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"Irisin Attenuates Collagen Deposition in Fibrotic Synoviocytes"

The paracrine potential of skeletal muscle to upkeep homeostasis and modulate disease pathophysiology of musculoskeletal systems has recently gained interest. Various myokines, cytokines, and growth factors produced by contracting muscle can be secreted to synovial fluid (SF) and modulate signaling pathways in knee osteoarthritis (KOA). Irisin, a novel hormone like paracrine factor in muscle, is a fibronectin type III domain-containing protein that interferes with cardiomyopathy, preserves skeletal bioenergetics, prevents denervation-induced myofiber atrophy, is chondroprotective, and its dysregulation has been implicated in pathological KOA. In vitro administration of irisin has been shown to exert an anti-fibrotic effect on stellate cells in liver fibrosis and chronic pancreatitis, and perivascular fibroblasts infarcted myocardium. Furthermore, synovial fibrosis (SFb) severity has been linked to range of motion (ROM) deficits and race-related disparities in structural and symptomatic KOA. Therefore, our overarching goal for this project is to challenge fibrotic synoviocytes with physiological concentrations of irisin and measure collagen output. We hypothesize that irisin treated synoviocytes will have less collagen deposition than those without treatment. Testing irisin's anti-fibrotic properties on these synoviocytes will allow us to potentially develop a non-operative alternative for attenuating KOA stiffness.

We measured collagen output from validated human fibroblast-like synoviocytes (HFLS) cell line derived from normal synovium. Naïve HFLS were thawed, plated in synoviocyte growth medium and growth to 70% confluency. HFLS were trypsinized and subcultured into 6-well plates at 7,000 cells/cm2. 25, 50, 100 ng/mL of r-irisin, 4 ng/mL of TGFB1, or vehicle was used to supplement the medium at 24hrs after subculturing and at every 48hr media change for the 7-day experimental timeline to effectively generate fibrotic (f-) HFLS, unstimulated (u-) HFLS, and irisn (t-) HFLS. f-HFLS and u-HFLS were treated with 25, 50, 100 ng/ML of irisin at 48hrs and at every 48hr media change for the rest of the experimental timeline. Cells were homogenized to measure soluble Col1a1 concentrations by ELISA. Data were analyzed using one-way ANOVA with $\alpha=0.05$ via Prism Graphpad.

There was a significant increase of 889.15% in collagen deposition when comparing u-HFLS and f-HFLS groups ($p<0.0003$). When comparing the u-HFLS and t-HFLS groups there was no significant difference in collagen deposition measured. Moreover, we measured an 80.70% ($p<0.0006$), 65.12%($p<0.0027$), and 59.78%($p<0.0046$) decrease in collagen deposition when comparing f-HFLS to f-HFLS treated with 25, 50, and 100 ng/mL of irisin, respectively. In the presence of TGFB1 stimulation irisin seems to have anti-fibrotic properties on HFLS.

This study has revealed the potential use of irisin as an attenuator of SFb during KOA to possibly delay the need of surgical intervention. Further studies are needed to fully understand the effects of irisin on synoviocytes and its possible therapeutic usage for SFb.