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Hyaluronic acid inhibited the upregulation of heat shock protein 70 in human chondrocytes from osteoarthritis and Kashin-Beck disease

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Abstract: This study aimed to investigate the effect of hyaluronic acid (HA) on the expression of heat shock protein 70 (HSP70) in chondrocytes isolated from patients with osteoarthritis (OA) and Kashin-Beck disease (KBD). The chondrocytes were collected from OA and KBD patients, and chondrocytes isolated from patients of accident injuries were used as the control. The chondrocytes were treated with HA at different doses. HSP70 expression in chondrocytes at both mRNA and protein levels was tested by PCR and Western blot analysis. Compared with control, both mRNA and protein levels of HSP70 were higher in chondrocytes from KBD and OA. However, HA at the dose of 500 μg/mL significantly inhibited HSP70 expression levels in both KBD and OA groups ($P < 0.05$). In conclusion, HSP70 is highly expressed in chondrocytes of patients of OA and KBD. HA intervention inhibits the upregulation of HSP70 in chondrocytes of OA and KBD patients and could be a promising agent for treatment of OA and KBD.

Introduction

Kashin-Beck disease (KBD) is a chronic endemic joint disease that seriously affects people's health. However, the pathogenesis of KBD remains to be elucidated. Although the etiology and pathogenesis are different between KBD and osteoarthritis (OA), the clinical manifestations are similar in many aspects between the end-stage KBD and OA. The commonly utilized therapies for OA include articular cavity injection of hyaluronic acid (HA) (Xu, 2004; Yu *et al.*, 2014). Therefore, we hypothesized that HA may be also beneficial for KBD.

Heat shock proteins are a group of proteins produced under heat stimulation to help cells cope with various external physical and chemical stimuli (Munoz, 2018). Heat shock protein 70 (HSP70) is a key member of the heat shock protein family that participates in the pathogenesis of OA, and HSP70 expression was positively associated with the severity of joints degeneration (Takahashi *et al.*, 1997; Grossin *et al.*, 2004; Ngarmukos *et al.*, 2018). HSP70 expression increased in OA patients which could inhibit the apoptosis of chondrocytes (Terauchi *et al.*, 2003).

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However, the role of HSP70 in KBD patients has not been investigated. This study aimed to investigate the expression of HSP70 in chondrocytes isolated from KBD patients and evaluate the therapeutic effect of HA on HSP expression.

Materials and Methods

Patients

The patients were diagnosed according to the KBD diagnostic criteria (GB16003-1995) and the OA diagnostic criteria defined by American Rheumatism Association (version 1995), and all collected cartilage tissues were classified into three groups: normal control (NC) group, KBD group, and OA group. The six patients of KBD (three men and three women between ages 35 and 47 years) were from the Institute of Endemic diseases, Shaanxi, China, and underwent the extirpation of the loose bodies of the knee joint. The chondrocytes were isolated from loose body cartilage. Another six patients of OA (three men and three women between ages 57 and 73 years) were from Xi'an Red Cross Hospital who underwent total knee arthroplasty. The normal cartilages were from six people (four men and two women between ages 27 and 39) who encountered an accident or died of an accident, and the cells were isolated from fresh joint cartilages. Those who had genetic OA and rheumatoid arthritis were excluded from the study. All participants gave written informed consent according to the Declaration of

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Helsinki. Ethics approval for the study was obtained from the Xi'an Jiaotong University Ethics Committee.

Chondrocytes isolation and culture

The joint cartilage tissues were transferred under sterile condition into a DEME/F12 serum-free medium, and cut into granules of 1 × 1 mm^2 dimension on a super-clean bench, and 10 mL of 0.2% trypsin was added to these granules for digestion at 30°C for 1 h, followed by digestion with hyaluronidase and type II collagenase for 1 and 4 hours, respectively. The separated cells were then collected by centrifugation, and filtered through a 200-mesh sieve and re-suspended with the DMEM/F12 medium containing 10% calf serum. The primary cells were then cultured at the seeding density of 4 × 10^5 cells/bottle with 4 mL of the DMEM/F12 medium containing 10% calf serum (supplemented with 100 U/mL penicillin and streptomycin), and incubated with 5% CO_2 at 37°C. The growth of cells was monitored by an inverted microscope, and the culture medium was refreshed every 48 hours. The single-layer cells could be obtained in 3-4 weeks.

