Irisin attenuates mitochondrial dysfunction by regulating biogenesis in human osteoarthritic chondrocytes in vitro

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INTRODUCTION: Osteoarthritis (OA) is a prevalent degenerative joint disorder characterized by progressive cartilage degradation, leading to pain, functional impairment, and reduced quality of life. While current treatments primarily focus on symptom management, they fail to halt or reverse disease progression. Irisin, a myokine released upon muscle contraction, has demonstrated to yield anabolic effects on different cell types, including chondrocytes[1]. Mitochondrial dysfunction downregulates chondrocyte activity, accelerating the development of OA[2]. In this study, we hypothesized that irisin protects against mitochondrial dysfunction in OA by mitigating inflammatory damage in human osteoarthritic chondrocytes (hOACs).

METHODS: The study was approved by our local institutional ethics committee (Approval No. 215.24 CET2 CBM). hOACs were isolated from osteochondral tissues of patients undergoing total knee arthroplasty (n=5, 3 females and 3 males). hOACs were expanded in vitro and treated with recombinant irisin (r-IR) in the presence of interleukin (IL)-1β. The following parameters were assessed: glycosaminoglycan (GAG) content (DMMB assay), cell proliferation (Trypan blue exclusion), apoptosis-related proteins (Western blot), metabolic activity (MTT assay), nitrite levels (Griess assay), cellular senescence (β-galactosidase assay), and mitochondrial DNA (mtDNA) gene expression (qPCR). Mitochondrial morphology was examined via transmission electron microscopy (TEM), and mitochondrial biogenesis was evaluated using confocal microscopy.

RESULTS SECTION: r-IR significantly enhanced hOAC proliferation, metabolic activity, and GAG synthesis, while attenuating IL-1β–driven nitrite accumulation. Irisin also reduced senescence-associated β-galactosidase staining and downregulated p16 and p21 expression. At the molecular level, r-IR upregulated key mitochondrial biogenesis markers including TFAM, TFBM1, mt-CO1, and D-loop, and increased PPARγ, NRF1, and TFAM protein levels. Microscopic analyses confirmed that r-IR preserved mitochondrial network integrity and ultrastructure under inflammatory conditions.

DISCUSSION: These findings suggest that irisin exerts a protective role in OA by enhancing mitochondrial function, counteracting oxidative stress, and reducing chondrocyte senescence. Given its ability to modulate cartilage metabolism, irisin may represent a promising therapeutic target for OA management and cartilage regeneration.

SIGNIFICANCE/CLINICAL RELEVANCE: Due to the lack of effective disease-modifying treatments for OA, the identification of agents capable of restoring mitochondrial function in chondrocytes is of high clinical importance. By acting on a key pathogenic mechanism, irisin has translational potential as a new therapeutic approach for the management of OA and cartilage regeneration.

REFERENCES: 1. Vadala et al. Cells. 2020;9(6):1478; 2. Wang et al. Antioxidants (Basel). 2020;9(9):810.

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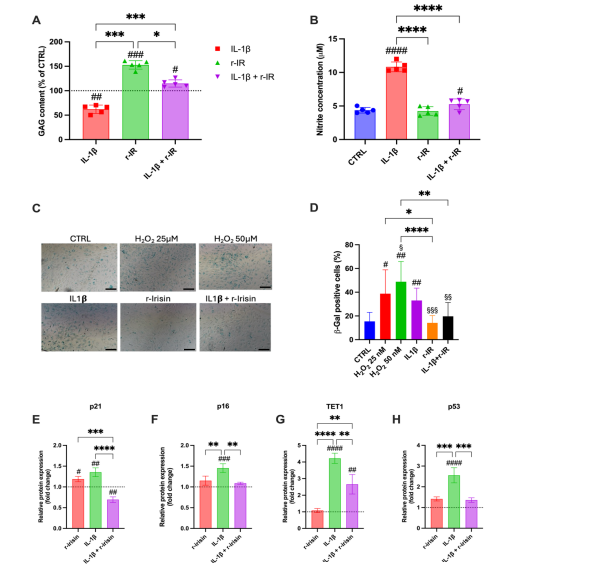

Fig. 1. Irisin enhances ECM synthesis, reduces oxidative stress, and attenuates hOAC senescence. (A) GAG content normalized to DNA. r-IR significantly increased GAG levels and fully reversed IL-1β–induced GAG loss (n = 5). (B) Nitrite accumulation in culture supernatants, measured by the Griess assay. IL-1β markedly increased nitrite release, whereas co-treatment with r-IR reduced nitrite to near-baseline (n = 5). (C) Representative images of SA-β-gal staining. Blue-stained cells indicate senescent hOACs. 10x magnification. Scale bars = 100 µm. (D) Quantification of SA-β-gal–positive cells. IL-1β and H_2O_2 treatments elevated senescence, while r-IR alone and IL-1β + r-IR significantly reduced the percentage of senescent cells to levels comparable to control (n = 3). Protein concentration of senescence markers p21 (E), p16 (F), TET1 (G), p53 (H) demonstrating significant upregulation in IL-1β alone-treated groups, with r-IR exerting a protective effect (n=3). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; #p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001 compared to the control; §p<0.05, §§p<0.01, §§§p<0.001 compared to IL-1β.

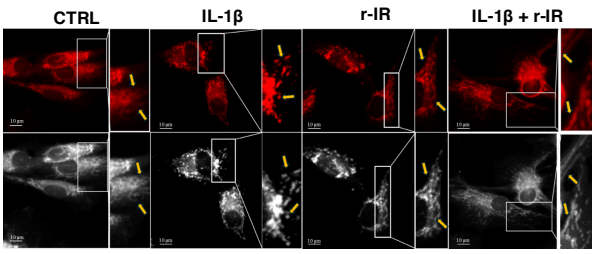

Fig. 2. Irisin preserves mitochondrial network integrity in hOACs under inflammatory stress. Live-cell confocal images of MitoTracker™ Deep Red–stained hOACs showing mitochondrial networks in control, IL-1β, r-IR, and IL-1β + r-IR conditions. Continuous, reticular networks (yellow arrows) are evident in control, r-IR, and IL-1β + r-IR groups, whereas IL-1β alone induces fragmentation and focal disruptions (n ≥ 5 fields per group). Scale bar: 10 µm