Activation of SIRT6 regulates mitochondrial function and reduces the severity of age-associated osteoarthritis in mice

Matheus M. Perez[1], Pranay Ramteke[1], Bahiyah Watson[1], Eirene Choi[1], Ashley Coleman[1], Owen Haslam[1], Susan Chubinskaya[3], Ryan E. Tomlinson[1], Mandy J. Peffers[4], Richard F. Loeser[2], Makarand V. Risbud[1], Theresa A. Freeman[1], John A. Collins[1,2]
[1]Thomas Jefferson University, Philadelphia, PA, USA; [2]University of North Carolina, Chapel Hill, NC, USA; [3] Rush Medical College, Chicago, IL, USA; [4] University of Liverpool, Liverpool, UK.

Disclosures: Matheus M. Perez (N), Pranay Ramteke (N), Bahiyah Watson (N), Eirene Choi (N), Ashley Coleman (N), Owen Haslam (N), Susan Chubinskaya (Y-5), Ryan E. Tomlinson (N), Mandy J. Peffers (N), Richard F. Loeser (Y-8), Makarand V. Risbud (N), Theresa A. Freeman (N), John A. Collins (N).

INTRODUCTION: Sirtuin 6 (SIRT6) is a nuclear localized histone deacetylase that epigenetically regulates several age-associated pathways, including senescence, longevity, inflammation, redox balance, metabolism, and genomic stability. Using human chondrocytes, we have previously demonstrated that SIRT6 activity significantly declines with age, promoting oxidative stress conditions and catabolic signaling events implicated in osteoarthritis (OA) (1). *In vivo*, we recently reported that cartilage-specific loss of *Sirt6* repressed pro-anabolic Insulin Growth Factor 1 (IGF-1) signaling and increased surgery-induced and age-related OA severity in mice (2). The **objective** of this study was to determine whether systemic administration of MDL-800, a small molecule activator of SIRT6, reduced the severity of age-associated OA in mice.

METHODS: All animal studies were approved by the Thomas Jefferson University Animal Care and Use Committee and were performed using male C57BL/6 mice. 18 month old mice received weekly intraperitoneal injections of MDL-800 (75 mg/kg) or vehicle control for six months, and OA severity was analyzed at 24 months of age (vehicle control group; n=5, MDL-800 group, n=4). Micro computed tomography (MicroCT) was used to analyze the tissue volume (TV) and bone volume (BV) to determine the bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), and subchondral bone plate thickness (SCBP.th). Sectioned limbs underwent histological analysis (mid-coronal), and toluidine blue and hematoxylin and eosin (H&E) staining was used to assess Articular Cartilage Structure (ACS), proteoglycan content, and osteophyte formation on the medial and lateral tibial plateaus (MTP, LTP) and medial and lateral femoral condyles (MFC, LFC). Detailed histomorphometry was also performed on H&E stained sections to assess articular cartilage, calcified cartilage, and SCBP area and thickness on MTP and LTP. RNA sequencing was conducted on primary human chondrocytes treated in the presence or absence of MDL-800 (12.5 uM, 24 hours), and the effect of MDL-800 to regulate mitochondrial function was analyzed using the MitoCarta 3.0 database and gene set enrichment analysis (GSEA). Ingenuity Pathway Analysis (IPA) was used to assess the effect of MDL-800 to regulate the OA disease process.

