Expression of matrix metalloproteinase–2 and –9 in human ligamentum flavum cells treated with tumor necrosis factor– α and interleukin-1 β

Bum-Joon Kim, MD, PhD,¹ Junseok W. Hur, MD, PhD,¹ Jong Soo Park, MD,²
Joo Han Kim, MD, PhD,¹ Taek-Hyun Kwon, MD, PhD,¹ Youn-Kwan Park, MD, PhD,¹ and
Hong Joo Moon, MD, PhD¹

¹Department of Neurosurgery, Korea University College of Medicine; and ²Department of Neurosurgery, Thejoeun Hospital, Seoul, Korea

OBJECT An in vitro study was performed to understand the potential roles of matrix metalloproteinase (MMP)–2 and MMP-9 in the elastin degradation of human ligamentum flavum (LF) cells via treatment with tumor necrosis factor– α (TNF α) and interleukin-1 β (IL-1 β). Previous studies have identified a decreased elastin to collagen ratio in hypertrophic LF. Among the extracellular matrix remodeling endopeptidases, MMP-2 and MMP-9 are known to have elastolytic activity. The hypothesis that activated LF cells exposed to inflammation would secrete MMP-2 and MMP-9, thereby resulting in elastin degradation, was examined.

METHODS To examine MMP-2 and MMP-9 expression in human LF, cells were isolated and cultured from LF tissues that were obtained during lumbar disc surgery. Isolated LF cells were equally divided into 3 flasks and subcultured. Upon cellular confluency, the LF cells were treated with TNF α , IL-1 β , or none (as a control) and incubated for 48 hours. The conditioned media were collected and assayed for MMP-2 and MMP-9 using gelatin zymography and Western blot analysis. The electrophoresis bands were compared on densitometric scans using ImageJ software.

RESULTS The conditioned media from the isolated human LF cells naturally expressed 72-kD and 92-kD gelatinolytic activities on gelatin zymography. The IL-1 β -treated LF cells presented sustained increases in the proenzyme/zymogen forms of MMP-2 and -9 (proMMP-2 and proMMP-9), and activeMMP-9 expression ($p = 0.001$, 0.022 , and 0.036 , respectively); the TNF α -treated LF cells showed the most elevated proMMP9 secretion ($p = 0.006$), as determined by Western blot analyses. ActiveMMP-2 expression was not observed on zymography or the Western blot analysis.

CONCLUSIONS TNF α and IL-1 β promote proMMP-2 and proMMP-9 secretion. IL-1 β appears to activate proMMP-9 in human LF cells. Based on these findings, selective MMP-9 blockers or antiinflammatory drugs could be potential treatment options for LF hypertrophy.

<http://thejns.org/doi/abs/10.3171/2015.6.SPINE141271>

KEY WORDS elastin degradation; ligamentum flavum cells; interleukin-1 β ; tumor necrosis factor– α ; matrix metalloproteinase–2; matrix metalloproteinase–9

HYPERTROPHIED ligamentum flavum (LF) is common in patients with lumbar spinal stenosis. A comparative decrease in the elastin to collagen ratio associated with hypertrophied LF has been described in several research studies.^{12,28,31} However, the exact mechanism of elastin degradation has not yet been established. The de-

creased elasticity of LF may result in folding within the spinal canal and contribute to spinal stenosis.^{12,31}

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases that are thought to play important roles in extracellular matrix (ECM) remodeling. These proteolytic effectors are involved in in vivo processes such as wound

ABBREVIATIONS ECM = extracellular matrix; EDTA = ethylenediaminetetraacetic acid; FBS = fetal bovine serum; IL-1 β = interleukin-1 β ; HBSS = Hank's balanced saline solution; LF = ligamentum flavum; MMP = matrix metalloproteinase; proMMP = proenzyme/zymogen form of MMP; P/S = penicillin/streptomycin; SDS-PAGE = sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TIMP = tissue inhibitor of the metalloproteinase; TNF α = tumor necrosis factor– α .

SUBMITTED December 17, 2014. **ACCEPTED** June 11, 2015.

INCLUDE WHEN CITING Published online November 13, 2015; DOI: 10.3171/2015.6.SPINE141271.

