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Matrilin-3 alleviates extracellular matrix degradation of nucleus pulposus cells *via* induction of IL-1 receptor antagonist

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Abstract. – OBJECTIVE: The intervertebral disc contains abundant extracellular matrix (ECM) imbued with proteoglycans, collagens, and water. With the development of intervertebral disc degeneration (IVDD), the ECM undergoes changes characterized by loss of water content, proteoglycans, and collagen content. The purpose of this study was to explore the vital role of Matrilin-3, an ECM protein involved in the progress of IVDD.

MATERIALS AND METHODS: NP cells were isolated from the patients' disc samples and exposed to recombinant human (rh)-Matrilin-3 protein (MATN3), and IL-1β is used as a reducer of nucleus pulposus (NP) cells degeneration. Matrilin-3 and IL-1 receptor antagonist (IL-1Ra) were knocked down by siRNA transfection. Messenger RNA expressions of IL-1Ra, Collagen II, aggrecan, MMP-13, and ADAMTS-5 were determined using Real-Time quantitative Polymerase Chain Reaction (RT-qPCR). Later, the protein levels of IL-Ra, Collagen II, and aggrecan were also detected by Western blot. The IL-1Ra, MMP-13, and ADAMTS-5 dose of the supernatants in the culture medium was determined by enzyme linked immunosorbent assay (ELISA). Finally, immunofluorescence was used to expose the expression of Collagen II, aggrecan, and Collagen X.

RESULTS: It was found that the expression of IL-1Ra was markedly increased in the present of MATN3 or IL-1β, especially these two at once. Besides, MATN3 could upregulate Collagen II and aggrecan expressions, as well as inhibit the MMP-13 and Collagen X production of NP cells. However, the protective effects of Collagen II and aggrecan were abolished after Matrilin-3 silenced. Furthermore, IL-1β downregulated the Collagen II and aggrecan but promoted the MMP-13 and Collagen X levels of NP cells, which were antagonized by the action of MATN3. Surprisingly, silencing of IL-1Ra significantly abolished the MATN3-induced the protective effects of ECM in NP cells.

CONCLUSIONS: This study provides a novel viewpoint of Matrilin-3 in the ECM stability of NP due to its ability to activate IL-1Ra. It is considered that MATN3 efficiently protects ECM degeneration of human NP cells related to maintain the content of Collagen II and aggrecan, as well as inflammatory inhibition.

Key Words:
Matrilin-3, Nucleus pulposus cells, Intervertebral disc degeneration, Extracellular matrix, IL-1 receptor antagonist.

Introduction

Intervertebral disc degeneration (IVDD) is the pathological basis of degenerative diseases of the spine, which can cause disc herniation, spinal instability, spinal stenosis, low back pain, and other common diseases[1]. At present, IVDD is caused by many factors *in vitro* and *in vivo*, including genetic factors, intervertebral disc dystrophies, immune factors, matrix metalloproteinases, inflammatory mediators, extracellular matrix (ECM) disorders, and mechanical load[2,3]. Nucleus pulposus (NP) cell, the major cell type in the intervertebral disc tissue, is only 1% of the total volume, while the remaining 99% is ECM. Although the number of NP cells is small, they play an important role in the homeostasis of the intervertebral disc. Degeneration of the intervertebral disc begins in the NP. The decrease in the number of NP cells and the abnormality of the extracellular matrix are the key factors for the degeneration of the intervertebral disc[4]. In addition, the most important function of the NP cells is to produce a functional ECM and maintain the matrix components stable. Moreover, NP cells

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mainly express proteoglycans (mainly aggrecan) and Collagen II, which reflect the phenotype of chondrocyte-like cells[5].

Decreased number of NP cells in the intervertebral disc leads to a decreased synthesis of the functional matrix, such as aggrecan and Collagen II. Due to the imbalance between anabolism and catabolism, some degradative enzymes, such as matrix metalloproteinases (MMPs), cause accelerated degradation of the matrix, resulting in decreases in proteoglycan content and water content. At the same time, the Collagen II content is gradually decreased, and the NP tissue gradually loses its original gel-like morphology and hydrostatic properties. Matrilin-3 is one of the four members of the Matrilin family of non-collagenous oligomeric ECM proteins[6,7]. As an extracellular matrix-binding protein, Matrilin-3 regulates the interaction between tissue components, such as collagen and proteoglycan in a collagen-dependent or non-dependent manner to form an intercellular network[8,9]. IL-1β acts as one of the major inflammatory cytokines related to NP damage, having abilities to promote the expression of proteinases, including MMPs and aggrecans and to suppress the expression of matrix components containing Collagen II and aggrecan[10,11]. Inhibition of the IL-1β pathway presents a promising means of preventing IVDD, and one of the major endogenous inhibitors of the IL-1β pathway is IL-1 receptor antagonist (IL-1Ra)[12].

The purpose of this study was to explore whether Matrilin-3 plays a positive role in NP homeostasis genes to explain its protective function in the progress of IVDD. In addition, whether these novel regulatory properties of Matrilin-3 depend on its induction of IL-1Ra were tested, so as to further clarify the molecular mechanism of ECM changes in the IVDD process and provide a theoretical basis for Matrilin-3 as a new therapeutic target for IVDD.

Materials and Methods

Human NP Tissue Collection

The present project was approved by the Ethics Committee of the First Hospital of Shanxi Medical University. All patients provided written informed consent. This study was conducted in accordance with the Declaration of Helsinki. A total of 7 patients (5 males and 2 females) aged 35-53 years old, with an average age of 42 years old who underwent lumbar disc herniation surgeries in our hospital from November 2018 to March 2019 participated in the study. The degenerated magnitude of the disc in each segment was mildly based on the Pfirrmann classification score according to the Magnetic Resonance Imaging (MRI) before surgery, of which only grades I or II were chosen in the study. After that, the tissues were conserved in a sterile cell culture medium immediately after cutting from patients for the following NP cell isolation.

