SIRT6 Activation by MDL-800 Reduces DNA Damage in Synovial Fibroblasts

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INTRODUCTION: Osteoarthritis (OA), a leading cause of disability worldwide, is characterized by progressive dysfunction of joint tissues such as cartilage and synovium. Aging is the strongest risk factor for OA and prior work has shown that chondrocytes accumulate DNA damage with age [1]. Cellular senescence in response to DNA damage is one potential driver of OA, as senescent cells release pro-inflammatory and matrix-degrading factors that accelerate disease. Sirtuin 6 (SIRT6), a member of the sirtuin family of deacetylases, functions in part by facilitating DNA repair through the removal of acetyl groups from amino acid residues within histone proteins such as H3K9. Prior studies in chondrocytes show that SIRT6 activation reduces DNA damage, limits inflammatory signaling, and protects against cartilage degradation [2]. The small molecule MDL-800, a potent SIRT6 activator, has been shown in chondrocytes to enhance DNA repair efficiency and compensate for the decreased SIRT6 activity that occurs with aging [3]. However, it is unclear whether synovial fibroblasts also accumulate a significant amount of DNA damage and whether this burden can be reduced by SIRT6 activation. This study evaluates whether MDL-800 can modulate SIRT6 activity and DNA damage in both human and murine synovial fibroblasts, providing insight into the therapeutic potential of SIRT6 activation for OA.

METHODS: Human synovial tissue was isolated during total knee replacement (70- and 74-year-old males; 58-, 64-, 70-, and 73-year-old females). Use of de-identified surgical waste was confirmed by institutional IRB. Dissected synovium was digested with pronase (1 mg/mL, 45 min) and collagenase P (1 mg/mL, 3–5 h) in 10% FBS DMEM-HG, then filtered, centrifuged, washed, and resuspended. Cells were plated and treated at passage 0 or 1. Murine fibroblasts were isolated under institutional ethics approval using DNase (0.4 mg/mL), Liberase TM (0.4 mg/mL), and collagenase IV (0.4 mg/mL) in 10% FBS DMEM-HG for 1–2 h. To assess age effects, cells were collected from young (4–5 mo) and aged (24–28 mo) cohorts. Human and murine fibroblasts were treated for 24 h with 20 µM MDL-800, 40 µM MDL-800, or DMSO. DNA damage was assessed by comet assay: cells were suspended in low-melt agarose, layered, lysed, electrophoresed under alkaline conditions, stained, and ~100 cells per group analyzed with OpenComet. Donor averages reflect the mean of quantifiable cells. Flow cytometry of human fibroblasts measured H3K9ac (normalized to DMSO controls; reduced acetylation indicates higher SIRT6 activity). Cells were fixed in 4% paraformaldehyde, incubated with primary antibodies for H3K9ac and total H3 overnight at 4 °C, followed by secondary antibodies, and analyzed on an Attune NXT. Statistical analysis for human DNA damage experiments was performed by repeated measures one-way ANOVA with Tukey's post-hoc test. Analysis of H3K9ac was a one-sample t-test compared to the expected value of 1, as the average H3K9ac/total H3 ratio across all H3-positive cells was normalized to that same value of the DMSO control for each donor. Analysis for murine DNA damage experiments was by two-way repeated measures ANOVA (age, treatment) with Tukey's post-hoc test.

RESULTS SECTION: Six independent human synovial fibroblast donors were used for analysis in both the comet assay and flow cytometry experiments. MDL-800 significantly reduced DNA damage in human synovial fibroblasts compared to DMSO controls at both 20 µM and 40 µM ($p = 0.02$ and $p<0.001$, respectively) (Fig. A). Consistent with enhanced SIRT6 activity, flow cytometry revealed a significant reduction in H3K9ac levels following MDL-800 treatment. Decreases were observed in both the 20 µM and 40 µM groups ($p = 0.0498$ and $p < 0.0001$), with the higher dose producing a greater effect (Fig. B). Murine experiments included four cohorts of old mice and four cohorts of young mice, with each cohort representing pooled synovial fibroblasts from four mice. Baseline DNA damage represented by the DMSO control treatment group was significantly higher in old fibroblasts compared to young fibroblasts ($p < 0.001$) (Fig C). In old mice, DNA damage was significantly reduced by 20 µM and 40 µM MDL ($p = 0.037$ and $p=0.0107$). In young mice, MDL-40 also significantly reduced DNA damage relative to baseline ($p = 0.0026$, one-way ANOVA). Notably, DNA damage levels in old fibroblasts treated with MDL-40 were not significantly different from baseline levels in young fibroblasts ($p =0.2532$, Welch's t test).

DISCUSSION: Our findings demonstrate that pharmacological activation of SIRT6 with MDL-800 reduces the extent of DNA damage in synovial fibroblasts. In both human and murine cells, MDL-800 treatment reduced the percentage of DNA present in comet assay tails, while flow cytometry confirmed decreased H3K9ac levels, consistent with enhanced SIRT6 deacetylase activity. Aged murine fibroblasts treated with MDL-800 exhibited DNA damage levels comparable to those of young fibroblasts, suggesting a restorative effect on genomic stability. These results extend the protective role of SIRT6 beyond chondrocytes to include synovial fibroblasts, a critical joint cell type implicated in osteoarthritis. Synovial fibroblasts contribute to disease progression by secreting pro-inflammatory cytokines and matrix-degrading enzymes, processes that are amplified when cells become senescent. If confirmed that reduced DNA damage limits synovial fibroblast senescence, SIRT6 activation may attenuate the pathological influence of synovial fibroblasts and dampen joint inflammation. From a translational perspective, our study supports the potential of SIRT6 as a disease-modifying therapeutic target in osteoarthritis. A limitation is that we focused on short-term cell culture, which may not fully recapitulate the complexity of the in vivo joint environment.

SIGNIFICANCE/CLINICAL RELEVANCE: This study demonstrates that SIRT6 activation in synovial fibroblasts can reduce DNA damage that accumulates with aging and OA, similar to what was previously shown with chondrocytes. The consistency of MDL-800's protective effects across multiple joint cell types suggests that enhancing SIRT6 activity could yield broad joint-level benefits, including reduced inflammatory signaling, preservation of extracellular matrix, and improved genomic stability.

REFERENCES: [1] Copp…Diekman, *Aging Cell*, 2022. [2] Ji…Lu, *Nature Communications*, 2022. [3] Copp…Diekman, *Aging*, 2023