

## Primary Human Chondrocytes Exhibit a Decline in DNA Damage Repair with Age

Oliver B. Hansen<sup>1</sup>, Michaela E. Copp<sup>1</sup>, Jacqueline Shine<sup>1</sup>, Susan Chubinskaya<sup>2</sup>, John A. Collins<sup>3</sup>, Richard F. Loeser<sup>1</sup>, Brian O. Diekman<sup>1</sup>  
<sup>1</sup>University of North Carolina, Chapel Hill, NC, USA <sup>2</sup>Rush University, Chicago, IL, USA <sup>3</sup>Thomas Jefferson University, Philadelphia, PA, USA  
 oliver\_hansen@med.unc.edu

**Disclosures:** Oliver B. Hansen (N), Michaela E. Copp (N), Jacqueline Shine (N), Susan Chubinskaya (5 - AlloSource), John A. Collins (N), Richard F. Loeser (8 - Arthritis & Rheumatology, Osteoarthritis & Cartilage), Brian O. Diekman (8 - Connective Tissue Research)

**INTRODUCTION:** The leading risk factor for osteoarthritis (OA) is older age and there is evidence that senescence of chondrocytes and other cell types of the joint may be one link between aging and OA pathogenesis. As assessed at the single-cell level by the “comet assay”, chondrocytes from older cadaveric donors harbor DNA damage that is equivalent to a senescence-inducing dose of 10 Gy irradiation (IR) [1]. Sirtuin 6 (SIRT6) is a nuclear-localized NAD(+)-dependent deacetylase that has been shown to play an important role in DNA damage repair, and the enzymatic activity of SIRT6 declines with age in human chondrocytes [2]. The small molecule MDL-800 is a potent allosteric activator of SIRT6 [3] and can be used as a tool to probe the mechanisms by which SIRT6 modification may be of therapeutic benefit in OA. The objective of this study was to determine whether DNA repair efficiency declines with age and whether enhancing SIRT6 activity is sufficient to enhance DNA repair in chondrocytes from older individuals.

**METHODS:** Human cadaveric ankle tissue from de-identified donors was obtained under an exemption granted by the institutional review board. Chondrocytes were isolated by enzymatic digestion, allowed to recovery for approximately 7 days in culture, and then frozen, allowing for subsequent plating and analysis of multiple donors in parallel. The repair rate of young ( $\leq 45$  years old), middle-aged (50-65), and older ( $\geq 70$ ) donors was tested via the alkaline comet assay by embedding chondrocytes in low melt agarose on a microscope slide, irradiating the gels with 10 Gy, and incubating the slides in media baths for repair until lysis at the appropriate time point. For SIRT6 activation experiments, 20  $\mu$ M MDL-800 (or DMSO control) was given to cells for two hours in monolayer as a pre-culture period as well as during the repair phase. DNA damage was quantified by staining and microscopy in approximately 100 cells per group via OpenComet software, with the percentage of DNA signal in the comet “tail” as the output; donor averages represent the mean of all quantifiable cells. Statistical analysis was performed using two-way ANOVA with Tukey’s multiple comparisons test.

**RESULTS SECTION:** The bolus of immediate damage caused by IR generated equivalent levels of DNA damage regardless of donor age (n=3-4 per age group, Fig. A). Chondrocytes from younger donors had less damage than older donors at 1, 2, and 4 hours of repair; younger donors had less damage than middle-aged donors at 2 and 4 hours; middle-aged donors had less damage than older donors at 2 hours (Fig. A). Allowing for overnight recovery demonstrated that most chondrocytes of all donor ages repaired DNA and achieved near baseline levels of damage. Analyzing the individual cells combined from two young, two middle-aged, and two older donors showed that 94.1%, 88.3%, and 87.5% of cells, respectively, exhibited less than 15% of “tail DNA” after overnight repair (Fig. B, left column with purple dots shows damage immediately after IR; right column with red dots shows damage after overnight repair). To determine whether activation of SIRT6 accelerated the repair of DNA in chondrocytes from older donors (n=4), 20  $\mu$ M MDL-800 or DMSO vehicle control were provided for 2h and during repair. MDL-800 caused a trend towards reduced baseline and immediate damage, and significantly less damage at 30, 60, 120 and 240 minutes (Fig. C, \*p<0.05 \*\*p<0.01 \*\*\*p<0.001, Tukey’s post-hoc).

**DISCUSSION:** Chondrocytes become dysfunctional with age and lose the capacity to balance cartilage matrix synthesis and degradation. The current study shows that DNA repair efficiency declines with age, which could explain the increased accumulation of DNA damage with age [1]. Further, the subset of chondrocytes that are unable to fully repair after a bolus of DNA damage may represent a population at risk of apoptosis or cellular senescence. Enhancing DNA damage repair to mitigate senescence is one possible strategy to prevent the secretion of pro-inflammatory and matrix-degrading enzymes by senescent cells. This study showed that activation of SIRT6 by MDL-800 can enhance DNA damage repair in chondrocytes from older cadaveric donors. Chondrocyte-specific deletion of Sirt6 in mice increases post-traumatic and age-related OA [4,5], and intra-articular delivery of MDL-800 decreases markers of senescence during the post-injury period [5]. In addition to DNA damage repair, SIRT6 plays a role in the response to oxidative stress and metabolic regulation. Thus, future work seeking to harness SIRT6 activation for OA therapy will need to clarify the relative contributions of this multi-functional protein.

**SIGNIFICANCE/CLINICAL RELEVANCE:** We show that the DNA repair efficiency of chondrocytes deteriorates throughout life but can be enhanced by activating SIRT6. The long-term goal of this work is to mitigate the high incidence of OA with aging by limiting the accumulation of DNA damage that may lead to senescence and joint dysfunction.

**REFERENCES:** [1] Copp...Diekman, *Aging Cell*, 2022. [2] Collins...Loeser, *Free Radic Biol Med*, 2021. [3] Huang...Zhang, *Nat Cell Bio*, 2018. [4] Collins...Loeser. *Ann Rheum Dis*, 2023. [5] Ji...Lu, *Nat Commun*, 2022.

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