

Original Article

HtrA1 up-regulates expression of MMPs via Erk1/2/Rock-dependent pathways

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Abstract: Background: There are few studies that have identified the potential role of a high temperature requirement A1 (HtrA1) in intervertebral disc degeneration (IDD). This study was undertaken to investigate the regulatory role of HtrA1 in the pathogenesis of IDD. Material and Methods: The mRNA levels of HtrA1 and matrix metalloproteinases (MMPs) of human intervertebral disc degeneration tissues were measured by real-time quantitative PCR, and a correlation between the expression level of HtrA1 and MMPs was also investigated. Human nucleus pulposus cells (HNPCs) were challenged with rHtrA1, and expression of MMPs was measured by real-time quantitative PCR, Western blotting, and ELISA. Moreover, to analyze the mechanism by which HtrA1 up-regulates MMPs, ERK1/2/ROCK signaling pathway inhibitors were also used. Results: We found significant increases in mRNA expression of HtrA1 and MMP1, 3, 9, and 13 in IDD tissues compared with control. HtrA1 expression level was associated with the levels of MMP1, 3, and 13. Expression of MMP1, 3, and 13 mRNA and protein were significantly increased in HNPCs treated by rHtrA1. Moreover, administration of the ERK1/2 signaling pathway inhibitor or ROCK signaling pathway inhibitor decreased rHtrA1-induced MMPs production. Therefore, changes in HtrA1 expression could be involved in the pathogenesis of IDD. Conclusion: Our findings indicate that HtrA1 can induce increases in MMPs in HNPCs via the ERK1/2/ROCK signaling pathway, thus providing new insights into the role of HtrA1 in the pathogenesis of IDD.

Keywords: Intervertebral disc, intervertebral disc degeneration, matrix metalloproteinases, extracellular matrix

Introduction

Low-back pain (LBP) is among the leading causes of the costliest musculoskeletal problems in adults worldwide, and causes severe social and economic burdens. Investigators have shown that intervertebral disc degeneration (IDD) is one of the most common disorders reported in LBP [1]. The issue is intensified by the increasing elderly adult population. Advances in research on pathogenesis of IDD have been made and possible etiological factors in the pathogenesis of IDD have been identified as aberrant, cell-mediated, and age- and genetic-dependent molecular degeneration processes [2]. An intervertebral disc (IVD) consists mainly of: (a) the highly hydrated nucleus pulposus (NP), composed mainly of proteoglycan, hyaluronan, and type II collagen; and (b)

the radially aligned type I collagen fibrils of the annulus fibrosus (AF) [3]. Partly because the IVD is the largest avascular tissue and has poor self-healing potential, tissue-regenerative therapy for IDD has not been achieved [4]. Previous studies have demonstrated that expression of matrix metalloproteinases (MMPs) has essential roles in human IDD. During the pathogenesis of IDD, degradation of the extracellular matrix (ECM) is initiated by proteolytic enzymes, including MMP1, 3, 7, 9, and 13 [5-10].

HtrA1 (High temperature requirement A1) belongs to the HtrA family of serine proteases. In humans, HtrA1 was originally isolated from fibroblasts as a transformation-sensitive protein due to its downregulation by SV40 [11]. There is increasing evidence that HtrA1 regulates several physiological and pathological

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Table 1. Primer list

Gene	Primer F	Primer R
HtrA1	GACTACATCCAGACCGACGC	GCTTTTCCTTTGGCCTGTCA
MMP1	TGACACCCAGCATGAACC	GTTGTCCCGATGATCTCCCC
MMP3	TGACACCCAGCATGAACC	ACTTCGGGATGACAGGAAAG
MMP7	GGAGCTCATGGGGACTCCTA	TCCAGCGTTCATCCTCATCG
MMP9	CATCCGGCACCTCTCTATGGTA	CATCGTCCACCGGACTCAA
MMP13	CATGAGTTCGGCCACTCCTT	CCTCGGAGACTGGTAATGGC

processes, including tumor development [12-15], Alzheimer's disease (AD) [16], placental [17], age-related macular degeneration [18], and osteoarthritis [19]. HtrA1 can act on different targets, including extracellular matrix proteins [20]. Our previous study [21] found the HtrA1 plays an important role in the pathological process of IDD. However, the mechanism(s) by which it regulates these processes that have not been fully elucidated in IDD.

Drawing on this background, in this study we hypothesized that the serine protease HtrA1 plays a critical role in IDD, involving modifications of the MMPs. Therefore, the present work was performed to determine whether HtrA1 can contribute to IDD development via degrading numerous extracellular matrix proteins. Our results clearly demonstrate a critical role for HtrA1 in IDD that is based on its ability to stimulate MMPs production, associated with extracellular signal-regulated kinase (ERK), and Rho-associated protein kinase (ROCK) signaling pathways.

Material and methods

Human intervertebral disc tissues and patient information

The study included 30 IDD patients and 20 healthy volunteers with spinal fractures as controls from the Affiliated Hospital of Jiangsu University. The median age of patients (11 male and 19 female) was 54.83 years (range, 52-67 years). The average age of controls (11 males and 9 females) was 22.3 years, ranging from 16 to 27 years. All the patients were untreated for their condition at the time of tissue collection and diagnosed based on commonly accepted clinical and laboratory criteria. The current study was approved by the Ethics Committee of the Affiliated Hospital of Jiangsu University (JDFYLL-2015016), and was conducted in ac-

cordance with provisions of the Declaration of Helsinki, and written informed consent was obtained from all individuals.

Cell culture and treatment

Human NP cells (HNPCs) were obtained from ScienCell (USA), and cultured in 6-well plates using nucleus pulposus cell medium (ScienCell, USA) with 10% fetal bovine serum supplemented with antibiotics to enable the cells grow to 80% confluence. After the cells reached confluence, 1 group of cells was challenged with 5 µg/ml/10 µg/ml exogenous recombinant HtrA1 (rHtrA1, Cloud-Clone Corp) with and without ERK signaling pathway inhibitor SCH772984 (10 µmol/l) or ROCK signaling pathway inhibitor Y27632 (10 µmol/l), and untreated groups were used as a control. The cells were harvested at 1 time point (after 24 h) from HtrA1 challenge, as well as at different time points (0, 24, and 48 h) from HtrA1 challenge, and then stored at -80°C to observe the temporal expression pattern of mRNAs and proteins. In addition, the cell culture supernatant was collected for measurement of MMP1, MMP3, MMP7, MMP9, and MMP13 using specific ELISA kits.

