CD10-Bound Human Mesenchymal Stem/Stromal Cell-Derived Small Extracellular Vesicles Possess Immunomodulatory Cargo and Maintain Cartilage Homeostasis under Inflammatory Conditions

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INTRODUCTION: The onset and progression of human inflammatory joint diseases are strongly associated with the activation of resident synovium/infrapatellar fat pad (IFP) pro-inflammatory and pain-transmitting signaling. We recently reported that intra-articularly injected IFP-derived mesenchymal stem/stromal cells (IFP-MSC) acquire a potent immunomodulatory phenotype and actively degrade substance P (SP) via neutral endopeptidase CD10 (neprilysin). Our hypothesis is that IFP-MSC robust immunomodulatory therapeutic effects are largely exerted via their CD10-bound small extracellular vesicles (IFP-MSC sEVs) by attenuating synoviocyte pro-inflammatory activation and articular cartilage degradation.

METHODS: Herein, IFP-MSC sEVs were isolated from CD10High- and CD10Low-expressing IFP-MSC cultures and their sEV miRNA cargo was assessed using multiplex methods. Functionally, we interrogated the effect of CD10High and CD10Low sEVs on stimulated by inflammatory/fibrotic cues synoviocyte monocultures and cocultures with IFP-MSC-derived chondropellets. Finally, CD10High sEVs were tested in vivo for their therapeutic capacity in an animal model of acute synovitis/fat pad fibrosis.

RESULTS: Our results showed that CD10High and CD10Low sEVs possess distinct miRNA profiles. Reactome analysis of miRNAs highly present in sEVs showed their involvement in the regulation of six gene groups, particularly those involving the immune system. Stimulated synoviocytes exposed to IFP-MSC sEVs demonstrated significantly reduced proliferation and altered inflammation-related molecular profiles compared to control stimulated synoviocytes. Importantly, CD10High sEV treatment of stimulated chondropellets/synoviocyte cocultures indicated significant chondro-protective effects. Therapeutically, CD10High sEV treatment resulted in robust chondroprotective effects by retaining articular cartilage structure/composition and PRG4 (lubricin)-expressing cartilage cells in the animal model of acute synovitis/IFP fibrosis (Figure 1).

DISCUSSION: Our study suggests that CD10High sEVs possess immunomodulatory miRNA attributes with strong chondroprotective/anabolic effects for articular cartilage in vivo.

SIGNIFICANCE/CLINICAL RELEVANCE: The results could serve as a foundation for sEV-based therapeutics for the resolution of detrimental aspects of immune-mediated inflammatory joint changes associated with conditions such as osteoarthritis (OA).

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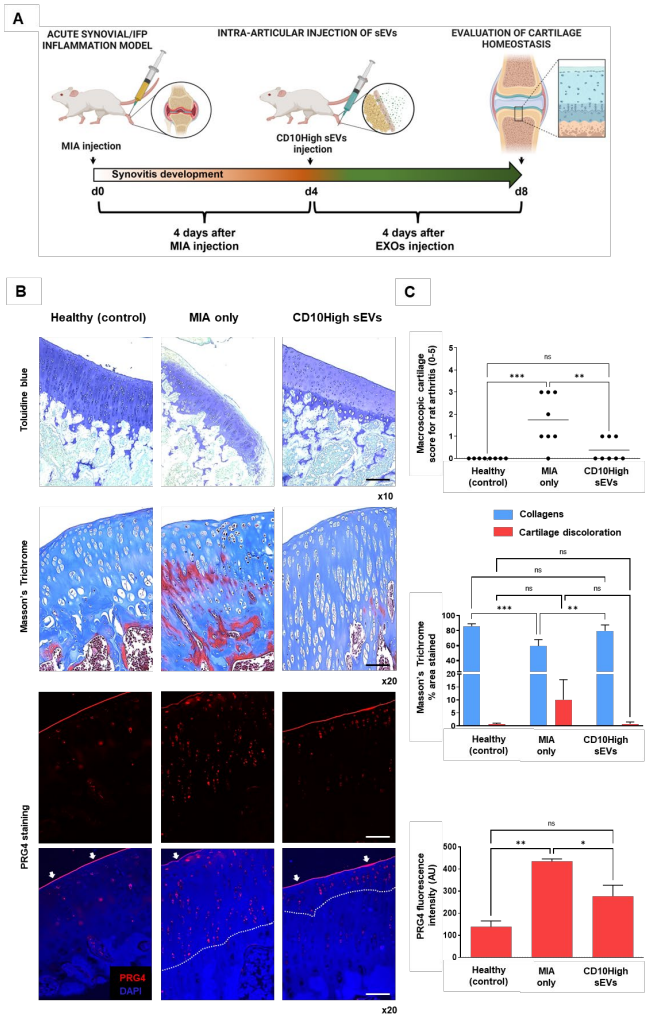

Figure 1. Effects of CD10High sEVs on articular cartilage homeostasis in vivo. (A) Schematic indicating the generation of acute synovitis/IFP fibrosis rat model, IFP-MSC sEVs' therapeutic intervention and chronological evaluation. (B,C) Toluidine blue staining (top panel), Masson's trichrome staining (middle panel), and PRG4 immunolocalization (lower panels) in sagittal-sectioned knees of representative rats for healthy, diseased (MIA only), or CD10High sEV treated groups. The diseased group demonstrated strong cartilage degeneration findings exemplified by reduced staining for sulfated proteoglycans. In contrast, CD10High sEV intra-articular infusion resulted in significantly reduced cartilage degeneration with only minor cartilage de-pressions and significantly increased collagen composition. Compared to the diseased group, the CD10High sEV group showed preservation of PRG4 expression on the upper cartilage surface and only minor expression from the intermediate zone chondrocytes (indicated by white arrows and dotted lines, ns: non-significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$). Toluidine blue scale bar: 200 µm, Masson's trichrome and PRG4 staining scale bars: 100 µm.