INTRODUCTION: Proteoglycan 4 (PRG4) is a mucinous glycoprotein that fulfills a homeostatic role in the joint. In joints lacking PRG4 expression, synovial hyperplasia, fibrosis, and accumulation of pro-inflammatory macrophages progressed with age and these pathological changes were partially reversed with PRG4 re-expression. PRG4 regulates macrophage activation via binding to its cognate receptor, CD44 and activation of protein phosphatase 2A (PP2A) and downstream inhibition of xanthine oxidase (XO) expression. In this context, XO-derived reactive oxygen species (ROS) was shown to drive NLRP3-dependent IL-1β secretion by macrophages. We aimed to characterize early metabolic changes in the synovium with respect to XO modulation and downstream synovial macrophage immune activation in a novel conditional knockout mouse model, where animals are born PRG4 competent and PRG4 expression can be inactivated with tamoxifen (TAM). We hypothesized that XO upregulation in synovium contributes to exaggerated inflammatory activation of these macrophages. METHODS: PRG4^<fl/fl> R26<sup>lox/lox</sup>Tg;Tot-<sup>Cre</sup> and PRG4<sup>fl/fl</sup>Frt/GT<sup>Frt</sup>/Cre;25<sup>mM</sup> or corn oil (Veh) (100 µl) administration occurred in 4 weeks-old animals for 10 days and histological analyses and synovial tissue collection for RNA and synovial macrophage (SM) isolations were performed 6 weeks later. Histological analyses included PRG4 and XO immunohistostains by Mab 86.79 and anti-XO antibody (Abcam) (1:100 dilutions for both antibodies), respectively followed by DAB staining as well as hematoxylin and eosin (H&E). In TAM and Veh administered animals, RNA was isolated from synovial tissues of both knee joints and pooled together to represent one independent biological sample and multiplexed gene expressions were performed using pre-validated panels for murine immune activation status and cell metabolism using the nCounter technology (NanoString). In addition, Synovial tissues from 2-3 animals were pooled together and subjected to SM isolation. Isolated SMs (500,000 cells per well) were activated using LPS (100 ng/ml) and IFNγ (50 ng/ml) and intracellular ROS levels were quantified using the DCFDA/3HDCFDA kit (Abcam). Alternatively, glycolytic activation of SMs was monitored in real time using a Seahorse Analyzer displaying proton efflux rate (PER), a marker of pro-inflammatory SM activation. Pharmacological treatments included febuxostat (Feb; XO inhibitor; 25 µM) (Cayman Chemicals) and echinomycin (HIF-1α inhibitor; 50 nM). Statistical analyses included Student’s t-test and ANOVA followed by post-hoc Tukey’s test.

RESULTS: Abolishment of PRG4 expression was evident in TAM mice, and this was associated with enhanced XO staining and synovial hyperplasia (Fig. 1A & 1B). In TAM synovia (n=3), glycolysis, hypoxia and oxidative stress pathways were upregulated compared to Veh synovia (n=3) (Fig. 2A) and upregulated individual genes of interest included Xdh (gene symbol for XO; p<0.01) and Hif1α (p<0.008) (Fig. 2B). ROS levels were higher in TAM SMs at baseline and following LPS+IFNγ activation (Fig. 3A). Feb treatment reduced ROS level in TAM SMs (Fig. 3B, p<0.001). TAM SMs more readily switch to aerobic glycolysis (Fig. 3C & 3D) and this switch was inhibited by Feb (Fig. 3E; p<0.01) and HIF-1α inhibitor (Fig. 3F, p<0.001) treatments.

DISCUSSION: PRG4 inactivation induced synovial hyperplasia and XO upregulation. Immune activation, glycolysis and hypoxia pathways were also activated in the synovium due to PRG4 inactivation. SMs from PRG4 deficient animals had a higher ROS burden likely due to XO upregulation and XO and HIF-1α contributed to exaggerated inflammatory activation of these macrophages. SIGNIFICANCE: PRG4 is a significant modulator of SM phenotypic switching and restoring PRG4-mediated synovial homeostasis is potentially therapeutic in chronic synovitis.

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