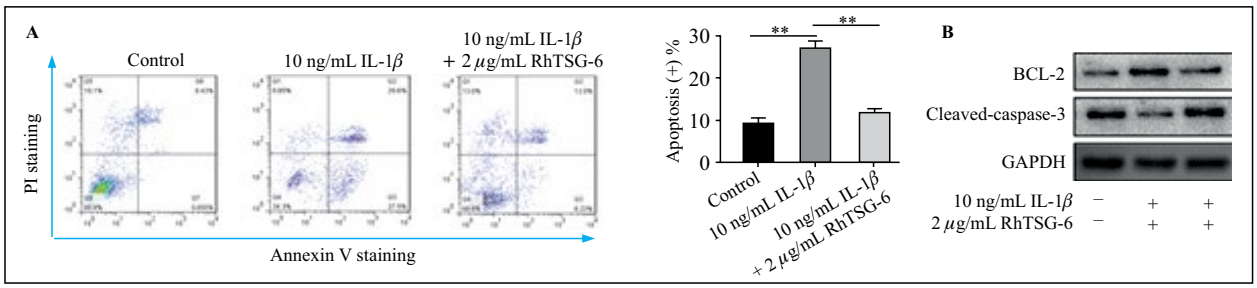

Figure 3. RhTSG-6 protects NP cells against IL-1β-induced apoptosis. **A.** Flow cytometry was used to detect the cell apoptosis of NP cells by the Annexin V-FITC Apoptosis Detection kit. **B.** Western blot analysis was used to determine the protein expression of Bcl-2 and cleaved caspase-3. GAPDH served as an internal control. Data are expressed as mean ± SD, $^{**}P < 0.01$.

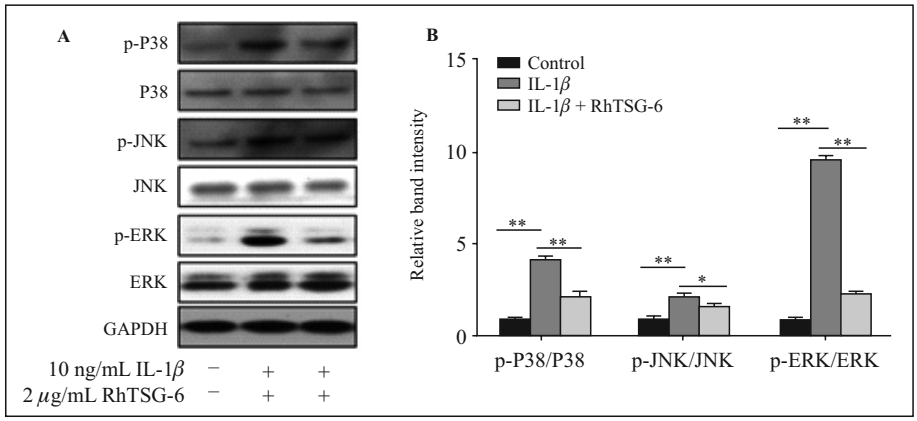

Figure 4. Effects of rhTSG-6 on the IL-1β-induced activation of p38, JNK and ERK in NP cells. **A.** The effects of rhTSG-6 on p38, p-P38, JNK, p-JNK, ERK and p-ERK were determined by western blot analysis. GAPDH served as an internal control. Data are expressed as mean ± SD, $^{*}P < 0.05$, $^{**}P < 0.01$.

increased apoptosis of NP cells and hyperactive ECM degradation [11]. Current evidence suggests that decreasing NP cell apoptosis and increasing expression of some important ECM components may provide a therapeutic strategy for IDD [31–33]. In this study, in vitro model of IDD degeneration was successfully constructed by IL-1β-induced NP cells. We provided the first evidence that in human IL-1β-treated NP cells rhTSG-6 has inhibitory effects on cell apoptosis and expression of ECM-degrading molecules, and that potential regulatory mechanism is mediated by the activation of p38, JNK and ERK pathways.

