

Taurine attenuates ER stress-associated apoptosis and catabolism in nucleus pulposus cells

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Abstract. Nucleus pulposus (NP) apoptosis and subsequent excessive degradation of the extracellular matrix (ECM) are key pathological characteristics of intervertebral disc degeneration (IDD). The present study aims to examine the signaling processes underlying the effects of taurine on IDD, with specific focus on endoplasmic reticulum (ER) stress-mediated apoptosis and ECM degradation, in NP cells. To clarify the role of taurine in IDD, NP cells were treated with various concentrations of taurine and IL-1 β or thapsigargin (TG). Cell Counting Kit-8, western blotting, TUNEL, immunofluorescence assays and reverse transcription-quantitative PCR were applied to measure cell viability, the expression of ER stress-associated proteins (GRP78, CHOP and caspase-12), apoptosis and the levels of metabolic factors associated with ECM (MMP-1, 3, 9, ADAMTS-4, 5 and collagen II), respectively. Taurine was found to attenuate ER stress and prevent apoptosis in NP cells induced by IL-1 β treatment. Additionally, taurine significantly decreased the expression of ER stress-activated glucose regulatory protein 78, C/EBP homologous protein and caspase-12. TUNEL results revealed that taurine decreased the number of apoptotic TG-treated NP cells. TG-treated NP cells also exhibited characteristics of increased ECM degradation, supported by observations of increased MMP-1, MMP-3, MMP-9 and A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-4 and ADAMTS-5 expression in addition to decreased collagen-II expression. However, taurine treatment significantly reversed all indicators of excessive ECM catabolism aforementioned. These data suggest that taurine can mediate protection against apoptosis and ECM degradation in NP cells by inhibiting ER stress, implicating therapeutic potential for the treatment of IDD.

Introduction

Intervertebral disc degeneration (IDD) is a degenerative condition that is primarily associated with age (1). IDD is a major cause of pain in the lower back, which reduces the quantity of life and wellbeing of the patient (2). In addition, IDD applies pressure on the global healthcare structure and inflicts substantial financial costs (2). Numerous external and patient-specific factors have been reported to contribute to the occurrence and progression of IDD, including mechanical stress, age and genetic predispositions (3-5). However, the precise molecular mechanism underlying the pathogenesis of IDD remains poorly understood. As a result, effective long-term treatment strategies for this condition remains elusive.

The intervertebral disc (IVD) is an avascular organ that is comprised of three main structures: Central nucleus pulposus (NP); adjacent annulus fibrosus; and the bony endplate. Collectively, these entities guarantee the precise mechanical functionality of the disc, relieving any compressive axial forces that act on the spinal column to confer multiaxial flexibility (6,7). Various stimuli, such as mechanical stress and senescence, have been found to trigger the death of cells in the IVD by activating numerous signal transduction pathways (including the ER and death receptor pathways) (8). It has been proposed that cell death can serve an instrumental role in the progression of IDD (8,9). Indeed, excessive NP cell apoptosis can trigger IDD and has been previously reported to be a node of therapeutic intervention for treating IDD (8,10).

Under physiological conditions, in a healthy IVD the NP is abundantly hydrated and produces copious quantities of aggrecan and collagen II (11). In addition, sufficient quantities of extracellular matrix (ECM) maintains the internal pressure within the IVD, which provide a stable hydrodynamic structure to facilitate IVD functionality (12,13). A specialized catabolic enzyme system in IVD primarily regulates ECM metabolism. The system includes MMPs, a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) (14). Previous studies reported these enzymes to serve key roles in the progression of degenerative cartilaginous diseases (15,16). In particular, MMPs 1, 2, 3 and 9 were found to be expressed at high levels in NP cells from degenerate discs (17). In addition, another study found that compared with those in healthy tissues, the expression of ADAMTS-4 and ADAMTS-5 was considerably elevated in degenerated disc tissues (18).

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Taurine is a free amino acid that is abundant in mammalian tissues, particularly in myocardial tissues (19). However, the plasma levels of taurine typically decrease with age in both humans and rats (20,21). This age-related reduction in plasma taurine levels may result in adverse effects on the regulation of blood pressure, cardiovascular function and CNS function (20). Due to its numerous reported physiological properties, taurine has been applied as an anti-stress supplement, with effects including the regulation of Ca^{2+} homeostasis, antioxidant and anti-apoptotic action (22,23). There is accumulating evidence that taurine can also regulate energy metabolism and endoplasmic reticulum (ER) stress-induced cell damage (23). Furthermore, taurine deficiency has been previously reported in various diseases, such as Parkinson's disease and epilepsy (24,25). Adequate taurine levels was only noted in healthy disc tissues, whilst it was only found in small amounts in degenerated discs (21). This suggests a potential role of taurine on IDD. However, there is an insufficient number of studies that aimed to characterize the effects of taurine on IDD and its associated mechanism. Therefore, the present study attempted to examine the effects of IL-1 β on NP cells *in vitro* and investigate the functional characteristics of ER stress. In addition, the present study explored the possible mechanism of taurine on NP cells following exposure to thapsigargin (TG), which is a classical inducer of ER stress.

Materials and methods

Chemicals and materials. TG and taurine were purchased from Sigma-Aldrich; Merck KGaA. IL-1 β was purchased from R&D Systems, Inc. Caspase-12 antibodies (cat. no. sc-21747) were acquired from Santa Cruz Biotechnology, Inc. C/EBP homologous protein (CHOP; cat. no. 2895), Bax (cat. no. 14796), cleaved caspase-3 (cat. no. 9664) and β -actin (cat. no. 3700) antibodies were purchased from Cell Signaling Technology, Inc. Collagen-II (cat. no. ab34712), Bcl-2 (cat. no. ab194583), caspase-9 (cat. no. ab184786) and glucose regulatory protein 78 (GRP78; cat. no. ab21685) antibodies were purchased from Abcam. HRP-conjugated anti-rabbit IgG (cat. no. ab288151), Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG H&L (cat. no. ab150077), Dylight[®] 488-conjugated goat anti-mouse IgG H&L (cat. no. ab96879) and goat anti-rat IgG H&L (Alexa Fluor 647; cat. no. ab150167; Abcam) secondary antibodies were purchased from Abcam. The *In Situ* Cell Death Detection Kit was purchased from Roche Diagnostics (cat. no. 11684817910).

NP cell culture. Each empirical procedure involving animals performed in the present study adhered to the rules in the Animal Care and Use Committee of Shanghai Jing'an District Zhabei Central Hospital. The present study was approved by the Animal Care and Use Committee of Shanghai Jing'an District Zhabei Central Hospital (Shanghai, China). Collectively, 15 male Sprague-Dawley rats (mean weight, 201.4 \pm 12.4 g; weight range, 180–220 g, 5–7 weeks; Animal Center of Chinese Academy of Sciences) were housed under specific pathogen-free laboratory conditions (18–29°C; 40–70% humidity), with free access to food and water and controlled lighting (12 h light/dark cycle). All rats were aseptically collected after euthanasia by CO_2 inhalation (40% vol/min for

5 min). Death was confirmed when the rat lacked a heartbeat or respiratory activity. The annulus fibrosus was first secluded to separate the gelatinous NP. The NP cells of the rats were then isolated using protocols described previously (26). Briefly, the lumbar disc was exposed and the annulus fibrosus was cut open using a pair of ophthalmic scissors under aseptic conditions. The gelatinous NP was then isolated, which was cut into 1–3 mm-thick slices in sterile D-Hanks solution (MilliporeSigma). This tissue was incubated with 0.1% collagenase type II (2 h at 37°C, MilliporeSigma) and 10 U/ml hyaluronidase (2 h at 37°C, MilliporeSigma). The cells were centrifuged (300 x g; 3 min; 37°C) and cultured in DMEM-F12 medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 15% FBS (Gibco; Thermo Fisher Scientific, Inc.) at 37°C and 5% CO_2 . In general, the intervertebral discs of three to four rats were collected and pooled into one culture. Subsequently, 1×10^5 cells were transferred into each well of six-well plates before they were pre-treated with various concentrations of taurine [0 (Control), 5, 10, 20, 40, 80 nmol/l] for 2 h at 37°C prior to treating the cells with 10 ng/ml IL-1 β or 10 μM TG for 24 h at 37°C (27).

