Int J Clin Exp Pathol 2020;13(9):2240-2248
www.ijcep.com /ISSN:1936-2625/IJCEP0106217

Original Article

HMGB1-induced autophagy promotes extracellular matrix degradation leading to intervertebral disc degeneration

Biao Fu[1], Xia Lu[1], Er-Yang Zhao[1], Ji-Xin Wang[2], Si-Min Peng[1]

[1]*Department of Neurosurgery, The People Hospital of Xin Chang, No. 117 Gushan Middle Road, Nanming Street, Xin Chang 312500, Zhejiang, China;* [2]*Department of Spinal Surgery, Zhejiang Quhua Hospital, No. 62 Wenchang Road, Quzhou 475000, Zhejiang, China*

Received December 10, 2019; Accepted January 19, 2020; Epub September 1, 2020; Published September 15, 2020

Abstract: Intervertebral disc degeneration (IDD) remains a leading cause of adult disability. High mobility group box I (HMGB1) is a nuclear DNA-binding protein and acts as a central mediator of inflammation. The purpose of this study was to investigate the effect of HMGB1 in IDD. In our study, IDD intervertebral disc tissues were collected and nucleus pulposus cells (NPCs) were primarily cultured. The HMGB1 expression and the effect of HMGB1 on NPCs and extracellular matrix and autophagy were all evaluated. Results showed that HMGB1 was markedly over-expressed in IDD ($P<0.05$), and upregulated expression of HMGB1 can inhibit NPC proliferation and promote NPC apoptosis ($P<0.05$), promote extracellular matrix degradation, and activate cell autophagy ($P<0.05$). Therefore, we concluded that HMGB1 was up-regulated in IDD and HMGB1-induced autophagy can promotes extracellular matrix degradation and thus lead to intervertebral disc degeneration. In brief, HMGB1 may serve as a novel diagnostic biomarker and therapeutic target for IDD.

Keywords: IDD, HMGB1, extracellular matrix, autophagy

Introduction

Intervertebral disc degeneration (IDD) remains a common cause of neck and low back pain and adult disability [1]. Age and injury (including cumulative injury) are two important factors leading to IDD [2]. Current studies suggest that the pathogenesis of IDD is largely due to internal disc nucleus pulposus cells (NPC) loss, and extracellular matrix components of change, and inflammatory factor infiltration [3]. Also increased inflammatory factors can lead to reduction of NPCs and degradation of extracellular matrix, and in turn, apoptosis of NPCs extracellular matrix degradation can promote the release of inflammatory factors [4]. IDD is also regarded as an inflammation-related diseases [5].

High mobility group (HMG) is a nuclear DNA-binding protein and widely distributed in eukaryotic cells, which can regulate the replication and recombination of genes and maintain the structural stability of nucleosomes [6]. High mobility group box I (HMGB1) is a member of the HMG family and is involved in DNA recombination, autophagy, and inflammatory damage process [7]. HMGB1 contains a C-terminal that can regulate transcription (aa186-215) and two DNA-binding domains (A box aa1-79 and B box aa89-163). It can binds to Toll-like receptors and regulates the release of a variety of inflammatory factors [8]. Recent studies [9-11] have shown that HMGB1 is closely related to the occurrence and development of many inflammatory diseases, such as septicemia, autoimmune diseases, and osteoarthritis. However, the role of HMGB1 in the pathogenesis of IDD has not been reported and still remains unknown.

Consequently, IDD intervertebral disc tissues were collected and nucleus pulposus cells (NPCs) were primarily cultured. The HMGB1

Table 1. Forward (F) and reverse (R) primer sequences for qRT-PCR

Gene		Primer sequence	Length
HMGB1	F	5'-GATCCCAATGCACCCAAGAG-3'	150 bp
	R	5'-GTGATGGGATTTCCATTGAT-3'	
Collagen II	F	5'-CCTGAAACTCTGCCACCCAG-3'	151 bp
	R	5'-CTGCAGCACGGTATAGGTGA-3'	
Agrecan	F	5'-GCTACGGAGACAAGGATGAGT-3'	124 bp
	R	5'-CGTAAAAGACCTCACCCTCCA-3'	
MMP-3	F	5'-AAACCCACCTTACATAC-3'	125 bp
	R	5'-TCCATAGAGGGACTGA-3'	
MMP-9	F	5'-TCCCTGGAGACCTGAGAACC-3'	130 bp
	R	5'-GCCACCCGAGTGTAACCAT-3'	
β-actin	F	5'-GGAGTCAACGGATTTGGT-3'	120 bp
	R	5'-GTGATGGGATTTCCATTGAT-3'	

expression and the effect of HMGB1 on NPCs and extracellular matrix and autophagy were all evaluated, respectively in our study. Our results showed that HMGB1 was up-regulated in IDD and HMGB1-induced autophagy can promote extracellular matrix degradation and thus lead to intervertebral disc degeneration. Indeed, to the best of our knowledge, this is the first report on the role of HMGB1 in the pathogenesis of IDD.

Materials and methods

Materials

Clinical specimens: Intervertebral disc tissue of 3 cases of IDD patients and 2 cases of spinal fracture patients were collected from department of Spinal Surgery, Zhejiang Quhua Hospital from November 2017 to May 2018. The degree of intervertebral disc degeneration was graded according to preoperative MRI Pfirrmann classification [12], and all 3 cases of IDD patients were grade IV. 2 cases of spinal fracture patients had no history of low back pain. All participants or guardians gave written informed consent and the study was approved by the institutional ethical review board of Zhejiang Quhua Hospital.

