Depletion of *Prg4*-expressing cells in adult mice enhances synovial ossification and does not protect from cartilage degeneration following surgically induced post-traumatic osteoarthritis.

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INTRODUCTION: *Prg4* (encodes the protein lubricin) is widely expressed in the synovial joint including cells at the surface of articular cartilage (AC) and in the synovial lining. Previous work[1] has shown that genetic depletion of *Prg4*-expressing cells in a mouse model had little impact on cartilage homeostasis with age and, paradoxically, protected AC from degeneration following surgically induced post-traumatic osteoarthritis (destabilization of the medial meniscus, DMM). However, Prg4+ cell depletion in that study was induced in growing 3-week-old mice potentially resulting in structural/organizational compensation that diminished degeneration following DMM. Would depletion of *Prg4*-expressing cells in adult animals (with fully organized tissues) produce the same results? What is the impact of Prg4-cell depletion in the synovial lining? To answer these questions, we used a transgenic *Prg4CreER* allele[2] combined with the Rosa26-driven diphtheria toxin antigen (DTA) mice used in the previous study (commercially available) to induce Prg4-cell depletion in adult mice (12 weeks old) and induced post-traumatic osteoarthritis (PTOA) using the DMM surgical model.

METHODS: IACUC approved all animal work. Mouse Models: *Rosa-DTA* (Jax#09669) was crossed to *Prg4CreER* (transgenic) mice[2]. *Prg4iDTA* (Cre+) animals and control (Cre-) littermates were treated with 10 doses of Tamoxifen (150mg/kg) over 12 days starting at 12 weeks of age. Due to possible differences in *Prg4CreER* lines (the previous study[1] used a knock-in), we treated a separate group of controls and mutants starting at 3 weeks of age. For both ages, a set of animals (n=4-7 per group) was used to assess normal joint homeostasis (Fig. 1A) and a second set (n=5-8 per group) underwent DMM surgery to induce PTOA[3]. Both sexes were used throughout but males and females were analyzed separately in PTOA due to known sex differences. To maintain consistency between the two age groups, surgery was performed 5-6 weeks following start of tamoxifen treatments and collected for analysis 12 weeks following surgery (Fig. 2A). For all collections, animals were euthanized by CO_2 inhalation. Histologic Analyses: Knee joints were fixed in 10% formalin, decalcified in 20% EDTA, and processed into paraffin. Density was measured with Sox9/DAPI staining. SafraninO/Fast Green/Hematoxylin was used for modified mankin[4] and synovitis scoring[5]. microCT Analyses: Knee joints were in a Scanco µCT 35 instrument at 14.9 µm. Analysis was carried out using 3D Slicer (open source). Data were analyzed using Graphpad Prism by 2way ANOVA and Uncorrected Fisher's LSD. P-value: *<0.05, **<0.002, ***<0.001.

RESULTS: First, we assessed disruption of cartilage phenotypes. *Prg4*-expressing cell ablation with *Rosa-DTA* effectively reduced cells in the superficial zone (where *Prg4* expression is highest) of AC at both induction ages (Fig. 1B). Total AC cell density was also significantly reduced in *Prg4iDTA* animals at 3 weeks immediately following induction whereas adult *Prg4iDTA* animals showed delayed decrease (Fig. 1C). Immunostaining for Sox9 showed no changes in *Prg4iDTA* animals induced at 3 weeks but a significant decrease (40%) in *Prg4iDTA* animals induced at 12 weeks (Fig. 1D). Following DMM surgery, male and female *Prg4iDTA* animals induced at 12 weeks and males induced at 3 weeks were not protected from cartilage damage (Fig. 2B). Interestingly, all female animals that underwent DMM surgery at 8-9 weeks of age were protected from cartilage damage (Fig. 2B, arrow). Next, we assessed the response to Prg4-cell depletion in the synovial lining where it is also highly expressed. Here, we found a differential response that is age dependent. *Prg4iDTA* animals induced at 12 weeks of age show ectopic cartilage formation (Fig. 3A, brackets) and significantly increased synovial hyperplasia that does not resolve with time (Fig. 3A, arrows and 3B). This was exacerbated following DMM surgery to include osteophyte formation (Fig. 3C, red arrow) significantly increasing in *Prg4iDTA* animals treated with tamoxifen at 12 weeks in both DMM-and SHAM-operated groups (Fig. 3D).

