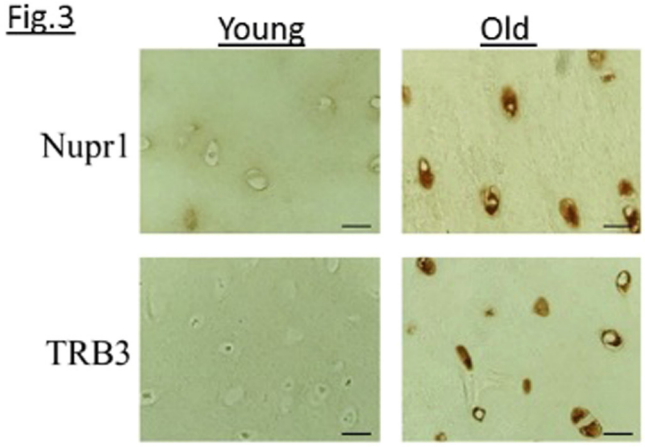

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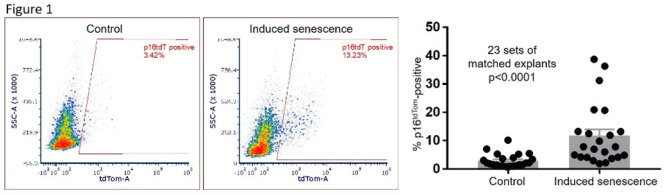
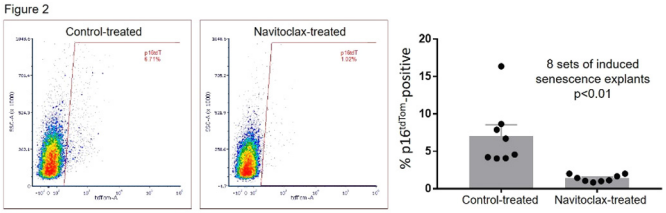
CONTROLLED INDUCTION AND TARGETED ELIMINATION OF P16^{INK4A}-HIGH CHONDROCYTES TO INVESTIGATE SENESCENCE-MEDIATED CARTILAGE DYSFUNCTION

G.A. Sessions [1], M.A. Sinkler [2,1], M.E. Copp [1,3], B.O. Diekman [1,3]. [1] *Univ. of North Carolina Chapel Hill, Chapel Hill, NC, USA;* [2] *Med. Coll. of Georgia at Augusta Univ., Augusta, GA, USA;* [3] *North Carolina State Univ., Raleigh, NC, USA*