NP Cells Isolation and Treatment

Each intervertebral disc sample was washed three times with sterile phosphate-buffered saline (PBS) solution and the NP tissues out of endplates were diced into small fragments. NP fragments were digested in 0.25% trypsin (Gibco, Rockville, MD, USA) for 10 min and 0.25% type II collagenase (Sigma-Aldrich, St. Louis, MO, USA) for 12 h under shaking conditions. Then, the digestion solution was filtered using a 100 μm cell strainer (BD, Franklin Lakes, NJ, USA) to collect NP cells. Cell pellets were re-suspended in cell culture medium Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) and 0.2% streptomycin (Life Technologies, Gaithersburg, MD, USA). Subsequently, NP cells of passage one were used in the following experiment. The culture medium was replaced to serum-free and incubated for 2 hours before recombinant human (rh)-Matrilin-3 protein (MATN3, 100 ng/ml or 200 ng/ml; R&D Systems, Minneapolis, MN, USA) treatment with or without IL-1β (10 ng/ml; PeproTech, Rocky Hill, NJ, USA).

Western Blot Analysis

Western blot analysis was performed using standard protocols. Briefly, after NP cells underwent different treatments, the total protein was isolated using the radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China). Equal protein sample of each group was added in the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto the polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA), and then blocked with 5% milk to avoid nonspecific binding. Afterwards, membranes were probed with desired primary antibodies: IL-1Ra (1:1000, Cell Signaling, Danvers, MA, USA), Collagen II (1:1000, Cell Signaling, Danvers, MA, USA), aggrecan (1:1000, Santa Cruz, Santa Cruz, CA, USA), and β-actin (1:1000, Cell Signaling, Dan-

vers, MA, USA) as loading control overnight at 4°C. The membranes were then washed three times with PBST and incubated with HRP-conjugated secondary antibody for 1 h at room temperature. Finally, the membranes were incubated in enhanced chemiluminescence (ECL) substrate (Beyotime, Shanghai, China), and exposed using developing film. Band intensities were measured using ImageJ software (Rawak Software, Inc., Hamburg, Germany).

Immunofluorescence

Briefly, NP cells were seeded on glass coverslips in 6-well plates. NP cell monolayers on coverslips were washed three times with PBS, fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100 both at room temperature for 15 min. Later, 5% bovine serum albumin (BSA) was used to block the coverslips for at least 1 h at room temperature. Afterwards, the coverslips were washed again and incubated with IL-1Ra (1:200, Abcam, Cambridge, MA, USA) at 4°C overnight. After washing, the coverslips were then incubated with Alexa Fluor488 conjugated goat anti-rabbit IgG (Invitrogen, Carlsbad, CA, USA) with 4',6-diamidino-2-phenylindole (DAPI) at room temperature in the dark for at least 1 h. The staining intensity proceeded using the Image-Pro Plus software (Version 5.1, Media Cybernetics, Inc. Silver Springs, MD, USA).

Real Time-Quantitative Polymerase Chain Reaction (RT-qPCR) Analysis

The total RNA was isolated from human NP cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. For RT-qPCR, 1 μg of RNA was reversely transcribed using a reverse transcription kit (Roche, Basel, Switzerland), according to the manufacturer's instructions. The cDNA of each sample was subjected to RT-qPCR using SYBR Green Master (TOYOBO, Osaka, Japan). The human-specific primers sequences for genes encoding ADAMTS-5, Collagen II, aggrecan, MMP-13, and IL-1Ra can be found in Table I. Moreover, the relative gene expression was achieved by normalization to the amount of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and calculated according to the $2^{-\Delta\Delta Ct}$ method.

Small Interfering RNA (siRNA)-Based Silencing of MATN3 and IL-1Ra in Human NP Cells

NP cells were seeded at 2×10^5 cells/well in 6-well culture plates 1 day before siRNA transfection. Then, they were transfected with a MATN3 or IL-1Ra-siRNA (Cell Signaling Technology, Danvers, MA, USA) for 48 hours at approximately 60% cell density using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) based on the manufacturer's instructions. After that, the siRNA targeted and suppressed the mRNA and protein expression of all four endogenous isoforms of human MATN3 or IL-1Ra. A non-silencing siRNA (Cell Signaling Technology, Danvers, MA, USA) was used as control. Next, the cells were cultured in a 37°C incubator and media was changed after 8 hours. After 48 hours' transfection, NP cells silencing of IL-1Ra were treated with MATN3 and/or IL-1β for the next experiments.

Enzyme-Linked Immunosorbent Assay (ELISA)

The levels of IL-1Ra, MMP-13, and ADAMTS-5 in the supernatant of NP cell culture medium were investigated using commercial ELISA kits (Abcam, Cambridge, MA, USA) according to the manufacturer's instructions. All assays were performed in duplicate.

Statistical Analysis

SPSS 22.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Mean

Table I. Primer sequences of the genes for RT-qPCR.

