Evaluating the Paracrine Release of Myokines Relative to Myopenia and Fibrosis in Osteoarthritis

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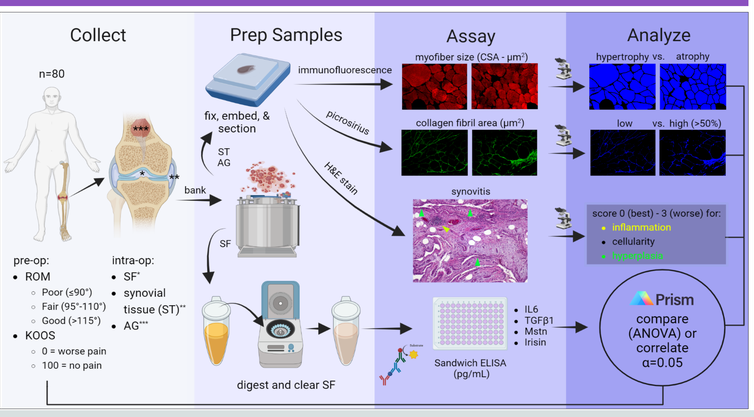
Introduction

Peri-articular myopenia and fibrosis are secondary to post-traumatic and chronic knee osteoarthritis (KOA).[1] The definitive treatment for KOA is total knee arthroplasty (TKA), which primarily replaces the cartilaginous articular surfaces and a fraction of subchondral bone segments. However, disuse-mediated muscle wasting of the quadriceps femoris (QF) complex, synovitis, and synovial fibrosis (SFb), which increases contracture of the joint capsule, are difficult to treat peri-operatively and can persist after TKA, contributing to myopathy and stiffness that effectively limit full range of motion (ROM).[2] Cytokines interleukin 6 (IL6) and transforming growth factor (TGF)β1 are well known drivers of inflammation and fibrogenesis, respectively, that are highly expressed in articular soft tissues such as the synovium and elevated in the synovial fluid (SF) of KOA patients. As paracrine structures, peri-articular muscles such as the QF release dysregulated concentrations of myokines during KOA that can alter the inflammatory severity of the disease and progression of arthrofibrosis.[3] The myokines myostatin (Mstn) and irisin are paracrine effectors differentially secreted in response to both exercise and disease.[4-7] The main role of Mstn, a member of the TGF family, is to inhibit the Akt kinase in myocytes that is responsible for muscle hypertrophy, but is also known to trigger synthesis of IL6 and has been reported to increase muscle fibrosis.[8] Irisin, on the other hand, is a recently discovered, exercise-mediated hormone that facilitates glucose uptake by muscles and lipid metabolism but can exert an anti-fibrotic effect by modulating the canonical TGFβ1 pathway.[9,10] The intra-articular Articularis genu (AG) extensor muscle of the knee is continuous to the QF and has been shown to undergo similar disuse-mediated structural changes such as myofiber type switching, atrophy, and fibrosis as the *Vastus lateralis* of the QF.[11] To that effect, the structural integrity of the AG can potentially be analyzed as a surrogate for the health status of the QF relative to clinical metrics such as ROM angles and values form the patient-reported Knee Osteoarthritis Outcome Scores (KOOS) questionnaire. This study aims 1) to understand the association between well-known pro-inflammatory and pro-fibrogenic cytokines in KOA with muscle-mediated paracrine release of Mstn and irisin into the SF to refine assessment of KOA soft tissue status and 2) to compare structural changes to the AG partly modulated by these myokines between patients grouped by ROM deficits. We predict that lower concentrations of irisin with higher concentrations of Mstn, IL-6, and TGFβ1 in the SF will be associated to aberrant KOA-attributable myopenia and fibrosis, both of which worsen with decreasing ROM and lower (worse) KOOS scores.

Hypothesis

We predict that lower concentrations of irisin with higher concentrations of Mstn, IL-6, and TGFβ1 in the SF will be associated with aberrant KOA-attributable myopenia and fibrosis, both of which increase in severity with decreasing ROM and KOOS. Overall, understanding the effect of differential myokine release in association to symptomatic and structural KOA will help tailor novel patient-centered, soft tissue rehabilitation strategies to attenuate KOA severity, maximize joint function, and improve patient satisfaction before and after TKA.