Quantitative RT-PCR (RT-qPCR)

HtrA1, MMP1, MMP3, MMP7, MMP9, and MMP13 mRNA levels were assessed by RT-qPCR according to the method previously described. Briefly, mRNA was extracted from human intervertebral tissues and cells. Cells were pooled from 3 wells to be able to collect sufficient mRNA for 1 sample. Total RNA was extracted from cells and tissues using Trizol (Life Technologies) reagent according to the manufacturer's instructions. RNA was then reverse-transcribed to cDNA with oligo (dT) primer from 500 (ng) total RNA according to the manufacturer's instructions by the use of aRT reagent kit (TaKaRa, Ohtsu, Japan). For quantitative real-time PCR, cDNA (1 µL) was amplified with the SYBR Green Premix EX Taq kit (TaKaRa, Ohtsu, Japan) by real-time PCR. Each sample was analyzed in triplicate with the CFXA96 Cyclor (Thermal) and the relative mRNA expression quantification was calculated with the comparative threshold cycle (Ct) method. β-actin was used as an internal control. All primer sequences are shown in **Table 1**.

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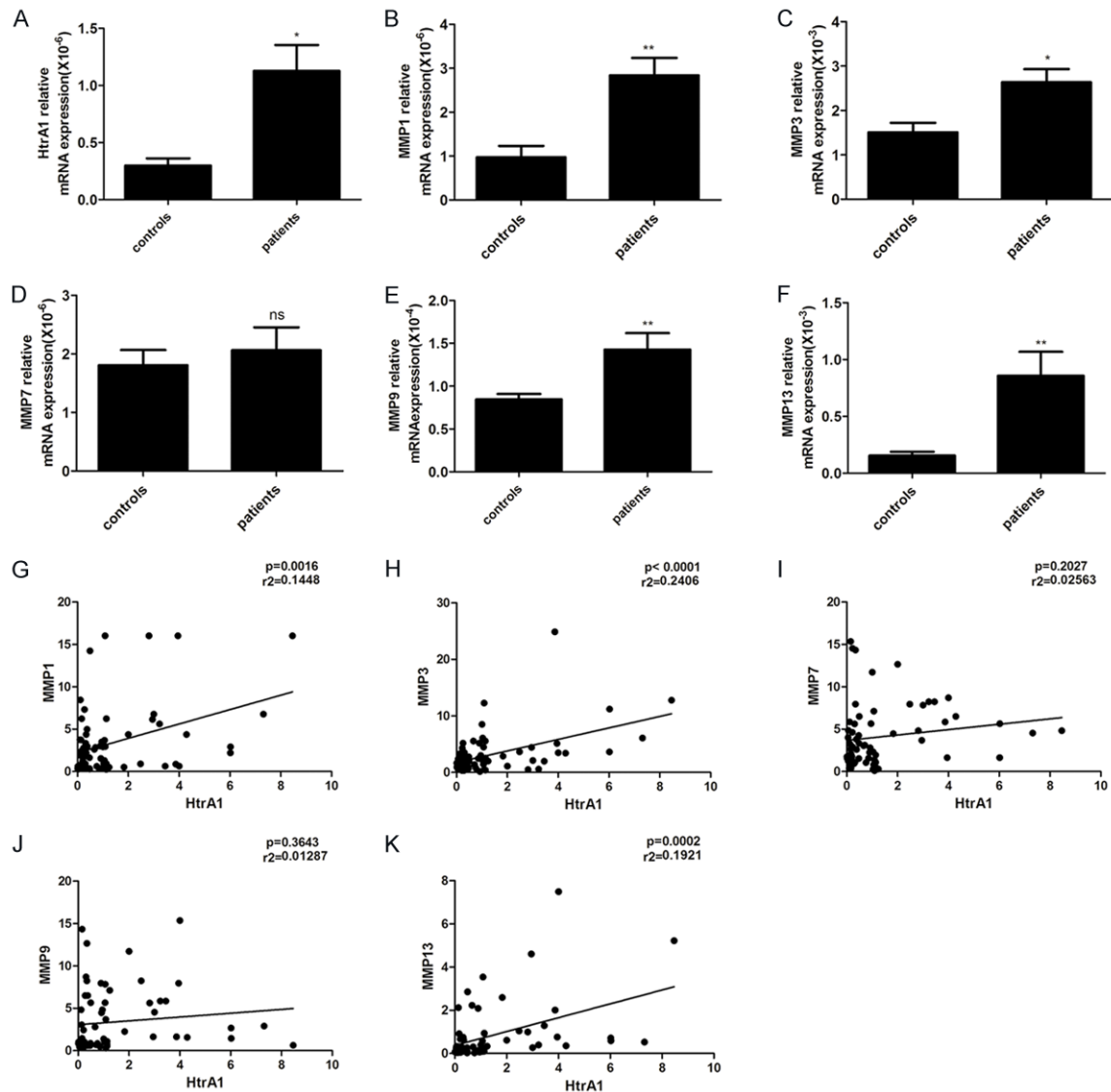


Figure 1. Elevation of MMP1, MMP3, and MMP13 was correlated with overexpression of HtrA1 in IDD patients. A-F. Representative diagrams of quantitative analysis the expression of HtrA1, MMP1, 3, 9, and 13 showing up-regulation in IDD patients. D. Representative diagrams of quantitative analysis of MMP7. G, H, K. There were positive correlations between expression of HtrA1 and MMP1, MMP3, and MMP13. I, J. No significant correlation was observed between expression of HtrA1 and MMP7, or MMP9. (***) $P < 0.001$, (**) $P < 0.01$, (*) $P < 0.05$, ns, no significant).

Western blotting analysis

HNPCs were homogenized and lysed in RIPA buffer supplemented with proteinase inhibitors. Equal amounts of total protein were loaded and separated on 12% SDS-polyacrylamide gel. Following electrophoresis, the proteins were transferred to a PVDF membrane (Millipore, USA), blocked in 5% (w/v) non-fat milk, and incubated with the primary antibodies. Membranes were incubated with monoclonal antibody against β -actin (CWBI0, CW0096), MMP1 (Bioworld Technology, BS1229), MMP3 (Abcam, ab52915), MMP7 (Santa Cruz, sc-8832), MMP9 (Abcam, ab58803), MMP13 (Abcam, ab-

39012), p-ERK1/2 (Santa Cruz, sc-101760), and p-ROCK (Santa Cruz, sc-17794) at 4°C overnight. The membrane was washed 3 times with Tris-buffered saline/Tween (TBS/T). After washing, HRP-conjugated secondary antibody was added for 1 h at 37°C. Detection was performed with enhanced chemiluminescence (ECL) and relevant blots were quantified by densitometry by using the accompanying computerized image analysis program.