DISCUSSION: These studies provide new insights on the function of *Prg4*-expressing cells in growing and mature mouse synovial joints. Results provide evidence that Prg4 cell depletion is more disruptive to cartilage homeostasis (Sox9 expression) in adult animals compared to young animals but does not impact cartilage damage at either age following DMM surgery. This surprising latter result conflicts with protection observed in the previous study Prg4 cell depletion study[1] and the differing *Prg4CreER* model systems (knock-in versus transgenic alleles) is a current limitation. However, our results at homeostasis add to growing evidence that chondrocyte death may not, by itself, induce significant degeneration[1,6]. Importantly, we also show that *Prg4* cell depletion increases ectopic ossification in synovial lining only in adult animals providing new evidence that this tissue loses plasticity rapidly in adult animals.

SIGNIFICANCE: This study provides new, preclinical evidence that depletion of *Prg4*-expressing cells disrupts homeostasis in mature (no longer developing) synovial joints, particularly in the synovial lining, and does not protect against cartilage damage in a surgical model of PTOA.

REFERENCES: [1]Zhang, et al.(2016) [2]Decker, et al.(2017) [3]Glasson, et al.(2007) [4]Hensen & Vincent (2008) [5]Krenn, et al.(2002) [6]Masson, et al.(2025).

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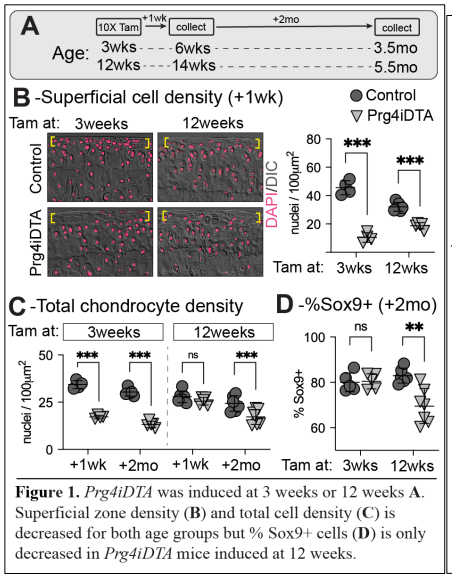

Figure 1. *Prg4iDTA* was induced at 3 weeks or 12 weeks **A**. Superficial zone density (**B**) and total cell density (**C**) is decreased for both age groups but % Sox9+ cells (**D**) is only decreased in *Prg4iDTA* mice induced at 12 weeks.

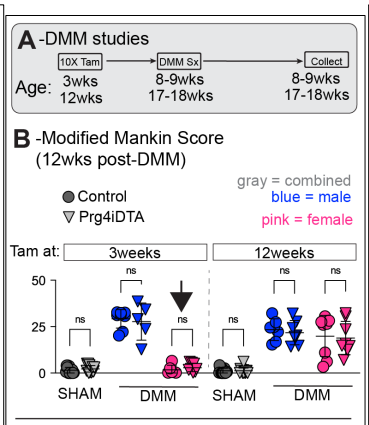

Figure 2. Experiment design (**A**) of DMM-induced PTOA in *Prg4iDTA* induced at 3 weeks or 12 weeks of age. Modified mankin scoring (**B**) shows no protection from DMM surgery based on *Prg4iDTA*, but significant protection in all females that underwent DMM surgery at 8-9 weeks of age (arrow).

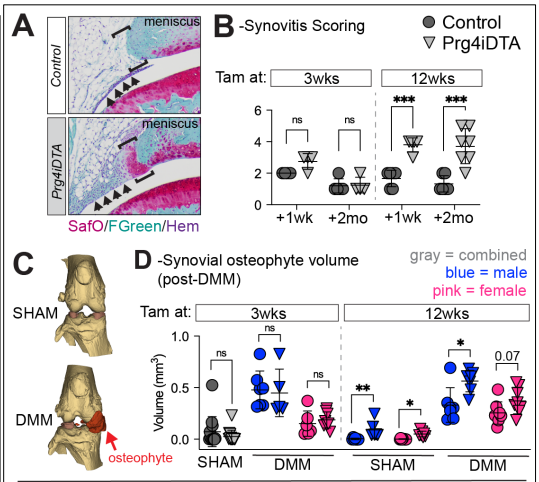

Figure 3. Staining for SafO (**A**) shows ectopic cartilage (brackets) and synovial hyperplasia (arrows) in *Prg4iDTA* animals induced at 12 weeks (quantified in **B**). µCT quantification of osteophyte formation following DMM (**C**) shows increase in *Prg4iDTA* animals induced at 12 weeks (**D**).