Gene name	Forward (5'>3')	Reverse (5'>3')
Aggrecan	GGTGAACCAGTTGTGTTGTC	CCGTCCTTTCCAGCAGTC
Collagen II	TGGACGATCAGGCGAAACC	GCTGCGGATGCTCTCAATCT
IL-1Ra	CATTGAGCCTCATGCTCTGTT	CGCTGTCTGAGCGGATGAA
MMP-13	ACTGAGAGGCTCCGAGAAATG	GAACCCCGCATCTTGGCTT
ADAMTS-5	GAACATCGACCAACTCTACTCCG	CAATGCCCACCGAACCATCT
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC

RT-qPCR, quantitative reverse-transcription polymerase chain reaction.

values were calculated and presented as mean ± standard deviation (SD). The differences between the two groups were analyzed by using the Student's *t*-test., and comparisons among multiple groups were done using One-way ANOVA test followed by post-hoc test (Least Significant Difference). Statistical significance was accepted at $p<0.05$ for all analyses. All analysis was performed independently at least three times.

Results

MATN3 Induced IL-1Ra Expression

IL-1Ra is an effective natural endogenous inhibitor of the IL-1 pathway. To determine whether MATN3 affects IL-1Ra synthesis, human NP cells were treated with MATN3 protein (100 ng/ml) for 12 h. Western blot and RT-qPCR were used to analyze IL-1Ra expression under MATN3 stimulation, and ELISA assay was used to determine the IL-1Ra dose in the supernatant of culture medium. As shown in Figure 1A and 1B, MATN3 upregulated protein expression of IL-1Ra. An increase of IL-1Ra mRNA level was also observed under the treatment of MATN3 especially in the presence of IL-1β compared with the control group (Figure 1C). Besides, NP cells treated with MATN3 in the presence of IL-1β significantly increased IL-1Ra expression in the supernatant compared with that treated without or with IL-1β alone (Figure 1D). These results suggest that MATN3 induces the protein and mRNA expressions of IL-1Ra in human NP cells.

MATN3 Upregulated Collagen II and Aggrecan Expression

To determine whether MATN3 induces Collagen II and aggrecan content in human NP cells, NP cells were treated with MATN3 protein for 12 hours. A significant induction of Collagen II and aggrecan protein expressions was observed under MATN3 treatment with or without IL-1β stimuli (Figure 2A, 2B). What's more, Collagen II and aggrecan were upregulated in NP cells under MATN3 stimulation according to RT-qPCR

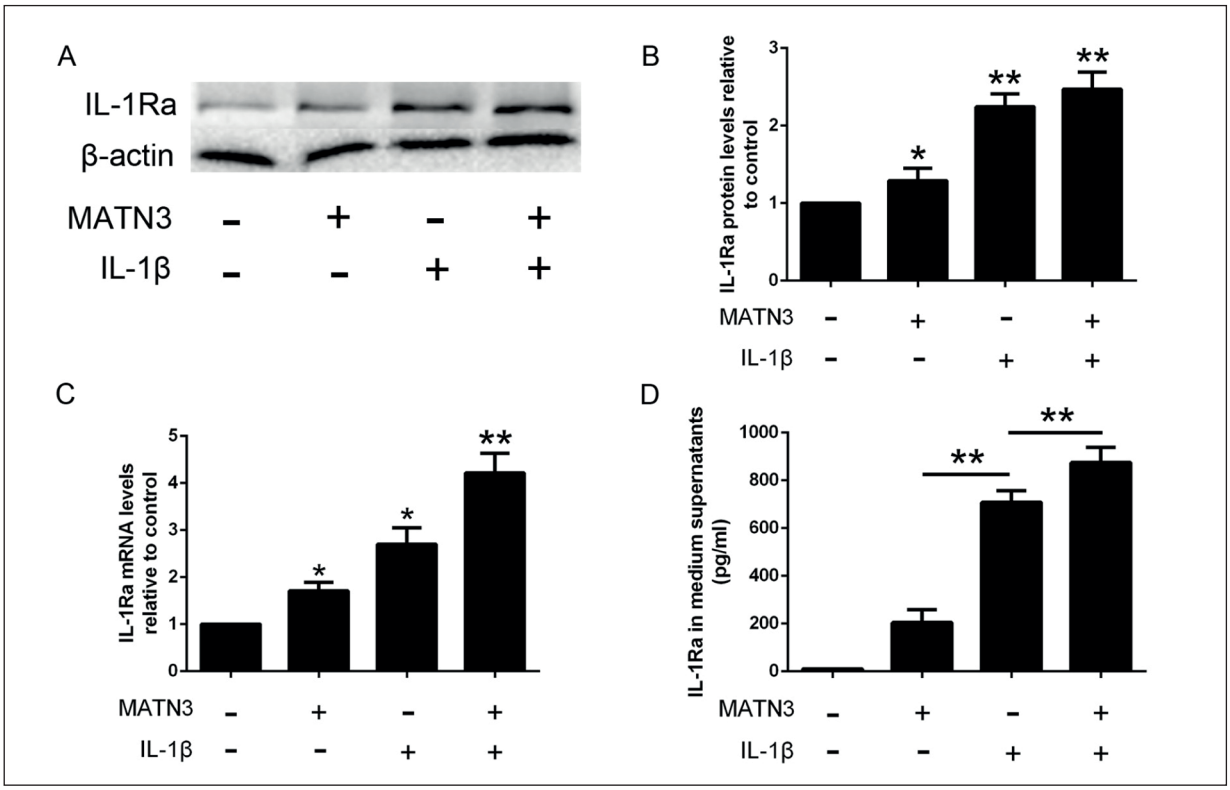

Figure 1. MATN3 enhances IL-1Ra expression in human NP cells. **A, C,** NP cells treated with both MATN3 (200 ng/ml) and IL-1β (10 ng/ml) for 24 hours exhibit significantly higher expression levels of IL-1Ra protein (**A**) and mRNA (**C**) compared with treated with MATN3 or IL-1β alone. **B,** Western blot quantification analysis. **D,** The concentrations of IL-1Ra protein in their cell media were determined using ELISA. The values are mean ± SD of three independent experiments. *$p<0.05$, **$p<0.01$ compared with control group.

results. However, the increment by MATN3 in NP cells was partly inhibited after IL-1β treatment (Figure 2C). To verify whether the lack of MATN3 affects the expression of Collagen II and aggrecan in NP cells, immunofluorescence was adopted to analyze the Collagen II and aggrecan content of MATN3 silencing NP cells and the control cells. The MATN3 silencing NP cells showed a significant reduction of Collagen II and aggrecan levels compared with the control (Figure 2D, 2E), which indicates that the absence of MATN3 downregulates the expression of these two vital genes during the progression of IVDD.

