Nicotinamide Riboside Supplementation and CD38 Loss of Function Each Modulate Pro-fibrotic Gene Expression in a Murine Model of Post-traumatic Osteoarthritis

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Introduction: Within the adult synovial joint, articular cartilage is maintained by a long-lived population of chondrocytes. Upon onset of osteoarthritis (OA) due to aging or prior joint injury, articular chondrocytes become metabolically disturbed, leading some to die while others acquire a pro-fibrotic phenotype that cannot sustain cartilage biomechanical function. The mechanisms underlying chondrocyte dysfunction are not well understood, partly explaining the lack of disease-modifying therapies to prevent cartilage loss. Nicotinamide adenine dinucleotide (NAD) is an essential mediator of electron exchange during cellular metabolism and a substrate for vital cellular processes. Decreased tissue NAD levels have been implicated in several diseases of aging and fibrosis. Tissue NAD pools are sustained through a combination of synthesis from vitamin precursors, including nicotinamide riboside (NR), as well as recycling of locally-consumed NAD via a salvage pathway. Recently, we demonstrated the unique dependence of articular chondrocytes for NAD salvage during joint development [1]. Tissue NAD pools are also determined by local consumption activity. In particular, the elevated activity of cyclic ADP-ribose hydrolase, also known as CD38, has been linked to NAD deficiency in several tissues during aging and fibrosis. We hypothesize that inadequate intra-articular NAD levels through inadequate recycling and/or elevated CD38 consumption disrupts articular chondrocyte function and contributes to cartilage loss during OA.

Methods: Animal studies were pre-approved by the local Institutional Animal Care and Use Committee. Post-traumatic OA was modeled in the right knee joints of 12 week old male Cd38$^{-/-}$ and wild type littermates (C57BL6 background) by surgical destabilization of the medial meniscus (DMM). Control mice received sham surgery consisting of arthrotomy alone [2]. A portion of wild type mice were administered NR in their drinking water (1000 mg/kg/day; refreshing water daily) starting the day before DMM or sham surgery. Mice were euthanized at 4 or 8 weeks post-injury (wpi) for histological scoring of cartilage degeneration according to OARSI recommendations [3]. At 2 wpi, cells were enzymatically isolated from the distal femur and proximal tibia of 2 joints per condition, hematopoietic cells were depleted using magnetic beads, and remaining mesenchymal-lineage cells were submitted for single cell RNA sequencing (scRNA-seq) using a 10X Genomics workflow. Sequencing data was harmonized and integrated following the method described in our previous work [1]. Articular chondrocytes and synovial fibroblasts identified through unbiased cluster analysis were compared between experimental groups. Differential gene expression between clusters was determined using a standard negative binomial statistic. Expression localization for transcripts of interest was confirmed by in situ hybridization using RNAscope.

Results: CD38$^{-/-}$ mice displayed improved histological scores of articular cartilage erosion compared to littermate controls. In situ hybridization for *Cd38* demonstrated expression primarily within the synovium. To determine any shared mechanisms of action between NAD supplementation and CD38 loss of function in modulating cartilage degeneration, we performed additional DMM or sham surgery in CD38$^{-/-}$ male mice and wild type littermates, treating a subgroup of the wild type animals with NR in their drinking water. Two weeks post-DMM, mesenchymal-lineage cells were analyzed by scRNA-seq (Figure 1). Pro-fibrotic genes shown to be upregulated in human OA cartilage were also upregulated in Prg4-expressing chondrocytes from DMM mouse knees compared to Sham controls. Strikingly, these markers were downregulated by both NR treatment and CD38 knockout. We observed similar patterns of regulation within intra-articular fibroblasts, including synovial fibroblasts that mediate fibrosis during OA progression (Figure 1C).

Discussion: Our results show a protective effect of global CD38 deletion on cartilage degeneration following joint injury in mice, confirming published findings [4]. Strikingly, we found that CD38 deletion and NR treatment each reduced the pro-fibrotic gene expression activated within articular chondrocytes after injury. These results suggest that an intra-articular NAD decline may promote cellular dysfunction within both cartilage and synovium during PTOA. The sources of CD38-mediated NAD consumption within the OA joint require further investigation.

Significance: OA is a leading cause of disability associated with advanced age as well as prior joint trauma. The goal of this work is to better understand how these risk factors lead to articular cartilage loss, a central feature of OA with no viable treatment options, by studying how they alter steady-state levels of essential metabolite nicotinamide adenine dinucleotide (NAD) within the joint. Results should inform the development of disease-modifying OA treatments that maintain NAD homeostasis.

References: [1] Warren et al. Nature Comm 2023 14(1): 3616; [2] Glasson et al. Osteoarthritis Cartilage 2007 15(9):1061; [3] Glasson et al. Osteoarthritis Cartilage 2010 18 Suppl 3:S17. [4] Gil Alabarse et al. Arthritis Rheumatol 2023 75(3):364.

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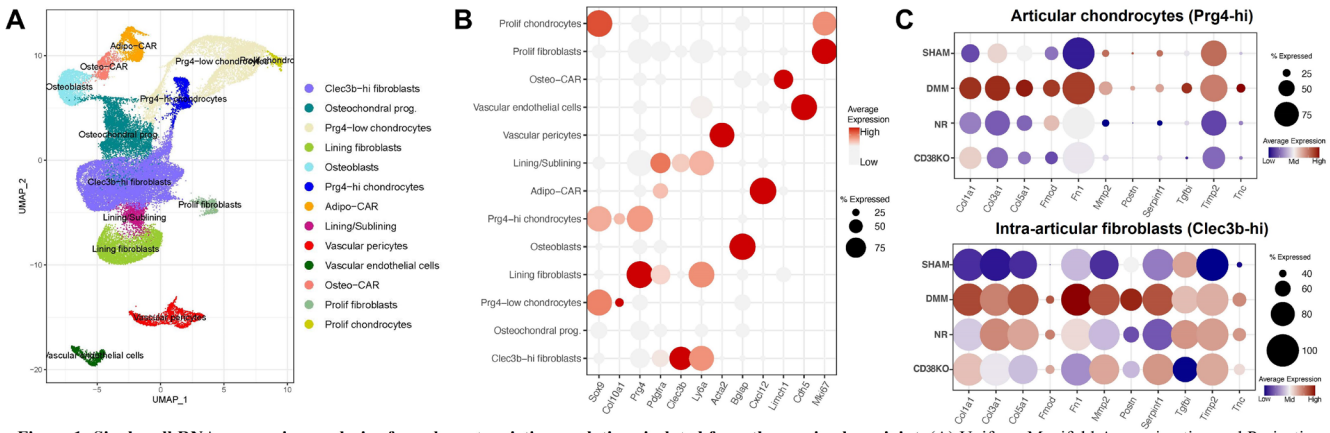

Figure 1: Single cell RNA sequencing analysis of non-hematopoietic populations isolated from the murine knee joint. (**A**) Uniform Manifold Approximation and Projection (UMAP) based visualization of the non-hematopoietic cells isolated from the distal femur and proximal tibia of sixteen young adult C57BL/6 male mice (one joint per animal). (**B**) Dot plot displaying expression of key marker genes for the principal clusters from panel A. (**C**) Dot plots representing differential gene expression of fibrosis-related genes within individual clusters isolated from knee joints harvested 2 weeks after Sham or DMM surgery in wild type or DMM in CD38KO mice. Some mice were treated with NR (1000 mg/kg/day) between DMM surgery and cell isolation.