TSG-6 is a pleiotropic regulatory protein secreted by pro-inflammatory mediator-induced (including TNF-α and IL-1β) immune cells (such as neutrophils, monocytes, macrophages, medullary dendritic cells), and stromal cells (such as fibroblasts and smooth muscle cells) [34–36]. TSG-6 is quickly activated early in the inflammatory process and plays an anti-inflammatory role. The protective effects of TSG-6 secreted by bone marrow mesenchymal stem cells in IDD has been proved by a recent study, which focused on the mechanism of reducing inflammation, especially its role in inhibiting the expression of inflammatory cytokines *via* TLR2/NF-κB signaling [28]. Since the mechanism of inhibiting IDD may involve many aspects, the present study explored the effects of exogenous addition of rhTSG-6 on the changes of some important ECM components' expression and cell apoptosis in IL-1β-induced NP cells. Consistent with the results of the previous study [28], rhTSG-6 decreased the expression of MMP3 and MMP13, and increased the expression of collagen II in IL-1β-induced NP cells. In addition, ECM-catabolic proteinases (ADAMTS-4 and ADAMTS-5) were also inhibited by exogenous rhTSG-6. Furthermore, the inhibitory effect of rhTSG-6 on IL-1β-induced NP cell apoptosis was demonstrated by flow cytometry and western blot analysis. These results reveal that rhTSG-6 has the potential to regulate the progression of IDD by inhibiting ECM degradation and cell apoptosis of NP cells.

IDD-associated inflammation is capable of activating various intracellular signaling pathways that mediate the production of downstream effectors that are closely related to the progression of IDD [37]. P38, JNK and ERK, as important members of mitogen-activated protein kinase (MAPK) pathway, have been reported as key signaling pathways regulating IDD [38–42]. Hua *et al.* demonstrated that icariin, an anti-inflammatory drug isolated from Epimedium brevicornum, inhibits IL-1β-induced inflammatory response and ECM reduction through suppressing the p38/MAPK pathway in human NP cells [43]. Lin *et al.* suggested that Propionibacterium acnes induces IDD by promoting NP cell apoptosis *via* the TLR2/ /JNK/mitochondrial-mediated pathway [44]. Besides, a recent study proved that simvastatin suppresses IL-1β-induced cell apoptosis and ECM degradation by inhibiting the p38, JNK and ERK phosphorylation [16]. Therefore, the activation of p38, JNK and ERK signaling pathway may exacerbate IDD. RhTSG-6 has been reported to inhibit p38, JNK and ERK pathways in various disease models [28, 45–47]. Hence, we supposed that p38, JNK and ERK pathways might be involved in the regulatory effects of rhTSG-6 on degradation of ECM and cell apoptosis in the *in vitro* IDD model. To confirm our hypothesis, western blot was performed to detect the expression changes of p-p38, p-JNK, and p-ERK. The results revealed that rhTSG-6 could inhibit the P38, JNK and ERK pathway, thus explaining the effects of rhTSG-6 on NP cell apoptosis and possible ECM degradation described above.

In conclusion, this study suggested that rhTSG-6 could inhibit the IL-1β-induced ECM degradation and cell apoptosis through inhibiting the P38, JNK and ERK pathways and should be further investigated as a possible novel therapeutic approach treatment for IDD.

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Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Southern Medical University, Guangzhou, China.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

RDK and PSS conceived and designed the experiments, YJW and ZY analyzed and interpreted the results of the experiments, SLH and CS performed the experiments.

References

1. Hoy D, March L, Brooks P, et al. Measuring the global burden of low back pain. Best Pract Res Clin Rheumatol. 2010; 24(2): 155–165, doi: 10.1016/j.berh.2009.11.002, indexed in Pubmed: 20227638.
2. Jensen C, Riis A, Petersen K, et al. Economic evaluation of an implementation strategy for the management of low back pain in general practice. PAIN. 2017; 158(5): 891–899, doi: 10.1097/j.pain.0000000000000851, indexed in Pubmed: 28114182.
3. Chen YC, Su WY, Yang SH, et al. In situ forming hydrogels composed of oxidized high molecular weight hyaluronic acid and gelatin for nucleus pulposus regeneration. Acta Biomater. 2013; 9(2): 5181–5193, doi: 10.1016/j.actbio.2012.09.039, indexed in Pubmed: 23041783.
4. Mead TJ, Apte SS. ADAMTS proteins in human disorders. Matrix Biol. 2018; 71-72: 225–239, doi: 10.1016/j.matbio.2018.06.002, indexed in Pubmed: 29885460.
5. Wu B, Meng C, Wang H, et al. Changes of proteoglycan and collagen II of the adjacent intervertebral disc in the cervical instability models. Biomed Pharmacother. 2016; 84: 754–758, doi: 10.1016/j.biopha.2016.09.077, indexed in Pubmed: 27716589.
6. Li Y, Li K, Han X, et al. The imbalance between TIMP3 and matrix-degrading enzymes plays an important role in intervertebral disc degeneration. Biochem Biophys Res Commun. 2016; 469(3): 507–514, doi: 10.1016/j.bbrc.2015.12.020, indexed in Pubmed: 26686417.
7. Tang BL. ADAMTS: a novel family of extracellular matrix proteases. Int J Biochem Cell Biol. 2001; 33(1): 33–44, doi: 10.1016/s1357-2725(00)00061-3, indexed in Pubmed: 11167130.
8. Jabłońska-Trypuć A, Matejczyk M, Rosochacki S. Matrix metalloproteinases (MMPs), the main extracellular matrix (ECM) enzymes in collagen degradation, as a target for anticancer drugs. J Enzyme Inhib Med Chem. 2016; 31(sup1): 177–183, doi: 10.3109/14756366.2016.1161620, indexed in Pubmed: 27028474.
9. Yang W, Yu XH, Wang C, et al. Interleukin-1β in intervertebral disk degeneration. Clin Chim Acta. 2015; 450: 262–272, doi: 10.1016/j.cca.2015.08.029, indexed in Pubmed: 26341894.
10. Chen J, Xuan J, Gu YT, et al. Celastrol reduces IL-1β induced matrix catabolism, oxidative stress and inflammation in human nucleus pulposus cells and attenuates rat intervertebral

