Senescent Macrophages Induce Chondrocyte Catabolism and Accelerate Cartilage Degeneration in Osteoarthritis

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INTRODUCTION: Osteoarthritis (OA) is a common degenerative joint disease associated with aging, characterized by progressive cartilage degradation, synovial inflammation, and subchondral bone remodeling. While chondrocyte senescence has been implicated in OA pathogenesis, the contribution of senescence in other joint-resident cells remains poorly understood. In particular, immunosenescence the aging-related functional decline of immune cells has recently emerged as a driver of chronic inflammatory diseases. Synovial macrophages, key mediators of OA-associated inflammation, may undergo senescence and adopt a senescence-associated secretory phenotype (SASP), thereby amplifying cartilage damage. However, the specific role of senescent macrophages in OA progression has not been fully elucidated. This study investigates how senescent macrophages influence chondrocyte catabolism and compromise cartilage integrity.

METHODS: I Murine peritoneal macrophages were harvested from 8-week-old C57BL/6 mice and exposed to senescence-inducing stimuli—doxorubicin (200 nM), etoposide (20 μM), or hydrogen peroxide (100 μM). Senescence was confirmed by SA-β-galactosidase staining and Western blot detection of phosphorylated p53 and p21.Macrophages were then co-cultured with primary murine chondrocytes using a 1.0 μm Transwell system for 48 hours. Post co-culture, chondrocytes were collected for qRT-PCR and RNA-seq analysis to assess inflammatory and catabolic gene expression.
In ex vivo experiments, femoral head cartilage explants from 4-week-old C57BL/6 mice were co-cultured with senescent macrophages for 72 hours. Cartilage degradation was evaluated by quantifying glycosaminoglycan (GAG) release into the culture medium via the dimethylmethylene blue (DMMB) assay.

RESULTS: Among the stimuli tested, doxorubicin significantly increased the expression of phosphorylated p53 ($p = 0.03$) and p21 ($p = 0.02$), confirming the induction of senescence in macrophages. When co-cultured with these senescent macrophages, chondrocytes exhibited marked upregulation of MMP-3 ($p = 0.04$) and IL-6 ($p = 0.003$), indicating enhanced catabolic and inflammatory activity. RNA sequencing analysis further revealed activation of inflammatory signaling pathways, including Hepatitis B, as well as oxidative stress–related pathways such as oxidative phosphorylation. At the same time, chondrocytes shifted toward cell cycle arrest and a senescent phenotype. In ex vivo cartilage explants, co-culture with senescent macrophages resulted in increased glycosaminoglycan (GAG) release, suggesting accelerated cartilage matrix degradation.

DISCUSSION: These findings highlight the deleterious role of senescent macrophages in OA. Senescent macrophages acquire a SASP-like phenotype that can disrupt cartilage homeostasis by driving catabolic gene expression in chondrocytes. Given the expanding understanding of immune aging in chronic disease, our study suggests that senescent macrophages may serve as key amplifiers of inflammation and cartilage destruction in OA. Therapeutically targeting these cells or modulating their secretory phenotype could open new avenues for OA treatment, particularly in aging populations.

SIGNIFICANCE/CLINICAL RELEVANCE: Senescent macrophages promote cartilage degeneration by enhancing catabolic activity in chondrocytes. Targeting immune cell senescence may offer a novel and effective strategy for halting OA progression and preserving joint integrity.

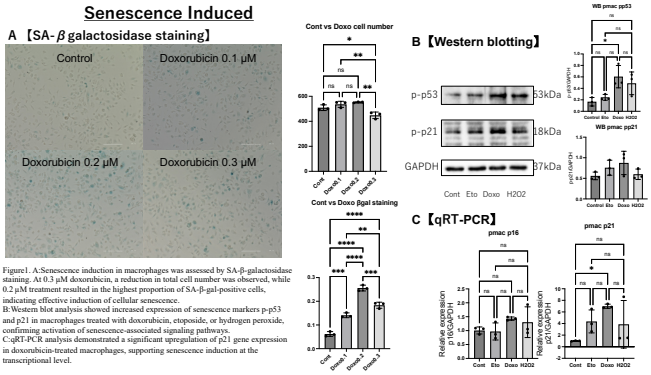

Figure1. A:Senescence induction in macrophages was assessed by SA-β-galactosidase staining. At 0.3 μM doxorubicin, a reduction in total cell number was observed, while 0.2 μM treatment resulted in the highest proportion of SA-β-gal-positive cells, indicating effective induction of cellular senescence.
B:Western blot analysis showed increased expression of senescence markers p-p53 and p21 in macrophages treated with doxorubicin, etoposide, or hydrogen peroxide, confirming activation of senescence-associated signaling pathways.
C:qRT-PCR analysis demonstrated a significant upregulation of p21 gene expression in doxorubicin-treated macrophages, supporting senescence induction at the transcriptional level.

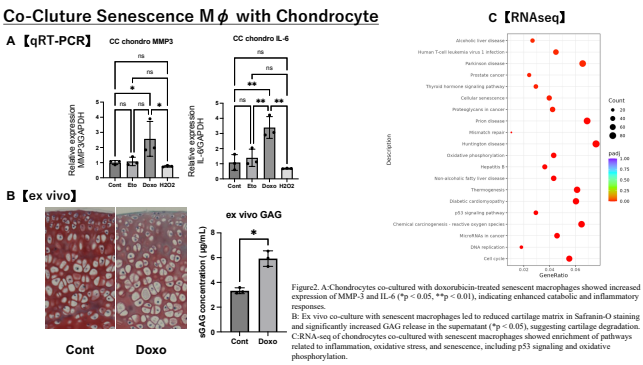

Figure2. A:Chondrocytes co-cultured with doxorubicin-treated senescent macrophages showed increased expression of MMP-3 and IL-6 (*p < 0.05, **p < 0.01), indicating enhanced catabolic and inflammatory responses.
B: Ex vivo co-culture with senescent macrophages led to reduced cartilage matrix in Safranin-O staining and significantly increased GAG release in the supernatant (*p < 0.05), suggesting cartilage degradation.
C:RNA-seq of chondrocytes co-cultured with senescent macrophages showed enrichment of pathways related to inflammation, oxidative stress, and senescence, including p53 signaling and oxidative phosphorylation.