Cell viability assay. NP cells ($5\text{--}6 \times 10^3$ cells per well) were seeded into 96-well plates. Different dosages of taurine were used for treating the cells (0, 5, 10, 20, 40, 80 nmol/l) alongside IL-1 β (10 ng/ml) for 24 h at 37°C. However, the control group were not treated with IL-1 β . The NP cells were then incubated for 2 h at 37°C with the Cell Counting Kit-8 reagent (CCK8; 10 μl per well; Dojindo Molecular Technologies, Inc.). Subsequently, a microplate reader (Molecular Devices, LLC) was utilized to measure the absorbance at 450 nm.

Immunofluorescence staining. Following treatments, the NP cells were first seeded into six-well plates (5×10^5 on cover glass). They were then fixed with 4% (v/v) paraformaldehyde for 1 h at room temperature, then incubated with 0.5% (v/v), Triton X-100 for 10 min and incubation with 5% (w/v) BSA (MilliporeSigma) for 1 h at 37°C. Primary antibodies against collagen II (1:50) and caspase-12 (1:50) were then added to the cells followed by incubation for 12 h at 4°C. Next, [Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG H&L (cat. no. ab150077; 1:500) and goat anti-rat IgG H&L (Alexa Fluor 647; ab150167; 1:500)] were utilized to treat all cells for a 60 min at room temperature. DAPI (Abcam; cat. no. ab285390) was then added to incubate the cell nuclei for 5 min at room temperature (28), which allowed for the visualization of the NP cells using a Nikon Eclipse Ti confocal microscope (Nikon Corporation; magnification, x40).

Western blotting. Following treatment, protein samples were extracted from the NP cells using RIPA buffer (MilliporeSigma). Bicinchoninic acid method was used to measure the protein concentrations in each sample. SDS-PAGE (8–12%) was used to separate the proteins (40 μg) before transferring onto PVDF membranes. The membranes were then incubated in 5% non-fat milk for 1 h at room temperature before incubation with primary antibodies. Specifically, anti-Bax (1:1,000), anti-Bcl-2 (1:1,000), cleaved-caspase-3 (1:1,000), caspase-9 (1:1,000), anti-CHOP (1:1,000), anti-GRP78 (1:1,000) and anti-collagen II (1:1,000)

Table I. Primer sequences of the targeted genes.

Genes	Primer sequences
MMP-1	Forward: 5'-GCTTAGCCTTCCTTTGCTGTTGC-3' Reverse: 5'-GACGTCTTCACCCAAGTTGTAGTAG-3'
MMP-3	Forward: 5'-CTGGGCTATCCGAGGTCATG-3' Reverse: 5'-TGGACGGTTTCAGGGAGGC-3'
MMP-9	Forward: 5'-CCACCGAGCTATCCACTCAT-3' Reverse: 5'-GTCCGGTTTCAGCATGTTTT-3'
ADAMTS-4	Forward: 5'-AAGCATCCGAAACCCTGTCAACG-3' Reverse: 5'-AGCCATACCCAGAGCGTCAC-3'
ADAMTS-5	Forward: 5'-AGAGTCCGAACGAGTTTACG-3' Reverse: 5'-GTGCCAGTTCTGTGCGTC-3'
SRY-box transcription factor 9	Forward: 5'-GCGACGTCATCTCCAACATC-3' Reverse: 5'-ATGCCGTAGCTGCCAGTGTA-3'
Aggrecan	Forward: 5'-GACCCAAACAGCAGAAACAGC-3' Reverse: 5'-CGGGAAAGTGGCGATAACAG-3'
Collagen II	Forward: 5'-CACCCAGAGTGGAAGAGCG-3' Reverse: 5'-TCAGTGGACAGTAGACGGAGGA-3'
β -actin	Forward: 5'-TCAGGTCATCACTATCGGCAAT-3' Reverse: 5'-AAAGAAAGGGTGTAACGCA-3'

ADAMTS, A disintegrin and metalloproteinase with thrombospondin motifs.

antibodies were used, with incubations for 12 h at 4°C. Subsequently, the membranes were incubated with secondary antibodies [HRP-conjugated anti-rabbit IgG (1:5,000) and Dylight 488-conjugated goat anti-mouse IgG H&L (1:5,000)] for a further 1 h. A ChemiDoc™ XRS+ imaging system (v4.0, Bio-Rad Laboratories, Inc.) and visualization reagent (Thermo Fisher Scientific, Inc.; cat. no. 32106) was used to quantify the intensities of the blots.

TUNEL assay. Following treatment, NP cells (5×10^5 on cover glass) were incubated in 4% paraformaldehyde for 30 min at room temperature, 3% (v/v) H_2O_2 for 10 min and 0.1% Triton X-100 for 7 min. The TUNEL reagent was then applied to the NP cells for 15 min at room temperature before the nuclei were stained with DAPI for 7 min at room temperature. After washing with PBS, the slides were sealed with sealing agent (Thermo Fisher Scientific, Inc.; cat. no. P36970). Images in five fields were captured using a Nikon Eclipse Ti confocal microscope (Nikon Corporation, magnification, x40) to evaluate the apoptotic activity (v5.0, GraphPad Prism; GraphPad Software, Inc.).

Reverse transcription-quantitative PCR. Following treatment, TRIzol® reagent (Thermo Fisher Scientific, Inc.) was used to isolate total RNA from the extracted NP cells, before being reverse transcribed using a cDNA Synthesis kit (cat. no. 6130, Takara Biotechnology Co., Ltd.) using the following conditions: 15 min 37°C and 5 sec 85°C. Subsequently, SYBR Premix Ex Taq mixture (cat. no. B110032; Sangon Biotech Co., Ltd.) was used for qPCR using the following thermocycling conditions: 10 min at 95°C, followed by 40 cycles of 15 sec at 95°C and 1 min at 60°C. The $2^{-\Delta\Delta C_q}$ method (29) was adopted to quantify

the expression levels of mRNA associated with the targeted gene in NP cells, and the primer sequences of targeted genes are listed in Table I. All process were performed according to the protocols outlined by the manufacturers (30).

Statistical analysis. All data were analyzed using GraphPad Prism 7 software (GraphPad Software, Inc.). Data were presented as the mean \pm standard deviation. Each assay was repeated at least three times. One-way ANOVA followed by Tukey's test was used to compare the data in the present study. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Taurine elevates the viability of NP cells induced by IL-1 β . CCK-8 assay was used for assessing the effects of taurine on NP cells. Ascending doses of taurine were used to treat the NP cells. No cytotoxic effects could be observed at concentrations $\leq 40 \mu M$ (Fig. 1A). Compared with that in the control group, the viability of IL-1 β -induced NP cells was significantly lower (Fig. 1B), which was reversed by taurine treatment (10–40 μM , Fig. 1B). Collectively, these findings suggest that taurine can mediate protective effects on the NP cells.