Methods



Results

Mstn is elevated in KOA disuse-mediated myopenia

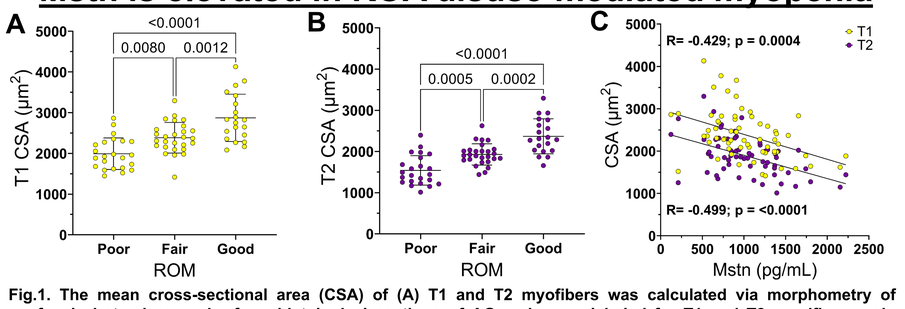


Fig.1. The mean cross-sectional area (CSA) of (A) T1 and T2 myofibers was calculated via morphometry of confocal photomicrographs from histological sections of AG co-immunolabeled for T1 and T2-specific myosin heavy chains. Values were compared between AG samples from patients grouped by poor, fair, and good ROM by one-way ANOVA. (C) Mstn in the SF, which was elevated by an average of 19% in poor compared to the good ROM group, (C) showed a negatively moderate association to T1 and T2 CSA.

Mstn associates with synovitis grade but not fibrosis

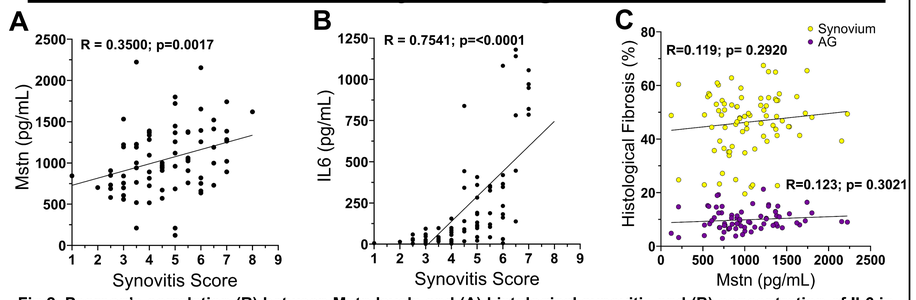


Fig.2. Pearson's correlation (R) between Mstn levels and (A) histological synovitis and (B) concentration of IL6 in SF. (C) Mstn levels showed a weak association to fibrosis metrics from synovium and AG tissues.

AG and ST fibrosis are proportional, increase with worse symptoms, and associate to TGFβ1 in the SF

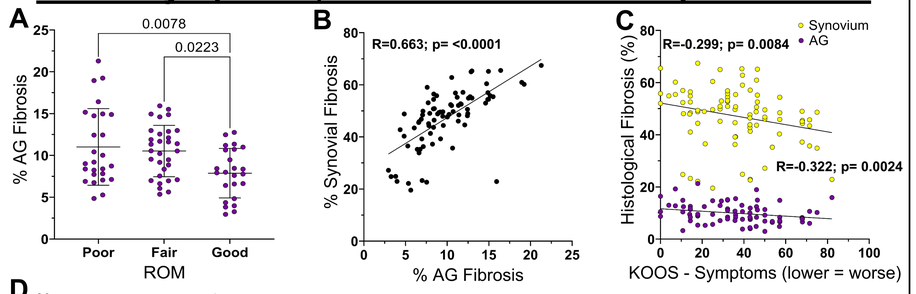


Fig.3. Fibrosis of the AG endomysium and synovial subintima was measured from regions of picrosirius (PS)-stained tissue sections captured by confocal. Labeled collagen fibrils within connective tissue areas were automatically measured and percentages calculated over total tissue area using software-assisted thresholding. (A) Mean metrics of AG fibrosis were compared between ROM groups using one-way ANOVA with Tukey's multiple comparison test. (B) A moderately high Pearson's correlation was observed between synovial and AG histological fibrosis. To confirm its relation to stiffness, (C) synovial and AG fibrosis measures were analyzed against the symptoms subscale of the KOOS survey. Further, (D) measured concentrations of free TGFβ1 in SF were evaluated to test for an association with synovial and AG fibrosis.