Purpose: Cellular senescence is a phenotypic state that emerges in response to stress after injury and during aging. Senescent cells are characterized by increased expression of the cell cycle inhibitor p16^{INK4a} (p16), high lysosomal activity, and the production of pro-inflammatory and matrix-degrading proteins known as the senescence associated secretory phenotype (SASP). Expression of p16 increases in both murine and human chondrocytes with age and high p16 expression correlates with SASP factor production. Furthermore, treatment with "senolytic" compounds that specifically target senescent cells for apoptosis was shown to mitigate post-traumatic OA in a murine model. The aim of this study was to establish a cartilage explant model system to explore the functional effects of selectively eliminating p16-expressing (senescent) chondrocytes.
Methods: A knock-in murine genetics approach was used to replace the coding region of p16 with the fluorescent molecule tdTomato in order to quantify p16 expression on a single-cell basis with flow cytometry. Femoral cap cartilage explants were isolated from mice with one intact copy of p16 and one copy of the p16^{tdTomato} allele. Explants were cultured for three weeks either in control media or senescence induction media containing 1 ng/ml transforming growth factor beta-1 and 5 ng/ml basic fibroblastic growth factor. Explants were digested and the percentage of p16^{tdTomato}-high cells was determined by flow cytometry. Cell sorting was used to separate chondrocytes into p16^{tdTomato}-high and p16^{tdTomato}-low fractions and these populations were analyzed for senescence features such as senescence-associated beta galactosidase (SABG) activity. Senescent cells upregulate anti-apoptotic pathways to maintain survival despite significant cellular damage. The ability to selectively eliminate p16-high cells with a drug that inhibits this apoptotic block is both a potential treatment and a way to further confirm the senescent nature of the p16^{tdTomato}-high cell fraction. In this study, senolytic treatment was performed using navitoclax, a BCL pathway inhibitor, at a concentration of 5 μM for 72 hours. As an initial assessment of how senolytic clearance affects cartilage health, the glycosaminoglycans (GAGs) content was measured in femoral cap explants that were cultured for an additional week following control or navitoclax treatment.
Results: The treatment of murine cartilage explants with growth factors for three weeks induces a significant fraction of p16^{tdTomato}-high chondrocytes (Figure 1). This p16^{tdTomato}-high cell population demonstrates higher SABG activity as compared to the p16^{tdTomato}-low fraction (n=4 sorts, mean = 39.1% vs. 20.5% positive, p=0.03 by paired t-test). Following treatment with the senolytic navitoclax, explants showed a significant reduction in the percentage of p16^{tdTomato}-high cells (Figure 2). As compared to control treatment, clearing senescent cells with navitoclax shows a trend towards increasing the GAG content per cell in cartilage explants (n=4 matched explants, mean = 57.6 vs. 41.3 μg GAG/μg DNA, p=0.06 by paired t-test).
Conclusions: This work utilized a p16-based reporter allele to quantify the induction and elimination of senescent chondrocytes. As consistent with known conditions for senescence induction, the percentage of p16^{tdTomato}-high cells increased upon growth factor stimulation in the context of growth restraint provided by the extra-cellular matrix. These p16^{tdTomato}-high cells showed features of senescence and were selectively eliminated upon treatment with navitoclax. Analysis in a set of matched cartilage explants indicates that senolytic treatment is capable of increasing GAG levels, which suggests that senescence clearance may improve matrix homeostasis and limit cartilage degradation associated with aging and joint injury.





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KNOCKDOWN OF SIRTUIN3 IN CARTILAGE PROTECTS MALE MICE AGAINST HIGH-FAT DIET-INDUCED OSTEOARTHRITIS

S. Zhu [1], E.L. Donovan [1,2], E.B. Lopes [1], M. Kinter [1], A. Simmons [1,3], D. Makosa [1], D. Cortassa [1], M. West [1], T.M. Griffin [1,4]. [1] *Oklahoma Med. Res. Fndn., Oklahoma City, OK, USA;* [2] *Oregon Inst. of Technology, Klamath Falls, OR, USA;* [3] *Univ. of Wisconsin, Madison, WI, USA;* [4] *Univ. of Oklahoma Hlth.Sci. Ctr., Oklahoma City, OK, USA*

Purpose: Aging impairs cellular processes that promote metabolic and oxidative homeostasis in chondrocytes. Many of these changes affect the mitochondria and are associated with an increased risk of osteoarthritis (OA). We hypothesized that these age-related mitochondrial impairments synergize with other OA risk factors, such as obesity, to further increase OA severity. We tested this hypothesis by genetically deleting the mitochondrial enzyme Sirtuin3 (Sirt3) in chondrocytes of young mice fed a control or high-fat diet. Sirt3 is a NAD^+-dependent deacetylase enzyme that regulates the activity of metabolic and antioxidant enzymes in the mitochondria under conditions of cellular stress. Sirt3 is an attractive enzyme to target for testing this hypothesis because it decreases in articular cartilage during aging.
Methods: Following IACUC approval, we bred *Aggrecan-Cre^{ERT2}* and *Sirt3$^{fl/fl}$* mice to generate littermate wild-type (WT) and inducible cartilage Sirt3 KO mice (Cart-Sirt3KO). Tamoxifen was administered at 5 wks to induce Sirt3 excision, and mice were fed a control (D12450Ji) or high-fat (HF) (D12492i) diet (Research Diets) from 6 wks until the study endpoint at 26 wks of age. Knee cartilage was dissected and processed in TRIzol for RNA and protein isolation, which were used for quantitative gene expression (90 OA-related target genes; IFC Delta Gene Assay) and targeted proteomic analyses (>100 metabolic and antioxidant proteins; Triple Quadrupole LC-MS). The contra-lateral knee was processed for OA histopathology using standard methods and blinded semi-quantitative OARSI and modified Mankin scoring. Cartilage Sirt3 immuno-staining and excised:intact floxed allele DNA content were