Taurine reverses ER stress and apoptosis in NP cells treated with IL-1 β . CCK-8 results and a previous report (28) revealed that IL-1 β (10 ng/ml) reduced cell viability by 50%, which is a quantifiable level of cell injury. Therefore, IL-1 β (10 ng/ml) was selected for treating the NP cells. In the present study, the expression of ER stress-associated protein CHOP and GRP78 was measured to determine the

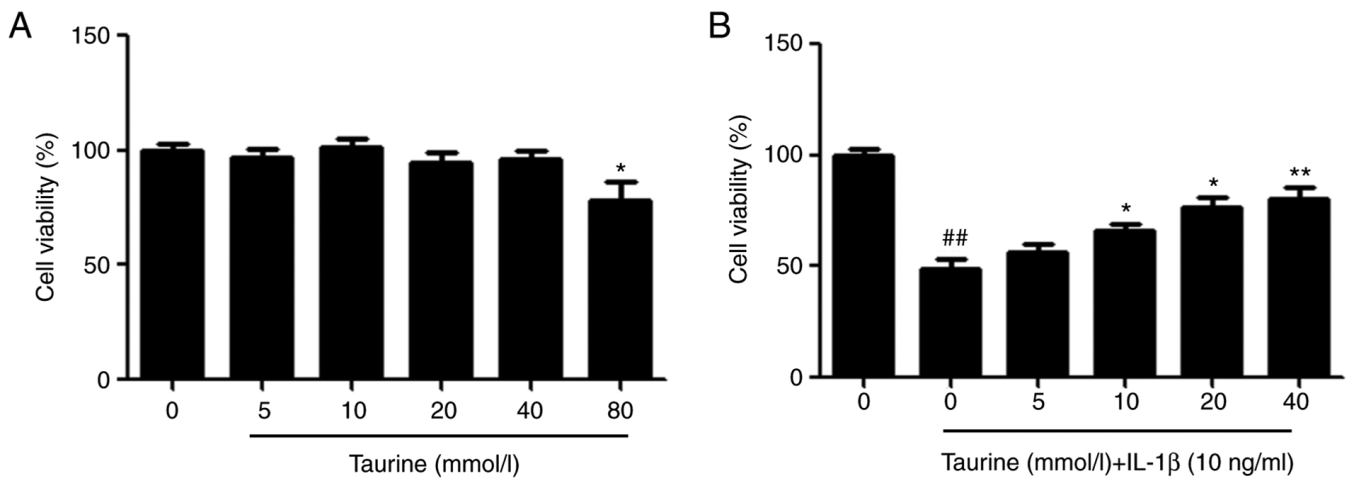


Figure 1. Taurine promotes the survival of IL-1 β -treated nucleus pulposus cells. Cell Counting Kit-8 results of (A) cells treated with different dosages of taurine for 24 h and (B) cells treated with IL-1 β after pre-treatment with taurine. n=5. *P<0.05, **P<0.01 vs. IL-1 β group and ##P<0.01 vs. Control group.

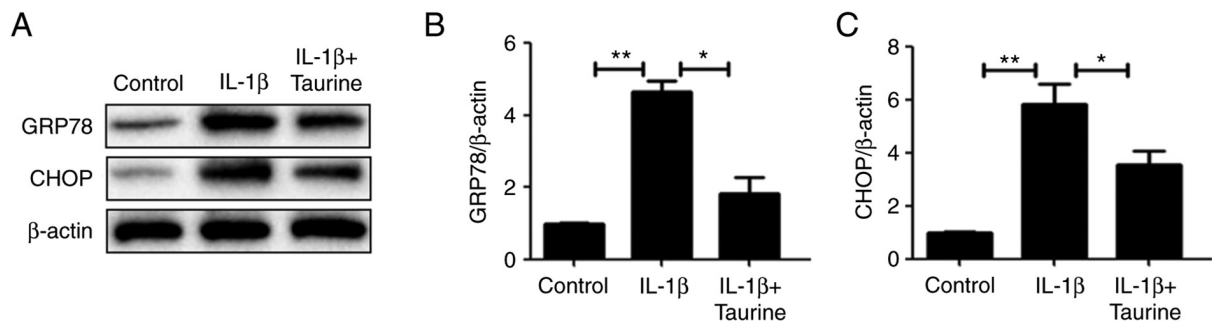


Figure 2. Taurine relieves IL-1 β -triggered ER stress and apoptosis in nucleus pulposus cells. (A) Expression of GRP78 and CHOP after IL-1 β and taurine treatment was measured using western blotting. (B) GRP78 and (C) CHOP expression was quantified (n=5). *P<0.05 and **P<0.01 as indicated. GRP78, glucose regulatory protein 78; CHOP, C/EBP homologous protein.

extent of ER stress in the IL-1 β -treated cohort in response to taurine treatment. The expression levels of all two proteins exhibited significantly elevated expression levels in response to IL-1 β compared with those in the control group. However, taurine treatment significantly reversed this increment (Fig. 2).

Taurine reverses TG-triggered ER stress and apoptosis in NP cells. TG, suppressor of the sarcoplasmic reticulum/ER Ca²⁺ ATPase, can increase cytoplasmic Ca²⁺ by inhibiting the ability of the cell to pump Ca²⁺ back into the ER. A previous study (27) showed that TG at 10 μ M could reduce cell viability of PC12 cells by 50%, which is a quantifiable level of cell injury. In addition, other concentrations of TG either failed to induce cell injury or induced ~100% cell death. Therefore, 10 μ M TG was chosen for treating the NP cells. The NP cells were treated with TG in the present study to assess if ER stress had an association with the cytoprotective effects of taurine. Compared with the control groups, TG significantly elevated GRP78 and CHOP expression, a downstream apoptotic protein. Taurine partially but significantly reversed this increase in GRP78 and CHOP expression (Fig. 3A-C). In addition, immunofluorescence staining of cleaved caspase-12 also supported the notion that taurine inhibited TG-activated apoptosis (Fig. 3D).

Taurine suppresses TG-induced apoptosis in NP cells. To further explore the effects of taurine on the apoptosis of TG-treated NP cells, TUNEL and western blotting were performed to measure the apoptotic activity of NP cells. TUNEL results demonstrated that taurine significantly decreased the number of apoptotic TG-induced NP cells (Fig. 4A and B), suggesting that taurine exerted anti-apoptotic effects on TG-induced NP cells. Administration of TG also increased the protein expression of cleaved caspase-3 and caspase-9, whilst decreasing that of Bcl-2 (Fig. 4C-G). By contrast, these TG-induced effects on the expression of proteins associated with apoptosis were significantly reversed by treatment with taurine (Fig. 4C-G). These findings suggest that taurine suppressed TG-mediated apoptosis in NP cells.

Taurine attenuates the ER stress-induced expression of catabolic enzymes of the ECM in NP cells. Previous studies have reported catabolic changes (such as MMP-1, MMP-3, MMP-9, ADAMTS-4 and ADAMTS-5) in the degenerative disc (31,32). Therefore, the expression ECM-degrading enzymes MMP-1, MMP-3, MMP-9, ADAMTS-4 and ADAMTS-5 was measured by RT-qPCR. Accordingly, TG treatment triggered significant elevations in the mRNA and protein expression of MMP-1, MMP-3, MMP-9, ADAMTS-4