Pilot data on irisin SF levels suggest negative trends with TGFβ1, fibrosis, and ROM deficits

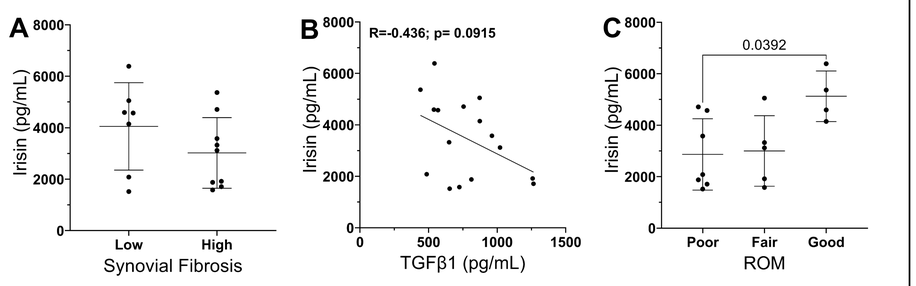


Fig.4. To test the relevance of irisin as a negative modulator of fibrosis and TGFβ1 synthesis, concentrations of irisin in the SF from 16 of the 80 patients in the study were compared between patients grouped by (A) low or high synovial fibrosis by one-way ANOVA and (B) evaluated against TGFβ1. (C) Irisin levels were also compared between patients grouped by ROM status, since functional limitations are associated to fibrosis status.

Summary

- Our team measured significant alterations to myofiber distribution, increased myofiber atrophy, and severe endomysial fibrosis of the articular musculature that worsen with decreasing ROM (i.e., increased joint disuse).
- Structural changes to the intra-articular AG extensor are associated to the differential expression of inflammatory and fibrogenic cytokines that can be secreted from various joint tissues[12], but also effector myokines Mstn and irisin that play an indirect role on inflammatory status, muscle growth control, and modulation of fibrosis within articular muscle and adjacent soft tissues such as the synovial membrane.[4-8]
- Although Mstn is reported to exert an anti-hypertrophic, inflammatory, and pro-fibrotic effect[8] beyond muscle, our data suggest that elevated local levels of Mstn during KOA are mostly related to progression of muscular atrophy, a moderate but indirect effect on synovitis, but no effect on inter-compartmental fibrosis, which is mostly driven by TGFβ1.
- Metrics of fibrosis in the synovial subintima and the endomysium of the AG are proportional, with a strong association to local levels of TGFβ1, which may act as a common fibrogenic effector between these two soft tissue compartments. The severity of fibrosis, evaluated by measuring the density of deposited collagen fibrils, is relative to ROM deficits and patient-reported stiffness, which supports similar published data.[13]
- Irisin supplementation has been shown to relieve pressure overload in a mouse model of transverse aortic constriction through inhibition of TGFβ1-Smad2/3 signaling triggered by angiotensin-II mediated oxidative stress.[14] Through a similar mechanism, irisin supplementation also attenuates fibrosis in models of liver,[9] chronic kidney disease, and pancreatitis. Consistent with this paradigm, pilot measures of local irisin in a fraction of our KOA samples indicate lower levels in patients with high fibrosis and/or ROM deficits with negative association to TGFβ1.

Conclusion

- Aberrant thickening and contracture of the synovium contribute to functional limitations in KOA, which lead to joint disuse-mediated myopenia. Therefore, associating the structural status of the peri-articular musculature and synovium in KOA relative to local concentrations of Mstn, IL-6, TGFβ1, and irisin can potentially inform on the severity of myopenia, inflammation, and fibrosis around the joint from synovial fluid harvested by arthrocentesis.
- Underpinned by the negative association of irisin levels in SF with metrics of fibrosis presentation, further exploring the role, mechanism of action, and effectiveness of targeted irisin supplementation as an anti-fibrotic is critical for the development of conservative patient-centric prophylaxis and intervention against pre-operative arthritis-attributable fibrosis and post-operative arthrofibrosis complication.

Limitations

- Irisin was evaluated from the SF of 16/80 patients in the total sample due to problems with supply chain of the irisin ELISA kit.
- Data are not adjusted for potential confounding variables such as body mass index (BMI), age, sex, and co-morbidities such as diabetes, which can skew inflammatory and wound healing responses.
- Correlations between SF analyte levels with clinical presentation do not suggest causation or explain mechanism of action.
- Although validated, the KOOS questionnaire and compiled values are general but not accurate representations of symptomatic KOA.

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This research project was supported through an award from the Research Enhancement Program of the LSU Health Sciences Center – N.O. School of Medicine.