Reagents and instruments: RPMI1640 DMEM Medium, Penicillin/Streptomycin Double Antibody Reagent, fetal bovine serum and trypsase were bought from Sigma Co. Ltd (USA). MTT Cell Proliferation and Cytotoxicity Assay Kit (GM01-500T), TRIzol kit (GC001-50T), AnnexinV-FITC/PI Apoptosis Detection Kit (GA02-02-50T), BCA Protein Assay Kit (GM03-200T), Transfection reagent Lipofectamine 3000 (GC002-50T), First Strand cDNA Synthesis Kit (GM03-50T) were purchased from Shanghai GEFAN Bio-chemistry Co. Ltd (China). Rabbit polyclonal to HMGB1 (1:1000 dilution, catalogue number: ab18-256, Abcam Co. Ltd, USA), Rabbit polyclonal to Collagen II (1:1000 dilution, catalogue number: ab34712, Abcam Co. Ltd, USA), Rabbit polyclonal to Aggrecan (1:1000 dilution, catalogue number: ab6276, Abcam Co. Ltd, USA), Rabbit polyclonal to MMP-3 (1:500 dilution, catalogue number: ab53-015, Abcam Co. Ltd, USA), Rabbit polyclonal to MMP-9 (1:500 dilution, catalogue number: ab73734, Abcam Co. Ltd, USA), Rabbit polyclonal to Beclin 1 (1:500 dilution, catalogue number: ab62557, Abcam Co. Ltd, USA), Rabbit polyclonal to LC3 II/I (1:500 dilution, catalogue number: ab515204, Abcam Co. Ltd, USA) were all bought from Abcam Co. Ltd (USA). HMGB1 Overexpression vector (HMGB1 OE) and pcDNA3.1 empty vector, small interfering RNA targeting HMGB1 (si-HMGB1) and a control siRNA (si-NC), were designed and synthesized and purchased from Shanghai GEFAN Bio-chemistry Co. Ltd (China). The sequence as follows: si-HMGB1-1: 5'-GGAGAUCCUAAGAAGCCGATT-3'; si-HMGB1-2: 5'-GGGAGGAGCAUAAGAAGAATT-3'; si-NC: 5'-UUCUCCGAACGUGUCACG UTT-3'; HMGB1 OE vector: 5'-TCGCTGAGGAAAAACAACT-3'; pcDNA3.1: 5'-AAAACTGCGCTAGAACCAACTTAT3'. The Forward (F) and reverse (R) primers sequence of HMGB1, Collagen II, Agrecan, MMP-3 and MMP-9 for qRT-PCR were designed and synthesized and purchased from Shanghai GEFAN Biochemistry Co. Ltd (China), the sequence showed in **Table 1**.

Methods

Immunohistochemistry: The intervertebral disc tissues from degenerative and normal groups were fixed with 4% polyformaldehyde and embedded in paraffin. Gradient alcohol dewaxing and dehydration were used. After high temperature retrieval, 5% BSA was sealed at room temperature for 30 min. HMGB1 Rabbit anti-Human Monoclonal antibody was added, overnight at 4°C. Goat anti-rabbit horseradish peroxidase labeled second antibody was added after rewarming and washing the next day. Finally, the DAB stain was applied, tissue was slightly stained with haematoxylin and followed by neutral balata covering.

Primary cell culture: The primary culture of degenerated NPCs was carried out by the enzymatic digestion method. After thoroughly washing the intervertebral disc tissues with PBS, they were cut by ophthalmic scissors and digested with 2.5 g/L trypsin at 37°C for 30 min. After centrifugation and discarding the supernatant, digested with 2 g/L collagenase II at 37°C for 3-5 h, till the tissues features in floc. Then, it was filtered with 200 mesh cell sieve and centrifuged to give a cell precipitation. DMEM medium containing 10% fetal bovine serum was added to the cell precipitate and cultured in a cell culture box containing 5% CO_2 at 37°C. Primary and secondary generation cells were identified by H&E, toluidine blue, and collagen II staining.

Quantitative real-time PCR: Total RNA was extracted by Trizol method and reverse transcribed to cDNA. The desired gene and GAPDH were amplified by real-time fluorescent quantitative PCR. Then relative expression quantity of objective gene mRNA was analyzed. The expression value of the target gene mRNA was calculated by $2^{-\Delta\Delta Ct}$ method. Reaction conditions: Pre-denaturation at 95°C for 3 min, denaturation at 95°C for 20 s, annealing at 60°C for 30 s, extension at 72°C for 20 s, 35 cycles. The Forward (F) and reverse (R) primers sequence of HMGB1, Collagen II, Agrecan, MMP-3 and MMP-9 for qRT-PCR were designed and synthesized and purchased from Shanghai GEFAN Biochemistry Co. Ltd (China).

Cell transfection: Cell transfections were performed by Lipofectamine 3000 reagent (Invitrogen Co. Ltd, USA) according to the manufacturer's instruction. The sequence of siHMGB1 and a control siRNA (siNC), HMGB1 overexpression vector and empty vector pcDNA3.1 vector (pcDNA3.1) were synthesized and further transfected by Shanghai GEFAN Biochemistry Co. Ltd (China). After 48 h transfection, GFP expression of each groups were observed through fluorescence microscopy. The transfection efficiency was assessed with qRT-PCR assay.