Enzyme-linked immunosorbent assay (ELISA)

The concentrations of MMP1, MMP3, MMP7, MMP9, and MMP13 in the cell culture superna-

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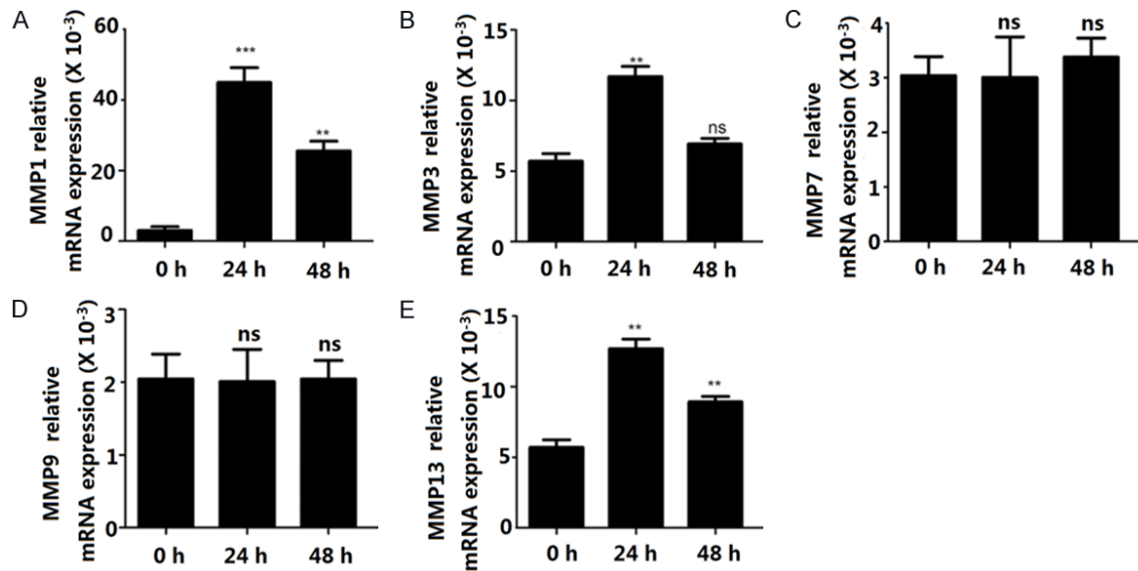


Figure 2. rHtrA1 up-regulated the gene expression of MMP1, 3, and 13, but not MMP7 and MMP9. A, B, and E. Representative diagrams of quantitative analysis the expression of MMP1, 3, and 13 show up-regulation in HNPCs treated by exogenous rHtrA1. C, D. Representative diagrams of quantitative analysis the expression of MMP7 and 9 show no change in HNPCs treated by exogenous rHtrA1. All samples were measured in triplicate. (***) $P < 0.001$, (**) $P < 0.01$, ns, no significant).

tant from groups pre-treated with rHtrA1 and control at 24 h and 48 h time points were measured following the manufacturer's protocols (eBioscience, San Diego, CA, USA) by ELISA. All samples were run in batches and in triplicate, and the optical density (OD) value was detected using an ELISA microplate reader (Labequip Ltd, Ontario; Canada) at 450 nm wavelengths using an ELISA microplate reader (Labequip Ltd, Ontario; Canada).

Statistical analysis

All statistical analyses were performed by using Prism 5 (Graph Pad Software, La Jolla, CA, USA). Comparisons between groups were performed by using the unpaired *t* test. The association between 2 clinicopathological variables was tested using Spearman test. A *p*-value < 0.05 was considered to indicate a statistically significant difference.

Results

Elevation of MMP1, MMP3, and MMP13 were correlated with overexpression of HtrA1 in IDD patients

To determine whether HtrA1 participates in the progress of IDD, we performed a real-time PCR to determine the mRNA level of HtrA1 in degen-

erative NP tissues. As shown in **Figure 1A**, significantly increased HtrA1 mRNA expression was found in degenerative NP tissues compared with the control group. Real-time quantitative PCR indicated a similar expression pattern at the mRNA levels of MMP1, MMP3, MMP9, and MMP13 (**Figure 1F**). The correlation between HtrA1 and MMP1, MMP3, MMP7, MMP9, and MMP13 in mRNA level was further analyzed. Our data show that the expression level of HtrA1 was associated with the expression levels of MMP1, MMP3, and MMP13.

HtrA1 up-regulated the gene expression of MMP1, 3, and 13

To determine the effects of HtrA1 on MMPs expression, HNPCs were treated with exogenous rHtrA1 (5 $\mu\text{g/ml}$ and 10 $\mu\text{g/ml}$) and cells were harvested at different time points (0, 24, and 48 h). The expression of MMPs was examined by real-time PCR. It is noteworthy that we found that expression of MMPs was induced by exogenous rHtrA1 and increased in a dose-dependent manner in HNPCs. We observed that the mRNA levels peaked at 24 h after a 5 $\mu\text{g/ml}$ dose of exogenous rHtrA1 was used to challenge the cells (**Figure 2**). Intriguingly, MMP1, 3, and 13, but not MMP7, 9 were remarkably increased at 24 h in the presence of exogenous rHtrA1 (**Figure 2**), indicating effec-