healing, inflammation, bone remodeling, metastasis, and angiogenesis.^{18,47} MMPs can be classified by their substrate affinity as collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs, or others.^{7,38,44} Among these, MMP-2 and MMP-9—also referred to as gelatinase A and gelatinase B—respectively, have elastolytic activity.^{10,18,36} Many studies related to cardiovascular disease have addressed the relationship between MMP-2 and MMP-9 with elastin degradation.^{1,26,27} Researchers identified that MMP-2 and MMP-9 knockout mice did not develop elastin degradation when subjected to CaCl₂-mediated aortic injury.^{1,16} Additionally, doxycycline, a nonspecific MMP inhibitor, has a preventive effect against the development of aortic aneurysm by elastin protection.^{2,27} Although the exact role of MMPs in inflammatory reactions is not clear, an increase or dysregulation of MMPs is frequently observed during inflammatory response.^{26,44,46} In lumbar degenerative diseases, inflammatory cytokines are thought to be involved in the pathomechanism of degeneration. An increase in inflammatory cytokines, including tumor necrosis factor- α (TNF α) and interleukin-1 β (IL-1 β), has been identified in herniated intervertebral discs.^{15,41} Additionally, Sairoyo et al. reported the expression of TNF α and IL-1 β genes in LF.³²

Thus, this study hypothesizes that MMPs play an important role in the elastin degradation of LF during the inflammation of LF cells. The expression and activity of MMP-2 and MMP-9 in cultured human LF cells in response to proinflammatory cytokines were examined using an in vitro model.

Methods

LF Cell Isolation and Culture

We conducted this study in accordance with the regulations of the institutional review board at Korea University Guro Hospital, and appropriate informed consent was obtained from all participating patients. Tissue samples were collected from 7 patients (5 men and 2 women) during their lumbar disc herniation surgeries. Human LF cells were isolated from the LF tissues of patients who did not show any hypertrophy on previous MR images. Patients who did not require a ligamentum flavectomy, patients with a previous history of surgery or steroid injection, patients with a suspected infection, and pediatric patients were excluded.

Tissue specimens were washed 3 times with sterile Hank's balanced saline solution (HBSS; Gibco-BRL) containing 1% penicillin/streptomycin (P/S; Gibco-BRL) in order to achieve pure isolation of LF cells. After removing contaminants like blood clots, the specimens were minced and digested for 60 minutes in Ham's F-12 media (Gibco-BRL) containing 1% P/S, 5% heat-inactivated fetal bovine serum (FBS; Gibco-BRL), and 0.2% pronase (Calbiochem); the specimens were then incubated overnight in 0.025% collagenase I (Roche Diagnostics). The LF cells were filtered through a sterile 70- μ m-pore nylon mesh to remove any remnant tissue debris and centrifuged at 224 g for 5 minutes. Approximately 3.0×10^5 LF cells were resuspended in Ham's F-12 culture media containing 10% FBS and 1% P/S and placed in a tissue culture flask (VWR

Scientific Products). The LF cells were incubated at 37°C in a humidified atmosphere of 5% carbon dioxide, and the culture medium was changed twice per week.

Treating LF Cells With Proinflammatory Cytokines

After an average incubation of 3 weeks, the LF cells were grown to full confluence and removed from the flask by treatment with 0.05% trypsin/ethylenediaminetetraacetic acid (EDTA; Gibco-BRL) for subculturing. The pellet aggregates were formed by centrifugation (5 minutes at 224 g) and resuspended in culture medium. At the second passage, the culture medium containing the isolated LF cells was equally divided into 3 flasks and reincubated for 2 more weeks until almost full confluence was reached. The culture flasks were washed 3 times with HBSS. Then, the control LF cells were reincubated for 48 hours in Ham's F-12 culture medium that contained 1% FBS and 1% P/S in the absence of proinflammatory cytokines. The other 2 flasks of LF cells were incubated for 48 hours in Ham's F-12 culture media containing 1% FBS and 1% P/S in the presence of 10 ng/ml recombinant human TNF α (R&D Systems) and 1 ng/ml recombinant human interleukin-1 β (R&D Systems), respectively. The concentration and exposure time of the proinflammatory cytokines were determined based on previous experimental data.²⁰

Collection of Conditioned Media and Protein Measurement

Following incubation with proinflammatory cytokines for 48 hours, the conditioned media were collected from the 3 LF cell culture flasks. The LF cells were removed from the flasks by treatment with 0.05% trypsin/EDTA. Approximately 5.0×10^6 LF cells were counted per sample. The conditioned media were centrifuged at 1578 g at 4°C for 5 minutes to remove cellular debris. The protein content of each supernatant was determined using the bicinchoninic acid assay (23227; Thermo Scientific). The conditioned media were stored at -80°C for Western blot analysis and gelatin zymography. Because the preliminary assay of the LF cell lysates revealed no notable difference in the expression levels of MMP-2 and MMP-9, further analysis of the cell lysates was not performed.