Western blotting: After washing homogenized tissue or cells with PBS, RIPA lysate containing PMSF was added, and total protein was collected after reaction on ice for 30 min. Cell concentration was detected by BCA assay. 50 μg sample was subjected to SDS-PAGE gel electrophoresis and transferred to PVDF membrane. After blocking with 5% BSA at room temperature for 1 h, and adding Rabbit polyclonal to HMGB1 (1:1000 dilution, catalogue number: ab18256, Abcam Co. Ltd, USA), Rabbit polyclonal to Collagen II (1:1000 dilution, catalogue number: ab34712, Abcam Co. Ltd, USA), Rabbit polyclonal to Aggrecan (1:1000 dilution, catalogue number: ab6276, Abcam Co. Ltd, USA), Rabbit polyclonal to MMP-3 (1:500 dilution, catalogue number: ab53015, Abcam Co. Ltd, USA), Rabbit polyclonal to MMP-9 (1:500 dilution, catalogue number: ab73734, Abcam Co. Ltd, USA), Rabbit polyclonal to Beclin 1 (1:500 dilution, catalogue number: ab62557, Abcam Co. Ltd, USA), Rabbit polyclonal to LC3 II/I (1:500 dilution, catalogue number: ab515204, Abcam Co. Ltd, USA) separately, at 4°C for the night. The corresponding second antibody was added on the 2nd day and incubated at 37 C for 1 h. Blot bands were visualized by ECL systems. Relative protein expression was analyzed by using chemiDoc XRS+Imaging System.

MTT assay: Transfected cells in logarithmic growth stage were collected and seeded as 1×10^5 cells into a 96-well plate. After culturing for 0, 12, 24, 48, 72 h, each group of cells was stained with 20 μl methyl thiazolyl tetrazolium (MTT) reagent for 4 h. After washed twice with PBS liquid, the crystals were dissolved by 100 μl dimethyl sulfoxide (DMSO). The absorbance value (OB) was measured and calculated at 490 nm by enzyme label.

Annexin V/PI double staining: Cell apoptotic rate were detected by Annexin V/PI double staining kit. Transfected cells in logarithmic growth stage were collected and resuspended 1×10^5 cells in binding buffer at 20% cytocrit. Then, 5 μl Annexin PE and PI dyes were added respectively and incubated at room temperature for 15 min avoid light. The apoptotic rate was estimated by flow cytometry.

Immunofluorescence: Transfected cells in logarithmic growth stage were digested and cell climbing sheets were collected after 24 h cultured, then, the cells were fixed with 4% PFA. Membrane was blocked with 10 g/L Triton-X100 for 30 minutes at 37°C. Inactivation of endogenous peroxidase was conducted by 3% hydrogen peroxide and blocking with 5% BSA. Incubation was overnight at 4°C with antibody. Fluorescent antibodies were added the next day after washing twice with PBS liquid. After incubated for 1 h at 37°C avoid light, Finally,

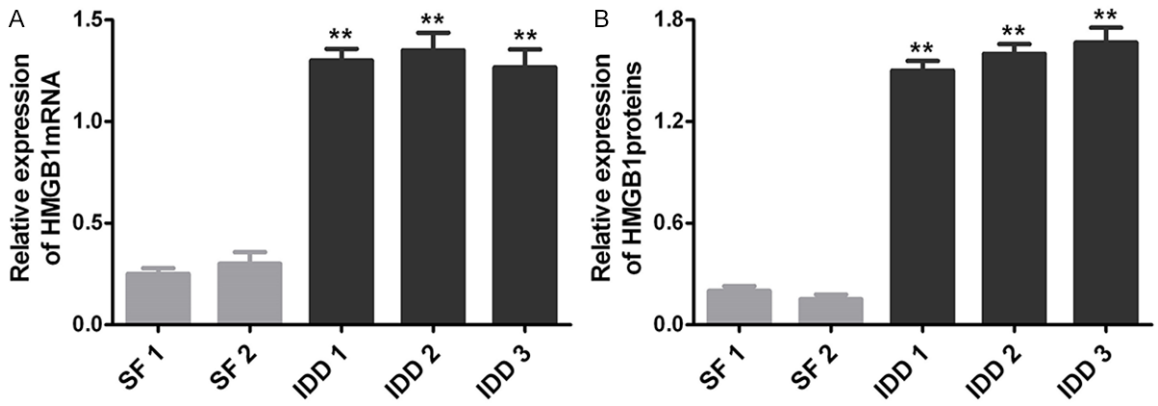

Figure 1. HMGB1 was markedly overexpressed in IDD. (A) qRT-PCR and (B) western blot results showed that HMGB1 was markedly overexpressed in 4 cases of IDD tissues, **P*<0.05, ***P*<0.01 versus 2 cases of spinal fracture tissues (n=2). IDD: Intervertebral disc degeneration. SF: spinal fracture.

DAB stain, haematoxylin slightly stain, and neutral balata coverslipping. The fluorescence intensity was observed and photographed under immunofluorescence laser confocal microscopy.

Statistical analysis

SPSS17.0 statistics software (version 7.0) and GraphPad Prism (version 5.01) were used in our study. Paired *t*-test and independent *t*-test were performed among two groups comparison, whereas comparisons among groups were analyzed by one-way analysis of variance followed by Tukey's multiple comparison test. The *p* values were designated as $^{*}P<0.05$, $^{**}P<0.01$, $\alpha=0.05$.

Results

HMGB1 was markedly overexpressed in IDD

In this study, intervertebral disc tissue of 3 cases of IDD patients and 2 cases of spinal fracture patients were collected. qRT-PCR and western blot results exhibited that HMGB1 mRNA (**Figure 1A**, *P*<0.05) and HMGB1 proteins (**Figure 1B**, *P*<0.05) were markedly overexpressed in 4 cases of IDD tissues (n=4), compared with 2 cases of spinal fracture tissues (n=2).