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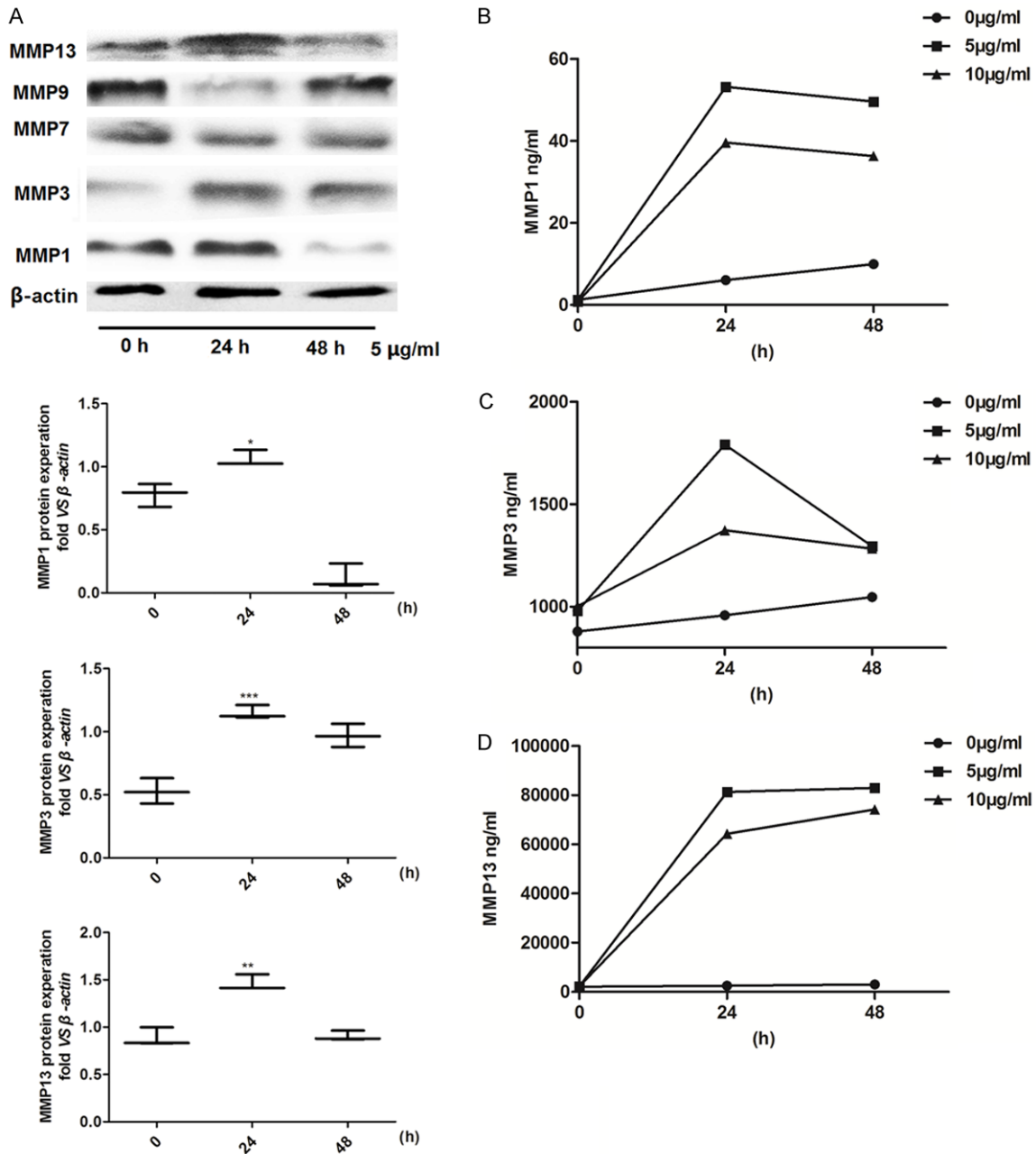


Figure 3. Protein levels of MMP1, 3, and 13 were increased in human NP cells in response to exogenous rHtrA1. (A) Western blot analyses the protein of MMP1, 3, 7, 9, and 13 expressed in exogenous rHtrA1-treated HNPCs. The protein levels of MMP1, 3, and 13 were all elevated and peaked at 24 h at a dose of 5 µg/ml, as well as the protein released in the cell culture supernatants (B-D). (***) $P < 0.001$, (**) $P < 0.01$).

tive up-regulation of MMP1, 3, and 13 following challenge by exogenous rHtrA1 in HNPCs.

Protein levels of MMP1, 3, and 13 were increased in HNPCs in response to exogenous rHtrA1

Because exogenous rHtrA1 treatment induced increased mRNA levels of MMP1, 3, and 13, we then used Western blotting and ELISA to assess

whether exogenous rHtrA1 would increase the protein level of MMP1, 3, and 13 as well. As expected, and in line with the mRNA expression, elevated levels of MMP1, 3, and 13 proteins were observed in the post-treated HNPCs (Figure 3A), and similar data were also obtained in the cell culture supernatants (Figure 3B-D). Moreover, Western blotting analyses showed no detectable changes in MMP7 or MMP9 expression.

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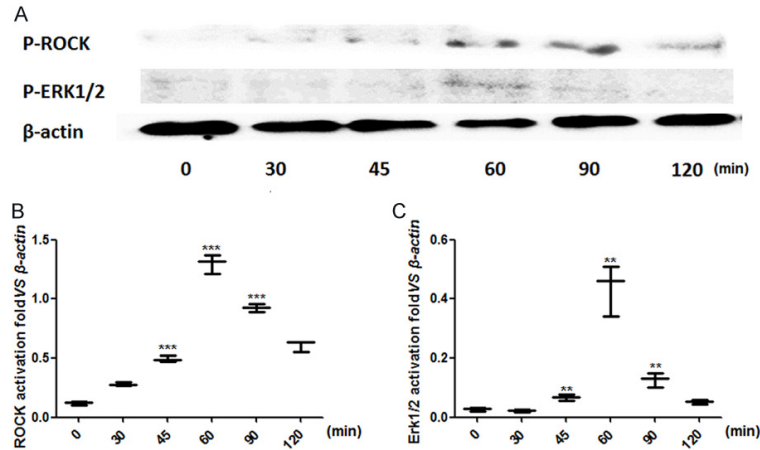


Figure 4. Exogenous rHtrA1 activated the Erk1/2/ROCK pathway in HNPCs. (A) HNPCs were treated with 5 μ g/ml rHtrA1. At the points indicated, HNPCs were harvested and phosphorylated Erk1/2 and ROCK levels were assessed by Western blot analysis. There were transient increases in the phosphorylation of ERK1/2 and ROCK within HNPCs (B and C). (*** $P < 0.001$, ** $P < 0.01$).