Gelatin Zymography

MMP-2 and MMP-9 enzymatic activity was measured in the conditioned media using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gelatin zymography. Equal amounts of protein from the conditioned media were diluted in nonreducing zymogram sample buffer (161-0764; Bio-Rad). The sample was then electrophoresed in 10% precast polyacrylamide gel with gelatin (161-1113; Bio-Rad) at 4°C. The gels were washed 3 times with 2.5% Triton X-100 and incubated in the presence of a renaturation buffer (161-0765; Bio-Rad) at 37°C for 1 hour with shaking. Subsequently, the gels were transferred to development buffer that contained 50 mmol/L Tris-HCl, 5 mmol/L CaCl₂, and 200 mmol/L NaCl (pH 7.5) and incubated overnight at 37°C. After rinsing in water the next day, each gel was stained with Coomassie blue (0.5% Coomassie blue in 30% ethanol/10% acetic acid) for 1 hour and de-stained for 10 minutes using an aque-

ous mixture of 30% ethanol and 10% acetic acid. The molecular size of each gelatinolytic band was estimated using known protein standards (Bio-Rad) and a positive control (HT1080 fibrosarcoma cells). MMP-2 and MMP-9 activity were determined by densitometric scanning of the bands using a flatbed photo scanner (Epson 1680 Pro), and analysis was performed using ImageJ 1.48p software (W. Rasband, National Institutes of Health; available at <http://rsb.info.nih.gov/ij/>).

Western Blot Analysis

Equal amounts of protein from the conditioned media (15 μ g) were heated, denatured in Laemmli sample buffer (161–0747; Bio-Rad), and subjected to reducing SDS-PAGE in precast gels (456–9034; Bio-Rad). After electrophoresis, the proteins were transferred to polyvinylidene fluoride membranes (Millipore Corp.) for 2 hours at 100 V. The membranes were blocked in 5% skim milk for 1 hour at room temperature and incubated overnight at 4°C with purified rabbit polyclonal antibody specific to MMP-2 (1:5000; ab37150, Abcam) and rabbit monoclonal antibody specific to MMP-9 (1:2000; ab76003, Abcam) in 5% skim milk in TBS-T (Tris-buffered saline with 0.1% Tween 20). After washing, the membranes were incubated with goat anti-rabbit polyclonal secondary antibody (1:20,000; ab6721, Abcam) for 1 hour at room temperature in 5% skim milk in TBS-T with shaking. The bands were visualized using an x-ray film processor (Konica SRX-101A). Each blot was repeated 2 times, and representative scans are presented.

Quantification and Statistical Analysis

The bands of the electrophoresed gels were digitized on a flatbed photo scanner and quantified using ImageJ 1.48p software. The integrated band densities were measured and analyzed using IBM SPSS Statistics 20 software (IBM Corp.). The box plots show the median, upper, and lower quartiles, as well as the maximum and minimum values. Repeated measures ANOVA was performed followed by paired t-test and Bonferroni correction to compare the differences between the stimulated and control groups, and p values < 0.05 were considered statistically significant.

Results

Identification of proMMP-2 and proMMP-9 in the Conditioned Media of LF Cells Using Gelatin Zymography

Gelatin zymography of the LF cell culture supernatants ($n = 7$) showed a sustained increase in proMMP-2 activity within TNF α - and IL-1 β -stimulated LF cells in comparison with the control samples. A representative zymography of LF samples is depicted in Fig. 1. An intense 72-kD digestive band, which is consistent with the proenzyme/zymogen form of MMP-2 (proMMP-2), was identified in every LF sample. The mean gelatinolytic activity of the proMMP-2 band measured by densitometric analysis was highest in IL-1 β -stimulated LF cells. LF cells treated with TNF α or IL-1 β also presented a faint 92-kD band, whereas the control showed only a single 72-kD band. In some stimulated LF cells, tiny 82-kD bands were visible to the naked eye. However, digitalization with the flatbed

FIG. 1. Representative gelatin zymography of human LF cells. An intense gelatinolytic 72-kD band was detected in every LF cell, and its intensity increased in response to inflammatory cytokines. LF cells that were treated with TNF α or IL-1 β showed an additional faint 92-kD band, which corresponds to proMMP-9.

scanner failed to detect the weak 82-kD band, and it was therefore not included in quantitative analyses.

The densitometric analysis shown in Fig. 2 shows the statistically significant variation in proMMP-2 expression as determined by repeated measures ANOVA ($p = 0.014$). However, the Bonferroni post hoc comparison failed to show statistical significance ($p = 0.062$). In contrast, the proMMP-9 bands were significantly different, and the IL-1 β -stimulated LF cells showed higher enzyme activity in comparison with the control cells ($p = 0.035$).