MATN3 Inhibits MMP-13, ADAMTS-5, and Collagen X Expression

Next, whether MATN3 could protect the extracellular matrix by inhibiting the IL-1β-induced matrix metalloproteinase and ADAMTS-5 expression was investigated. From the result of RT-qPCR, IL-1β stimulation increased MMP-13 mRNA expression and MATN3 treatment suppressed MMP-13 expression with or without the presence of IL-1β (Figure 3A). Likewise, the IL-1β-induced ADAMTS-5 level was reduced by MATN3 (Figure 3B), which was also verified using the ELISA methods (Figure 3C, 3D). Collagen X is one of the key molecules in endochondral bone growth and development, especially matrix calcification. With the IVDD progression, Collagen X showed an upregulation compared with the health disc tissues. From the immunofluorescence against Collagen X, IL-1β treatment increased Collagen X protein concentrations, while treatment with MATN3 significantly reduced this increase (Figure 3E, 3F).

MATN3 Upregulated Collagen II and Aggrecan Expression Depends on IL-1Ra

Then, the effects of MATN3 on Collagen II and aggrecan expressions in IL-1Ra gene silencing NP cells were detected. Immunofluorescence was used for double staining of Collagen II and IL-1Ra. The results indicated that MATN3 treatment rescued the IL-1β induced downregulation of Collagen II protein in NP cells transfected with a scrambled siRNA construct. However, MATN3 was not able to upregulate Collagen II expression in the cells transfected with the IL-1Ra siRNA (Figure 4A, 4B). Similarly, MATN3 reduced the extent of IL-1b-induced downregulation of Collagen II and aggrecan mRNA expressions in NP cells transfected with the scrambled siRNA construct but not in the cells transfected with the IL-1Ra siRNA (Figure 4C, 4D). These results suggest that MATN3 can upregulate Collagen II and aggrecan expressions during the progress of NP cells degeneration mainly depending on IL-1Ra.

MATN3 Inhibits MMP-13, ADAMTS-5 and Collagen X Expression Depends on IL-1Ra

Finally, whether MATN3 inhibited the degeneration of matrix depends on IL-1Ra was determined by analyzing the MMP-13 and ADAMTS-5 expressions in IL-1Ra gene silencing NP cells. Their mRNA levels in the IL-1Ra silenced NP cells were quantified. Control NP cells significantly increased the level of MMP-13 expression under the stimulation of IL-1β. At the presence of MATN3, MMP-13 was significantly downregulated in IL-1β induced NP cells (Figure 5A, left). However, this inhibition was not significant in IL-1Ra knock-down NP cells (Figure 5A, right). The same result was also verified on the ADAMTS-5 expression (Figure 5B). In addition, immunofluorescence double staining of Collagen X and IL-1Ra was used to detect whether MATN3 inhibits Collagen X expression which depended on IL-1Ra. The results showed that MATN3 treatment rescued the IL-1β induced overexpression of Collagen X protein in NP cells transfected with a scrambled siRNA construct. However, MATN3 was not able to inhibit Collagen X expression in cells transfected with the IL-1Ra siRNA (Figure 5C, 5D). These data suggest that MATN3 can mediate MMP-13, ADAMTS-5, and Collagen X gene expressions by IL-1Ra.

Discussion

NP plays a dominant role in the physiological function of the intervertebral disc, which is a jelly-like tissue composed of notochord cells, chondrocyte-like cells and a large number of extracellular matrices. The most important ECM components secreted by NP cells are the aggrecan and Collagen II. The glycosaminoglycan branch of aggrecan can adsorb water molecules and maintain the water content and hydrostatic pressure of the NP tissue, so as to facilitate the uniform dispersion of the load and avoid stress concentration[13]. The light lysine side chain of Collagen II can produce monosaccharide and disaccharide derivatives by saccharification so that proteoglycans and water molecules aggregate on them and form a linkage, and the Collagen II