Increased levels of MMP1, 3, and 13 induced by rHtrA1 was ameliorated by ERK1/2 or ROCK signaling pathway inhibition

Challenging HNPCs with exogenous rHtrA1 resulted in a transient increase in the phosphorylation of ERK1/2 and ROCK within HNPCs, peaking at 45-120 min and 45-90 min, respectively (Figure 4), and resulted in an increase in MMP1, 3, and 13 within HNPCs, peaking at 24 h (Figure 3). To further confirm that the transient increase was due to ERK1/2 signaling pathway or ROCK signaling pathway following exogenous rHtrA1 treatment, we used Y27632, a ROCK signaling pathway inhibitor and SCH-772984, a ERK1/2 signaling pathway inhibitor, respectively. We performed real-time PCR to examine expression of MMP1, 3, and 13 in HNPCs treated with exogenous rHtrA1. Our results showed the expression of these mRNA was significantly decreased after treatment with Y27632 or SCH772984 (Figure 6A-C). To further substantiate that ERK1/2 or ROCK signaling was essential for promoting up-regulation of MMP1, 3, and 13 by HtrA1 in HNPCs, we used Western blot analysis to compare the protein expression levels of Y27632 or SCH772984- HNPCs and control cells treated with exogenous HtrA1. HNPCs challenged with Y27632 or SCH772984 ameliorated the increase of these proteins induced by rHtrA1 (Figures 5, 6D-F), which showed a similar res-

ult with mRNA. Taken together, these results clearly demonstrate that HtrA1 directly led to increased expression of MMP1, MMP3, and MMP13, which was associated with the ERK1/2/ROCK signaling pathway.

Ameliorated protein level of MMP1, 3, and 13 in the cell culture supernatants

Our results have showed that exogenous rHtrA1 could up-regulate the expression of MMP1, 3 and 13 within the HNPCs. We also analyzed the protein level of these factors in the cell culture supernatants. Exogenous rHtrA1-treated HNPCs were exposed to ERK1/2/ROCK signaling pathway inhibitor. As Figure 6 shows, protein levels of MMP1, 3, and 13 in the cell culture supernatants were obviously decreased. These results clearly demonstrate that HtrA1 contributed to MMP1, 3, and 13 deposition via ERK1/2/ROCK activation.

Discussion

During exploration of the etiology and pathophysiology of IDD, many molecules have been identified as endogenous damage-associated molecules and may be responsible for the disease. Although the cause and pathophysiology of IDD remains unclear, it has been suggested that the degenerative process begins in the NP of the IVD and is associated with progressive loss of aggrecan (ACAN) and type II collagen (Col-2) from the ECM [22]. With aging, nucleus pulposus cells are subject to senescence and thereby lose their ability to proliferate and replace cells lost to necrosis or apoptosis [23], and decreased anabolism or increased catabolism of senescent cells that result in accumulation of ECM, which may facilitate IDD [24].

It is well accepted that MMPs have essential roles in the degradation of ECM [25]. HtrA1 can cleave numerous ECM, such as fibronectin, ecorin, fibromodulin, ACAN, Col-2, biglycan, clusterin, a disintegrin and metalloproteinase domain-containing 9 (ADAM9), vitronectin, α 2 macroglobulin, and the amyloid precursor pro-

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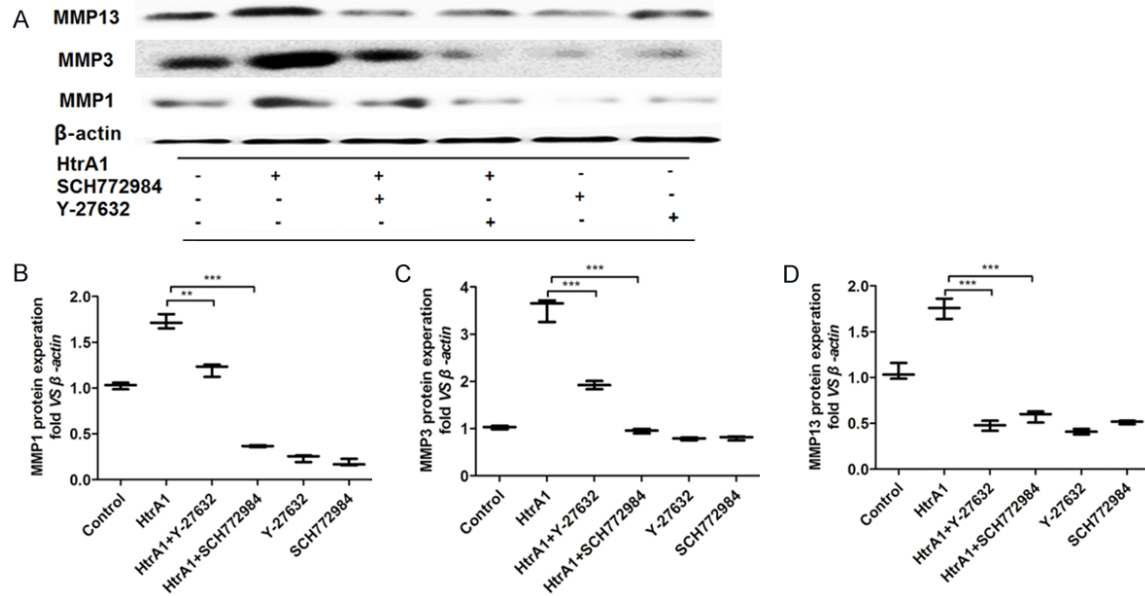


Figure 5. Increased levels of MMP1, 3, and 13 induced by rHtrA1 were ameliorated by Y27632 or SCH772984. (A) Western blot analyses of MMP1, 3, and 13 levels in HNPCs challenged by exogenous rHtrA1 with or without signaling pathway inhibitor. Representative blots are shown above and densitometric analyses, below (B-D). Blots were cropped from different gels, but all the gels had been run under the same experimental conditions. These data show that Y27632 and SCH772984 can ameliorate the transient increase caused by exogenous rHtrA1. All samples were measured in triplicate. (** $P < 0.01$, *** $P < 0.001$).

tein fragment A β [26-30]. This has encouraged further research into the potential role of HtrA1 in human diseases in which breakdown of the ECM is considered to be of significant importance. A mouse osteoarthritis model identified increased levels of HtrA1 in articular cartilage from diseased mice in association with both MMP13 and discoidin domain-containing receptor 2 (Ddr2) [31].

In the present study, we first showed a significantly increased level of HtrA1 in patients with IDD, which was consistent with higher MMP1, MMP3, and MMP13 transcript levels, indicating that HtrA1 participates in the progression of IDD and HtrA1 elevation correlated with elevation of MMP1, MMP3, and MMP13 in IDD patients.

