Irisin attenuates mitochondrial dysfunction and regulates biogenesis in human osteoarthritic chondrocytes in vitro

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INTRODUCTION: Osteoarthritis (OA) is a chronic, degenerative joint disease and is regarded as a worldwide cause of morbidity and disability. Physical activity favors weight loss and ameliorates joint pain and function in patients affected by OA. Irisin, a myokine released upon muscle contraction, has been demonstrated to yield anabolic effects on different cell types, including human osteoarthritic chondrocytes (hOACs)[1]. Previous studies have shown that irisin could significantly improve hOAC metabolism and proliferation while reducing catabolic and pro-inflammatory marker expression via inhibiting p38, Akt, JNK, and NFκB pathways[1]. Among major pathomecanisms of OA, mitochondrial dysfunction has been shown to downregulate chondrocyte metabolism hence accelerating the development of the disease[2]. The aim of this study was to investigate the putative effects of irisin on mitochondrial dysfunction in vitro. We hypothesized that this myokine would regulate mitochondrial biogenesis by rescuing hOAc from their dysfunction, ultimately improving cell metabolism.

METHODS: The current study was approved by the IRB of Campus Bio-Medico University of Rome. hOACs (n=4) were isolated from tissues of patients undergoing elective total knee replacement and expanded in monolayer in DMEM + 10% FBS + 1% P/S. Cells were treated with either PBS (control group), 10 ng/mL interleukin (IL)-1β, 25 ng/mL human recombinant irisin (r-irisin), or the combination of both (IL-1β followed by the addition of r-irisin for 24 h). Cell proliferation was assessed by flow cytometry. Glycosaminoglycan (GAG) content was evaluated with the 1,9-dimethylmethylene blue assay. Protein levels of apoptosis markers (caspase 3, cytochrome C, PARP) were assessed by western blot. Metabolic activity and nitrite concentration were measured by MTT assay and Griess reaction, respectively. Cell senescence was investigated with β-galactosidase staining. The expression levels of mitochondrial biogenesis markers (TFAM, TFBMI, mtCO1, D-LOOP) were evaluated via qPCR. Mitochondrial morphology was assessed using the MitroTracker Red CMXRos probe under confocal microscopy. The normality of data distribution was confirmed by the Wilk-Shapiro test. The analysis of the results was performed using one-way ANOVA.

RESULTS: Irisin increased hOAC proliferation ($p<0.05$), metabolic activity ($p<0.05$), and GAG content ($p<0.05$), while significantly decreasing nitrite concentration ($p<0.001$) and cell senescence ($p<0.01$) compared to cells stressed with IL-1β. The expression of mitochondrial biogenesis biomarkers TFAM, TFBMI, mtCO1, and D-LOOP significantly increased after treatment with irisin, even in the presence of IL-1β ($p<0.05$; Fig. 1). Irisin significantly rescued the increased protein levels of caspase 3 ($p<0.01$), cytochrome C ($p<0.05$), and PARP ($p<0.05$) following IL-1β stimulation. Under confocal microscopy, irisin preserved mitochondrial morphology and prevented fragmentation compared to IL-1β-treated hOACs (Fig. 2)

DISCUSSION: Irisin was demonstrated to promote hOAC metabolism and reduce mitochondrial dysfunction and cell apoptosis induced by IL-1β. Mitochondria are crucial to cell metabolism and mitochondrial dysfunction has been implicated in several OA-related mechanisms[3]. By rescuing mitochondrial morphology and function, irisin may reduce oxidative stress and mitochondrial apoptosis, thus attenuating hOAC catabolism and attenuating OA changes.

SIGNIFICANCE/CLINICAL RELEVANCE: The data from this study provide additional evidence of the protective role of irisin against OA in vitro. Apart from expanding the knowledge base regarding the effect of irisin on hOAC, these results support the preliminary concept of a cross-talk mechanism between the muscle and cartilage and further promote the role of irisin as a promising molecular biomarker of musculoskeletal health.

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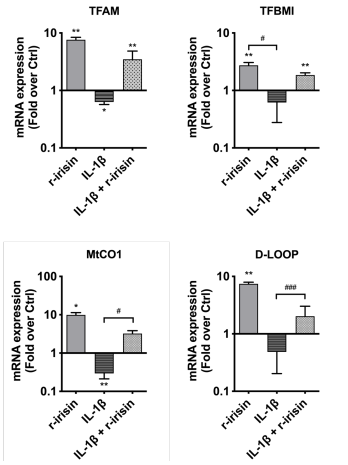

Fig. 1. Gene expression of mitochondrial biogenesis markers. Data have been normalized and shown as fold changes over the control group. *$p<0.05$, **$p<0.01$ compared to the control group. #$p<0.05$, ###$p<0.001$ compared to IL-1β.

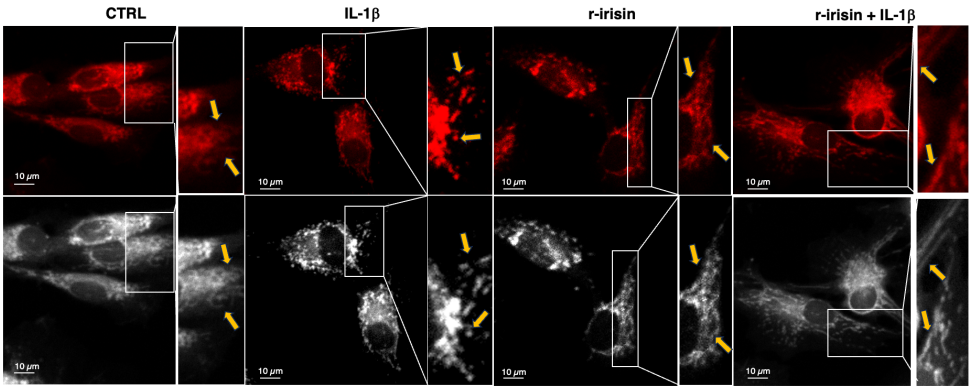

Fig. 2. MitoTracker Red staining of mitochondria shows retention of mitochondrial morphology and network in irisin-treated hOACs. Yellow arrows indicate a reticular, continuous mitochondrial network in the CTRL, r-irisin and r-irisin + IL-1β, while showing focal interruptions in the IL-1β group. Scale = 10 mm.