disc degeneration in vivo. Biomed Pharmacother. 2017; 91: 208–219, doi: 10.1016/j.biopha.2017.04.093, indexed in Pubmed: 28458159.
11. Risbud MV, Shapiro IM. Role of cytokines in intervertebral disc degeneration: pain and disc content. Nat Rev Rheumatol. 2014; 10(1): 44–56, doi: 10.1038/nrrheum.2013.160, indexed in Pubmed: 24166242.
12. Johnson ZI, Schoepflin ZR, Choi H, et al. Disc in flames: Roles of TNF-α and IL-1β in intervertebral disc degeneration. Eur Cell Mater. 2015; 30: 104–16; discussion 116, doi: 10.22203/ecm.v030a08, indexed in Pubmed: 26388614.
13. Lee J, Song J, Baek M, et al. Interleukin-1β induces angiogenesis and innervation in human intervertebral disc degeneration. Journal of Orthopaedic Research. 2010; 29(2): 265–269, doi: 10.1002/jor.21210, indexed in Pubmed: 20690185.
14. Hua W, Zhang Y, Wu X, et al. Icariin Attenuates interleukin-1β-induced inflammatory response in human nucleus pulposus cells. Curr Pharm Des. 2018; 23(39): 6071–6078, doi: 10.2174/1381612823666170615112158, indexed in Pubmed: 28619001.
15. Kang L, Yang C, Yin H, et al. MicroRNA-15b silencing inhibits IL-1βinduced extracellular matrix degradation by targeting SMAD3 in human nucleus pulposus cells. Biotechnol Lett. 2017; 39(4): 623–632, doi: 10.1007/s10529-016-2280-3, indexed in Pubmed: 28039556.
16. Tu Ji, Li W, Zhang Y, et al. Simvastatin inhibits IL-1β-induced apoptosis and extracellular matrix degradation by suppressing the NF-kB and MAPK pathways in nucleus pulposus cells. Inflammation. 2017; 40(3): 725–734, doi: 10.1007/s10753-017-0516-6, indexed in Pubmed: 28188410.
17. Lee TH, Wisniewski HG, Vilcek J. 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. 1992; 116(2): 545–557, doi: 10.1083/jcb.116.2.545, indexed in Pubmed: 1730767.
18. Nentwich H, Mustafa Z, Rugg M, et al. A Novel allelic variant of the humanTSG-6gene encoding an amino acid difference in the CUB module. J Biol Chem. 2002; 277(18): 15354–15362, doi: 10.1074/jbc.m110765200, indexed in Pubmed: 11854277.
19. Lee RH, Pulin AA, Seo MJ, et al. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell. 2009; 5(1): 54–63, doi: 10.1016/j.stem.2009.05.003, indexed in Pubmed: 19570514.
20. Parkar A, Kahmann J, Howat S, et al. TSG-6 interacts with hyaluronan and aggrecan in a pH-dependent manner via a common functional element: implications for its regulation in inflamed cartilage. FEBS Letters. 1998; 428(3): 171–176, doi: 10.1016/s0014-5793(98)00523-7.
21. Blundell CD, Mahoney DJ, Almond A, et al. The link module from ovulation- and inflammation-associated protein TSG-6 changes conformation on hyaluronan binding. J Biol Chem. 2003; 278(49): 49261–49270, doi: 10.1074/jbc.M309623200, indexed in Pubmed: 12972412.
22. Tuo J, Cao X, Shen D, et al. Anti-inflammatory recombinant TSG-6 stabilizes the progression of focal retinal degeneration in a murine model. J Neuroinflammation. 2012; 9: 59, doi: 10.1186/1742-2094-9-59, indexed in Pubmed: 22452753.
23. Li R, Liu W, Yin J, et al. TSG-6 attenuates inflammation-induced brain injury via modulation of microglial polarization in SAH rats through the SOCS3/STAT3 pathway. J Neuroinflammation. 2018; 15(1): 231, doi: 10.1186/s12974-018-1279-1, indexed in Pubmed: 30126439.
24. Bayliss MT, Howat SL, Dudhia J, et al. Up-regulation and differential expression of the hyaluronan-binding protein TSG-6 in cartilage and synovium in rheumatoid arthritis and osteoarthritis. Osteoarthritis Cartilage. 2001; 9(1): 42–48, doi: 10.1053/joca.2000.0348, indexed in Pubmed: 11178946.
