Targeting IL-6/MMP13 axis in infrapatellar fat pad ameliorates osteoarthritis in mice

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Introduction: There are unmet demands on R&D of innovative and effective disease-modifying treatments for osteoarthritis (OA). OA is a multifactorial disease and the detrimental role of infrapatellar fat pad (IPFP) on OA progression has been reported by our team [1]. Here we investigated the specific role of IPFP-involved IL-6/MMP13 axis on the destruction of OA progression and devised a therapeutic strategy to mitigate OA progression.

Methods: *C57BL/6* mice were subjected to the destabilization of medial meniscus (DMM) surgery. The knee joints of mice were collected on days 0, 3, 7, and 14 post-surgeries for histological and mRNA analysis, respectively. To evaluate the effect of inflamed IPFP on OA progression, IPFP was removed on day 14 post-sham or -DMM surgery, including Sham/Sham, Sham/IPFPx, DMM/Sham, and DMM/IPFPx. Gait analysis was performed by the Catwalk system. To validate the role of IL-6 in fibrosis of IPFP, healthy mice were injected with saline or recombinant mouse IL-6 protein (rmIL-6) into the subcapsular IPFP, while DMM mice were injected with saline or IL-6-neutralizing antibody (Neu Ab). The concentration of MMP13 in the conditioned medium cultured with sham- or DMM-derived IPFP was measured by ELISA. The rmIL-6 pre-treated *in vitro* IPFP was transfected with siRNA *Mmp13*, and then co-cultured with primary chondrocytes for 2 days. Core/shell nanogel was synthesized and modified with RGD peptide. The lyophilized ^{RGD}-Nanogel particles were resuspended in CL82198 (MMP13 inhibitor) solution to encapsulate CL82198 through a “breathing-in” method. Mice weekly treated ^{RGD}-Nanogel/CL82198 were sacrificed on day 56 post DMM surgery combined intra-IPFP injection. The specified experimental protocols were approved by the Animal Experiment Ethics Committee.

Results: The histopathological grade and stage of DMM group were slightly higher than that of sham group on day 14 post-surgery (Fig. 1A and 1B). The mRNA expression levels of chondrocyte hypertrophic markers in the cartilage were comparable between DMM group and sham group within the first 14 days post-surgery. The fibrosis in IPFP happened on day 3 post-DMM surgery and predated the damage to the articular cartilage surface (Fig. 1C and 1D). Removal of IPFP on day 14 post-DMM surgery significantly mitigated the articular cartilage destruction and improved the functional behaviors of knee joint. The expression of *Il-6* peaked in IPFP on day 7 post-DMM surgery, but the mRNA expression of *Mmp13* peaked on day 7 and kept until day 56. Intra-IPFP injection of rmIL-6 could induce IPFP fibrosis in otherwise healthy mice, and intra-IPFP injection of Neu Ab could compromise the fibrogenic process in DMM mice, with less expressions of alpha-smooth muscle actin and Collogen 1A1. The concentration of IPFP-secreted MMP13 in the conditioned medium was significantly higher under rmIL-6 treatment relative to control group. The rmIL-6 pre-treated *ex vivo* IPFP was transfected with the siRNA *Mmp13* that could revive the extracellular matrix synthesis and simultaneously arrest *Mmp13* expression in primary chondrocytes (Fig. 1E to 1G). ^{RGD}-Nanogel heightened the retention and efficiency of CL82198 in the joint cavity. Intra-IPFP injection of ^{RGD}-Nanogel/CL82198 biologically targeted and inhibited the expression of *Mmp13* in the IPFP, thus attenuating OA progression.

Discussion: This study demonstrates that IL-6/MMP13 axis in IPFP plays a crucial role in OA progression, including articular chondrocyte hypertrophy and IPFP itself fibrosis. Besides, our designed ^{RGD}-nanogel/CL82198 particles effectively attenuate the cartilage degeneration and IPFP inflammation, leading to the development of an innovative therapeutic strategy to mitigate OA progression (Fig. 2). However, additional concerns of investigations are unmet needs for the interpretation of our findings, such as the initial factors driving the homeostasis disorders of IPFP and a definitive clarification in specimens from patient remain elusive.

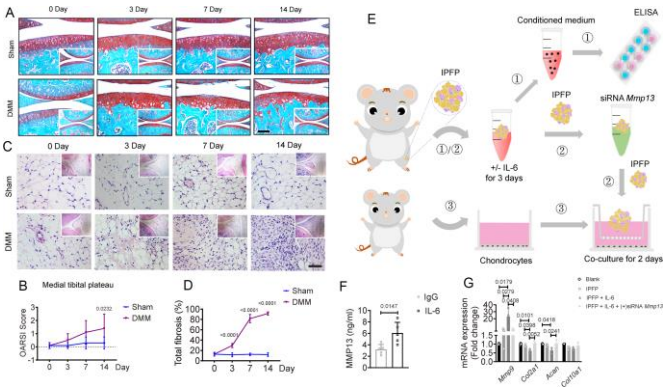

Figure. 1. (A and B) Representative staining and quantifications of OA severity by OARSI scores of knee joint sections from sham and DMM mice. Scale bar: 100 μm. (C and D) Representative staining and quantifications of IPFP from sham and DMM mice. (E to G) Schematic diagram illustrating the experimental outline for *ex vivo*.

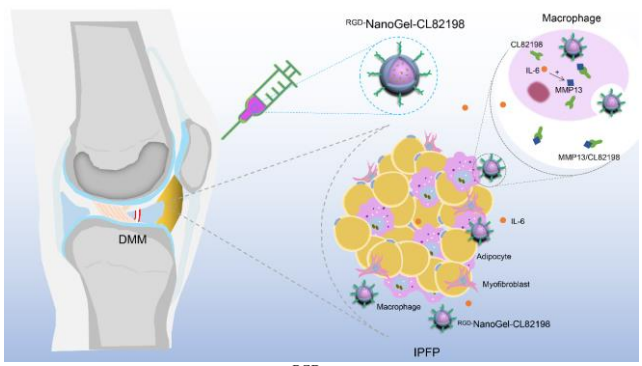

Figure 2. Schematic diagram of ^{RGD}-Nanogel/CL82198 for OA treatment by disturbing the signaling in IPFP-involved IL-6/MMP13 axis.

Reference

1. Dai, B., et al., *Blockage of Osteopontin-Integrin β3 Signaling in Infrapatellar Fat Pad Attenuates Osteoarthritis in Mice.* Adv Sci (Weinh), 2023: p. e2300897.

Disclosures: The authors declare no conflict of interest.