Effect of TNF α and IL-1 β on MMP-2 on Western Blot Analysis

Western blot analysis detected a single intense band with a molecular weight of 72 kD, consistent with the proMMP-2, in every LF cell sample. Both the TNF α - and IL-1 β -stimulated LF cells presented an overall increase in the expression of the proMMP-2 band in comparison with the control, which is consistent with the results of

FIG. 2. Densitometric analysis of gelatin zymography. The mean proMMP-2 and median proMMP-9 activities were highest in IL-1 β -stimulated LF cells. However, Bonferroni post hoc comparison was not significant ($p = 0.062$) for mean proMMP, and a significant difference was observed only between the IL-1 β and control groups ($p = 0.035$). For this box-and-whiskers plot, the *box* indicates the 25th to 75th percentile, the *middle line* indicates the median value, and the *whiskers* indicate the range after excluding outliers. *Diamonds* indicate outliers that are more than 1.5 times greater or less than the interquartile range.

gelatin zymography. For the densitometric analysis, the Bonferroni post hoc test revealed a significant difference between the control and IL-1 β -stimulated LF cells ($p = 0.001$). However, other comparisons did not show this statistical significance. The active form of MMP-2 was not detected on Western blot analysis.

Effect of TNF α and IL-1 β on MMP-9 on Western Blot Analysis

As shown in Fig. 3, 2 bands corresponding with proMMP-9 (92 kD) and activeMMP-9 (67 kD) were clearly identified in the majority of the LF samples. TNF α -stimulated LF cells showed the most intense proMMP-9 bands. The IL-1 β -stimulated LF cells presented 2 definite bands, with an activeMMP-9 band that was more intense than both that of the control and that of the TNF α -stimulated LF cells. In the densitometric analysis, the integrated density of proMMP-9 increased in both the TNF α - and IL-1 β -stimulated LF cells in comparison with the control cells ($p = 0.006$ and 0.022 , respectively), and the activeMMP-9 density was distinctly higher in IL-1 β -stimulated LF cells ($p = 0.036$), as shown in Fig. 4.

Discussion

The normal human LF is predominantly composed of elastic fibers and contains about 70% elastin.^{28,35,48} This elastin-rich tissue is hypertrophied by aging or pathological degeneration. LF hypertrophy exacerbates the tightness of the spinal canals in patients with spinal stenosis and can be partially responsible for sciatica in these patients.^{12,28,31} Currently, the only treatment for LF hypertrophy is resection. However, surgeries for spinal stenosis are a socioeconomic burden on patients and the community.

According to our literature review, MMP-2 and MMP-9 are thought to impact elastin degradation. Yasmin et al. identified a linear correlation between aortic stiffness and MMP-9 levels.⁴⁵ In cardiovascular diseases, many researchers have focused on the causal relationship between aortic aneurysms and MMP-2 and MMP-9 levels.^{10,27,39,45} In chronic obstructive lung diseases, Maclay et al. demonstrated that systemic elastin degradation, combined with the upregulation of MMP-2 and MMP-9, is associated with chronic obstructive lung diseases.¹⁷ Though human LF cells are composed of various cell types, cells from healthy LF are known to have fibroblast-like features,⁴⁰

FIG. 3. Representative Western blot of human LF cells. A single intense proMMP-2 band was detected in every LF cell sample, and its level was most elevated in IL-1 β -stimulated LF cells. Western blot revealed two 92-kD and 67-kD bands, which were most intense in TNF α - and IL-1 β -stimulated LF cells, respectively. Figure is available in color online only.

FIG. 4. Densitometric analysis of Western blots. IL-1 β -stimulated LF cells showed increased integrated density ($p = 0.001$). Compared with MMP-9, proMMP-9 was elevated in both TNF α - and IL-1 β -stimulated LF cells ($p = 0.006$ and 0.022 , respectively), and activeMMP-9 was higher in IL-1 β -stimulated LF cells ($p = 0.036$). For this box-and-whiskers plot, the box indicates the 25th to 75th percentile, the middle line indicates the median value, and the whiskers indicate the range after excluding outliers. Diamonds indicate outliers that are more than 1.5 times greater or less than the interquartile range.

and human fibroblasts secrete MMP-2 and MMP-9.¹¹ In this study, LF cells presented MMP-2 and MMP-9 activities, with a demonstrated increase in response to inflammatory cytokines. Based on these findings, it can be assumed that MMP-2 and MMP-9 play roles in elastin degradation in LF cells.

Inflammatory cytokines such as TNF α and IL-1 β can upregulate various ECM-degrading proteins and growth factors,^{9,33,34} even though the expression pattern differs for each cell type.¹³ Researchers have found that TNF α and IL-1 β activate MMP gene expression through the nuclear translocation of NF- κ B and mitogen-activated protein kinase stimulation of activator protein-1.⁴³ Inflammatory reaction is associated with elastin degradation in various in vivo situations. Previous research has demonstrated that TNF α and IL-1 β have inhibitory effects on elastin gene expression in lung fibroblasts.^{8,14} Considering that inflammatory cytokines are frequently observed in degenerative discs and LF,^{15,32,41} it could be inferred that TNF α and IL-1 β play roles in inflammatory ECM remodeling via both elastin degradation by inducing MMP-9 secretion and blocking elastin regeneration at the gene transcription level. Recent studies have identified that MMPs are related not only to ECM remodeling, but also angiogenesis and phagocytosis during inflammation,²⁶ and they regulate cellular functions and growth factors.²⁴ An MMP from a specific cell has multiple functions, and enzymatic functions may overlap with other MMPs, thereby making it difficult to define their exact roles.^{24,26}