25. Wisniewski HG, Colón E, Liublinska V, et al. TSG-6 activity as a novel biomarker of progression in knee osteoarthritis. Osteoarthritis Cartilage. 2014; 22(2): 235–241, doi: 10.1016/j.joca.2013.12.004, indexed in Pubmed: 24333293.
26. Mindrescu C, Thorbecke GJ, Klein MJ, et al. Amelioration of collagen-induced arthritis in DBA/1J mice by recombinant TSG-6, a tumor necrosis factor/interleukin-1-inducible protein. Arthritis Rheum. 2000; 43(12): 2668–2677, doi: 10.1002/1529-0131(200012)43:12<2668::AID-ANR6>3.0.CO;2-E, indexed in Pubmed: 11145024.
27. Tellier LE, Treviβo EA, Brimeyer AL, et al. Intra-articular TSG-6 delivery from heparin-based microparticles reduces cartilage damage in a rat model of osteoarthritis. Biomater Sci. 2018; 6(5): 1159–1167, doi: 10.1039/C8BM00010G, indexed in Pubmed: 29564448.
28. Yang H, Tian W, Wang S, et al. TSG-6 secreted by bone marrow mesenchymal stem cells attenuates intervertebral disc degeneration by inhibiting the TLR2/NF-κB signaling pathway. Lab Invest. 2018; 98(6): 755–772, doi: 10.1038/s41374-018-0036-5, indexed in Pubmed: 29483622.
29. Luoma K, Riihimäki H, Luukkonen R, et al. Low back pain in relation to lumbar disc degeneration. Spine (Phila Pa 1976). 2000; 25(4): 487–492, doi: 10.1097/00007632-200002150-00016, indexed in Pubmed: 10707396.
30. Fontana G, See E, Pandit A. Current trends in biologics delivery to restore intervertebral disc anabolism. Adv Drug Deliv Rev. 2015; 84: 146–158, doi: 10.1016/j.addr.2014.08.008, indexed in Pubmed: 25174310.
31. Lu L, Hu J, Wu Q, et al. Berberine prevents human nucleus pulposus cells from IL1-β-induced extracellular matrix degradation and apoptosis by inhibiting the NFκB pathway. Int J Mol Med. 2019; 43(4): 1679–1686, doi: 10.3892/ijmm.2019.4105, indexed in Pubmed: 30816449.
32. Wang Ke, Chen T, Ying X, et al. Ligustilide alleviated IL-1-β--induced apoptosis and extracellular matrix degradation of nucleus pulposus cells and attenuates intervertebral disc degeneration in vivo. Int Immunopharmacol. 2019; 69: 398–407, doi: 10.1016/j.intimp.2019.01.004, indexed in Pubmed: 30785069.
33. Chai X, Si H, Song J, et al. miR-486-5p inhibits inflammatory response, matrix degradation and apoptosis of nucleus pulposus cells through directly targeting FOXO1 in intervertebral disc degeneration. Cell Physiol Biochem. 2019; 52(1): 109––118, doi: 10.33594/000000008, indexed in Pubmed: 30790508.
34. Milner CM, Day AJ. TSG-6: a multifunctional protein associated with inflammation. J Cell Sci. 2003; 116(Pt 10): 1863––1873, doi: 10.1242/jcs.00407, indexed in Pubmed: 12692188.
35. Wisniewski HG, Vilček J. Cytokine-induced gene expression at the crossroads of innate immunity, inflammation and fertility: TSG-6 and PTX3/TSG-14. Cytokine Growth Factor Rev. 2004; 15(2-3): 129–146, doi: 10.1016/j.cytogfr.2004.01.005, indexed in Pubmed: 15110797.
36. Day AJ, Milner CM. TSG-6: A multifunctional protein with anti-inflammatory and tissue-protective properties. Matrix Biol. 2019; 78-79: 60–83, doi: 10.1016/j.matbio.2018.01.011, indexed in Pubmed: 29362135.
37. Wuertz K, Vo N, Kletsas D. Boos NJECM. Inflammatory and catabolic signalling in intervertebral discs: the roles of NF-KB and MAP kinases. 2012; 23: 103–19, doi: 10.22203/ecm.v023a08, indexed in Pubmed: 22354461.
38. Studer RK, Aboka AM, Gilbertson LG, et al. p38 MAPK inhibition in nucleus pulposus cells: a potential target for treating intervertebral disc degeneration. Spine (Phila Pa 1976). 2007;