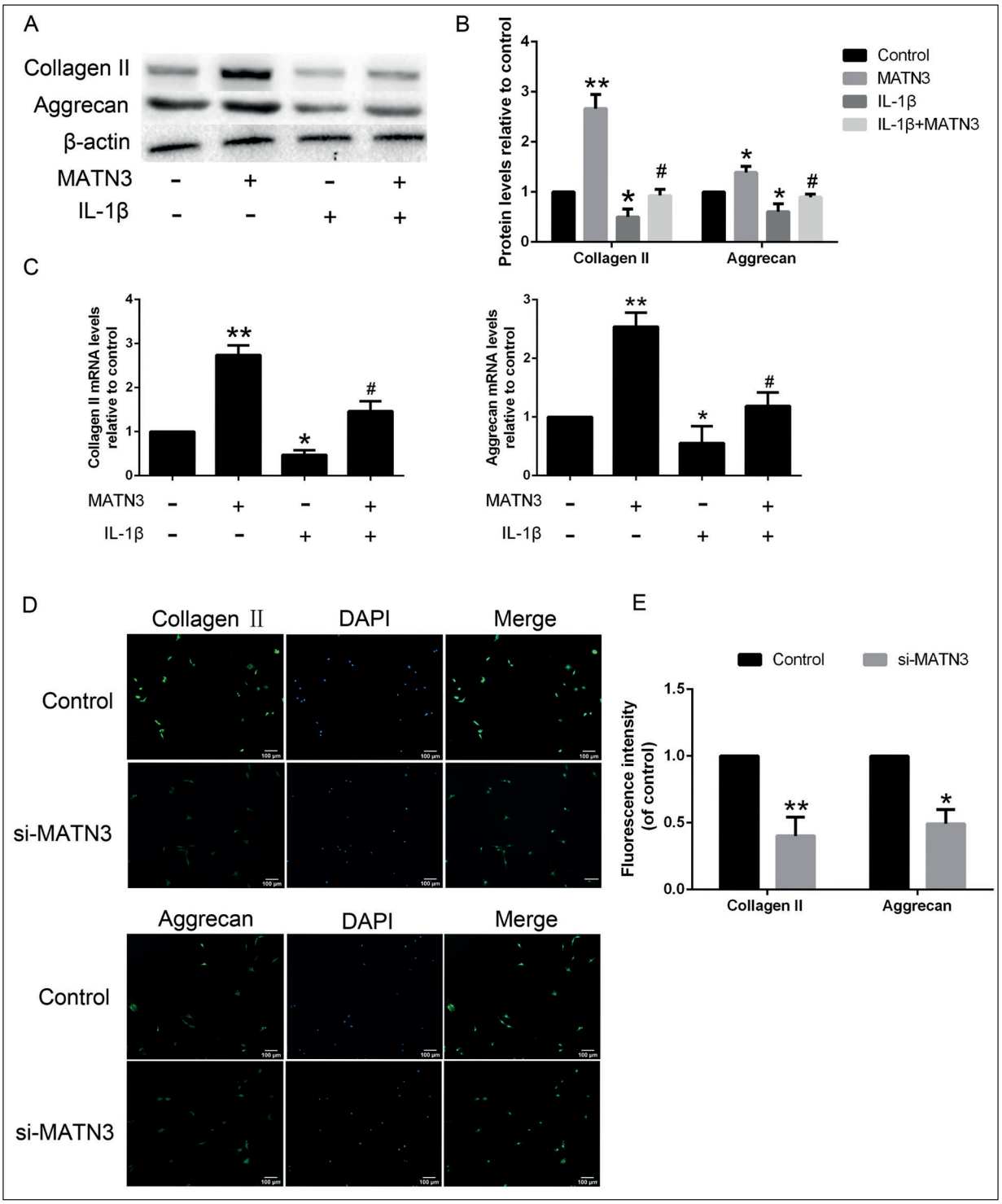

Figure 2. MATN3 enhances Collagen II and aggrecan expressions in human NP cells. NP cells are pretreated for 24 h with or without MATN3 (200 ng/ml) and IL-1β (10 ng/ml). **A, B,** The levels of Collagen II and aggrecan are assessed by Western blot (**A**) and quantification analysis (**B**). **C,** The mRNA expressions of Collagen II and aggrecan are assayed by RT-qPCR. **D, E,** The protein expression levels of Collagen II and aggrecan are determined by immunofluorescence and quantification analysis (magnification: 40×). The values are mean ± SD of three independent experiments. *p<0.05, **p<0.01 compared with control group; #p<0.05 compared with IL-1β group.