Cell transfection

HMGB1 OE vector, pcDNA3.1 and si-HMGB1, si-NC were transfected into NPCs. 48 h after transfection, Bright green fluorescence was visualized in each group of A431 and HSC-2 cells of each groups under fluorescence microscope (**Figure 2A**). qRT-PCR results showed that HMGB1 mRNA were dramatically enhanced in HMGB1 OE group and dramatically reduced in si-HMGB1 group, compared with corresponding negative control (NC) groups (**Figure 2B**, both *P*<0.05).

HMGB1 can inhibit NPCs proliferation and promote NPCs apoptosis

MTT and Annexin V/PI assay were used to estimate the effect of HMGB1 on NPCs proliferation and apoptosis. The MTT assay results showed that the proliferation activity were significantly decreased in the HMGB1 OE group and significantly increased in si-HMGB1 group, compared to with corresponding negative control groups (**Figure 3**, both *P*<0.05). The Annexin V/PI assay results demonstrated that the apoptotic rate was dramatically increased in HMGB1 OE group and markedly increased in si-HMGB1 group, compared with corresponding NC groups (**Figure 4**, both *P*<0.05). It was demonstrated that HMGB1 can inhibit NPC proliferation and promote NPC apoptosis.

HMGB1 promotes extracellular matrix degradation

qRT-PCR assay was used to assess the effect of HMGB1 on Collagen II and Agrecan expression. qRT-PCR results showed that Collagen II and Agrecan mRNA were significantly reduced in the HMGB1 OE group and enhanced in si-

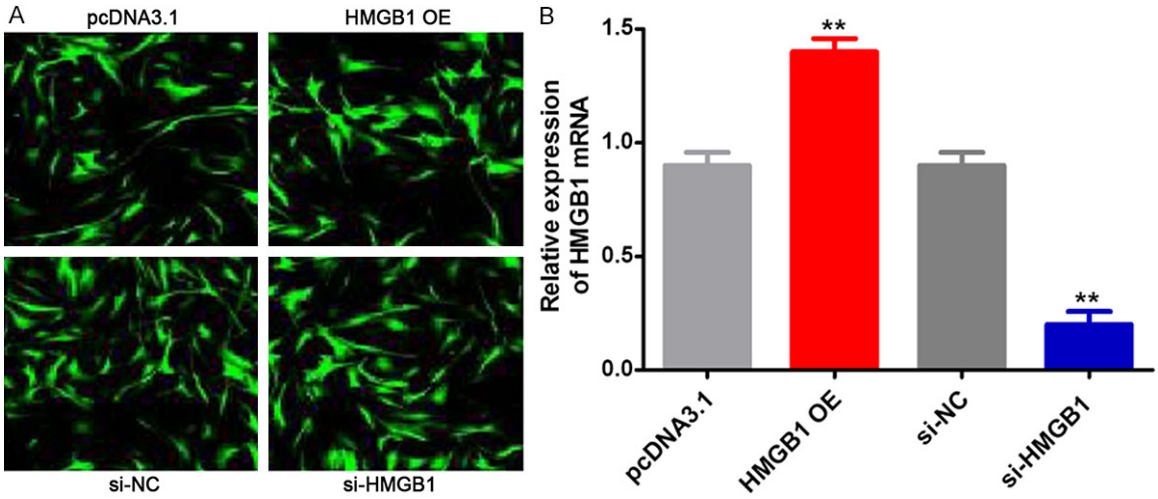

Figure 2. HMGB1 OE vector, pcDNA3.1 and si-HMGB1, si-NC were successfully transfected into NPCs. A. Bright green fluorescence were observed under fluorescence microscope. B. qRT-PCR results showed that HMGB1 mRNA were dramatically enhanced in HMGB1 OE group and dramatically reduced in si-HMGB1 group. $^{*}P<0.05$, $^{**}P<0.01$ versus corresponding NC group.

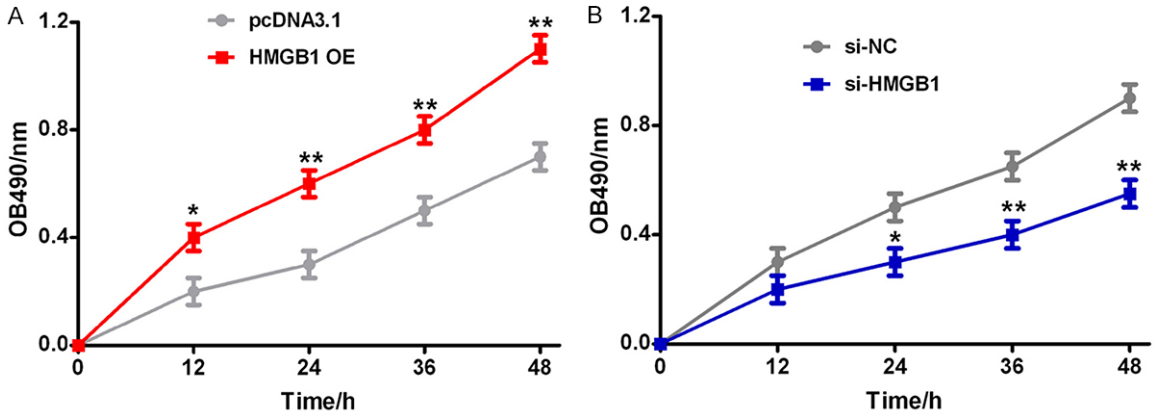

Figure 3. HMGB1 can inhibit NPC proliferation. MTT assay results showed that the proliferation activity were significantly decreased in HMGB1 OE group (A) and significantly increased in si-HMGB1 group (B). $^{*}P<0.05$, $^{**}P<0.01$ versus corresponding NC group.

