Targeting CD38-mediated NAD Metabolism in Synovial Fibroblasts to Modulate Gouty Arthritis

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INTRODUCTION: Gout is a common age-related autoinflammatory condition associated with recurrent monosodium urate (MSU) crystal-induced, painful flares and insufficient therapies in many patients. Nicotinamide adenine dinucleotide (NAD) facilitates vital cellular processes, and NAD decline is strongly associated with aging and degenerative diseases. The transmembrane ectoenzyme CD38 cleaves NAD, reducing overall availability. Blood CD38 levels are significantly associated with gout flare, and gout patients exhibit lower systemic NAD levels. While the role of immune cells in gout pathogenesis has been well studied, synovial fibroblasts (SFs) are now understood to orchestrate joint inflammation and pain. However, the contribution of SF NAD metabolism in gout is unknown. We hypothesized that CD38 plays a key role in SFs via regulating NAD homeostasis and inflammation upon MSU exposure.

METHODS: Primary mouse fibroblast-like synoviocytes (FLS) were isolated from C57/Bl6 (wild-type, WT) and global CD38 knockout (KO) mice (2 male+2 female pooled). All experiments used 200 µg/ml MSU crystals for 24 hours unless otherwise stated. The commercial NAD/NADH-Glo assay quantified intracellular NAD and NADH levels, normalized to Alamar Blue signal (n=12). Bulk RNA sequencing was performed on RNA isolated after 12 hours of MSU treatment (n=4) and analyzed in RStudio. Human SW982 synovial cells were treated with MSU and 5 nM MK-0159, followed by quantitative PCR (qPCR) and ELISA on supernatant media (n=6). For explant culture, synovial tissue was harvested from an RA patient (knee), cut into individual pieces, and cultured with no treatment, dexamethasone (Dex, positive control), or 10 nM MK-0159 (n=8) for 48 h, followed by ELISA of secreted IL-6.

RESULTS: MSU treatment significantly reduced NAD/NADH in WT but not CD38 KO FLS (**1A**). MSU also induced substantial changes in gene expression in both genotypes (**1B**). Of all MSU-induced genes, WT and KO cells exhibited 11 and 17% unique differentially expressed genes (DEGs), respectively, sharing 71% of response DEG (**1C**). Gene Set Enrichment Analysis (GSEA) demonstrated downregulation of inflammatory terms, Wnt signaling, and TGFβ production in KO cells, and upregulation of terms related to biomolecule synthesis and mitochondrial activity (**1D**). These changes were driven in part by *Il6*, *Wnt5a*, *Sfrp1*, Hif1a, Cxcl1, and *Tgfbr2* (**1E**). CD38 KO fibroblasts showed strong upregulation of terms related to oxidative phosphorylation compared to WT FLS (data not shown), demonstrating that CD38 is a key regulator of SF metabolism. Human SW982 cells treated with the CD38 inhibitor MK-0159 showed significantly lower MSU-induced expression of *Il6*, *Il1b*, and *Mmp1* (**1F**) and markedly blunted protein secretion of IL-6 (**1G**). MK-0159 also suppressed secretion of IL-6 from an arthritic intact human synovium, with comparable anti-inflammatory effects as Dex (**1H**).

DISCUSSION: Loss of CD38 protects SFs from MSU-induced NAD decline. Furthermore, CD38 KO FLS exhibit reduced inflammatory programming in response to MSU, while upregulating mitochondrial activity, indicating increased energy stores to adapt to MSU challenge. CD38 KO cells also showed downregulation of *Tgfbr2*, *Smad3*, and collagen genes, which could indicate a blunted fibrotic response. Wnt signaling, Hif1a, and Il6 appear to contribute to the differential response of CD38 KO cells. Notably, the NAD hydrolase activity of CD38 is also highly druggable by small molecules. Indeed, MK-0159 reduced cytokine and protease expression in MSU-treated SW982 cells, as well as in human RA synovial explants. Thus, our results indicate that small molecule CD38 inhibition is an exciting novel treatment strategy to reduce SF inflammation in gouty arthritis.