When the 100% confluence was obtained, the cells were washed once by phosphate-buffered saline (PBS), and digested with 2 mL 0.25% trypsin containing 0.02% EDTA. The digestion was terminated once the cells started to shrink into a round shape. The suspended cells were obtained after repeated pipetting and seeded at a density of 4 × 10^5 cells/bottle for continuous culture with 5% CO_2 at 37°C. The culture medium was refreshed every other day, and used for subsequent experiments once the 100% confluence was obtained. HA sodium was from Qisheng Biotech (Shanghai, China), and cells were treated with different dosages of HA (0, 100 and 500 μg/mL).

Reverse transcription-polymerase chain reaction

Total RNA was extracted from cells using FAST200RNA kit (Fasten Biotech, Shanghai, China), and used for cDNA synthesis using reverse transcription kit (MBI, USA). The primers for HSP70 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were synthesized by AuGCT Biotech (Beijing, China). PCR condition was as follows: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 54°C for 1 min, and extension at 70°C for 1 min.

Western blot analysis

Total proteins were extracted from cells by using Enhanced RIPA Lysis Buffer supplemented with protease inhibitor cocktail. Equal amounts of proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to the polyvinylidene fluoride (PVDF) membranes. The membranes were probed with primary and secondary antibodies, and developed with ECL kit. The densitometry was perforemd using the automated gel imaging analysis system.

Statistical analysis

The data were presented as the mean ± standard deviation, and the SPSS17 software was used for statistical analysis. All the data passed the normality test and variance homogeneity test. The analysis of variance was used for comparison among the three groups, and two-two comparison (least significant test) was conducted once there was any statistically significant difference. $P < 0.05$ was considered as significant.

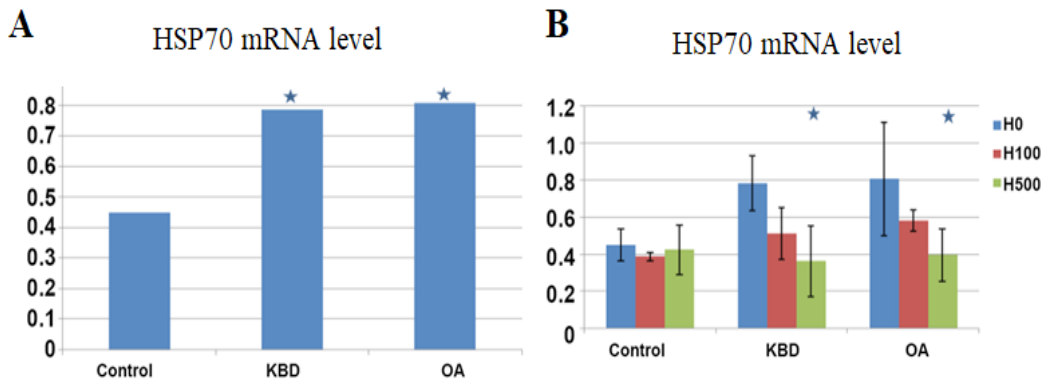

FIGURE 1. Real-time PCR analysis of HSP70 mRNA level in chondrocytes. A. The chondrocytes were isolated from control, KBD and OA patients and HSP70 mRNA levels were detected by real-time PCR. Data were presented as mean ± standard deviation (n = 3). * $P < 0.05$ *vs.* Control. B. The chondrocytes were isolated from control, KBD and OA patients and treated with HA at dose of 0, 100 and 500 μg/mL (H0, H100 and H500, respectively). HSP70 mRNA levels were detected by real-time PCR. Data were presented as mean ± standard deviation (n = 3). * $P < 0.05$ *vs.* H0 group.