HMGB1 group, compared with corresponding NC groups (**Figure 5**, both $P<0.05$). Also, qRT-PCR and western blot were used to assess the effect of HMGB1 on MMP-3 and MMP-9 expression. On the contrary, qRT-PCR and western blot results demonstrated that MMP-3 and MMP-9 proteins (**Figure 6A**, $P<0.05$) and MMP-3 and MMP-9 mRNA (**Figure 6B**, $P<0.05$) were dramatically increased in HMGB1 OE group and decreased in si-HMGB1 group, compared with with corresponding NC groups.

HMGB1 can activate cell autophagy

In our study, western-blot assay were used to detect the expression of Beclin 1 and LC3 II/I proteins. Western-blot results demonstrated that the relative expression of Beclin 1 protein and the ratio of LC3 II/I were significantly increased in the HMGB1 OE group and decreased in si-HMGB1 group, compare with corresponding NC groups (**Figure 7**, $P<0.05$).

Discussion

HMGB1 is a highly conserved nuclear DNA-binding protein and regarded as a central mediator of inflammation [13]. HMGB1 has two main parts: a box (aa1-79) and B box (aa89-163), and a C terminal tail (aa186-215). B box is the part of cytokine production induced by HMGB1,

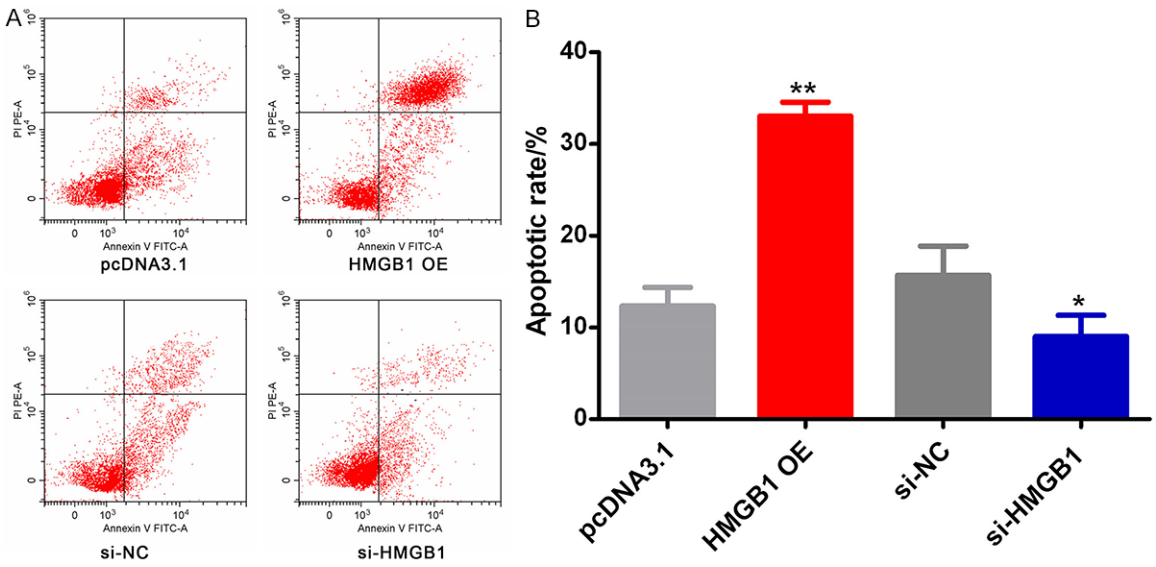

Figure 4. HMGB1 can promote NPC apoptosis. Annexin V/PI assay results demonstrated that the apoptotic rate was dramatically increased in the HMGB1 OE group and markedly increased in the si-HMGB1 group. A. Annexin V-FITC/PI image (×200); B. Apoptotic rate. *P<0.05, **P<0.01 versus corresponding NC group.

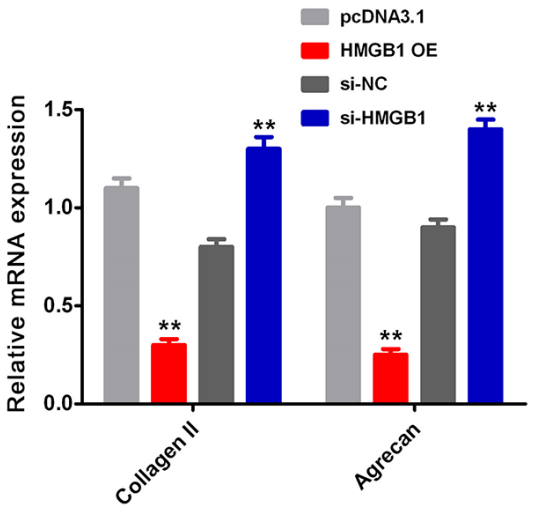

Figure 5. HMGB1 can inhibit Collagen II and Agrecan expression. Relative expression of Collagen II and Agrecan mRNA. **P<0.01 versus corresponding NC group.

and its first 20 amino acid residues are its active regions. Recombinant B box protein can stimulate the secretion of inflammatory cytokines such as TNF-α by monocytes/macrophages and neutrophils, while A box protein can antagonize the inflammation of B box protein [14]. HMGB1 can stimulate monocytes to secrete IL-1α, IL-8, MIP-1α, IL-1β, IL-6, MIP-1β and also can stimulate neutrophils to secrete IL-1β, TNF-α and IL-8 [15]. Recent studies [16, 17] have shown that HMGB1 is closely related to the occurrence and development of many inflammatory diseases, such as septicemia, autoimmune diseases, osteoarthritis, and ischemia-reperfusion.