SIGNIFICANCE/CLINICAL RELEVANCE: Increased understanding of gout pathomechanisms, particularly targeted at SFs to circumvent immunosuppression, could lead to improved disease management in patients with highly active disease for which standard of care is not sufficient.

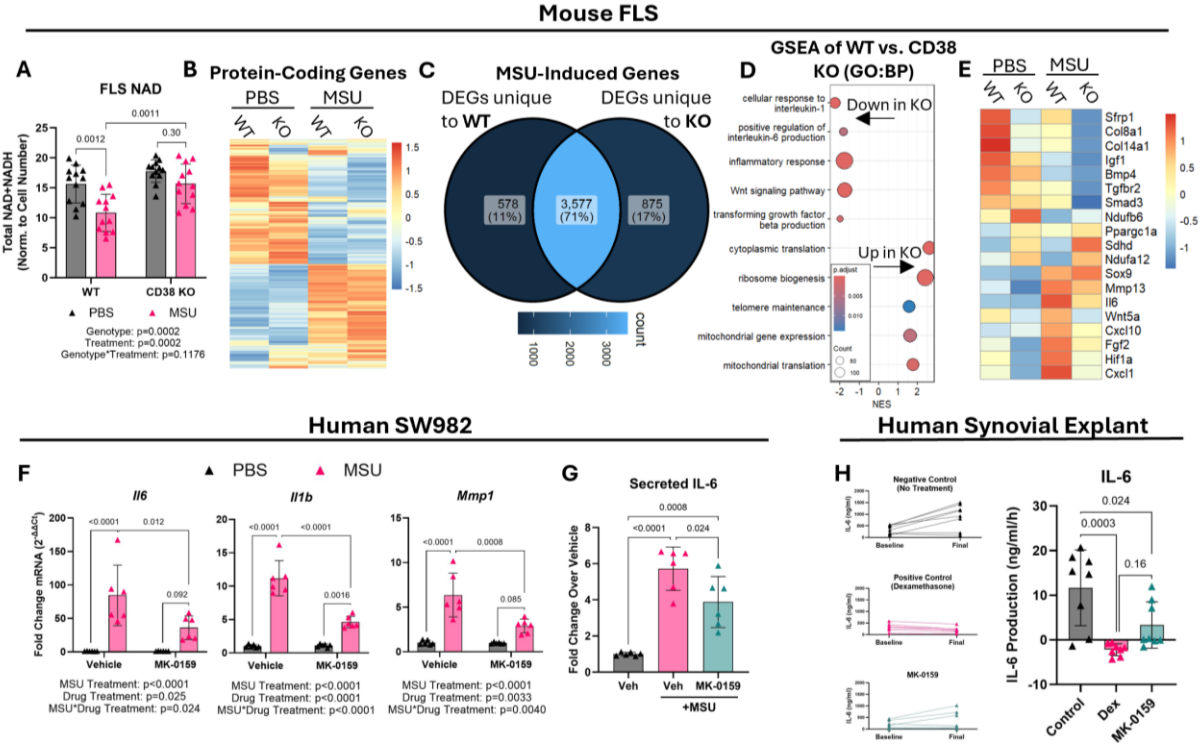

Figure 1: (**A-E**) Analysis of WT and CD38 KO primary mouse FLS treated with PBS or MSU crystals. (**A**) Measurement of NAD and NADH levels. (**B**) Heatmap of all protein-coding genes. (**C**) Venn diagram demonstrating shared and unique DEGs (MSU vs. PBS). (**D**) GSEA indicating differentially regulated pathways in MSU-treated cells. Positive NES indicates upregulation in CD38 KO cells. (**E**) Heatmap of key leading edge genes driving GSEA result. (**F-G**) Analysis of human SW982 cell line treated with PBS or MSU crystals +/- CD38 inhibitor MK-0159. (**F**) qPCR of disease-relevant genes. (**G**) ELISA of supernatant media IL-6 levels. (**H**) Human RA synovial explant secretion of IL-6 with MK-0159.