MATRIX METALLOPROTEINASE MMP12 IS ASSOCIATED WITH INTERVERTEBRAL DISC DEGENERATION

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INTRODUCTION: Intervertebral disc (IVD) degeneration is associated with low back pain. However, the molecular changes during the degeneration process is not entirely clear. The loss of nucleus pulposus (NP) integrity is one of the early events of the degeneration. Chondrogenic markers, such as SOX9 and aggrecan, have been commonly used to assess the degree of IVD degeneration. Recent transcriptomic studies have proposed several other candidates that may mark IVD degeneration. These include cartilage oligomeric matrix protein (COMP), matrix gla protein (MGP)[1], fibulin 1 (FBLN1) [2], cytokeratin 18 (KRT18) [3], cadherin-2 (CDH2) [3], cytokeratin 19 (KRT19) [2], and Runt-related transcription factor 2 (RUNX2) [4]. Studies also demonstrated that degenerated NP attains a fibrocartilaginous phenotype [5,6] with increased collagen stiffness and diameter, which can be rescued by the transplantation of mesenchymal stem cells [7], suggesting fibrotic events may play a role in IVD degeneration. This study aims to explore whether profibrotic and fibrosis markers, including COL1A1, COL3A1, FN1, HSP47, and MMP12, may serve as indicators of IVD degeneration in human through a comparative analysis with the conventional and recently proposed IVD degeneration markers. The association between IVD degeneration and activities of myofibroblasts, an important mediator of tissue fibrosis, was also investigated.

METHODS**:** Human IVD were collected from eleven patients with disc degeneration (graded IV-V at the Schneiderman scale) undergoing discectomy, and from eight patients undergoing corrective scoliosis surgery (as non-degenerative control) in the Duchess of Kent Children僂 Hospital in Hong Kong with informed patient consent and corresponding approval from institutional review board (IRB). RNA was isolated from human NP cells derived from degenerated discs graded IV-V at the Schneiderman scale (n=4) and non-degenerated (scoliosis) discs (n=3). Quantitative real-time PCR was performed on a Stepone Plus system (Applied Biosystems, USA) to determine the expression of three gene categories. The first group of traditional chondrogenic genes included ACAN and SOX9. The second group of potential NP degeneration indicators included CDH2, KRT19, KRT18, FBLN1, MGP and COMP. The third group of fibrosis related genes included COL1A1, COL3A1, FN1, HSP47, and MMP12. GAPDH was used as the endogenous control, and the relative quantification was achieved by comparative CT (cycle threshold) method. The co-expression of MMP12 and myofibroblast-related markers, including fibroblast specific protein 1 (FSP1) and ?-smooth muscle actin (?-SMA), were investigated by immunostaining in non-degenerated and degenerated human IVD, and in rodent IVD undergoing natural ageing (6m to 24m) or degeneration, induced by 25G needle puncture through annulus fibrosus (AF), from pre-injury to 8 weeks post injury. MMP12 positivity was determined as the number of MMP12 positive cells divided by the total number of cells per whole section.

RESULTS: KRT19 and MMP12 showed the most significant association with NP degeneration, where MMP12 was upregulated 6.7 fold (670%), and KRT19 was decreased -500 fold (0.002%) in degenerated NP cells compared to non-degenerated NP cells. The expression levels of

COL1A1, FBLN1 and MGP were upregulated, while ACAN, SOX9 and KRT18 were downregulated, in the degenerated NP cells compared to non-degenerated NP cells but the differences were not statistically significant. The expression level of CDH2 was found to be similar between the degenerated and non-degenerated samples.
Immunohistochemical analysis confirmed that both degenerated and non-degenerated human IVD have MMP12 positive cells (Figure 1A). Most of the MMP12 signal was intracellular, but was also occasionally detected in the territorial matrix. The percentage of MMP12 positive cells in degenerated human IVD was 3.7 fold of that in non-degenerated human IVD. Puncture-induced rat disc injury elevated the number of MMP12(+) cells in the peripheral NP and in the AF (Figure 1B). Aged mice also showed higher MMP12 positivity in the NP compared to young mice (Figure 1C). In addition, the positivity of ?-SMA, a marker of myofibroblast, was also increased in degenerated human IVD compared to non-degenerated human IVD (Figure 2A). Double immunofluorescence revealed that the MMP12 positive cells were co-expressed with ?-SMA and FSP1 (Figure 2B), indicating that the increased MMP12 positivity in the degenerated IVD could be related to the infiltration or activation of myofibroblasts.

DISCUSSION**:** Our study in human and animal models suggest that a loss of KRT19 expression and an increase of cellular MMP12 expression in the NP is characteristic of disc degenerative changes. MMP12 positivity in disc cells correlates with enhanced expression of the markers known to be expressed by myofibroblasts, including ?-SMA and FSP1, supporting that infiltration/ activation of myofibroblasts and fibrotic events are involved in IVD degeneration. MMP12 is found to be upregulated in many types of diseases, such as rheumatoid arthritis and chronic obstructive pulmonary disease. The over-expression or depletion of MMP12 has been shown to regulate the expression of other MMPs. Interestingly, MMP12 is recently found to be a transcription factor involved in cell immunity against viral infection. Our findings therefore imply an unidentified impact of MMP12 on disc cell homeostasis, which awaits further investigation.