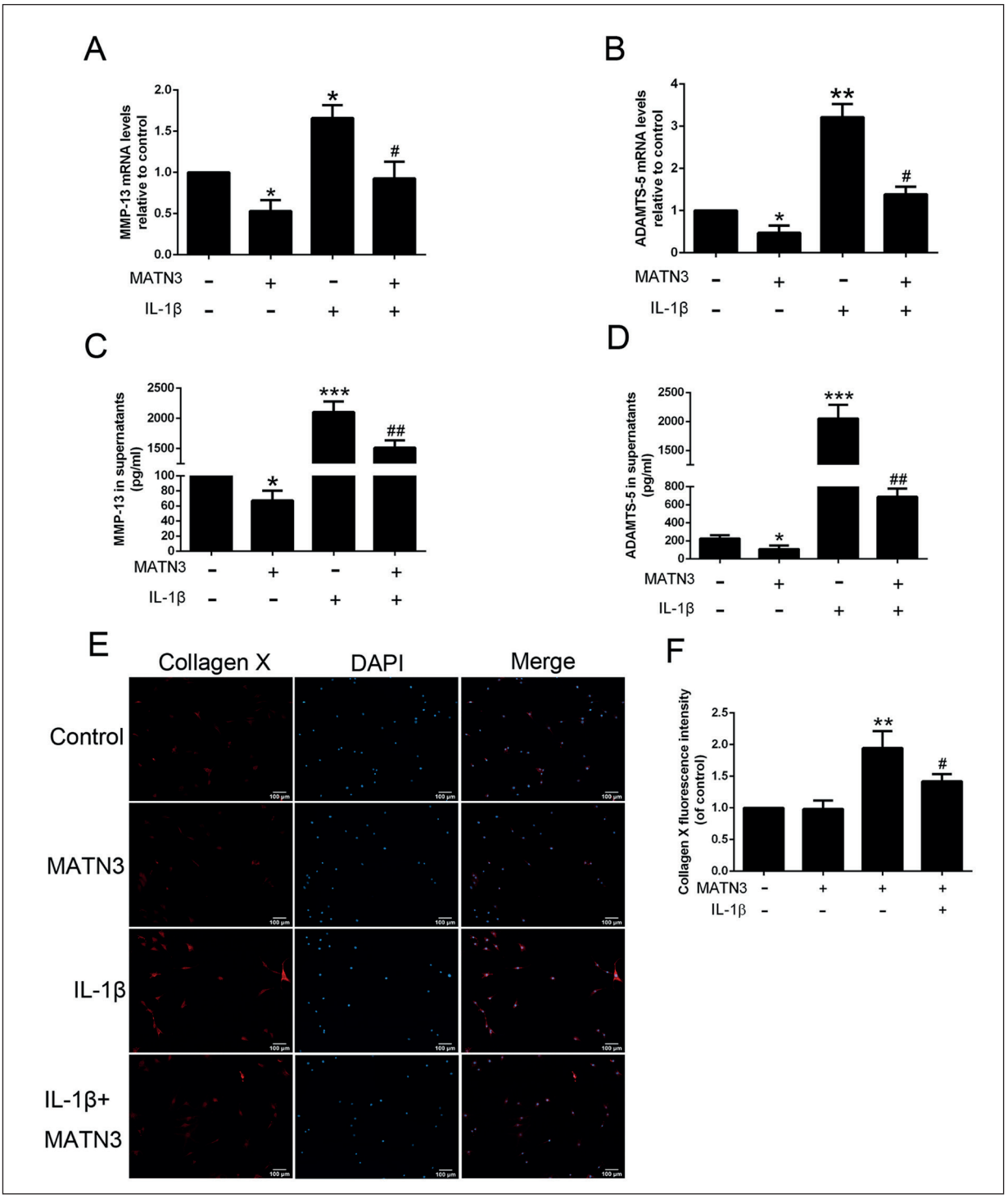

Figure 3.MATN3 inhibits MMP-13, ADAMTS-5 and Collagen X expression in human NP cells. NP cells are treated as mentioned above. **A-D,** The mRNA expression and protein levels in their cell media of MMP-13 and ADAMTS-5 are assayed by RT-qPCR (**A, B**) and ELISA (**C, D**), respectively. **E, F,** The protein expression level of Collagen X is determined by immunofluorescence and quantification analysis (magnification: 40×). The values are mean ± SD of three independent experiments. *p<0.05, **p<0.01, ***p<0.001 compared with control group; #p<0.05, ##p<0.01 compared with IL-1β group.