MMPs are regulated in multiple steps as follows: transcription, translation, enzyme trafficking, proMMP activation, inhibition, and degradation.^{4,5,26,42,44} MMPs are

secreted as proMMP, which is catalytically inactive. proMMP can be activated by the cleavage or destabilization of the interaction between the thiol group of a prodomain cysteine residue and the zinc ion of the catalytic site (the "cysteine switch").^{24,26,42,46} The activation of proMMPs is regulated via coordination with a tissue inhibitor of the metalloproteinase (TIMP) species. MMP-2 is known to be activated by the interaction between MT1-MMP and TIMP-2.³ proMMPs can also be activated by other MMPs, various proteases, and chemical agents such as plasmin, amino phenyl mercuric acid, and SDS.^{3,44}

According to previous studies, MMP-3 (stromelysin-1) activates proMMP-9,^{22,29,30} and Le Maitre et al. demonstrated in an in vitro study that IL-1 treatment enhances MMP-3 gene expression in disc cells.¹⁵ In this current study, IL-1 β treatment resulted in a marked increase in activeMMP-9. Thus, it is possible that IL-1 β activated proMMP-9 by inducing the secretion of MMP-3 in LF cells. In contrast, proMMP-2 expression substantially increased in response to both TNF α and IL-1 β . However, the active form of MMP-2 was not observed on zymography or Western blot analysis. Although proMMP-2 secretion increased in response to inflammation cytokines, it was not activated by stimulation using TNF α or IL-1 β alone. During proMMP-9 activation, cleavage of the 92-kD enzyme can result in an 82- to 83-kD active product or 64- to 67-kD final active product.^{23,37} In this study, only the 67-kD form was detected by our Western blot analysis. Moreover, other researchers have observed that MMP-2 can activate MMP-9.^{6,7} It is possible that MMP-9 can be completely activated without the activation of MMP-2 in human LF cells.

TABLE 1. Baseline characteristics of patients*

Case No.	Age (yrs), Sex	Level	Smoking	Nonsteroidal Antiinflammatory Drugs	Duration of Symptoms	Response to Inflammatory Cytokines
1	36, F	L4–5	No	4 mos	7 mos	Weak proMMP-2 band in response to TNF α & weak active MMP-9 band in response to IL-1 β
2	54, M	L2–3	No	1 wk	1 wk	
3	33, M	L4–5	3 pack-yrs	3 mos	3 mos	
4	45, M	L4–5	20 pack-yrs	No	3 mos	
5	51, M	L5–S1	20 pack-yrs	2 mos	1 yr	Weak proMMP-2 band in response to IL-1 β & weak active MMP-9 in response to IL-1 β
6	31, F	L4–5	No	2 mos	4 mos	Weak proMMP-9 in response to TNF α
7	28, M	L5–S1	No	1 mo	1 mos	

* No patient had experienced trauma, had diabetes, or was taking oral steroids.

Initially, we aimed to perform a comparative study between nonhypertrophied LF tissue from patients with lumbar disc herniation and hypertrophied LF tissue from patients with lumbar spinal stenosis. However, in the primary culturing of LF cells, the hypertrophied LF tissue was found to have scanty and unhealthy LF cells. After repeated failed attempts to isolate and culture pure, healthy LF cells from patients with stenosis, we realized that hypertrophied LF is not an appropriate source of LF cells for culturing. Therefore, although we agree that it would have provided interesting findings, the results of the LF cells from patients with stenosis were not included in this study.

In this study, it was determined that human LF cells constitutively secrete MMP-2 and MMP-9, which are up-regulated by inflammatory cytokines. Moreover, MMP-9 regulation appeared to be associated with TNF α and IL-1 β . TNF α -stimulated LF cells showed the highest expression of proMMP-9, and IL-1 β -stimulated LF cells showed the significantly increased expression of activeMMP-9 in comparison with control cells. These results demonstrate that TNF α stimulation can upregulate proMMP-9 secretion in human LF cells during the presecretion step, whereas IL-1 β plays a role in the activation cascade of proMMP-9 in LF cells. We coincidentally observed that LF cells obtained from patients who had preoperative symptoms for a relatively longer duration showed weak responses to inflammatory cytokines, although this finding could not be further analyzed because of the small sample size of our study (Table 1). A detailed study with a larger sample size should be conducted to confirm the changes in the sensitivity of LF cells to inflammatory cytokines according to the duration of preoperative symptoms.