Current studies suggest that the pathogenesis of IDD is largely due to internal disc nucleus pulposus cells (NPCs) loss, extracellular matrix components of change, and the inflammatory factors infiltration [18]. IDD is regarded as an inflammation-related disease. Also, the severity of IDD had a positive correlation with the inflammation factor level [18]. However, the role of HMGB1 in the pathogenesis of IDD has not been reported and still remains unknown. In our study, we found that HMGB1 was markedly overexpressed in IDD; upregulated expression of HMGB1 could inhibit NPC proliferation and promote NPC a apoptosis. It was indicated that HMGB1 important role in the pathogenesis of IDD.

A normal intervertebral disc consists of a nucleus pulposus tissue rich in extracellular matrix. Extracellular matrix has strong hydration ability and can play a role in carrying and buffering normal spinal physiological stress. Therefore, the degradation of extracellular matrix will lead to the disc being unable to withstand physiologic loads and the spine losing its stability [19].

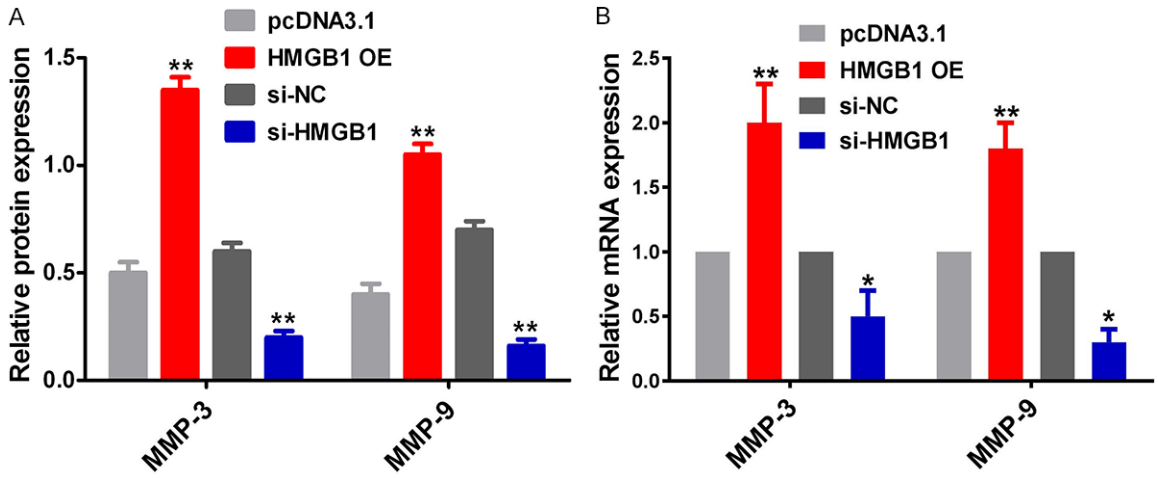

Figure 6. HMGB1 can promote MMP-3 and MMP-9 expression. A. Relative expression of MMP-3 and MMP-9 proteins; B. Relative expression of MMP-3 and MMP-9 mRNA. $^{*}P<0.05$, $^{**}P<0.01$ versus corresponding NC group.

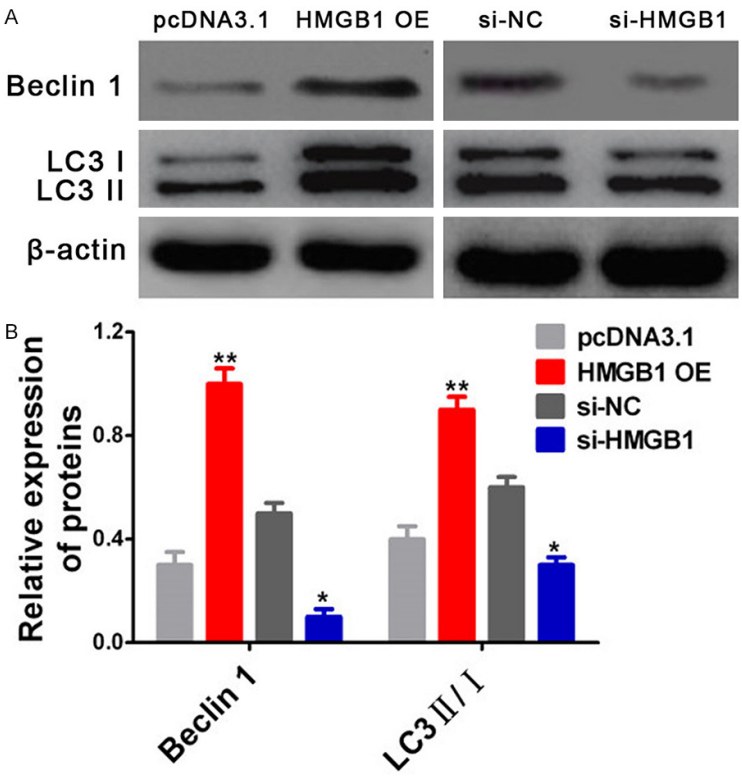

Figure 7. HMGB1 can activate cell autophagy. A. Wester bloting band; B. Relative expression of Beclin 1 protein and the ratio of LC3 II/I. $^{*}P<0.05$, $^{**}P<0.01$ versus corresponding NC group.