To further verify the potential role of HtrA1 in promoting the progression of IDD, we chose HNPCs as our research subject to elaborate the potential role of HtrA1 in the pathogenesis of IDD. We demonstrated that exogenous rHtrA1 can up-regulate MMP1, MMP3, and MMP13 mRNA and protein in HNPCs (Figure 2A, 2B, 2E). Western blot analysis and ELISA data further showed that exogenous rHtrA1 increased the protein level of these MMPs in HNPCs

(Figure 3). We also found that transient increases were dose- and time-dependent, peaking at 24 h at a dose of 5 $\mu\text{g}/\text{ml}$. However, our results did not show any change in MMP7 or MMP9 in mRNA or protein level (Figures 2C, 2D, 3A), which suggests that other molecules might participate in this process, such as IL-1 β , which has been proven to significantly increase MMP-9 expression in HNPCs in a NF- κB -dependent pathway in a previous study [32]. Moreover, there were no detectable levels of MMP7 and MMP9 in the cell culture supernatants.

Lakka et al reported that the ERK1/2 signaling pathway may be involved in regulating the expression of MMPs [33-38], and it has been shown *in vitro* that the Rho/ROCK signaling pathway plays a central role in the inflammatory response in nucleus pulposus cells [39-41]. Therefore, we postulated that ERK1/2 and ROCK signaling pathways may play critical roles in disease progression of IDD. Indeed, our results showed that HtrA1 directly led to ECM deposition, which was associated with the ERK1/2/ROCK signaling pathway, and inhibition of ERK1/2 or ROCK signaling pathway activation through the use of SCH772984 or Y27632

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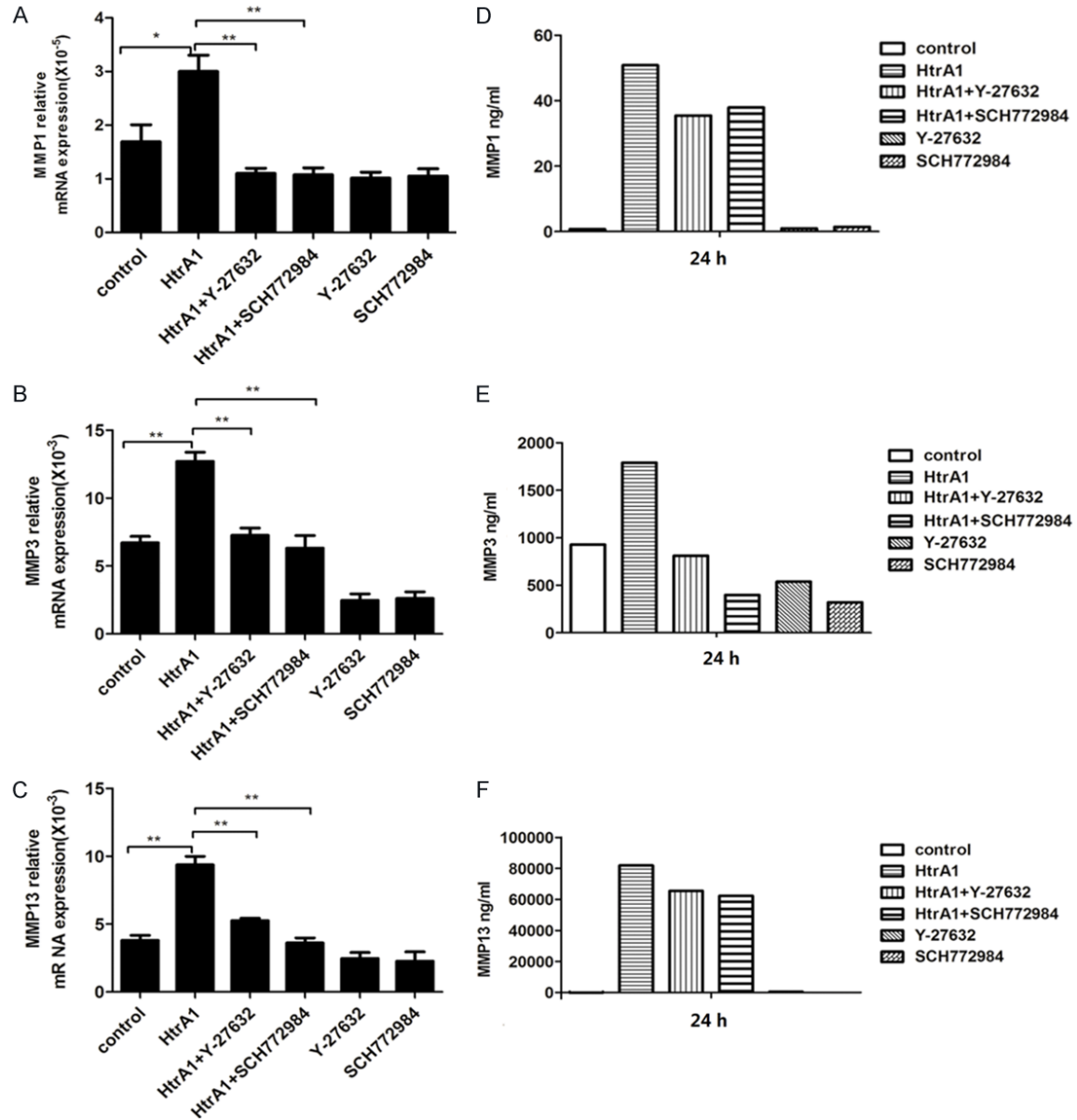


Figure 6. Ameliorated protein level of MMP1, 3, and 13 in the cell culture supernatants. (A-C) Representative diagrams of quantitative analysis of MMP1, 3, and 13. Data show the same tendency in HNPCs after treatment. Protein levels of MMP1, 3, and 13 in the supernatants (D-F) showed results similar to those of Western-blot analysis data. All samples were measured in triplicate. (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$).

mitigated the increase of MMP1, MMP3, and MMP13, among which MMP1 and MMP3 were more sensitive when treated by SCH772984 and MMP13 was more sensitive when treated by Y27632 (Figure 5). Furthermore, we showed that there were higher levels of MMP3 and MMP13 in the cell culture supernatants, which suggests more critical roles for MMP3 and MMP13 (Figure 6). Therefore, realizing the potential role of HtrA1 in regulating the expression of MMP1, MMP3, and MMP13 is crucial in

the search for a promising therapeutic target of IDD in future studies.