The results of this experiment must be carefully interpreted in light of several potential limitations, particularly because the function of MMP in vivo is quite complex and not yet fully understood. One explanation for the role of MMP-9 in the elastin degradation of LF cells is direct cleavage and remodeling by activeMMP-9, since MMP-9 itself has the ability to digest elastin. Another possible pathomechanism occurs by means of TGF- β 1. Both MMP-2 and MMP-9 enhance TGF- β 1 activity.^{19,24,49} TGF- β 1 is known to be associated with fibrosis and LF hypertrophy.^{21,25,31} Sairyo et al. demonstrated that LF hypertrophy is associated with scarring from inflammatory

reactions.^{31,32} It is important to understand the roles and cascades of MMPs in human LF cells because theoretically MMPs could be potential treatment targets for spinal stenosis. Additional histochemical and genotypic in vivo studies on inflammatory cytokines and MMPs are warranted in order to better understand the role of MMP-9 in elastin degradation.

Conclusions

The effects of TNF α and IL-1 β on MMP-2 and MMP-9 expression in human LF cells were evaluated using cell culture methods. The findings of this study provide evidence that human LF cells constitutively secrete MMP-2 and MMP-9 and demonstrate a sustained increase in proMMP-9 secretion in response to TNF α stimulation, whereas treatment using IL-1 β showed the distinct activation of MMP-9. This suggests that inflammatory cytokines such as TNF α and IL-1 β are involved in the MMP-9 pathway in human LF cells, which is a potential treatment strategy for LF hypertrophy.

References

- Basalyga DM, Simionescu DT, Xiong W, Baxter BT, Starcher BC, Vyavahare NR: Elastin degradation and calcification in an abdominal aorta injury model: role of matrix metalloproteinases. *Circulation* **110**:3480–3487, 2004
- Boyle JR, McDermott E, Crowther M, Wills AD, Bell PR, Thompson MM: Doxycycline inhibits elastin degradation and reduces metalloproteinase activity in a model of aneurysmal disease. *J Vasc Surg* **27**:354–361, 1998
- Brinckerhoff CE, Matrisian LM: Matrix metalloproteinases: a tail of a frog that became a prince. *Nat Rev Mol Cell Biol* **3**:207–214, 2002
- Chakraborti S, Mandal M, Das S, Mandal A, Chakraborti T: Regulation of matrix metalloproteinases: an overview. *Mol Cell Biochem* **253**:269–285, 2003
- Dreier R, Grässel S, Fuchs S, Schaumburger J, Bruckner P: Pro-MMP-9 is a specific macrophage product and is activated by osteoarthritic chondrocytes via MMP-3 or a MT1-MMP/MMP-13 cascade. *Exp Cell Res* **297**:303–312, 2004
- Fridman R, Toth M, Peña D, Mobashery S: Activation of progelatinase B (MMP-9) by gelatinase A (MMP-2). *Cancer Res* **55**:2548–2555, 1995
- Ghajar CM, George SC, Putnam AJ: Matrix metalloproteinase control of capillary morphogenesis. *Crit Rev Eukaryot Gene Expr* **18**:251–278, 2008