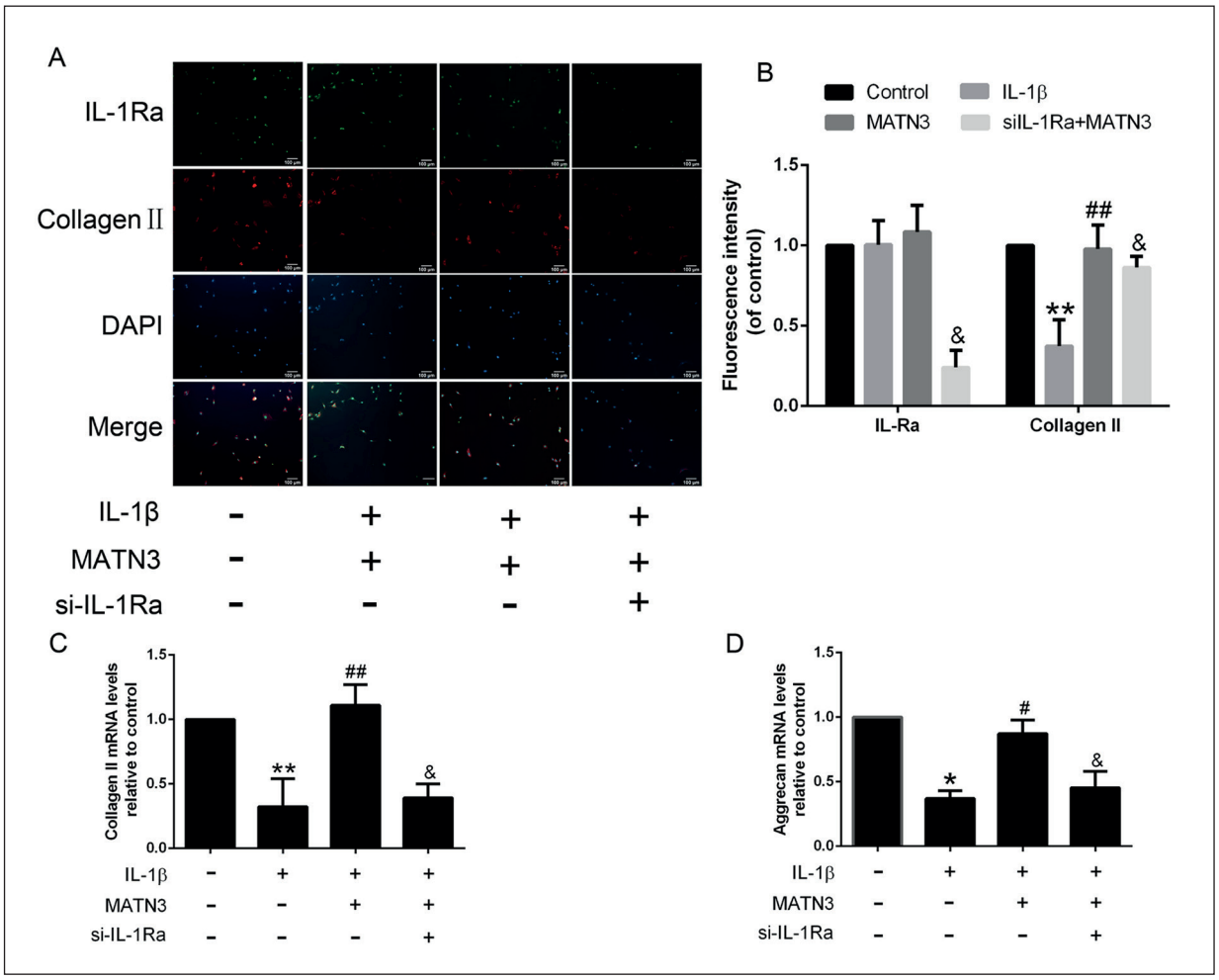

Figure 4. Silencing of IL-1Ra abolishes the protective effect of MATN3 on Collagen II and aggrecan in human NP cells. NP cells are pretreated with or without IL-1β (10 ng/ml) for 24 h followed by stimulation with MATN3 (200 nM) alone or MATN3 combined with IL-1Ra silenced NP cells. **A, B,** The protein expression levels of Collagen II and IL-1Ra are determined by immunofluorescence (magnification: 40×) (**A**) and quantification analysis (**B**). **C, D,** The mRNA levels of Collagen II and aggrecan are quantified by RT-qPCR. The values are mean ± SD of three independent experiments. *p<0.05, **p<0.01 compared with control group; #p<0.05, ##p<0.01 compared with IL-1β group; &p<0.05 compared with MATN3 group.

forms loose in the NP matrix by means of the joint. Reticulated structure resists the tension produced by proteoglycans and moisture. NP cells play a key leading role both in the normal physiological function of the intervertebral disc and in the pathological changes of IVDD. It is currently believed that disc degeneration begins in the NP, and the reduction in the number of NP cells and the abnormality of the ECM are key factors in the degeneration of the intervertebral disc[14]. At the level of cell biochemistry, the reduction of functional ECM reflects the imbalance between anabolism and catabolism of NP cells. With the decrease of proteoglycan in the matrix, the dehydration and high degree of NP reduce the bearing capacity of the intervertebral disc, thus affecting the biomechanics of the spine performance[15]. In-depth study on the biological characteristics of NP cells has important guiding significance for our research on the biological treatment of IVDD.

Matrilin-3, a member of Matrilin family, is a non-collagenous ECM protein that functions as an adaptor protein. Matrilin-3 protein is mainly detected in cartilaginous tissues, such as articular and epiphyseal cartilage, and in the cartilaginous anlage of developing bones[9,16]. In the cartilage matrix, Matrilin-3 binds to cartilage oligomeric matrix protein (COMP), Collagen II, Collagen XI, and proteoglycans, and acts as an extracellular matrix-binding protein to link various