The main components of extracellular matrix are Collagen II and Aggrecan. The Collagen II and Aggrecan content directly affect the homeostasis of extracellular matrix. Also, extracellular matrix is regulated By Matrix metalloproteinases (MMPs). MMPs can target and degrade extracellular matrix proteins [20]. Very limited data are available found about the effect of HMGB1 on extracellular matrix. Ojo *et al*. [21] reported that HMGB1 can promotes extracellular matrix synthesis and wound repair in human bronchial epithelial cells. In our study, upregulated expression of HMGB1 could cause the reduction of Collagen II and Aggrecan content, and the increase in MMP-3 and MMP-9 expression. It was suggested that HMGB1 can promote extracellular matrix degradation in IDD by the regulation of Collagen II, Aggrecan MMP-3, and MMP-9.

Autophagy is a lysosome-dependent degradation pathway in eukaryotic cells which plays important parts in the development, differentiation, reconstitution of organism and various cell physiologic and pathologic processes [22]. Studies [23, 24] have found that autophagy also participates in the pathogenesis of IDD. Also, studies [25] have shown that HMGB1 is a novel Beclin 1-binding protein and shared considerable sequence homology with Beclin 1, so HMGB1 plays a important role in sustaining autophagy and HMGB1 can regulate autophagy under conditions of oxidative stress.

Tang *et al*. [26] reported that HMGB1 release and redox can regulate cell autophagy in cancer cells. In cancer cells, anti-cancer agents enhanced autophagy as well as HMGB1 release. HMGB1 release may be a pro-survival signal for residual cells following various cytotoxic cancer treatments. Liu *et al.* [27] found that HMGB1-induced autophagy promotes chemotherapy resistance in leukemia cells. Autophagy-mediated HMGB1 release antagonizes apoptosis of gastric cancer cells induced by vincristine by transcriptional regulation of Mcl-1. Liu *et al.* [28] found that HMGB1-DNA complex-induced autophagy limits AIM2 inflammasome activation through RAGE. In our study, upregulated expression of HMGB1 could activates cell autophagy. After extensive literature review, this study appears to be the first about HMGB1- induced autophagy in the pathogenesis of IDD.

In summary, we first determined that HMGB1 was up-regulated in IDD. Upregulated expression of HMGB1 can inhibit NPCs proliferation and promote NPC apoptosis, promote extracellular matrix degradation, and activate cell autophagy. This study provides the first evidence that HMGB1-induced autophagy can promote extracellular matrix degradation and thus lead to intervertebral disc degeneration. In brief, HMGB1 may serve as a novel diagnostic biomarker and therapeutic target for IDD.

Acknowledgements

The project received support from Zhejiang Quhua Hospital. We also highly appreciate the kindly help and support from Shanghai GEFAN Bio-chemistry Co. Ltd (China).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Si-Min Peng, Department of Neurosurgery, The People Hospital of Xin Chang, No. 117 Gushan Middle Road, Xin Chang 312500, Zhejiang, China. E-mail: fubiaomail@yeah.net

References

[1] Huang X, Wang W, Meng Q, Yu L, Fan C, Yu J, Xue M and Ye X. Effect of needle diameter, type and volume of contrast agent on intervertebral disc degeneration in rats with discography. Eur Spine J 2019; 12: 1-9.

[2] Li Y, Cao L, Li J, Sun Z, Liu C, Liang H, Wang D and Tian J. Influence of microgravity-induced intervertebral disc degeneration of rats on expression levels of p53/p16 and proinflammatory factors. Exp Ther Med 2019; 17: 1367-1373.

[3] Li L, Zhou Z, Li J, Fang J, Qing Y, Tian T, Zhang S, Wu G, Scotti A, Cai K and Zhu W. Diffusion kurtosis imaging provides quantitative assessment of the microstructure changes of disc degeneration: an in vivo experimental study. Eur Spine J 2019; 28: 1005-1013.

[4] Jin H, Wang Q, Wu J, Han X, Qian T, Zhang Z, Wang J, Pan X, Wu A and Wang X. Baicalein inhibits the IL-1β-induced inflammatory response in nucleus pulposus cells and attenuates disc degeneration in vivo. Inflammation 2019; 42: 1032-1044.

[5] Gao C, Ning B, Sang C and Zhang Y. Rapamycin prevents the intervertebral disc degeneration via inhibiting differentiation and senescence of annulus fibrosus cells. Aging 2018; 10: 131-143.

[6] Paudel YN, Semple BD, Jones NC, Othman I and Shaikh MF. High mobility group box 1 (HMGB1) as a novel frontier in epileptogenesis: from pathogenesis to therapeutic approaches. J Neurochem 2019; 10: 510-519.

[7] Lee W, Cho SH, Kim JE, Lee C, Lee JH, Baek MC, Song GY and Bae JS. Suppressive effects of ginsenoside Rh1 on HMGB1-mediated septic responses. Am J Chin Med 2019; 5: 1-15.

[8] Kang R, Chen R, Zhang Q, Hou W, Wu S, Cao L, Huang J, Yu Y, Fan XG, Yan Z, Sun X, Wang H, Wang Q, Tsung A, Billiar TR, Zeh HJ 3rd, Lotze MT and Tang D. HMGB1 in health and disease. Mol Aspects Med 2014; 12: 1-116.

[9] Sims GP, Rowe DC, Rietdijk ST, Herbst R and Coyle AJ. HMGB1 and RAGE in inflammation and cancer. Annu Rev Immunol 2009; 28: 367-388.