8. Kähäri VM, Chen YQ, Bashir MM, Rosenbloom J, Uitto J: Tumor necrosis factor- α down-regulates human elastin gene expression. Evidence for the role of AP-1 in the suppression of promoter activity. **J Biol Chem** **267**:26134–26141, 1992
9. Kato T, Haro H, Komori H, Shinomiya K: Sequential dynamics of inflammatory cytokine, angiogenesis inducing factor and matrix degrading enzymes during spontaneous resorption of the herniated disc. **J Orthop Res** **22**:895–900, 2004
10. Katsuda S, Okada Y, Okada Y, Imai K, Nakanishi I: Matrix metalloproteinase-9 (92-kD gelatinase/type IV collagenase equals gelatinase B) can degrade arterial elastin. **Am J Pathol** **145**:1208–1218, 1994
11. Kobayashi T, Hattori S, Shinkai H: Matrix metalloproteinases-2 and -9 are secreted from human fibroblasts. **Acta Derm Venereol** **83**:105–107, 2003
12. Kosaka H, Sairyo K, Biyani A, Leaman D, Yeasting R, Higashino K, et al: Pathomechanism of loss of elasticity and hypertrophy of lumbar ligamentum flavum in elderly patients with lumbar spinal canal stenosis. **Spine (Phila Pa 1976)** **32**:2805–2811, 2007
13. Kossakowska AE, Edwards DR, Prusinkiewicz C, Zhang MC, Guo D, Urbanski SJ, et al: Interleukin-6 regulation of matrix metalloproteinase (MMP-2 and MMP-9) and tissue inhibitor of metalloproteinase (TIMP-1) expression in malignant non-Hodgkin's lymphomas. **Blood** **94**:2080–2089, 1999
14. Kuang PP, Goldstein RH: Regulation of elastin gene transcription by interleukin-1 beta-induced C/EBP beta isoforms. **Am J Physiol Cell Physiol** **285**:C1349–C1355, 2003
15. Le Maitre CL, Freemont AJ, Hoyland JA: The role of interleukin-1 in the pathogenesis of human intervertebral disc degeneration. **Arthritis Res Ther** **7**:R732–R745, 2005
16. Longo GM, Xiong W, Greiner TC, Zhao Y, Fiotti N, Baxter BT: Matrix metalloproteinases 2 and 9 work in concert to produce aortic aneurysms. **J Clin Invest** **110**:625–632, 2002
17. MacLay JD, McAllister DA, Rabinovich R, Haq I, Maxwell S, Hartland S, et al: Systemic elastin degradation in chronic obstructive pulmonary disease. **Thorax** **67**:606–612, 2012
18. Matrisian LM: The matrix-degrading metalloproteinases. **BioEssays** **14**:455–463, 1992
19. Mohan R, Chintala SK, Jung JC, Villar WV, McCabe F, Russo LA, et al: Matrix metalloproteinase gelatinase B (MMP-9) coordinates and effects epithelial regeneration. **J Biol Chem** **277**:2065–2072, 2002
20. Moon HJ, Kim JH, Lee HS, Chotai S, Kang JD, Suh JK, et al: Annulus fibrosus cells interact with neuron-like cells to modulate production of growth factors and cytokines in symptomatic disc degeneration. **Spine (Phila Pa 1976)** **37**:2–9, 2012
21. Nakatani T, Marui T, Hitora T, Doita M, Nishida K, Kurosaka M: Mechanical stretching force promotes collagen synthesis by cultured cells from human ligamentum flavum via transforming growth factor- β 1. **J Orthop Res** **20**:1380–1386, 2002
22. Ogata Y, Enghild JJ, Nagase H: Matrix metalloproteinase 3 (stromelysin) activates the precursor for the human matrix metalloproteinase 9. **J Biol Chem** **267**:3581–3584, 1992
23. Okada Y, Gonoji Y, Naka K, Tomita K, Nakanishi I, Iwata K, et al: Matrix metalloproteinase 9 (92-kDa gelatinase/type IV collagenase) from HT 1080 human fibrosarcoma cells. Purification and activation of the precursor and enzymic properties. **J Biol Chem** **267**:21712–21719, 1992
24. Page-McCaw A, Ewald AJ, Werb Z: Matrix metalloproteinases and the regulation of tissue remodelling. **Nat Rev Mol Cell Biol** **8**:221–233, 2007
25. Park JB, Chang H, Lee JK: Quantitative analysis of transforming growth factor-beta 1 in ligamentum flavum of lumbar spinal stenosis and disc herniation. **Spine (Phila Pa 1976)** **26**:E492–E495, 2001
26. Parks WC, Wilson CL, López-Boado YS: Matrix metalloproteinases as modulators of inflammation and innate immunity. **Nat Rev Immunol** **4**:617–629, 2004
27. Petrinec D, Liao S, Holmes DR, Reilly JM, Parks WC, Thompson RW: Doxycycline inhibition of aneurysmal degeneration in an elastase-induced rat model of abdominal aortic aneurysm: preservation of aortic elastin associated with suppressed production of 92 kD gelatinase. **J Vasc Surg** **23**:336–346, 1996
28. Postacchini F, Gumina S, Cinotti G, Perugia D, DeMartino C: Ligamenta flava in lumbar disc herniation and spinal stenosis. Light and electron microscopic morphology. **Spine (Phila Pa 1976)** **19**:917–922, 1994
29. Ramos-DeSimone N, Hahn-Dantona E, Siple J, Nagase H, French DL, Quigley JP: Activation of matrix metalloproteinase-9 (MMP-9) via a converging plasmin/stromelysin-1 cascade enhances tumor cell invasion. **J Biol Chem** **274**:13066–13076, 1999
30. Rosenberg GA, Cunningham LA, Wallace J, Alexander S, Estrada EY, Grossetete M, et al: Immunohistochemistry of matrix metalloproteinases in reperfusion injury to rat brain: activation of MMP-9 linked to stromelysin-1 and microglia in cell cultures. **Brain Res** **893**:104–112, 2001
31. Sairyo K, Biyani A, Goel V, Leaman D, Booth R Jr, Thomas J, et al: Pathomechanism of ligamentum flavum hypertrophy: a multidisciplinary investigation based on clinical, biomechanical, histologic, and biologic assessments. **Spine (Phila Pa 1976)** **30**:2649–2656, 2005
32. Sairyo K, Biyani A, Goel VK, Leaman DW, Booth R Jr, Thomas J, et al: Lumbar ligamentum flavum hypertrophy is due to accumulation of inflammation-related scar tissue. **Spine (Phila Pa 1976)** **32**:E340–E347, 2007
33. Sarén P, Welgus HG, Kovanen PT: TNF- α and IL-1 β selectively induce expression of 92-kDa gelatinase by human macrophages. **J Immunol** **157**:4159–4165, 1996
34. Sato T, Ito A, Ogata Y, Nagase H, Mori Y: Tumor necrosis factor α (TNF α) induces pro-matrix metalloproteinase 9 production in human uterine cervical fibroblasts but interleukin 1 α antagonizes the inductive effect of TNF α . **FEBS Lett** **392**:175–178, 1996
35. Schröder PK, Grob D, Rahn BA, Cordey J, Dvorak J: Histology of the ligamentum flavum in patients with degenerative lumbar spinal stenosis. **Eur Spine J** **8**:323–328, 1999
36. Senior RM, Griffin GL, Fliszar CJ, Shapiro SD, Goldberg GI, Welgus HG: Human 92- and 72-kilodalton type IV collagenases are elastases. **J Biol Chem** **266**:7870–7875, 1991
37. Shapiro SD, Fliszar CJ, Broekelmann TJ, Mecham RP, Senior RM, Welgus HG: Activation of the 92-kDa gelatinase by stromelysin and 4-aminophenylmercuric acetate. Differential processing and stabilization of the carboxyl-terminal domain by tissue inhibitor of metalloproteinases (TIMP). **J Biol Chem** **270**:6351–6356, 1995
38. Snoek-van Beurden PA, Von den Hoff JW: Zymographic techniques for the analysis of matrix metalloproteinases and their inhibitors. **Biotechniques** **38**:73–83, 2005
39. Southgate KM, Davies M, Booth RF, Newby AC: Involvement of extracellular-matrix-degrading metalloproteinases in rabbit aortic smooth-muscle cell proliferation. **Biochem J** **288**:93–99, 1992
40. Specchia N, Pagnotta A, Gigante A, Logroscino G, Toesca A: Characterization of cultured human ligamentum flavum cells in lumbar spine stenosis. **J Orthop Res** **19**:294–300, 2001
41. Takahashi H, Suguro T, Okazima Y, Motegi M, Okada Y, Kakiuchi T: Inflammatory cytokines in the herniated disc of the lumbar spine. **Spine (Phila Pa 1976)** **21**:218–224, 1996
42. Van Wart HE, Birkedal-Hansen H: The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. **Proc Natl Acad Sci U S A** **87**:5578–5582, 1990

