IGFBP7 secreted from DPP4 + senescent chondrocyte induces paracrine senescence in osteoarthritis.

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INTRODUCTION: It is known that the increase in senescent cells in various aging-related diseases, including osteoarthritis. Our previous study has shown that DPP4+ senescent chondrocytes can play an essential role in OA development, given that the removal of DPP4+ senescent cells increase cartilage regeneration capacity. In various tissue, paracrine senescence is considered as important factor in cell senescence. However, it is not clear how senescent chondrocytes are accumulated and contribute to OA development by paracrine senescence. In this study, isolated DPP4+ chondrocytes are used to study which factors secreted by senescent chondrocytes promote paracrine senescence and accumulate senescent chondrocytes.
METHODS: Human OA chondrocytes were isolated by sequential digestion from explants of total knee arthroplasty patients and non-OA chondrocytes were separated from the patients who underwent tumor removal in knee joint and were used as a control group. Senescence phenotypes, such as senescence-associated-galactosidase (SA-β-gal), DPP4, proliferation, senescence gene expression and OA phenotypes were evaluated. Human OA and non-OA chondrocytes were incubated with DPP4 antibody and FACS analysis was carried. Sorted DPP4- and DPP4+ chondrocytes, H_2O_2-induced senescence chondrocytes, OA patient's chondrocytes and non-OA patient's chondrocytes were cultured and conditioned medium was analyzed by ELISA. Human cartilage tissue and pellet were stained with Safranin-O, $p16^{INK4a}$ and DPP4.
RESULTS: We found DPP4 was selectively upregulated human OA chondrocytes. FACS mediated sorting of DPP4+ chondrocytes show increased mRNA expression of $p16^{INK4a}$ and $p21^{CIP1}$ compared with DPP4- chondrocytes from OA cartilage (Fig1). To confirm that factors secreted by senescent cells induce paracrine senescence, we exposed normal chondrocytes to conditioned medium (CM) from senescent chondrocytes and normal chondrocytes. Whereas cells exposed to CM from control cells grew normally, those treated with CM from senescent chondrocytes showed a senescent morphology, a decrease of proliferation, a higher percentage of SA-β-gal positive and activation of $p21^{CIP1}$ which suggested a paracrine senescence (Fig2A-B). Through gene expression profiling in DPP4- and DPP4+ chondrocytes by mRNA-seq analysis, we selected IGFBP7, as a paracrine senescence-mediating SASP and it is confirmed with ELISA results. In DPP4+ and OA cells IGFBP7 expression level is higher than DPP4- and non-OA cells (Fig2C-E). To confirm that IGFBP7 could directly induce paracrine senescence, we treated recombinant IGFBP7 to non-OA chondrocytes for 7days. The growth-arrested cells had an enlarged flat morphology, activation of $p21^{CIP1}$ and stained positively for SA-β-gal. When IGFBP7 was treated with non-OA chondrocytes, the shape of the pellet was unstable compared to the control group, and it was confirmed that the positive area also decreased when safranin-o staining. These results suggest that IGFBP7 secreted from senescent cells induces paracrine senescence of surrounding cells and increases the accumulation of senescent cells in cartilage.
DISCUSSION: The present study provides for the first time the possibility that IGFBP7 secreted by senescent cells mediate paracrine senescence in OA. It may aid in understanding the role of senescent chondrocytes in driving osteoarthritis pathology.
SIGNIFICANCE/CLINICAL RELEVANCE: We investigated which senescent chondrocyte-derived factors promote paracrine senescence and the accumulation of senescent chondrocytes in OA cartilage. Our data suggest that targeting IGFBP7 could be a therapeutic strategy for OA treatment.

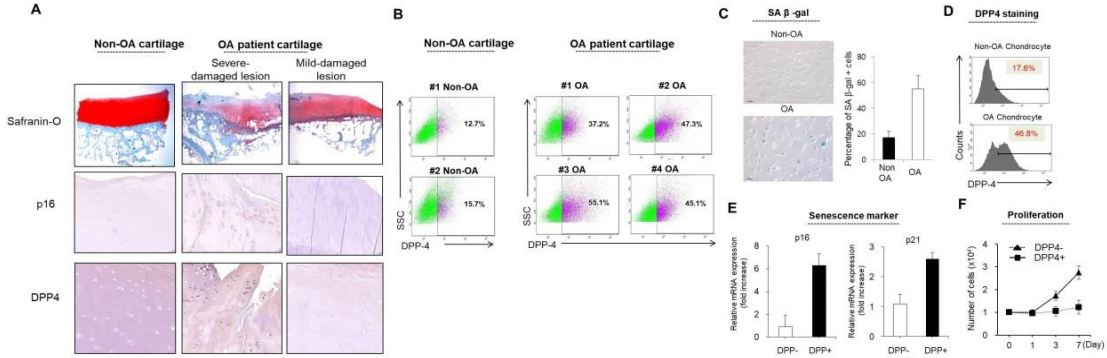

Figure1. DPP4+ senescent chondrocytes are significantly increased in human osteoarthritic cartilage.

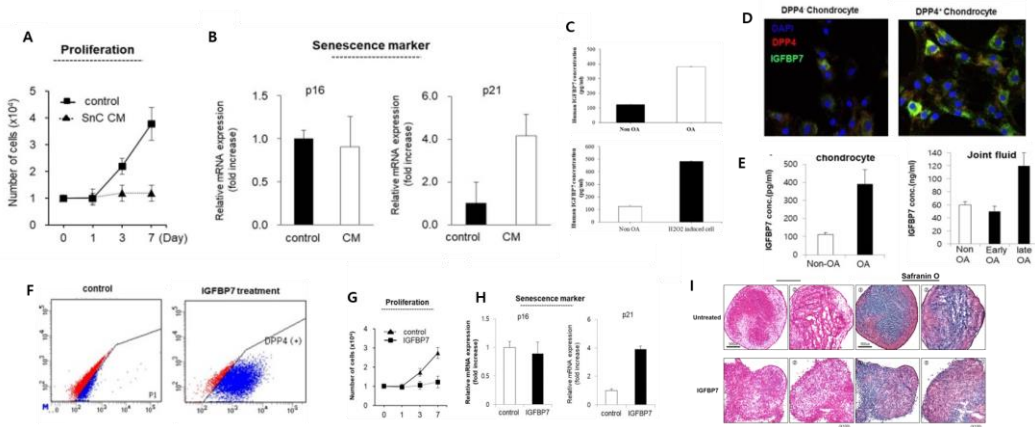

Figure2. IGFBP7 secreted from DPP4 + senescent chondrocytes induce paracrine senescence in OA.