[10] Bauzá MDR, Giménez CS, Locatelli P, De Lorenzi A, Hnatiuk A, Capogrossi MC, Crottogini A, Cuniberti L and Olea FD. High-dose intramyocardial HMGB1 induces long-term cardioprotection in sheep with myocardial infarction. Drug Deliv Transl Res 2019; 6: 1-10.

[11] Jin J, Gong J, Zhao L, Zhang H, He Q and Jiang X. Inhibition of HMGB1 attenuates podocyte apoptosis and epithelial-to-mesenchymal transition (EMT) by regulating autophagy flux. J Diabetes 2019; 12: 95-102.

[12] Urrutia J, Besa P, Campos M, Cikutovic P, Cabezon M, Molina M and Cruz JP. The Pfirrmann classification of lumbar intervertebral disc degeneration: an independent inter- and intra-observer agreement assessment. Eur Spine J 2016; 25: 2728-2733.

[13] Xue J, Ge H, Lin Z, Wang H, Lin W, Liu Y, Wu G, Xia J and Zhao Q. The role of dendritic cells

regulated by HMGB1/TLR4 signalling pathway in myocardial ischaemia reperfusion injury. J Cell Mol Med 2019; 23: 22-29.
[14] Paudel YN, Semple BD, Jones NC, Othman I and Shaikh MF. High mobility group box 1 (HMGB1) as a novel frontier in epileptogenesis: from pathogenesis to therapeutic approaches. J Neurochem 2019; 13: 145-151.
[15] Liu RZ, Li T and Zhao GQ. Cytosolic HMGB1 mediates autophagy activation in an emulsified isoflurane anesthesia cell model. Neurochem Res 2019; 5: 14-19.
[16] Yang H, Liu H, Zeng Q, Imperato GH, Addorisio ME, Li J, He M, Cheng KF, Al-Abed Y, Harris HE, Chavan SS, Andersson U and Tracey KJ. Inhibition of HMGB1/RAGE-mediated endocytosis by HMGB1 antagonist box A, anti-HMGB1 antibodies, and cholinergic agonists suppresses inflammation. Mol Med 2019; 25: 185-192.
[17] Tao X, Sun M, Chen M, Ying R, Su W, Zhang J, Xie X, Wei W and Meng X. HMGB1-modified mesenchymal stem cells attenuate radiation-induced vascular injury possibly via their high motility and facilitation of endothelial differentiation. Stem Cell Res Ther 2019; 10: 92-98.
[18] Feng C, Yang M, Lan M, Liu C, Zhang Y, Huang B, Liu H and Zhou Y. ROS: crucial intermediators in the pathogenesis of intervertebral disc degeneration. Oxid Med Cell Longev 2017; 15: 1-12.
[19] Wang AM, Cao P, Yee A, Chan D and Wu EX. Detection of extracellular matrix degradation in intervertebral disc degeneration by diffusion magnetic resonance spectroscopy. Magn Reson Med 2015; 73: 1703-1712.
[20] Zigouris A, Alexiou GA, Batistatou A, Voulgaris S and Kyritsis AP. The role of matrix metalloproteinase 9 in intervertebral disc degeneration. J Clin Neurosci 2011; 18: 1424-1425.
[21] Ojo OO, Ryu MH, Jha A, Unruh H and Halayko AJ. High-mobility group box 1 promotes extracellular matrix synthesis and wound repair in human bronchial epithelial cells. Am J Physiol Lung Cell Mol Physiol 2015; 309: L1354.
[22] Takahama M, Akira S and Saitoh T. Autophagy limits activation of the inflammasomes. Immunological Reviews 2018; 281: 62-68.
[23] Zhang SJ, Yang W, Wang C, He WS, Deng HY, Yan YG, Zhang J, Xiang YX and Wang WJ. Autophagy: a double-edged sword in intervertebral disc degeneration. Clinica Chimica Acta 2016; 457: 27-35.
[24] Jiang L, Zhang X, Zheng X, Ru A, Ni X, Wu Y, Tian N, Huang Y, Xue E, Wang X and Xu H. Apoptosis, senescence, and autophagy in rat nucleus pulposus cells: implications for diabetic intervertebral disc degeneration. J Orthop Res 2013; 31: 692-702.
[25] Tang D, Kang R, Livesey KM, Cheh CW, Farkas A, Loughran P, Hoppe G, Bianchi ME, Tracey KJ, Zeh HJ 3rd and Lotze MT. Endogenous HMGB1 regulates autophagy. J Cell Biol 2010; 190: 881-892.
[26] Tang D, Kang R, Cheh CW, Livesey KM, Liang X, Schapiro NE, Benschop R, Sparvero LJ, Amoscato AA, Tracey KJ, Zeh HJ and Lotze MT. HMGB1 release and redox regulates autophagy and apoptosis in cancer cells. Oncogene 2010; 29: 5299-310.
[27] Liu L, Yang M, Kang R, Wang Z, Zhao Y, Yu Y, Xie M, Yin X, Livesey KM, Lotze MT, Tang D and Cao L. HMGB1-induced autophagy promotes chemotherapy resistance in leukemia cells. Autophagy 2011; 25: 23-31.
[28] Liu L, Yang M, Kang R, Dai Y, Yu Y, Gao F, Wang H, Sun X, Li X, Li J, Wang H, Cao L and Tang D. HMGB1-DNA complex-induced autophagy limits AIM2 inflammasome activation through RAGE. Biochem Biophys Res Commun 2014; 450: 851-856.