Novel Transcriptional Roles of Matrix Metalloproteinase-13 in Osteoarthritis: More Than a Cartilage-Degrading Enzyme

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INTRODUCTION: Osteoarthritis (OA) is the most common degenerative joint disorder and a leading cause of disability in aging populations [1]. Progressive degradation of the articular cartilage represents the most recognized change in OA. Matrix metalloproteinase-13 (MMP-13) has been shown to be a primary collagenase and aggrecanase responsible for cartilage collagen turnover and has long been regarded as a secreted protease restricted to extracellular degradation [2]. Therefore, it has served as one of the promising targets for treating OA. Interestingly, our recent work discovered an increased nuclear localization of MMP-13 in OA chondrocytes, when compared to healthy chondrocytes. Given the fact that other MMPs, such as MMP-14, modulate gene expression by interacting with transcriptional factors [3], we hypothesize that elevated nuclear accumulation of MMP-13 plays a regulatory role and promotes the expression of pro-inflammatory cytokines and matrix-breaking enzymes, creating positive feedback that escalates cartilage degradation. We also explored the mechanism resulting in the nuclear translocation of MMP-13.

METHODS: (1) *Examine MMP-13 subcellular localization and endocytic entry.* Primary human chondrocytes were stimulated with interleukin (IL)-1β (1 ng/mL, 24 h) to induce inflammatory conditions. Immunofluorescence (IF) staining with anti-MMP-13 antibody and DAPI counterstaining was performed, and confocal microscopy was used to visualize subcellular localization. LDL receptor-related protein 1 (LRP1) was silenced in chondrocytes using siRNA, and protein expression was validated by Western blotting (WB). Subcellular fractionation was carried out to separate nuclear and cytoplasmic extracts, followed by western blot (WB) detection of MMP-13, Low-Density Lipoprotein Receptor-Related Protein 1(LRP1), Laminin Subunit Beta-1 (Laminin B1), and Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH). (2) *Assess MMP-13 nuclear activity*. To investigate the functional consequences of nuclear MMP-13, chondrocytes were transduced with lentiviral vectors encoding MMP-13 (OE-MMP-13) or negative control (NC), followed by treatment with recombinant MMP-3 protein (1 μg/mL, 48 h)(**Fig. 2A**). WB was used to detect pro- and active-MMP-13, while RT-qPCR was performed to measure *IL1B*, *COL2A1*, *ACAN*, and *MMP-13* expression levels. (3) *Investigate the degradation pathway of MMP-13 and its transcriptional regulatory interactions.* To examine the degradation and transcriptional regulatory role of MMP-13, co-immunoprecipitation (Co-IP) was performed using antibodies against MMP-13, LAMP1, and SMAD4 to assess protein–protein interactions. Confocal microscopy was employed to evaluate MMP-13 colocalization with LAMP1 and SMAD4, while molecular docking was conducted to predict binding sites between MMP-13 and SMAD4.

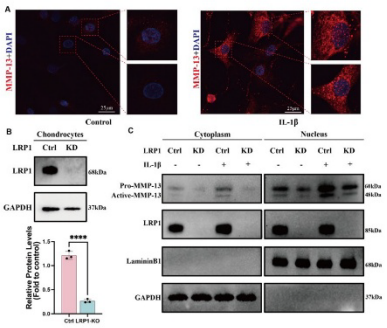

Fig. 1. LRP1 mediates endocytosis-dependent nuclear accumulation of MMP-13 in chondrocytes. (A) Immunostaining images showing increased nuclear localization of MMP-13 in IL-1β–stimulated chondrocytes, when compared with vehicle control. (scale bar: 25μm). (B) Successful knockdown of LRP1 was validated by western blot. GAPDH was used as the loading control. Ctrl: scrambled siRNA control; KD: LRP1 siRNA. ****, p<0.0001. (C) Subcellular fractionation and western blot revealed that LRP1 knockdown reduced cytoplasmic and nuclear accumulation of both