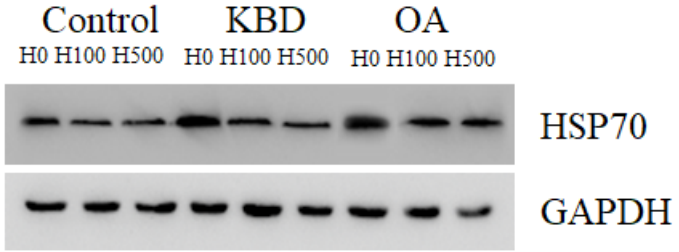

FIGURE 2. Western blot analysis of HSP70 protein level in chondrocytes. The chondrocytes were isolated from control, KBD and OA patients and treated with HA at dose of 0, 100 and 500 μg/mL (H0, H100 and H500, respectively). HSP70 protein levels were detected by Western blot analysis. GAPDH was loading control.

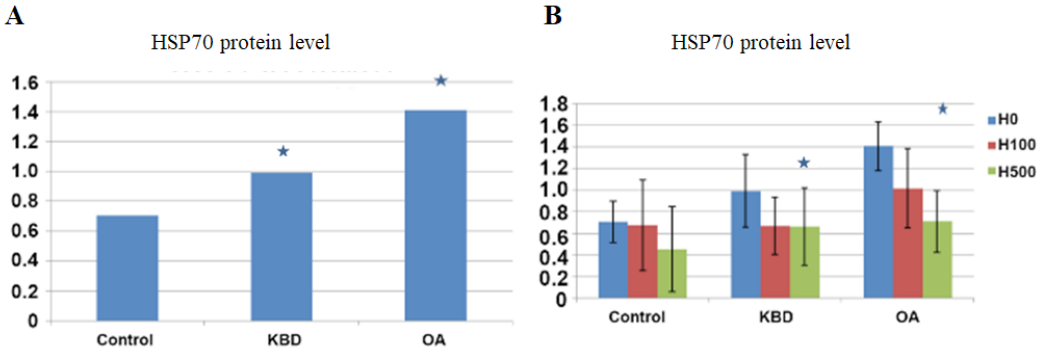

FIGURE 3. Densitometry analysis of HSP70 protein level in chondrocytes. A. The chondrocytes were isolated from control, KBD and OA patients and HSP70 protein levels were detected by Western blot analysis. Data were presented as mean ± standard deviation (n = 3). *$P < 0.05$ *vs.* Control. B. The chondrocytes were isolated from control, KBD and OA patients and treated with HA at dose of 0, 100 and 500 μg/mL (H0, H100 and H500, respectively). HSP70 mRNA levels were detected by real-time PCR. Data were presented as mean ± standard deviation (n = 3). *$P < 0.05$ *vs.* H0 group.

Results

As shown in Fig. 1, PCR analysis showed that HSP70 mRNA level was significantly higher in KBD and OA compared to control ($P < 0.05$). In addition, HA significantly reduced HSP70 mRNA level in KBD and OA group at the dose of 500 μg/mL ($P < 0.05$).

Furthermore, Western blot analysis showed that HSP70 protein level was higher in KBD and OA compared to control, and HA reduced HSP70 protein level in KBD and OA group at increasing dose (Fig. 2). Densitometry analysis showed that HSP70 protein level was significantly higher in KBD and OA compared to control ($P < 0.05$), and HA significantly reduced HSP70 protein level in KBD and OA group at the dose of 500 μg/mL ($P < 0.05$, Fig. 3).

Discussion

Heat shock proteins are a group of highly conserved proteins produced under unfavorable conditions, which have the functions of molecular chaperones and participate in the embryonic development, cell apoptosis, immune response, and cell cycle regulation. It has been reported that HSPs were highly expressed in chondrocytes under the physiological condition (Leonardi *et al.*, 2004; Ruiz-Romero *et al.*, 2005). Moreover, the expression of HSPs increased in chondrocytes subjected to external stress (Kaarniranta *et al.*, 1998). HSP 70 was considered to protect chondrocyte from apoptosis and necrosis induced by oxygen-free radicals. In the cartilage, high expression of HSP 70 induced at the early stage of OA was regarded as a protective response (Takahashi *et al.*, 1997). However, there is still no report on the expression and role of HSPs in KBD.

In this study, both PCR and Western blot analysis confirmed high expression of HSP70 in chondrocytes of KBD, similar to OA. The increased level of HSP70 may protect KBD chondrocyte from apoptosis induced by pathological causes, in accordance with the previous studies in OA (Takahashi *et al.*, 1997; Grossin *et al.*, 2004).

