Intra-articular Injection of a Fibroblast Activation Protein Inhibitor Mitigates Synovial Pathology in a Large Animal Model of PTOA

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Introduction: There are currently no disease-modifying therapeutics for osteoarthritis (OA).[1] Pathology of the synovium has been identified as a potential driver of disease. As OA progresses, the synovium stiffens, driving fibroblast-like synoviocytes (FLS) to differentiate into myofibroblasts.[2] These contractile cells lose key homeostatic functions, instead producing fibrotic matrix and inflammatory mediators.[3] Fibroblast activation protein (FAP) is a serine protease overexpressed in states of inflammation and fibrosis.[4] We recently showed that FAP is upregulated in OA synovium and contributes to FLS mechanoactivation *in vitro*.[5] In murine models, FAP inhibition ameliorates cartilage degeneration after joint injury;[6] however, the contribution of FAP to synovial pathology *in vivo* and the efficacy of FAP inhibition in a large animal model remain unknown. Thus, the objectives of this study were (1) to evaluate the relationship between FAP expression and progression of synovial pathology and (2) to determine whether FAP inhibition can slow joint degeneration in a porcine model of post-traumatic OA (PTOA).

Methods: All animal work was IACUC approved. Analysis of FAP expression in a large animal model of PTOA: Yucatan minipigs (n=15) underwent unilateral arthroscopic destabilization of the medial meniscus (DMM) to induce OA. Contralateral joints served as intact controls. As there are significant sex differences in progression of porcine PTOA,[7] only males were used to allow for comparisons to our previous datasets.[8,9] Synovium was harvested at 6 weeks (n=7) or 6 months (n=8) and scored for lining hyperplasia, inflammation, vascularity, and fibrosis[10] or stained for FAP and αSMA. Correlation between histological scores and FAP or αSMA staining was determined via linear regression. Evaluation of intra-articular delivery of a small molecular FAP inhibitor: An additional cohort of Yucatan minipigs (n=8) underwent unilateral arthroscopic DMM. Sham surgery was performed on the contralateral limbs. Synovial samples taken at the time of surgery served as naïve controls. Following DMM, minipigs received weekly bilateral intra-articular injections (1mL) with a small molecule FAP inhibitor (FAPi, 30μg/kg, n=4) or PBS (n=4). One animal had an allergic reaction after the first FAPi administration, and so was removed from the study. At 6 weeks, synovium was harvested for histology, immunofluorescence (IF), atomic force microscopy (AFM), and scRNA-seq. Osteochondral units were collected for indentation testing and histology. Outcomes were compared between sham and DMM groups using repeated-measures two-way ANOVAs with Fisher's LSD post-hoc tests.

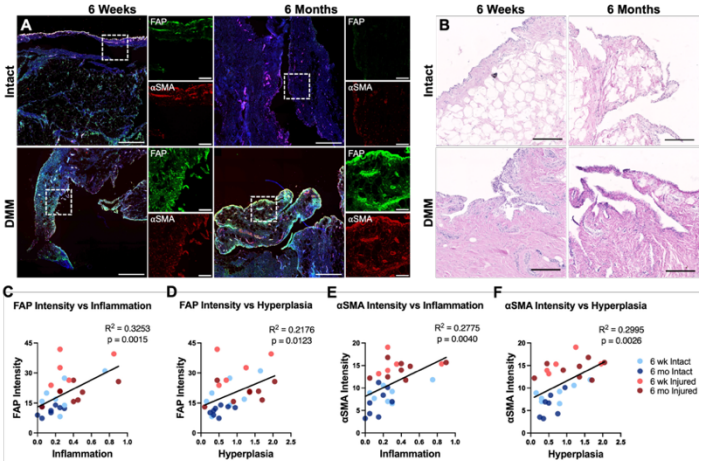

Figure 1. (A) Synovium from intact and DMM knees stained for FAP and αSMA. SB (merged images): 500μm. SB (individual channels): 100 μm. (B) Synovium stained with H&E. SB: 200μm. (C) Correlation between FAP staining and histological scores. (F) Correlation between αSMA staining and histological scores.