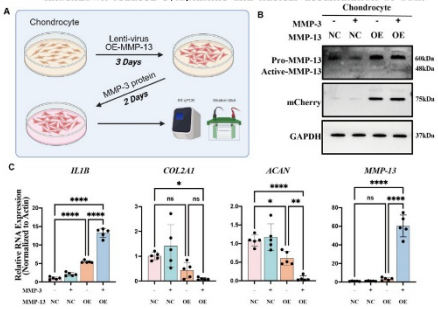

Fig. 2. Activation of extracellular precursors is required for MMP-13's transcriptional Roles. (A) Schematic illustration of the experimental design using lentiviral vectors to overexpress MMP13 (OE-MMP-13, OE group). The vectors carrying mCherry were used as the control group (NC group). Pro-MMP13 was activated by recombinant MMP-3 stimulation. (B) Western blot showing conversion of pro-MMP-13 to active MMP-13 upon MMP-3 treatment in chondrocytes from OE group. (C) qRT-qPCR results demonstrating that MMP-13 overexpression, after being activated by MMP-3 treatment, increased *IL-1B* and *MMP-13* expression while decreasing *COL2A1* and *ACAN*. (n=5).

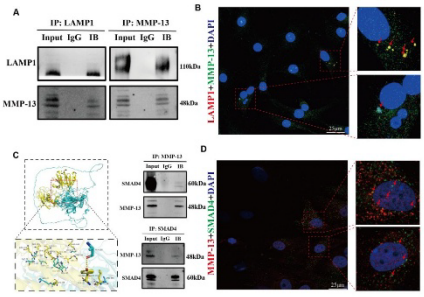

Fig. 3. Nuclear MMP-13 escapes lysosomal degradation and exerts transcriptional activity through SMAD4. (A) Co-IP showing interaction between MMP-13 and the lysosomal marker LAMP1; (B) Confocal microscopy confirming colocalization of MMP-13 with LAMP1 in chondrocytes. (scale bar, 25μm). (C) Structural modeling predicted binding between MMP-13 and SMAD4; Co-IP validated MMP-13–SMAD4 interactions. (scale bar: 25μm). (D) Colocalization of MMP-13 and SMAD4 in the nucleus.

RESULTS: (1) Nuclear accumulation of MMP-13 was markedly increased in IL-1β-stimulated chondrocytes (**Fig. 1A**). Silencing LRP1 significantly reduced both cytoplasmic and nuclear MMP-13 levels, confirming an endocytosis-dependent entry pathway (**Fig. 1B, C**). Interestingly, more MMP-13 was preserved in the nuclei than in the cytoplasm after LRP1 knockdown. **(2)** pro-MMP-13 was activated by the treatment of MMP-3 (**Fig. 2A, B**). Importantly, overexpression of MMP-13, after being activated by MMP-3 treatment, enhanced *IL1B* and *MMP-13* expression while suppressing collagen type IIα1 (*COL2A1)* and aggrecan (*ACAN)*, indicating a self-amplifying loop (**Fig. 2C**). (3) Co-IP and colocalization analyses revealed that MMP-13 interacts with lysosomal-associated membrane protein 1 (LAMP1) under basal conditions, suggesting lysosomal degradation as a clearance pathway (**Fig. 3A, B**). Using bioinformatic tools, we identified several factors that MMP-13 can bind to regulate gene expression. Additionally, nuclear MMP-13 was found to bind SMAD4, as confirmed by Co-IP and colocalization (**Fig. 3C, D**), and SMAD4 has been previously found to regulate MMP-13 expression [4].

DISCUSSION: We identify a novel pathway whereby the active form of MMP-13, traditionally viewed as an extracellular protease, translocates into the nucleus and functions as a transcriptional regulator. In particular, the nuclear translation of activated MMP-13 creates positive feedback to escalate the expression of its own gene, potentially accelerating ECM breakdown and pathological changes of chondrocytes. These findings expand our understanding and reveal intracellular MMP-13 as an important driver of OA progression.

SIGNIFICANCE/CLINICAL RELEVANCE: Our findings suggested that suppressing the matrix-breaking functions of MMP-13 is not sufficient to treat OA. Blocking nuclear import of MMP-13 or suppressing its transcriptional roles is also required when developing OA treatments targeting MMP-13.

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