Currently, intra-articular injection of HA remains to be one important therapy for OA (Bhadra *et al.*, 2017; O'Hanlon *et al.*, 2016).

In addition, the *in vitro* study indicated that HA could promote the synthesis of type II collagen and aggrecan in chondrocytes (Gao *et al.*, 2009). HA also upregulated the expression of osteopontin in chondrocytes (Zhou *et al.*, 2014). Meanwhile, an intra-articular injection of HA at an early stage of joints injury was demonstrated to effectively inhibit the apoptosis of chondrocytes, which was of great value for preventing OA after injury (Song *et al.*, 2013). In addition, HA as a major component of the extracellular matrix was found to promote the differentiation of MSCs toward chondrocyte (Wang *et al.*, 2011). Therefore, HA might be beneficial to OA treatment.

In this study, we found that HA inhibited HSP70 expression in chondrocytes isolated from both KBD and OA patients. These results suggest that HA would show similar effects to treat KBD and OA. Both KBD and OA are diseases involving chondrocytes, extracellular matrix, and cartilage bones' degradation and synthesis imbalance. HSPs as stress proteins are closely associated with chondrocyte maturation, differentiation, and metabolism (Carminati *et al.*, 2018). The integrity of extracellular matrix in chondrocytes is crucially implicated in the pathogenesis of KBD and OA. However, the role of HSPs in the pathogenesis of KBD and OA need further studies by genome and transcriptome analysis (Ataei *et al.*, 2017; Liu and Zhang, 2017). A recent study showed that HA exhibited anti-inflammatory and anti-apoptotic activities in osteoarthritic mice by inhibiting oxidative stress (Chiou *et al.*, 2018). Oxidative stress is known to induce the upregulation of HSP70. Therefore, we speculated that HA may inhibit oxidative stress to downregulate HSP70 expression and provide protection in chondrocytes. In future studies, we need analyze reactive oxygen species (ROS) in cells exposed to HA to explain the connection of ROS reduction and HSP70 downregulation.

In conclusion, HSP70 is highly expressed in chondrocytes isolated from patients of OA and KBD. HA intervention inhibits the upregulation of HSP70 in chondrocytes of OA and KBD patients and could be a promising agent for treatment of OA and KBD.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

References

Ataei R, Gholamhoseini M, Kamalizadeh M (2017). Genetic analysis for quantitative traits in bread wheat exposed to irrigated and drought stress conditions. *Phyton, International Journal of Experimental Botany* **86**: 228-235.

Bhadra AK, Altman R, Dasa V, Myrick K, Rosen J, Vad V, Vitanzo P Jr, Bruno M, Kleiner H, Just C (2017). Appropriate use criteria for hyaluronic acid in the treatment of knee osteoarthritis in the United States. *Cartilage* **8**: 234-254.

Carminati SA, Barbosa MC, Fader CM (2018). Platelet rich plasma (PRP) induces autophagy in osteoblast precursor 3T3-L1. *Biocell* **42**(1):13-16

Chiou CS, Wu CM, Dubey NK, Lo WC, Tsai FC, Tung TDX, Hung WC, Hsu WC, Chen WH, Deng WP (2018). Mechanistic insight into hyaluronic acid and platelet-rich plasma-mediated anti-inflammatory and anti-apoptotic activities in osteoarthritic mice. *Aging (Albany NY)* **10**(12): 4152-4165.

Gao ZQ, Guo X, Chen JC, Duan C, Ma WJ, Liu RY, Gu QS (2009). Effect of hyaluronic acid on collagen II and aggrecan mRNA expression in Kashin-Beck disease chondrocytes cultured in vitro. *Journal of Clinical Rehabilitative Tissue Engineering Research* **13**: 9061-9065.

Grossin L, Etienne S, Gaborit N, Pinzano A, Cournil-Henrionnet C, Gerard C, Payan E, Netter P, Terlain B, Gillet P (2004). Induction of heat shock protein 70 (Hsp70) by proteasome inhibitor MG 132 protects articular chondrocytes from cellular death in vitro and in vivo. *Biorheology* **41**: 521-534.