SIGNIFICANCE**:** This study identifies the profibrotic mediator MMP12 as a novel IVD degeneration indicator, and implies that pathogenesis of IVD degeneration involves an enhanced activity of myofibroblasts. Our findings signify a relationship between IVD degeneration and fibrotic events in NP.

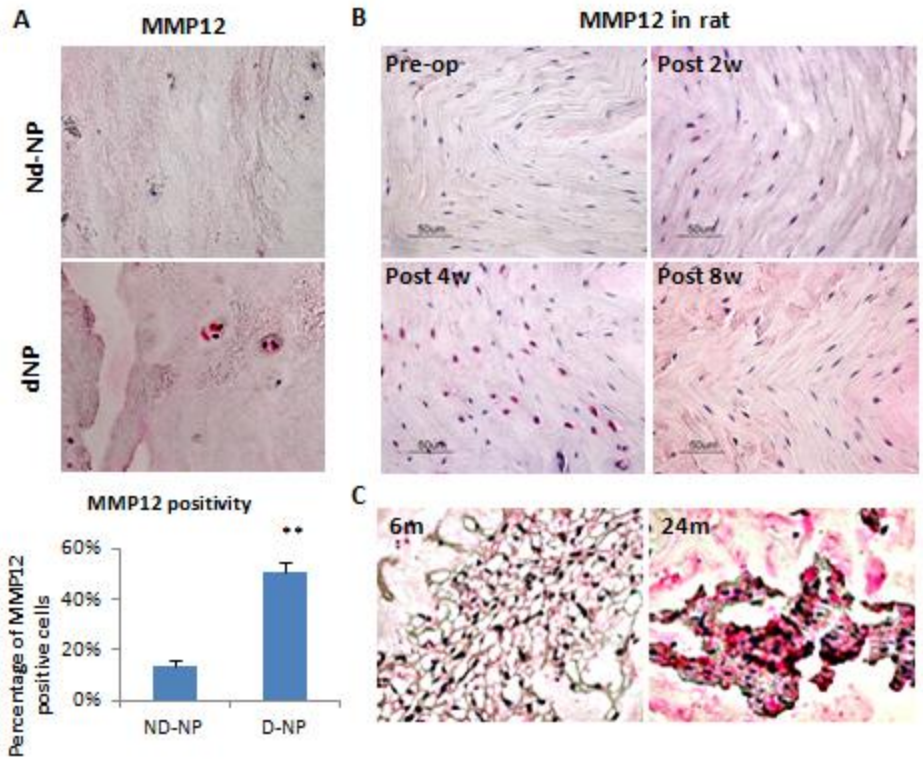

Figure 1. MMP12 expression is upregulated in degenerated IVD. (A) Detection of increased MMP12(+) cells in human degenerated compared to non-degenerated NP samples. The percentage of MMP12(+) cells is significantly higher in d-NP compared to Nd-NP. **(B)** MMP12 expression increased in the AF region in rats with induced IVD degeneration post needle puncture. The signals were visualized by Fast Red (rose color **(C)** Aged mice (24m) had increased MMP12 expression compared to young mice (6m). NP: nucleus pulposus. AF: annulsus fibrosus. Nd-NP: non-degenerated NP. dNP: degenerated NP. MMP12: matrix metalloproteinase 12.

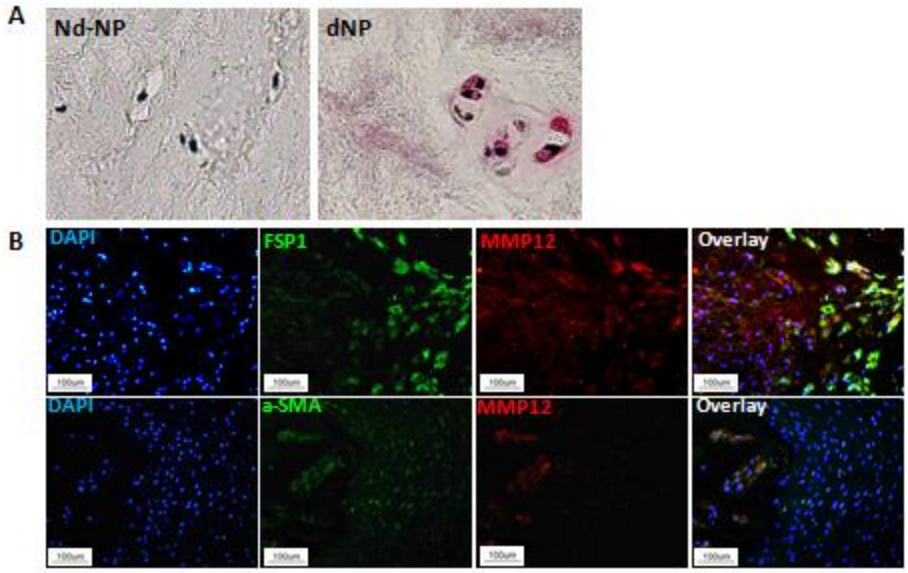

Figure 2. MMP12 expression is accompanied with increased a-SMA expression in degenerated IVD. (A) Detection of increased α-SMA(+) cells in human degenerated compared to non-degenerated NP samples. (B) Detection of co-expression of MMP12 with FSP1 and α-SMA in rat punctured IVD. NP: nucleus pulposus. Nd-NP: non-degenerated NP. dNP: degenerated NP. MMP12: matrix metalloproteinase 12. a-SMA: α-smooth muscle actin. FSP1: fibroblast specific protein 1.

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