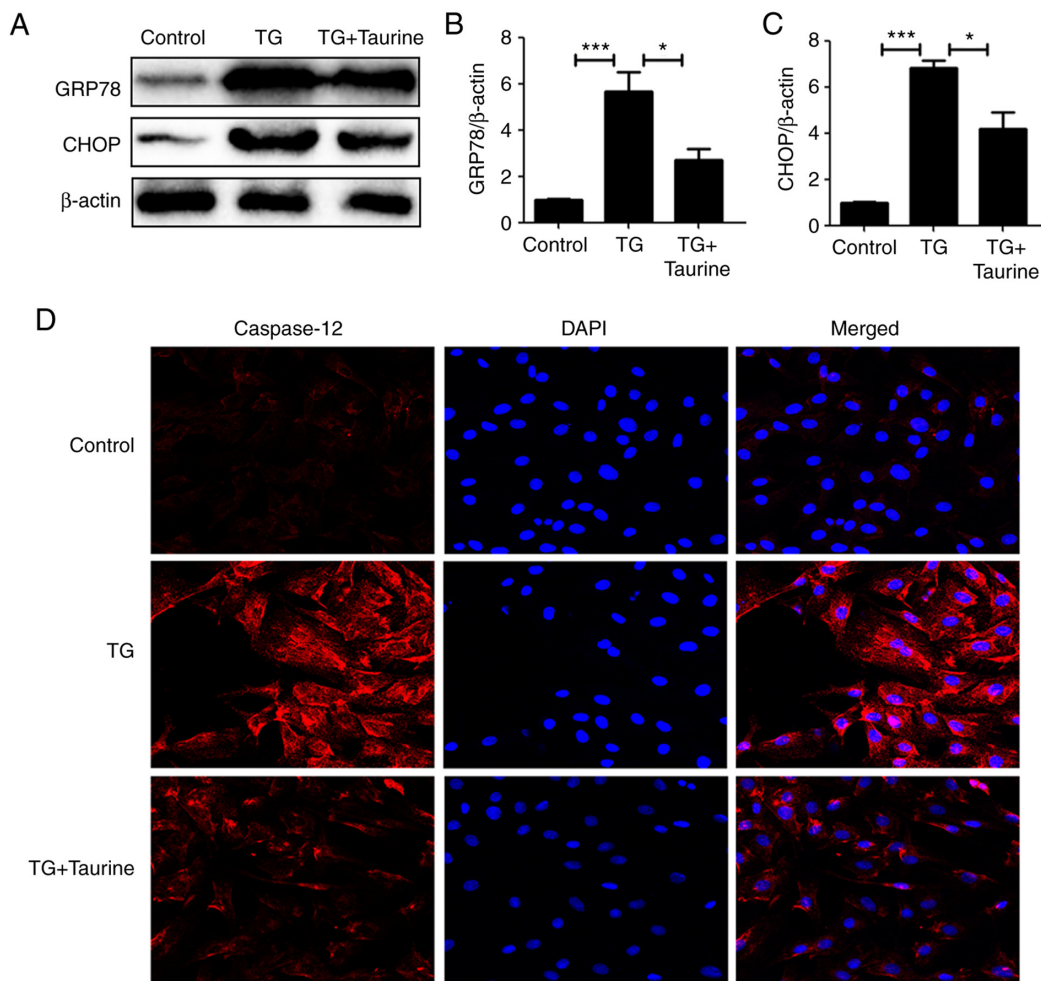


Figure 3. Taurine reverses TG-induced ER stress and apoptosis levels in nucleus pulposus cells. (A) Expression of GRP78 and CHOP after TG and taurine treatment was measured using western blotting. (B) GRP78 and (C) CHOP expression was then quantified. (D) Immunofluorescence analysis of cleaved caspase-12 expression (red). Blue represents nuclear DAPI staining (n=5). * $P<0.05$ and *** $P<0.001$ as indicated. TG, thapsigargin; GRP78, glucose regulatory protein 78; CHOP, C/EBP homologous protein.

and ADAMTS-5 enzymes (Fig. 5). However, taurine significantly reversed this TG-induced increase (Fig. 5).

Taurine restores the ECM in TG-treated in NP cells. Western blotting and immunofluorescence analysis were utilized to evaluate the extent of ECM metabolism in NP cells, which enabled the measurement of collagen-II and aggrecan. As shown in Fig. 6A and B, in the TG group, collagen-II expression were markedly suppressed by TG, indicating increased ECM catabolism in response to ER stress. Taurine appeared to have reversed this effect, consistent with results aforementioned in the present study. Immunofluorescence staining results of collagen-II were consistent with the western blot analysis results (Fig. 6C), whereby taurine treatment attenuated the TG-associated catabolism of the ECM in NP cells. In addition, RT-qPCR analysis showed that downregulation of aggrecan, Col II and Sox-9 by IL-1 β are attenuated by the treatment of Taurine (Fig. 6D-F).

Discussion

Recent reports have suggested that IDD is a major trigger of lower back pain (33,34). Conventional treatment methods for

IDD treatment include physical therapy and pain management medication (35). Surgical interventions are necessary if conventional methods cannot alleviate the symptoms, which can lead to a succession of sequelae (36). In addition, in the majority of cases, patients with IDD remain largely asymptomatic, such that early-stage IDD can only be revealed by MRI (37). Therefore, by exploiting all the available information on the pathophysiology of IDD, it is vital to explore novel treatment strategies to promote endogenous repair whilst halting IDD progression, particularly during early stages of the disease (38).

Accumulating evidence suggests that excessive ECM degradation and elevated NP cell apoptosis serve key roles in the pathology underlying IDD (11). Numerous biomechanical and biochemical activities can promote the deterioration of NP cells, which can in turn induce cell apoptosis to disrupt the equilibrium between ECM catabolism and anabolism (39). Since NP cells have limited capacities for resisting stress and self-repair (40), the molecular pathways underlying NP cell apoptosis require characterization for ameliorating NP degeneration in patients with IDD.

Aging and excessive mechanical loading are primary stressors that can cause NP cell apoptosis (41). Both can