Kaarniranta K, Elo M, Sironen R, Lammi MJ, Goldring MB, Eriksson JE, Sistonen L, Helminen HJ (1998). Hsp70 accumulation in chondrocyte cells exposed to high continuous hydrostatic pressure coincides with mRNA stabilization rather than transcriptional activation. *Proceedings of the National Academy of Sciences of the United States of America* **95**: 2319-2324.

Leonardi R, Barbato E, Paganelli C, Lo Muzio L (2004). Immunolocalization of heat shock protein 27 in developing jaw bones and tooth germs of human fetuses. *Calcified Tissue International* **75**: 509-516.

Liu M, Zhang X (2017). An integrated analysis of mRNA-miRNA transcriptome data revealed hub regulatory networks in three genitourinary cancers. *Biocell* **41**: 19-26.

Munoz EM (2018). Microglia-precursor cell interactions in health and in pathology. *Biocell* **42**: 41-45.

Ngarmukos S, Scaramuzza S, Theerawattanapong N, Tanavalee A, Honsawek S (2018). Circulating and synovial fluid heat shock protein 70 are correlated with severity in knee osteoarthritis. *Cartilage*.

O'Hanlon CE, Newberry SJ, Booth M, Grant S, Motala A, Maglione MA, FitzGerald JD, Shekelle PG (2016). Hyaluronic acid injection therapy for osteoarthritis of the knee: concordant efficacy and conflicting serious adverse events in two systematic reviews. *Systematic Reviews* **5**: 186.

Ruiz-Romero C, Lopez-Armada MJ, Blanco FJ (2005). Proteomic characterization of human normal articular chondrocytes: a novel tool for the study of osteoarthritis and other rheumatic diseases. *Proteomics* **5**: 3048-3059.

Song CZ, Zheng MQ, Zhou XY, Ji B, Yang SQ, Dong QR (2013). Experimental study on the effects of intra-articular injection of HA at early stage to provent chondrocyte apoptosis following cartilage injury. *Journal of Practical Orthopaedics* **19**: 41-43, 91.

Takahashi K, Kubo T, Arai Y, Imanishi J, Kawata M, Hirasawa Y (1997). Localization of heat shock protein in osteoarthritic cartilage. *Scandinavian Journal of Rheumatology* **26**: 368-375.

Takahashi K, Kubo T, Goomer RS, Amiel D, Kobayashi K, Imanishi J, Teshima R, Hirasawa Y (1997). Analysis of heat shock proteins and cytokines expressed during early stages of osteoarthritis in a mouse model. *Osteoarthritis and Cartilage* **5**: 321-329.

Terauchi R, Takahashi KA, Arai Y, Ikeda T, Ohashi S, Imanishi J, Mazda O, Kubo T (2003). Hsp70 prevents nitric oxide-induced apoptosis in articular chondrocytes. *Arthritis and Rheumatism* **48**: 1562-1568.

Wang CY, Yu L, Wang YZ, Kou JQ, Xia CS, Lv CY, Zhang HN, Xu H (2011). The effects of exogenous hyaluronic acid in different concentration on chondrogenic differentiation of rabbit bone marrow-derived mesenchymal stem cells. *Chinese Journal of Clinicians* **5**: 6213-6220.

Xu P, Guo X, Jin WZ, Yao JF, Wang JH, Zhang FQ, Zhang YG (2004). Curative effects of intra-articular injections of sodium hyaluronate to treat Kashin-Beck disease. *Chinese Journal of Endemiology* **23**: 588-590.

Yu FF, Xia CT, Fang H, Han J, Younus MI, Guo X (2014). Evaluation of the therapeutic effect of treatment with intra-articular hyaluronic acid in knees for Kashin-Beck disease: a meta-analysis. *Osteoarthritis Cartilage* **22**: 718-725.

Zhou B, Zhang FJ, Luo W, Gao SG, Zeng C, Xiong YL, Li YS, Lei GH (2014). Effects of hyaluronic acid on osteopontin mRNA and CD44 mRNA expression in human osteoarthritic chondrocytes. *Journal of Clinical Rehabilitative Tissue Engineering Research* **18**: 6172-6178.