Conclusions

Our data demonstrate that HtrA1 can up-regulate expression of MMP1, MMP3, and MMP13 via the ERK1/2 or ROCK signaling pathway. Using inhibitors of ERK or ROCK signaling ameliorated the increasing trend. Our study illustrates the fact that aberrant HtrA1 activation

can contribute to the pathogenesis of IDD, and the suppression of HtrA1 activity may be a useful target for treatment of IDD.

Acknowledgements

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Disclosure of conflict of interest

None.

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References

- [1] Choi H, Johnson ZI, Risbud MV. Understanding nucleus pulposus cell phenotype: a prerequisite for stem cell based therapies to treat intervertebral disc degeneration. *Curr Stem Cell Res Ther* 2015; 10: 307-16.
- [2] Kadow T, Sowa G, Vo N, Kang JD. Molecular basis of intervertebral disc degeneration and herniations: what are the important translational questions? *Clin Orthop Relat Res* 2015; 473: 1903-12.
- [3] Leung VY, Aladin DM, Lv F, Tam V, Sun Y, Lau RY, Hung SC, Ngan AH, Tang B, Lim CT, Wu EX, Luk KD, Lu WW, Masuda K, Chan D, Cheung KM. Mesenchymal stem cells reduce intervertebral disc fibrosis and facilitate repair. *Stem Cells* 2014; 32: 2164-77.
- [4] Iatridis JC, Nicoll SB, Michalek AJ, Walter BA, Gupta MS. Role of biomechanics in intervertebral disc degeneration and regenerative therapies: what needs repairing in the disc and what are promising biomaterials for its repair? *Spine J* 2013; 13: 243-62.
- [5] Tiaden AN, Klawitter M, Lux V, Mirsaidi A, Bahrenberg G, Glanz S, Quero L, Liebscher T, Wuertz K, Ehrmann M, Richards PJ. Detrimental role for human high temperature requirement serine protease A1 (HTRA1) in the pathogenesis of intervertebral disc (IVD) degeneration. *J Biol Chem* 2012; 287: 21335-45.
- [6] Wang P, Guan PP, Guo C, Zhu F, Konstantopoulos K, Wang ZY. Fluid shear stress-induced osteoarthritis: roles of cyclooxygenase-2 and its metabolic products in inducing the expression of proinflammatory cytokines and matrix metalloproteinases. *FASEB J* 2013; 27: 4664-77.
- [7] Wang S, Liu C, Sun Z, Yan P, Liang H, Huang K, Li C, Tian J. IL-1beta increases asporin expression via the NF-kappaB p65 pathway in nucleus pulposus cells during intervertebral disc degeneration. *Sci Rep* 2017; 7: 4112.
- [8] Sun Z, Yin Z, Liu C, Liang H, Jiang M, Tian J. IL-1beta promotes ADAMTS enzyme-mediated aggrecan degradation through NF-kappaB in human intervertebral disc. *J Orthop Surg Res* 2015; 10: 159.
- [9] Vo NV, Hartman RA, Patil PR, Risbud MV, Kletsas D, Iatridis JC, Hoyland JA, Le Maitre CL, Sowa GA, Kang JD. Molecular mechanisms of biological aging in intervertebral discs. *J Orthop Res* 2016; 34: 1289-306.
- [10] Graham JR, Chamberland A, Lin Q, Li XJ, Dai D, Zeng W, Ryan MS, Rivera-Bermudez MA, Flannery CR, Yang Z. Serine protease HTRA1 antagonizes transforming growth factor-beta signaling by cleaving its receptors and loss of HTRA1 in vivo enhances bone formation. *PLoS One* 2013; 8: e74094.
- [11] Kang S, Lee JS, Park J, Park SS. Staged lengthening and reconstruction for children with a leg-length discrepancy after excision of an osteosarcoma around the knee. *Bone Joint J* 2017; 99-B: 401-8.
- [12] Tossetta G, Avellini C, Licini C, Giannubilo SR, Castellucci M, Marzioni D. High temperature requirement A1 and fibronectin: two possible players in placental tissue remodelling. *Eur J Histochem* 2016; 60: 2724.
- [13] Filliat G, Mirsaidi A, Tiaden AN, Kuhn GA, Weber FE, Oka C, Richards PJ. Role of HTRA1 in bone formation and regeneration: in vitro and in vivo evaluation. *PLoS One* 2017; 12: e0181600.
- [14] Altobelli E, Marzioni D, Lattanzi A, Angeletti PM. HtrA1: its future potential as a novel biomarker for cancer. *Oncol Rep* 2015; 34: 555-66.
- [15] Lehner A, Magdolen V, Schuster T, Kotzsch M, Kiechle M, Meindl A, Sweep FC, Span PN, Gross E. Downregulation of serine protease HTRA1 is associated with poor survival in breast cancer. *PLoS One* 2013; 8: e60359.
- [16] Grau S, Baldi A, Bussani R, Tian X, Stefanescu R, Przybylski M, Richards P, Jones SA, Shridhar V, Clausen T, Ehrmann M. Implications of the serine protease HtrA1 in amyloid precursor protein processing. *Proc Natl Acad Sci U S A* 2005; 102: 6021-6.
- [17] Frochoux V, Hildebrand D, Talke A, Linscheid MW, Schluter H. Alpha-1-antitrypsin: a novel human high temperature requirement protease A1 (HTRA1) substrate in human placental tissue. *PLoS One* 2014; 9: e109483.
- [18] Tong Y, Liao J, Zhang Y, Zhou J, Zhang H, Mao M. LOC387715/HTRA1 gene polymorphisms and susceptibility to age-related macular de-