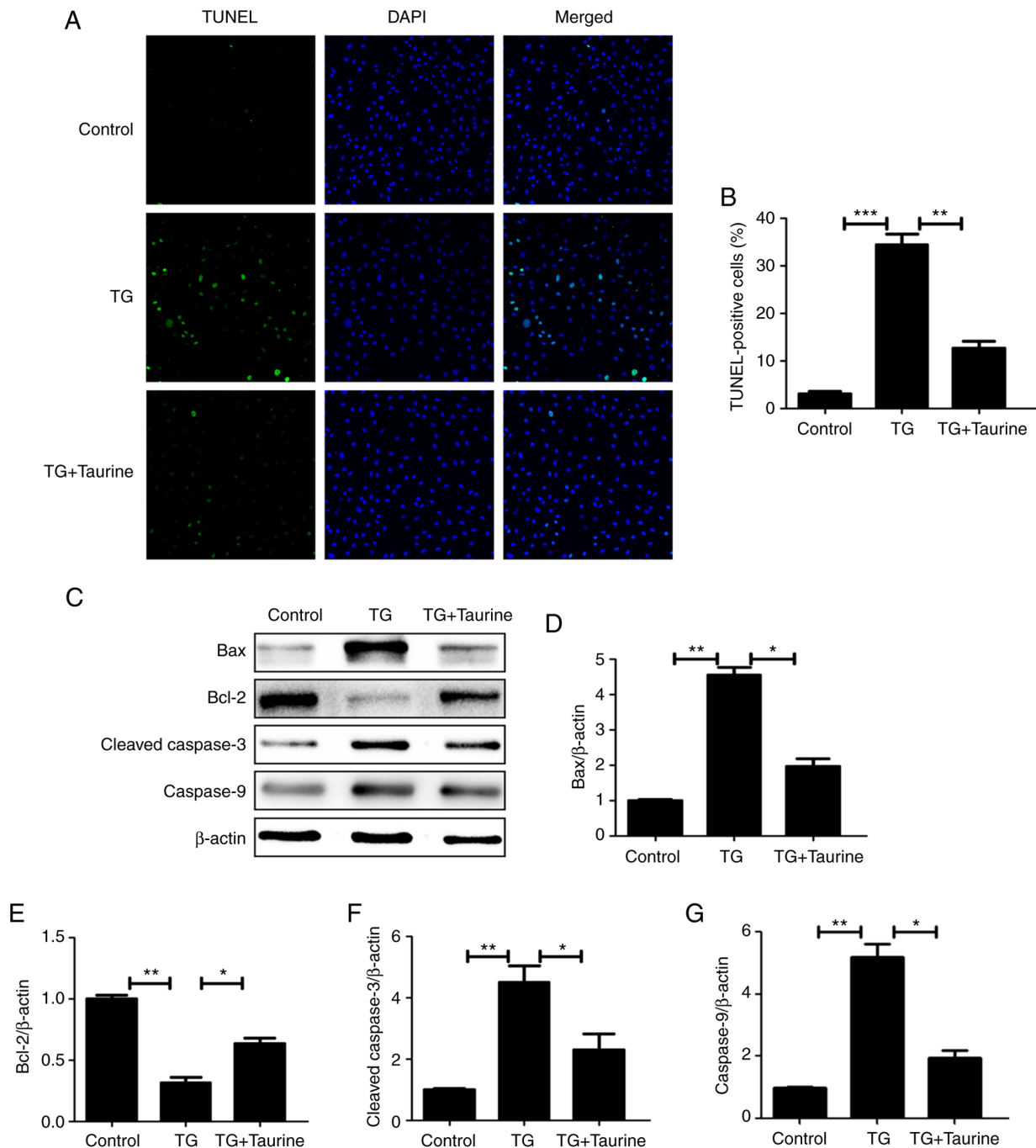


Figure 4. Taurine reverses TG-triggered apoptosis levels in nucleus pulposus cells. (A) TUNEL assay was used to measure apoptosis, (B) which was quantified. Blue represents nuclear DAPI staining. (C) Expression levels of Bax, Bcl-2, Cleaved caspase-3 and caspase-9 after TG and taurine treatment were measured by western blotting. Expression of (D) Bax, (E) Bcl-2, (F) cleaved caspase-3 and (G) caspase-9 was quantified (n=5). *P<0.05, **P<0.01 and ***P<0.001 as indicated. TG, thapsigargin.

activate numerous signaling pathways, including those such as the NLRX1 and NF- κ B pathways associated with inflammation (42). IL-1 β is a potent proinflammatory mediator that can trigger the apoptosis of NP cells by inducing mitochondrial dysfunction and activating caspase-3 (43). According to CCK-8 assay results, the viability of IL-1 β -induced NP cells was found to be lower compared with that in the control group. By contrast, taurine treatment reversed this IL-1 β -induced effect, suggesting that taurine can exert cytoprotective effects on NP cells.

ER stress and the unfolded protein response (UPR) downstream have been previously associated with the pathology of

a number of diseases, including cancer, skeletal disorders and neurodegeneration (44). In terms of musculoskeletal disorders, a previous study revealed that in human osteoarthritis, chondrocytes were chronically exposed to ER stress, where CHOP served a key role in mediating apoptosis and cartilage degeneration downstream of ER stress (45). By contrast, only a small number of studies have studied the potential contribution of ER stress to IDD (7). GRP78 is a 78-kDa glucose-regulated protein that is also known as immunoglobulin heavy chain binding protein (46). Eukaryotic translation initiation factor 2A (eIF2 α) phosphorylation is instrumental for regulating

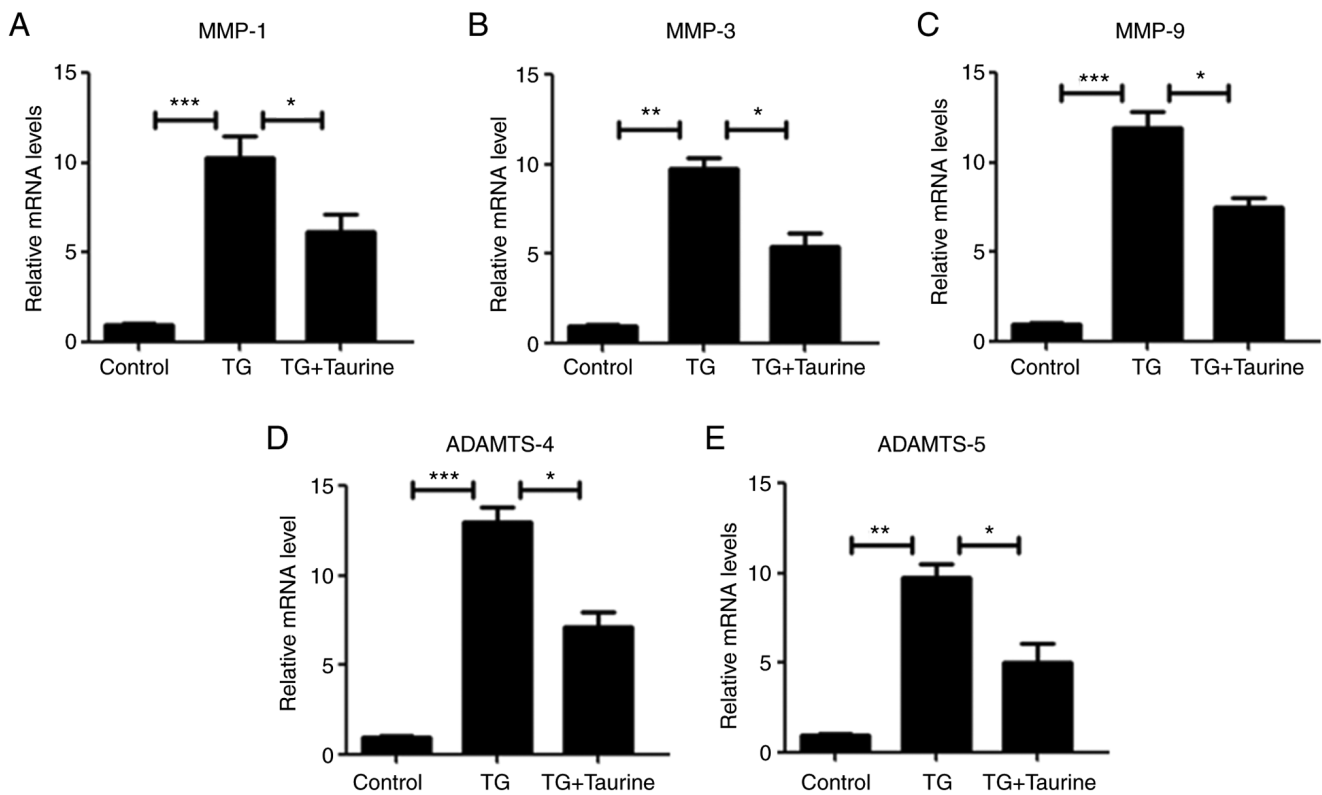


Figure 5. Taurine inhibits the TG-induced expression of catabolic enzymes in nucleus pulposus cells. Relative mRNA expression levels of (A) MMP-1, (B) MMP-3, (C) MMP-9, (D) ADADMTS-4 and (E) ADAMTS-5 were measured. n=5, *P<0.05, **P<0.01 and ***P<0.001. TG, thapsigargin; ADAMTS, A disintegrin and metalloproteinase with thrombospondin motifs.

the global rate of protein synthesis (47). In addition, protein kinase R-like ER kinase (PERK) has been reported to induce cell apoptosis by promoting the accumulation of CHOP under severe ER stress (48). ER stress can induce apoptosis by activating the UPR, upregulating the expression of GRP78 and activating PERK/eIF2 α signaling (47). After dissociation from PERK, eIF2 α is then phosphorylated. This eIF2 α phosphorylation then activate the regulatory factors of ER stress, such as activating transcription factor (ATF) 4 and CHOP (48). Specifically, caspase-12 also has a role in cell apoptosis resulting from ER stress (49). Taurine has been documented to protect cells by either inhibiting mitochondrial dysfunction or ER stress (50,51). Nonaka *et al* (52) previously found that taurine can alleviate homocysteine-triggered ER stress in vascular smooth muscle cells. According to the present study, the ER stress signaling pathway (GRP78 and CHOP) appeared to be more active in the IL-1 β groups compared with that in the control groups, suggesting that IL-1 β can trigger ER stress. Consequently, the administration of taurine can attenuate this ER stress, indicating the potential role of taurine in suppressing ER stress. In present study, TG was chosen to assess the extent to which ER stress associates with the cytoprotective effects of taurine in NP cells. TG can be extracted from the plant *Thapsia garganica* and is a classical inhibitor of ER Ca²⁺ ATPase (53). ER Ca²⁺ATPase dysfunction has been demonstrated to serve a role in various diseases, including IDD (54). By suppressing ER Ca²⁺ ATPase, TG interferes with Ca²⁺ flux in the ER lumen, which contributes to accumulation of cytoplasmic Ca²⁺ (55). This Ca²⁺ overload causes mitochondrial dysfunction and disrupts a number of metabolic pathways,