43. Vincenti MP, Brinckerhoff CE: Early response genes induced in chondrocytes stimulated with the inflammatory cytokine interleukin-1 β . **Arthritis Res** **3**:381–388, 2001
44. Visse R, Nagase H: Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. **Circ Res** **92**:827–839, 2003
45. Yasmin WS, McEniery CM, Wallace S, Dakham Z, Pulsalkar P, Maki-Petaja K, et al: Matrix metalloproteinase-9 (MMP-9), MMP-2, and serum elastase activity are associated with systolic hypertension and arterial stiffness. **Arterioscler Thromb Vasc Biol** **25**:372–378, 2005 (Erratum in **Arterioscler Thromb Vasc Biol** **25**:875, 2005)
46. Warner RL, Bhagavathula N, Nerusu KC, Lateef H, Younkin E, Johnson KJ, et al: Matrix metalloproteinases in acute inflammation: induction of MMP-3 and MMP-9 in fibroblasts and epithelial cells following exposure to pro-inflammatory mediators in vitro. **Exp Mol Pathol** **76**:189–195, 2004
47. Woessner JF Jr: Matrix metalloproteinases and their inhibitors in connective tissue remodeling. **FASEB J** **5**:2145–2154, 1991
48. Yahia LH, Garzon S, Strykowski H, Rivard CH: Ultrastructure of the human interspinous ligament and ligamentum flavum. A preliminary study. **Spine (Phila Pa 1976)** **15**:262–268, 1990
49. Yu Q, Stamenkovic I: Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF- β and promotes tumor invasion and angiogenesis. **Genes Dev** **14**:163–176, 2000

Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Moon. Acquisition of data: BJ Kim. Analysis and interpretation of data: BJ Kim. Drafting the article: BJ Kim. Critically revising the article: Hur, JS Park, JH Kim, Kwon, YK Park.

Correspondence

Hong Joo Moon, Department of Neurosurgery, Korea University College of Medicine, 148 Gurodong-ro, Guro-gu, Seoul 152-703, Korea. email: vagusmoon@gmail.com.