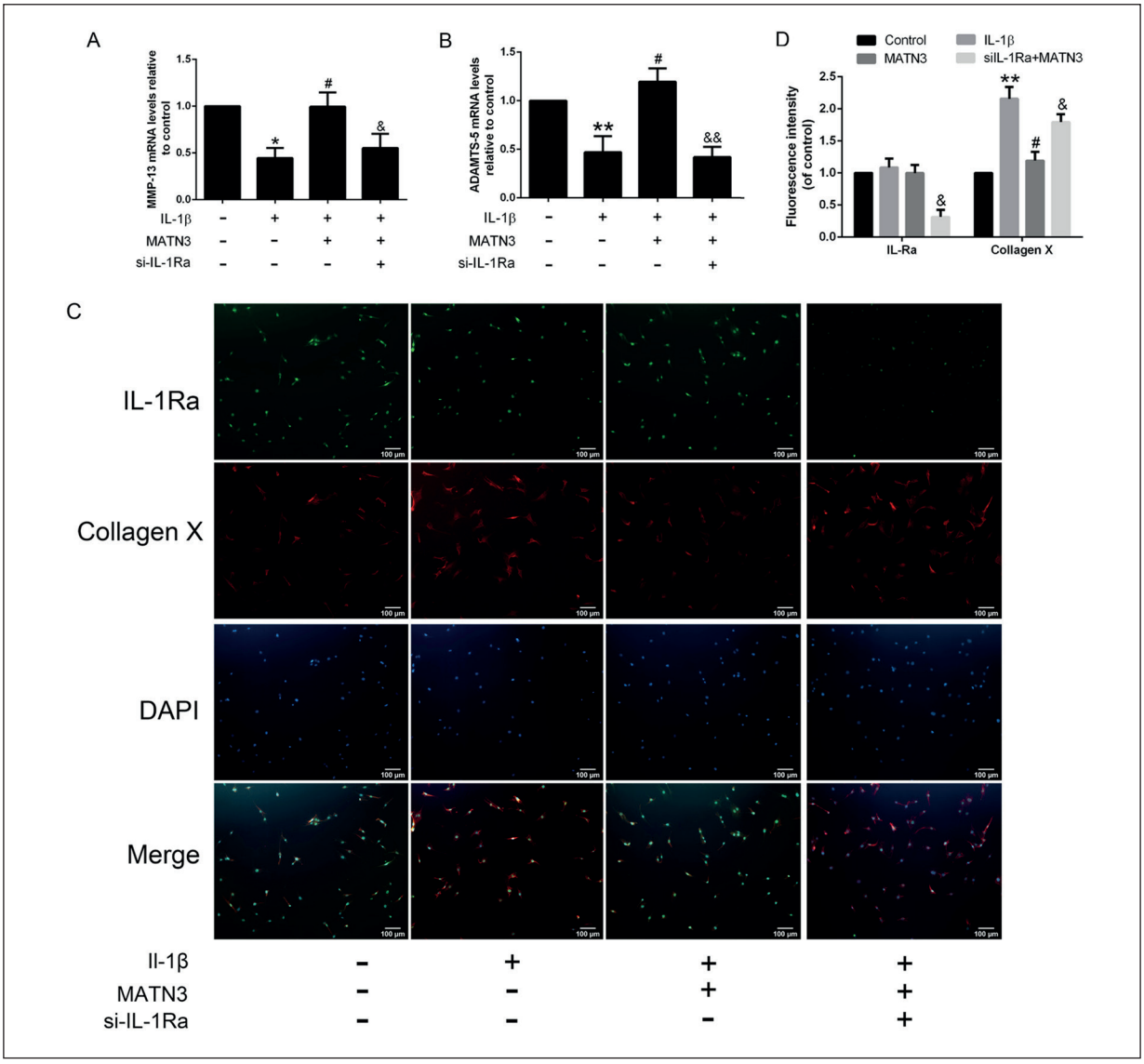

Figure 5. Silencing of IL-1Ra abolishes the suppression of MATN3 on MMP-13, ADAMTS-5 and Collagen X in human NP cells. NP cells are treated as mentioned previously. **A, B,** The mRNA levels of MMP-13 and ADAMTS-5 are determined using RT-qPCR. **C, D,** The protein expression levels of Collagen X and IL-1Ra are determined by immunofluorescence (**C**) and quantification analysis (magnification: 40×) (**D**). The values are mean ± SD of three independent experiments. *p<0.05, **p<0.01 compared with control group; #p<0.05 compared with IL-1β group; &p<0.05, &&p<0.01 compared with MATN3 group.

macromolecules to form a network structure[17]. The study of Matrilin-3 is mainly associated with the cartilage, but little is known about whether it also plays a role in the extracellular matrix of the intervertebral disc. In this study, it was hypothesized that Matrilin-3 may also be capable of preventing the IL-1β-induced NP cell degeneration. The results demonstrated that rhMATN3 protein induced gene expression of IL-1Ra in the human NP cells culture models. MATN3 promoted IL-1Ra expression with or without the presence of the inflammatory cytokine IL-1β. Besides, MATN3 stimulation increased the soluble IL-1Ra protein levels in NP cells culture medium which were determined by ELISA.

IL-1 induces the expression of a number of MMPs and ADAMTS family members and inhibits matrix synthesis[18]. IL-1Ra is potentially NP-protective since it inhibits IL-1β, which is a major stress and inflammatory cytokine that is closely associated with IVDD pathogenesis[19]. The concentration of soluble IL-1Ra decreases

with increasing grades. Therefore, finding a method to continuously stimulate IL-1Ra production in NP cells may provide a novel treatment strategy for IVDD by dampening inflammations. The results show here that MATN3 protein is capable of upregulating IL-1Ra levels in NP cells. To further test the protective effect of MATN3 in NP cells, the expression of ECM anabolic genes, such as Collagen II and aggrecan, and catabolic genes, such as MMP-13 and ADAMTS-5, in NP cells were examined. Recombinant human MATN3 protein increased Collagen II and aggrecan expression in spite of the presence of IL-1β. Conversely, the lack of MATN3 resulted in reduced basal expression of these ECM genes in Matrilin-3 silenced NP cells compared with the control one. Thus, Matrilin-3 is necessary to maintain normal expression of these genes in NP cells. In our experiments, MATN3 protein not only inhibited expression of catabolic genes induced by IL-1b, including MMP-13 and ADAMTS-5, but also suppressed the Collagen X level, which is closely related to IVDD pathogenesis. Collagen X is a member of the short-chain Collagens family, naturally presenting during endochondral ossification[20]. Collagen X mainly appears in degenerative discs associating with the calcification of the NP, while a young NP barely expresses Collagen X[21]. Since MATN3 inhibits the effects of IL-1β and upregulates IL-1Ra in NP cells, whether these two events are dependent were explored. IL-1Ra was knocked down by siRNA transfected. The Collagen II and aggrecan levels were significantly reduced in IL-1Ra silenced NP cells compared with the controls. Apart from this, treatment with MATN3 protein failed to significantly upregulate Collagen II and aggrecan under the stimuli of IL-1β. Besides, IL-1Ra silencing also abolished MATN3-induced decreases in MMP-13 and ADAMTS-5 mRNA levels in comparison with wild type. This suggests that the ability of Matrilin-3 to maintain the integrity of ECM depends on the presence of IL-1Ra.

The mechanism by which MATN3 regulates IL-1Ra is not totally understood. The findings of this study may provide evidence of several novel regulatory functions of Matrilin-3 in NP cells, including the promotion of IL-1Ra expression, upregulation of Collagen II, and aggrecan expression, and inhibition of the IL-1β-induced catabolic matrix proteases, which are potentially dependent on IL-1Ra. At the same time, this study provides a novel mechanism for IVDD treatment of Matrilin-3, which contributes to matrix synthesis and reduce inflammation, and it is possible to use MATN3 for inhibiting the development of IVDD in the human intervertebral disc.

Conclusions

The findings of this study point to a novel viewpoint of Matrilin-3 in the ECM stability of NP due to its ability to activate IL-1Ra. It is believed that MATN3 efficiently protects ECM degeneration of human NP cells related to maintain the content of Collagen II and aggrecan, as well as inflammatory inhibition.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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