including lipid turnover, which is reliant on lipase maturation factor 1, an ER chaperone (56). Furthermore, depletion of this Ca²⁺ store results in the rapid accumulation of unfolded proteins in the ER, promoting the dissociation of GRP78 from inositol-requiring enzyme 1 (IRE1), PERK and ATF6 pathways, thereby activating UPR signaling (47). Regulation of store-operated Ca²⁺ entry has also been associated with the expression of specific microRNAs (57) and may regulate the Ca²⁺-associated modulation of the UPR (IRE1 activity) in the ER (58). According to the present study, compared with that in the control groups, TG treatment elevated GRP78 expression in addition to increasing the expression of its downstream apoptotic mediator CHOP. Taurine partially reversed the upregulation of both GRP78 and CHOP. In addition, immunofluorescence staining results of cleaved caspase-12 were also consistent with the GRP78 and CHOP western blotting data. TUNEL and western blot analysis of apoptotic activity also revealed that taurine suppressed the TG-mediated apoptosis of NP cells. According to these results, in TG-treated NP cells, taurine partially reversed the activation of ER stress and ER stress-associated apoptosis.

Increasing the expression of ECM-degrading enzymes and proinflammatory mediators are conducive for IDD progression (59). Production of inflammatory factors, such as IL-1 β , in turn elevates the expression of catabolic proteins, such as MMP and ADAMTS, to promote continuous collagen and proteoglycan degradation (59). Numerous reports have presented evidence that ER stress is a key process in mediating inflammation and matrix degradation (54,60). A previous NP cell secretome analysis demonstrated an influence of ER stress

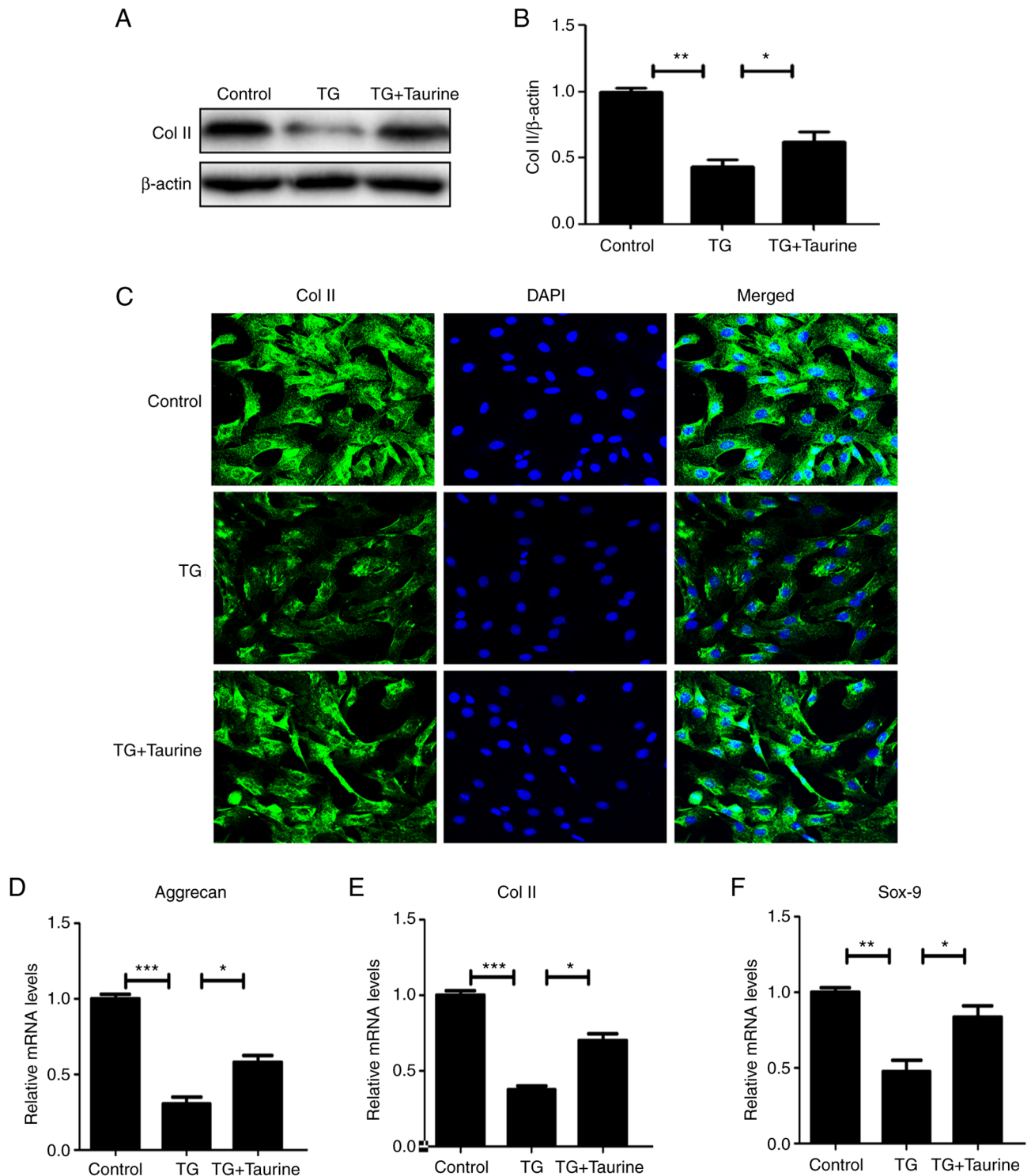


Figure 6. Taurine inhibits TG-induced extracellular matrix protein degradation in nucleus pulposus cells. (A) Relative Col II protein expression levels were measured by western blotting, (B) which was quantified. (C) Col II expression was detected using immunofluorescence. Green represents Col II, whereas blue represents nuclear DAPI staining. Relative (D) aggrecan, (E) Col II and (F) Sox-9 mRNA expression levels were measured (n=5). *P<0.05, **P<0.01 and ***P<0.001 as indicated. TG, thapsigargin; Col II, collagen; Sox-9, SRY-box transcription factor 9.

induction on the secretion of the ECM, which is accompanied with the reduction in the expression of collagen and cell adhesion-related proteins (60). Pharmacologically blocking ER Ca^{2+} release using Ca^{2+} antagonists was found to ameliorate ER stress and Ca^{2+} overload to prevent the apoptosis of NP cells and partially halt the development of IDD (54). The present study revealed that NP cells treated with TG exhibited

characteristics indicative of ECM degradation, including the upregulation of MMP-1, MMP-3, MMP-9 and ADAMTS-4, ADAMTS-5 expression and the downregulation of collagen-II expression. Taurine reversed these TG-induced effects on increasing the expression of these catabolic enzymes. Collectively, these results suggest that taurine attenuated the ER stress-associated metabolic processes of the NP cells.