32(25): 2827–2833, doi: 10.1097/BRS.0b013e31815b757a, indexed in Pubmed: 18246004.
39. Studer RK, Gilbertson LG, Georgescu H, et al. Kang JDJJoOR. p38 MAPK inhibition modulates rabbit nucleus pulposus cell response to IL-1. 2008; 26(7): 991–8, doi: 10.1002/jor.20604, indexed in Pubmed: 18302237.
40. Klawitter M, Quero L, Klasen J, et al. Curcuma DMSO extracts and curcumin exhibit an anti-inflammatory and anti-catabolic effect on human intervertebral disc cells, possibly by influencing TLR2 expression and JNK activity. J Inflamm (Lond). 2012; 9(1): 29, doi: 10.1186/1476-9255-9-29, indexed in Pubmed: 22909087.
41. Tsai TT, Guttapalli A, Agrawal A, et al. MEK/ERK signaling controls osmoregulation of nucleus pulposus cells of the intervertebral disc by transactivation of TonEBP/OREBP. J Bone Miner Res. 2007; 22(7): 965–974, doi: 10.1359/jbmr.070322, indexed in Pubmed: 17371162.
42. Marazza A, Tekari A, Roth E, et al. Investigation into ERK, JNK and p38 downstream signaling pathways: an anti-inflammatory approach against the Intervertebral Disc Degeneration; 2015.
43. Hua W, Zhang Y, Wu X, et al. Icariin Attenuates interleukin-1β-induced inflammatory response in human nucleus pulposus cells. Curr Pharm Des. 2018; 23(39): 6071–6078, doi: 10.2174/1381612823666170615112158.
44. Lin Y, Jiao Y, Yuan Ye, et al. Propionibacterium acnes induces intervertebral disc degeneration by promoting nucleus pulposus cell apoptosis via the TLR2/JNK/mitochondrial-mediated pathway. Emerg Microbes Infect. 2018; 7(1): 1, doi: 10.1038/s41426-017-0002-0, indexed in Pubmed: 29323102.
45. Zhang C, Zhang B, Wang H, et al. Tumor necrosis factor alpha-stimulated gene-6 (TSG-6) inhibits the inflammatory response by inhibiting the activation of P38 and JNK signaling pathway and decreases the restenosis of vein grafts in rats. Heart Vessels. 2017; 32(12): 1536–1545, doi: 10.1007/s00380-017-1059-3, indexed in Pubmed: 28975447.
46. Um S, Kim HY, Lee JH, et al. TSG-6 secreted by mesenchymal stem cells suppresses immune reactions influenced by BMP-2 through p38 and MEK mitogen-activated protein kinase pathway. Cell Tissue Res. 2017; 368(3): 551–561, doi: 10.1007/s00441-017-2581-4, indexed in Pubmed: 28247086.
47. Liu Yi, Yin Z, Zhang R, et al. MSCs inhibit bone marrow-derived DC maturation and function through the release of TSG-6. Biochem Biophys Res Commun. 2014; 450(4): 1409–1415, doi: 10.1016/j.bbrc.2014.07.001, indexed in Pubmed: 25014173.

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