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- generation: a HuGE review and meta-analysis. *Mol Vis* 2010; 16: 1958-81.
- [19] Larkin DJ, Kartchner JZ, Doxey AS, Hollis WR, Rees JL, Wilhelm SK, Draper CS, Peterson DM, Jackson GG, Ingersoll C, Haynie SS, Chavez E. Inflammatory markers associated with osteoarthritis after destabilization surgery in young mice with and without receptor for advanced glycation end-products (RAGE). *Front Physiol* 2013; 4: 121.
- [20] De Luca A, De Falco M, Fedele V, Cobellis L, Mastrogiacomo A, Laforgia V, Tuduca IL, Campioni M, Giraldo D, Paggi MG, Baldi A. The serine protease HtrA1 is upregulated in the human placenta during pregnancy. *J Histochem Cytochem* 2004; 52: 885-92.
- [21] Li D, Yue J, Jiang L, Huang Y, Sun J, Wu Y. Correlation Between expression of high temperature requirement serine protease a1 (htra1) in nucleus pulposus and t2 value of magnetic resonance imaging. *Med Sci Monit* 2017; 23: 1940-6.
- [22] Hangai M, Kaneoka K, Kuno S, Hinotsu S, Sakane M, Mamizuka N, Sakai S, Ochiai N. Factors associated with lumbar intervertebral disc degeneration in the elderly. *Spine J* 2008; 8: 732-40.
- [23] Wang F, Cai F, Shi R, Wang XH, Wu XT. Aging and age related stresses: a senescence mechanism of intervertebral disc degeneration. *Osteoarthritis Cartilage* 2016; 24: 398-408
- [24] Grunhagen T, Wilde G, Soukane DM, Shirazi-Adl SA, Urban JP. Nutrient supply and intervertebral disc metabolism. *J Bone Joint Surg Am* 2006; 88 Suppl 2: 30-5.
- [25] Kucukguven A, Khalil RA. Matrix metalloproteinases as potential targets in the venous dilation associated with varicose veins. *Curr Drug Targets* 2013; 14: 287-324.
- [26] Verlaan JJ, Somers I, Dhert WJ, Oner FC. Clinical and radiological results 6 years after treatment of traumatic thoracolumbar burst fractures with pedicle screw instrumentation and balloon assisted endplate reduction. *Spine J* 2015; 15: 1172-8.
- [27] An E, Sen S, Park SK, Gordish-Dressman H, Hathout Y. Identification of novel substrates for the serine protease HTRA1 in the human RPE secretome. *Invest Ophthalmol Vis Sci* 2010; 51: 3379-86.
- [28] Grau S, Richards PJ, Kerr B, Hughes C, Caterson B, Williams AS, Junker U, Jones SA, Clausen T, Ehrmann M. The role of human HtrA1 in arthritic disease. *J Biol Chem* 2006; 281: 6124-9.
- [29] Tsuchiya A, Yano M, Tocharus J, Kojima H, Fukumoto M, Kawaichi M, Oka C. Expression of mouse HtrA1 serine protease in normal bone and cartilage and its upregulation in joint cartilage damaged by experimental arthritis. *Bone* 2005; 37: 323-36.
- [30] Launay S, Maubert E, Lebeurrier N, Tennstaedt A, Campioni M, Docagne F, Gabriel C, Dauphinot L, Potier MC, Ehrmann M, Baldi A, Vivien D. HtrA1-dependent proteolysis of TGF-beta controls both neuronal maturation and developmental survival. *Cell Death Differ* 2008; 15: 1408-16.
- [31] Holt DW, Henderson ML, Stockdale CE, Farrell JT, Kooyman DL, Bridgewater LC, Seegmiller RE. Osteoarthritis-like changes in the heterozygous sedc mouse associated with the HtrA1-Ddr2-Mmp-13 degradative pathway: a new model of osteoarthritis. *Osteoarthritis Cartilage* 2012; 20: 430-9.
- [32] Csaki C, Mobasheri A, Shakibaei M. Synergistic chondroprotective effects of curcumin and resveratrol in human articular chondrocytes: inhibition of IL-1beta-induced NF-kappaB-mediated inflammation and apoptosis. *Arthritis Res Ther* 2009; 11: R165.
- [33] Eo SH, Choi SY, Kim SJ. PEP-1-SIRT2-induced matrix metalloproteinase-1 and -13 modulates type II collagen expression via ERK signaling in rabbit articular chondrocytes. *Exp Cell Res* 2016; 348: 201-8.
- [34] Lee WT, Lee TH, Cheng CH, Chen KC, Chen YC, Lin CW. Antroquinonol from antrodia camphorata suppresses breast tumor migration/invasion through inhibiting ERK-AP-1- and AKT-NF-kappaB-dependent MMP-9 and epithelial-mesenchymal transition expressions. *Food Chem Toxicol* 2015; 78: 33-41.
- [35] Lu KH, Chen PN, Hsieh YH, Lin CY, Cheng FY, Chiu PC, Chu SC, Hsieh YS. 3-Hydroxyflavone inhibits human osteosarcoma U2OS and 143B cells metastasis by affecting EMT and repressing u-PA/MMP-2 via FAK-Src to MEK/ERK and RhoA/MLC2 pathways and reduces 143B tumor growth in vivo. *Food Chem Toxicol* 2016; 97: 177-86.
- [36] Keld R, Guo B, Downey P, Cummins R, Gulmann C, Ang YS, Sharrocks AD. PEA3/ETV4-related transcription factors coupled with active ERK signalling are associated with poor prognosis in gastric adenocarcinoma. *Br J Cancer* 2011; 105: 124-30.
- [37] Taden AN, Richards PJ. The emerging roles of HTRA1 in musculoskeletal disease. *Am J Pathol* 2013; 182: 1482-8.
- [38] Lakka SS, Jasti SL, Gondi C, Boyd D, Chandrasekar N, Dinh DH, Olivero WC, Gujrati M, Rao JS. Downregulation of MMP-9 in ERK-mutated stable transfectants inhibits glioma invasion in vitro. *Oncogene* 2002; 21: 5601-8.
- [39] Ahmad N, Wang W, Nair R, Kapila S. Relaxin induces matrix-metalloproteinases-9 and -13 via RXFP1: induction of MMP-9 involves the

HtrA1 up-regulates MMPs

- PI3K, ERK, Akt and PKC-zeta pathways. *Mol Cell Endocrinol* 2012; 363: 46-61.
- [40] Bottino J, Gelaleti GB, Maschio LB, Jardim-Perassi BV, de Campos Zuccari DA. Immun-expression of ROCK-1 and MMP-9 as prognostic markers in breast cancer. *Acta Histochem* 2014; 116: 1367-1373.
- [41] Ishiguro M, Kawasaki K, Suzuki Y, Ishizuka F, Mishiro K, Egashira Y, Ikegaki I, Tsuruma K, Shimazawa M, Yoshimura S, Iwama T, Hara H. A Rho kinase (ROCK) inhibitor, fasudil, prevents matrix metalloproteinase-9-related hemorrhagic transformation in mice treated with tissue plasminogen activator. *Neuroscience* 2012; 220: 302-12.