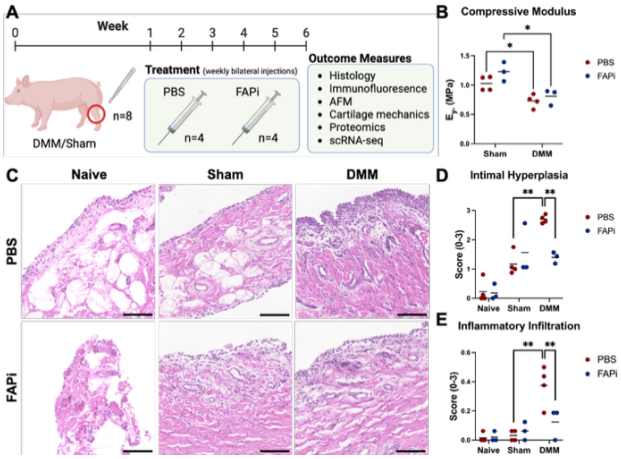

Figure 2. (A) Study design. (B) Cartilage compressive modulus. (C) Synovium from naïve, sham, and DMM knees. SB: 100μm. Histological scores for (D) hyperplasia and (E) inflammation. *p<0.05. **p<0.01.

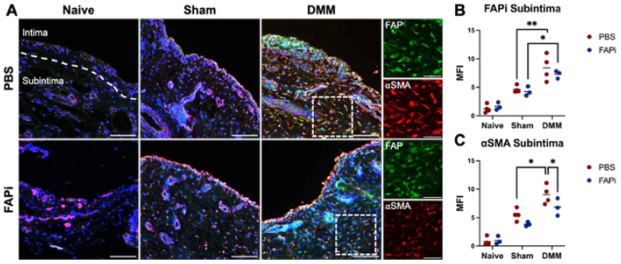

Figure 3. (A) Synovium from naïve, sham, and DMM knees treated with FAPi or PBS and stained for FAP and αSMA. SB: 100μm. Quantification of (B) FAP and (C) αSMA staining intensity in the subintima. *p<0.05. **p<0.01.

Results: FAP expression correlates with synovial pathology. FAP staining intensity positively correlated with inflammatory infiltration (R^2=0.3253, p=0.0015) and intimal hyperplasia (R^2=0.2176, p=0.0123) (**Fig 1A-D**). αSMA staining positively correlated with inflammatory infiltration (R^2=0.2775, p=0.004), intimal hyperplasia (R^2=0.2995, p=0.0026), and vascularity (R^2=0.2330, p=0.0093) (**Fig 1A-B, E-F**). FAP inhibition mitigates synovial pathology after joint injury. All joints showed marked changes in joint structure and function following injury, with cartilage compressive modulus decreasing after DMM in both PBS (p=0.0307) and FAPi (p=0.0115) groups (**Fig 2B**). Osteochondral histology and OARSI scoring are pending. With respect to the synovium, DMM increased hyperplasia (p=0.0024), inflammation (p=0.0037), vascularity (p=0.0018), and fibrosis (p=0.023) in PBS-treated animals. In the FAPi-treated group, however, there was no increase in synovial pathology compared to sham, and a significant reduction in hyperplasia (p=0.0038) and inflammation (p=0.0062) compared to PBS-treated controls (**Fig 2C-E**). As in the prior cohort, DMM increased expression of FAP (p=0.0041) and αSMA (p=0.0196). While FAP inhibition did not change FAP staining, it significantly reduced αSMA staining in the subintima (p=0.0487) (**Fig 3A-C**). AFM, synovial fluid proteomics, and scRNA-seq are pending.

Discussion: We previously showed that FAP expression increases in OA synovium.[5] Here we demonstrate that FAP expression positively correlates with histological scores for synovial pathology, further implicating FAP in joint pathology following injury. Based on these results, we proceeded with a large animal pre-clinical trial of intra-articular FAPi injection. Although we did not detect cartilage preservation (as was observed in murine models), we did find a significant attenuation of synovial pathology. Given that synovitis is a strong predictor of patient pain and symptoms, irrespective of cartilage loss,[11] these large animal findings are promising. Future studies will include longer-term time points to assess the durability of treatment effects, as well as further investigation of tissue and cell-level mechanisms by which FAPi exerts its positive effect.

Significance/Clinical Relevance: This study demonstrates that intra-articular FAP inhibition can attenuate synovial inflammation and fibrosis in a clinically relevant large animal model, highlighting FAPi as a promising therapeutic for modification of disease progression in PTOA.

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