RESULTS : Mice receiving MDL-800 displayed significantly lower summed ACS scores (control 28.6±16.1; MDL-800, 5.0±1.4; p=0.0159), toluidine blue scores (control, 25.8±16.4; MDL-800, 3.0±1.6; p=0.0159, **Fig. 1**), and osteophyte scores (control, 5.8±1.9; MDL-800, 2.0±1.4; p=0.0238). Accordingly, the histomorphometric analysis showed that mice receiving MDL-800 displayed increased articular cartilage area (p=0.0317) and thickness (p=0.0159) on MTP, as compared to controls. MicroCT analysis on the MTP demonstrated that mice receiving MDL-800 displayed lower BV/TV (p=0.0008) and Tb.Th values (p=0.0003), and higher Tb.Sp (p=0.0471) values when compared to controls, indicating a decrease in subchondral bone sclerosis in the MDL-800 treated group. Analysis of transcriptomic data from RNA sequencing of MDL-800 treated samples revealed 2250 differentially expressed genes (log2FC cutoff of ±2 and FDR<0.05; 1419 upregulated, 831 downregulated), as compared to controls. GSEA analysis revealed that MDL-800 treatment was associated with significant enrichment of mitochondrial pathways, including 'detoxification', which primarily involved the upregulation of mitochondrial antioxidant genes, and 'small molecule transport', which consisted of mitochondrial genes involved in import and export across the inner mitochondrial membrane. Other significantly enriched mitochondrial pathways included mitochondrial 'protein homeostasis', 'metabolism', 'ribosome', and 'mitochondrial dynamics and surveillance'. IPA analysis demonstrated that MDL-800 treatment is predicted to decrease cartilage catabolism and the OA disease process.

DISCUSSION: These preliminary results demonstrate that chronic administration (weekly for six months) of the SIRT6 activator, MDL-800, reduces age-associated OA severity in mice. MDL-800 treated mice displayed a decrease in cartilage damage, osteophyte formation, and subchondral bone sclerosis, as compared to controls. RNA sequencing analysis revealed that SIRT6 may regulate key mitochondrial pathways critical for response to stress, import and export of mitochondrial proteins, metabolism, and mitochondrial protein quality control. These findings build on our previous work, which demonstrated that overexpression of SIRT6 increased antioxidant capacity and that MDL-800-induced activation of SIRT6 enhanced pro-anabolic IGF-1 signaling in human chondrocytes (2). Our current and ongoing work aims to comprehensively define the role of SIRT6 as a regulator of nuclear-mitochondrial signaling and mitochondrial function in mouse and human joint tissues.

SIGNIFICANCE/CLINICAL RELEVANCE: Our findings show that *in vivo* administration of a SIRT6 activator decreases the severity of age-associated OA and may be an important regulator of mitochondrial function. Mitochondrial dysfunction is a hallmark of aging and is well documented to contribute to the development and progression of OA. Thus, targeted therapies that activate SIRT6 may represent a novel strategy to slow or stop OA progression by preserving homeostatic mitochondrial function during aging.

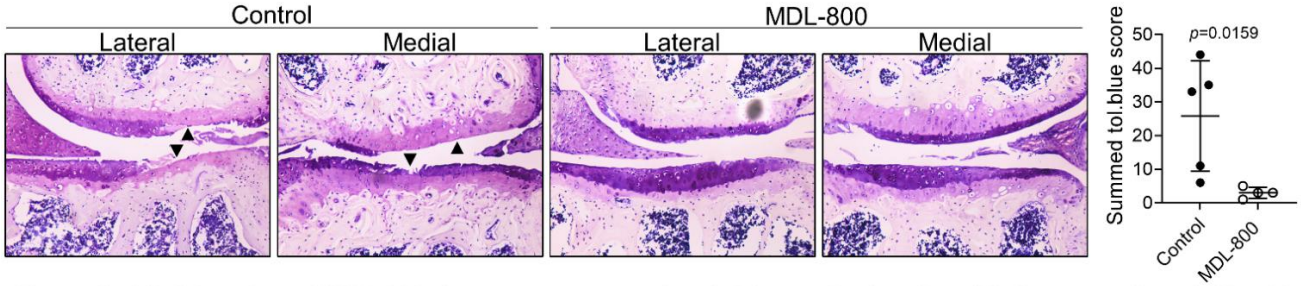

Figure 1. Administration of MDL-800 decreases age-associated OA severity in mice. (A) Representative toluidine blue staining of knee joints derived from mice receiving weekly injections of vehicle control or MDL-800 from 18-24 months of age. Arrows show areas of complete cartilage loss. **(B)** Summed toluidine blue scores for vehicle control and MDL-800 treated mice.

References: (1) Collins et al., *Free Radical Biology and Medicine*, 2021. (2) Collins et al., *Annals of the Rheumatic Diseases*, 2023.