TGFβ/IGF1 Signaling Regulates Glucose Metabolism in Articular Chondrocytes

Cuicui Wang[1#], Xiaofei Li[1#], Kevin Cho[2], Qiuyuan Guo[2], Gary Patti[2], Jie Shen[1], Regis O'Keefe[1]
[1] Department of Orthopaedic Surgery, Washington University School of Medicine, St. Louis, MO 63110, USA
[2] Department of Chemistry, Washington University in St. Louis, St. Louis, MO 63130, USA
These authors contributed equally: Cuicui Wang, Xiaofei Li
Email of Presenting Author: xiaofei.li@wustl.edu

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INTRODUCTION: Osteoarthritis (OA) is a prevalent degenerative joint disease characterized by progressive articular cartilage loss, subchondral bone sclerosis, and synovial inflammation, leading to pain and disability [1]. Once viewed primarily as a mechanical "wear-and-tear" condition, OA is increasing recognized as a disease with abnormal glucose metabolism [2]. Articular chondrocytes rely primarily on glycolysis for energy and on hexosamine biosynthetic pathway (HBP) for generating precursors required for extracellular matrix (ECM) synthesis, particularly glycosaminoglycan (GAG) [3]. Impairment of these pathways leads to diminished matrix synthesis, increased oxidative stress, and catabolic activation. Given the central role of glucose metabolism in chondrocyte function, this study aimed to elucidate the molecular mechanisms linking TGFβ signaling to glucose metabolism and identify the contribution of IGF1 as a downstream mediator in maintaining chondrocyte homeostasis and cartilage integrity.

METHODS: All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC). Articular chondrocytes were isolated from the femoral heads of 2-week-old *Tgfβr2$^{f/f}$*, *Igf1r$^{f/f}$*, *Rosa-Igf1$^{f/f}$* pups, transduced with adenoviral GFP or Cre (MOI = 50) in 10 μg/ml polybrene, and analyzed by qPCR or RNA-seq. Glucose uptake and lactate production were measured using commercial assay kits. For *in vivo* studies, 2-month-old male *Igf1r$^{f/f}$* , *Igf1r^{Agc1ERT2}*, *Rosa-Igf1$^{f/f}$* ,*Rosa-Igf1^{Agc1ERT2}*, and 2-week-old *Tgfβr2$^{f/f}$, Col2Cre^{ERT2};Tgfβr2$^{f/f}$*, *Tgfβr2$^{f/f}$;Rosa-Igf1$^{f/f}$* , *Col2Cre^{ERT2};Tgfβr2$^{f/f}$;Rosa-Igf1$^{f/f}$* received tamoxifen (1 mg/10 g body weight) intraperitoneally for five days to induce recombination. Medial meniscal ligament injury (MLI) was performed at 3-month-old *Igf1r$^{f/f}$* , *Igf1r^{Agc1ERT2}*, *Rosa-Igf1$^{f/f}$* ,*Rosa-Igf1^{Agc1ERT2}*male mice, as MLI-induced OA is more evident in male mice. Knee joints were harvested at indicated time for the following histological analysis. Data are shown as mean ± SD, with $p < 0.05$ considered significant.

RESULTS and DISCUSSION: TGFβ1 treatment significantly enhanced glucose uptake and lactate production in articular chondrocytes, as shown in Fig.1 A-D, indicating a robust increase in glycolytic flux induced by TGFβ1 treatment. Moreover, RNA-seq analysis confirmed enrichment of glycolytic and HBP pathways following TGFβ1 treatment, accompanied by marked upregulation of glycolytic and biosynthetic genes. Among these, Igf1 emerged as one of the most significantly induced genes, implicating that TGFβ1 transcriptionally activates Igf1 expression (Fig. 2 A-C). To determine whether IGF1 mediates TGFβ1-induced metabolic reprogramming, *Igf1r* were genetically ablated in articular chondrocytes. Loss of *Igf1r* abolished TGFβ1-induced glucose uptake and lactate production (Fig. 2 D-E), demonstrating that IGF1 acts as a critical downstream mediator of TGFβ1-driven glycolytic enhancement. In mice, cartilage-specific Igf1r deletion impaired GAG production and increased susceptibility to OA, with higher OARSI scores and proteoglycan depletion following MLI injury (Fig 2 F-G, n=7, $p<0.05$). Conversely, IGF1 overexpression promoted glucose consumption, elevated lactate production, and increased GAG production (Fig. 3 A-C), These metabolic effects translated into preserved cartilage integrity, and rescued cartilage degeneration in both MLI-injured mice (Fig. 3 D-E) and Tgfβr2-deficient mice (Fig. 3 F-G). Together, these results indicate that TGFβ1 promotes chondrocyte anabolic activity by enhancing glycolysis and HBP flux, thereby supporting ECM synthesis in cartilage. Our findings further identify IGF1 as an essential effector of TGFβ1 signaling that reprograms chondrocyte glucose metabolism to support anabolic function and maintain cartilage homeostasis. Disruption of this TGFβ/IGF1 signaling could contribute to the impaired anabolism and cartilage degeneration in OA, whereas overexpression of IGF1 could restore glucose metabolism and protect cartilage integrity, highlighting its potential as a therapeutic target for joint homeostasis.

SIGNIFICANCE/CLINICAL RELEVANCE:

(1) TGFβ/IGF1 signaling is essential for maintaining chondrocyte metabolism and cartilage homeostasis.

(2) Targeting IGF1 signaling may offer a promising therapeutic strategy to restore chondrocyte metabolism and prevent OA progression.

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