In conclusion, the present study revealed that taurine can promote NP cell viability whilst inhibiting apoptosis. These protective effects were associated with the prevention of ER stress in NP cells *in vitro*. In addition, taurine was able to attenuate ER stress-associated ECM catabolic activity in NP cells. Therefore, taurine should be investigated for its potentially feasibility as a therapeutic agent for preventing NP degeneration patients diagnosed with IDD.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LY and YO designed the study. LY and ZL contributed to experiments and statistical analysis. LY and YO confirm the authenticity of all the raw data. All authors read and approved the version of the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Animal Care and Use Committee of Shanghai Jing'an District Zhabei Central Hospital (Shanghai, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Wang F, Cai F, Shi R, Wang XH and Wu XT: Aging and age related stresses: A senescence mechanism of intervertebral disc degeneration. *Osteoarthritis Cartilage* 24: 398-408, 2016.
- Simon J, McAuliffe M, Shamim F, Vuong N and Tahaei A: Discogenic low back pain. *Phys Med Rehabil Clin N Am* 25: 305-317, 2014.
- Petersen T, Laslett M and Juhl C: Clinical classification in low back pain: Best-evidence diagnostic rules based on systematic reviews. *BMC Musculoskelet Disord* 18: 188, 2017.
- Barrey CY and Le Huec JC; French Society for Spine Surgery: Chronic low back pain: Relevance of a new classification based on the injury pattern. *Orthop Traumatol Surg Res* 105: 339-346, 2019.
- Liang D, Hong D, Tang F, Wang Y, Li J, Li L and Chen H: Upregulated lnc-HRK-2:1 prompts nucleus pulposus cell senescence in intervertebral disc degeneration. *Mol Med Rep* 22: 5251-5261, 2020.
- Pattappa G, Li Z, Peroglio M, Wismer N, Alini M and Grad S: Diversity of intervertebral disc cells: Phenotype and function. *J Anat* 221: 480-496, 2012.
- Sakai D and Grad S: Advancing the cellular and molecular therapy for intervertebral disc disease. *Adv Drug Deliv Rev* 84: 159-171, 2015.
- Ding F, Shao ZW and Xiong LM: Cell death in intervertebral disc degeneration. *Apoptosis* 18: 777-785, 2013.
- Xu S, Liang T and Li S: Correlation between polymorphism of TRAIL gene and condition of intervertebral disc degeneration. *Med Sci Monit* 21: 2282-2287, 2015.
- Zhao Z, Zheng J, Ye Y, Zhao K, Wang R and Wang R: MicroRNA-25-3p regulates human nucleus pulposus cell proliferation and apoptosis in intervertebral disc degeneration by targeting Bim. *Mol Med Rep* 22: 3621-3628, 2020.
- Tsingas M, Ottone OK, Haseeb A, Barve RA, Shapiro IM, Lefebvre V and Risbud MV: Sox9 deletion causes severe intervertebral disc degeneration characterized by apoptosis, matrix remodeling, and compartment-specific transcriptomic changes. *Matrix Biol* 94: 110-133, 2020.
- Borrelli C and Buckley CT: Injectable disc-derived ECM hydrogel functionalised with chondroitin sulfate for intervertebral disc regeneration. *Acta Biomater* 117: 142-155, 2020.
- Ohnishi T, Novais EJ and Risbud MV: Alterations in ECM signature underscore multiple sub-phenotypes of intervertebral disc degeneration. *Matrix Biol Plus* 6-7: 100036, 2020.
- Le Maitre CL, Pockert A, Buttle DJ, Freemont AJ and Hoyland JA: Matrix synthesis and degradation in human intervertebral disc degeneration. *Biochem Soc Trans* 35: 652-655, 2007.
- Wang WJ, Yu XH, Wang C, Yang W, He WS, Zhang SJ, Yan YG and Zhang J: MMPs and ADAMTSs in intervertebral disc degeneration. *Clin Chim Acta* 448: 238-246, 2015.
- Ding F and Li X: Apigenin mitigates intervertebral disc degeneration through the amelioration of tumor necrosis factor α (TNF- α) signaling pathway. *Med Sci Monit* 26: e924587, 2020.
- Roberts S, Caterson B, Menage J, Evans EH, Jaffray DC and Eisenstein SM: Matrix metalloproteinases and aggrecanase: Their role in disorders of the human intervertebral disc. *Spine (Phila Pa 1976)* 25: 3005-3013, 2000.
- Pockert AJ, Richardson SM, Le Maitre CL, Lyon M, Deakin JA, Buttle DJ, Freemont AJ and Hoyland JA: Modified expression of the ADAMTS enzymes and tissue inhibitor of metalloproteinases 3 during human intervertebral disc degeneration. *Arthritis Rheum* 60: 482-491, 2009.
- Yamori Y, Nara Y, Ikeda K and Mizushima S: Is taurine a preventive nutritional factor of cardiovascular diseases or just a biological marker of nutrition? *Adv Exp Med Biol* 403: 623-629, 1996.
- Wallace DR and Dawson R Jr: Decreased plasma taurine in aged rats. *Gerontology* 36: 19-27, 1990.
- Pacholczyk-Sienicka B, Radek M, Radek A and Jankowski S: Characterization of metabolites determined by means of 1H HR MAS NMR in intervertebral disc degeneration. *MAGMA* 28: 173-183, 2015.
- Goodman CA, Horvath D, Stathis C, Mori T, Croft K, Murphy RM and Hayes A: Taurine supplementation increases skeletal muscle force production and protects muscle function during and after high-frequency in vitro stimulation. *J Appl Physiol* (1985) 107: 144-154, 2009.
- Ghosh S, Chowdhury S, Das AK and Sil PC: Taurine ameliorates oxidative stress induced inflammation and ER stress mediated testicular damage in STZ-induced diabetic Wistar rats. *Food Chem Toxicol* 124: 64-80, 2019.
- Rainesalo S, Keränen T, Palmio J, Peltola J, Oja SS and Saransaari P: Plasma and cerebrospinal fluid amino acids in epileptic patients. *Neurochem Res* 29: 319-324, 2004.
- Engelborghs S, Marescau B and De Deyn PP: Amino acids and biogenic amines in cerebrospinal fluid of patients with Parkinson's disease. *Neurochem Res* 28: 1145-1150, 2003.
- Gao Z, Lin Y, Zhang P, Cheng Q, Ye L, Wu F, Chen Y, Fu M, Cheng C and Gao Y: Sinomenine ameliorates intervertebral disc degeneration via inhibition of apoptosis and autophagy in vitro and in vivo. *Am J Transl Res* 11: 5956-5966, 2019.
- Liu D, Gu Y, Wang W and Chen W: Astragalin alleviates ischemia/reperfusion-induced brain injury via suppression of endoplasmic reticulum stress. *Mol Med Rep* 22: 4070-4078, 2020.
- Chen J, Xuan J, Gu YT, Shi KS, Xie JJ, Chen JX, Zheng ZM, Chen Y, Chen XB, Wu YS, *et al*: Celastrol reduces IL-1 β induced matrix catabolism, oxidative stress and inflammation in human nucleus pulposus cells and attenuates rat intervertebral disc degeneration in vivo. *Biomed Pharmacother* 91: 208-219, 2017.

29. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
30. Pfaffl MW: A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29: e45, 2001.
31. Martirosyan NL, Patel AA, Carotenuto A, Kalani MY, Belykh E, Walker CT, Preul MC and Theodore N: Genetic alterations in intervertebral disc disease. *Front Surg* 3: 59, 2016.
32. Yurube T, Takada T, Suzuki T, Kakutani K, Maeno K, Doita M, Kurosaka M and Nishida K: Rat tail static compression model mimics extracellular matrix metabolic imbalances of matrix metalloproteinases, aggrecanases, and tissue inhibitors of metalloproteinases in intervertebral disc degeneration. *Arthritis Res Ther* 14: R51, 2012.
33. Ekşi MŞ, Özcan-Ekşi EE, Özmen BB, Turgut VU, Huet SE, Dinç T, Kara M, Özgen S, Özek MM and Pamir MN: Lumbar intervertebral disc degeneration, end-plates and paraspinal muscle changes in children and adolescents with low-back pain. *J Pediatr Orthop B* 31: 93-102, 2022.
34. Khan AN, Jacobsen HE, Khan J, Filippi CG, Levine M, Lehman RA Jr, Riew KD, Lenke LG and Chahine NO: Inflammatory biomarkers of low back pain and disc degeneration: A review. *Ann N Y Acad Sci* 1410: 68-84, 2017.
35. Zhang B, Xu H, Wang J, Liu B and Sun G: A narrative review of non-operative treatment, especially traditional Chinese medicine therapy, for lumbar intervertebral disc herniation. *Biosci Trends* 11: 406-417, 2017.
36. Jacobs WC, van der Gaag NA, Kruijt MC, Tuschel A, de Kleuver M, Peul WC, Verbout AJ and Oner FC: Total disc replacement for chronic discogenic low back pain: A Cochrane review. *Spine (Phila Pa 1976)* 38: 24-36, 2013.
37. Ogon I, Takashima H, Morita T, Oshigiri T, Terashima Y, Yoshimoto M, Fukushi R, Fujimoto S, Emori M, Teramoto A, *et al*: Relevance between schmorl's node and lumbar intervertebral disc degeneration quantified with magnetic resonance imaging T2 mapping in chronic low back pain. *Asian Spine J* 14: 621-628, 2020.
38. Cherif H, Bisson DG, Mannarino M, Rabau O, Ouellet JA and Haglund L: Senotherapeutic drugs for human intervertebral disc degeneration and low back pain. *Elife* 9: e54693, 2020.
39. Wang D, Chen Y, Cao S, Ren P, Shi H, Li H, Xie L, Huang W, Shi B and Han J: Cyclic mechanical stretch ameliorates the degeneration of nucleus pulposus cells through promoting the ITGA2/PI3K/AKT signaling pathway. *Oxid Med Cell Longev* 2021: 6699326, 2021.
40. Mobasheri A, Csaki C, Clutterbuck AL, Rahmanzadeh M and Shakibaei M: Mesenchymal stem cells in connective tissue engineering and regenerative medicine: Applications in cartilage repair and osteoarthritis therapy. *Histol Histopathol* 24: 347-366, 2009.
41. Zhao CQ, Wang LM, Jiang LS and Dai LY: The cell biology of intervertebral disc aging and degeneration. *Ageing Res Rev* 6: 247-261, 2007.
42. Xu H, Ji L, Yu C, Chen Q, Ge Q and Lu Y: MiR-423-5p regulates cells apoptosis and extracellular matrix degradation via nucleotide-binding, leucine-rich repeat containing X1 (NLRX1) in interleukin 1 beta (IL-1 β)-induced human nucleus pulposus cells. *Med Sci Monit* 26: e922497, 2020.
43. Guo HT, Yang SD, Zhang F, Liu S, Yang DL, Ma L, Wang H and Ding WY: 17 β -Estradiol protects against interleukin-1 β -induced apoptosis in rat nucleus pulposus cells via the mTOR/caspase-3 pathway. *Mol Med Rep* 20: 1523-1530, 2019.
44. Horiuchi K, Tohmonda T and Morioka H: The unfolded protein response in skeletal development and homeostasis. *Cell Mol Life Sci* 73: 2851-2869, 2016.
45. Uehara Y, Hirose J, Yamabe S, Okamoto N, Okada T, Oyadomari S and Mizuta H: Endoplasmic reticulum stress-induced apoptosis contributes to articular cartilage degeneration via C/EBP homologous protein. *Osteoarthritis Cartilage* 22: 1007-1017, 2014.
46. Shimizu A, Kaira K, Yasuda M, Asao T and Ishikawa O: Clinical and pathological significance of ER stress marker (BiP/GRP78 and PERK) expression in malignant melanoma. *Pathol Oncol Res* 23: 111-116, 2017.
47. Chern YJ, Wong JCT, Cheng GSW, Yu A, Yin Y, Schaeffer DF, Kennecke HF, Morin G and Tai IT: The interaction between SPARC and GRP78 interferes with ER stress signaling and potentiates apoptosis via PERK/eIF2 α and IRE1 α /XBP-1 in colorectal cancer. *Cell Death Dis* 10: 504, 2019.
48. Fu YF, Liu X, Gao M, Zhang YN and Liu J: Endoplasmic reticulum stress induces autophagy and apoptosis while inhibiting proliferation and drug resistance in multiple myeloma through the PI3K/Akt/mTOR signaling pathway. *Oncotarget* 8: 61093-61106, 2017.
49. Lee W, Kim DH, Boo JH, Kim YH, Park IS and Mook-Jung I: ER stress-induced caspase-12 activation is inhibited by PKC in neuronal cells. *Apoptosis* 10: 407-415, 2005.
50. Yang Y, Zhang Y, Liu X, Zuo J, Wang K, Liu W and Ge J: Exogenous taurine attenuates mitochondrial oxidative stress and endoplasmic reticulum stress in rat cardiomyocytes. *Acta Biochim Biophys Sin (Shanghai)* 45: 359-367, 2013.
51. Yang YJ, Han YY, Chen K, Zhang Y, Liu X, Li S, Wang KQ, Ge JB, Liu W and Zuo J: TonEBP modulates the protective effect of taurine in ischemia-induced cytotoxicity in cardiomyocytes. *Cell Death Dis* 6: e2025, 2015.
52. Nonaka Y, Tsujino T, Watari Y, Emoto N and Yokoyama M: Taurine prevents the decrease in expression and secretion of extracellular superoxide dismutase induced by homocysteine: Amelioration of homocysteine-induced endoplasmic reticulum stress by taurine. *Circulation* 104: 1165-1170, 2001.
53. Li Z, Guo J, Bian Y and Zhang M: Intermedin protects thapsigargin-induced endoplasmic reticulum stress in cardiomyocytes by modulating protein kinase A and sarco/endoplasmic reticulum Ca²⁺-ATPase. *Mol Med Rep* 23: 107, 2021.
54. Luo R, Song Y, Liao Z, Yin H, Zhan S, Wang K, Li S, Li G, Ma L, Lu S, *et al*: Impaired calcium homeostasis via advanced glycation end products promotes apoptosis through endoplasmic reticulum stress in human nucleus pulposus cells and exacerbates intervertebral disc degeneration in rats. *FEBS J* 286: 4356-4373, 2019.
55. Canová NK, Kmoníčková E, Martínek J, Zídek Z and Farghali H: Thapsigargin, a selective inhibitor of sarco-endoplasmic reticulum Ca²⁺-ATPases, modulates nitric oxide production and cell death of primary rat hepatocytes in culture. *Cell Biol Toxicol* 23: 337-354, 2007.
56. Mao HZ, Ehrhardt N, Bedoya C, Gomez JA, DeZwaan-McCabe D, Mungro IN, Kaufman RJ, Rutkowski DT and Péterfy M: Lipase maturation factor 1 (lmf1) is induced by endoplasmic reticulum stress through activating transcription factor 6 α (Atf6 α) signaling. *J Biol Chem* 289: 24417-24427, 2014.
57. Finger F and Hoppe T: MicroRNAs meet calcium: Joint venture in ER proteostasis. *Sci Signal* 7: re11, 2014.
58. Groenendyk J, Peng Z, Dudek E, Fan X, Mizianty MJ, Dufey E, Urrea H, Sepulveda D, Rojas-Rivera D, Lim Y, *et al*: Interplay between the oxidoreductase PDIA6 and microRNA-322 controls the response to disrupted endoplasmic reticulum calcium homeostasis. *Sci Signal* 7: ra54, 2014.
59. Lama P, Zehra U, Balkovec C, Claireaux HA, Flower L, Harding IJ, Dolan P and Adams MA: Significance of cartilage endplate within herniated disc tissue. *Eur Spine J* 23: 1869-1877, 2014.
60. Novais EJ, Choi H, Madhu V, Suyama K, Anjo SI, Manadas B, Shapiro IM, Salgado AJ and Risbud MV: Hypoxia and hypoxia-inducible factor-1 α regulate endoplasmic reticulum stress in nucleus pulposus cells: Implications of endoplasmic reticulum stress for extracellular matrix secretion. *Am J Pathol* 191: 487-502, 2021.



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