

# GFPT-Mediated Regulation of the Hexosamine Biosynthesis Pathway in Articular Chondrocytes

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**INTRODUCTION:** Osteoarthritis (OA) is a common debilitating disorder characterized by the progressive deterioration of articular cartilage, synovial inflammation, and subchondral bone sclerosis, leading to chronic pain, joint stiffness, and diminished mobility [1]. It imposes a substantial burden on health systems, with an increasing impact in the aging population [1]. Although OA is traditionally viewed as a wear-and-tear disease, the interplay between cellular metabolism and OA has garnered significant attention. Emerging research shows that metabolic pathways contribute to the pathogenesis of OA. Among these, less is known about the hexosamine biosynthesis pathway (HBP). The HBP serves a metabolic crossroads during glycolysis in which a portion of glucose is shifted away from energy production into a pathway resulting in the synthesis of UDP-N-acetylglucosamine (UDP-GlcNAc). UDP-GlcNAc is necessary for synthesis of glycosaminoglycans (GAGs) and proteoglycans in the extracellular matrix of cartilage [2]. The first enzymes in the pathway, the Glutamine: fructose-6-phosphate amidotransferases (GFPTs), likely have a key role regulating metabolic processes within HBP pathway [3]. However, the precise mechanisms underlying the regulatory effect of GFPTs on the HBP pathway remain elusive. This study aims to investigate the role of the GFPTs in the HBP pathway in articular chondrocytes, and to determine if this pathway is involved in OA and a potential therapeutic target.

**METHODS:** All animal experiments in this study were performed in accordance with approval of the Committees on Animal Resources in Washington University in St. Louis. *Gfpt1*<sup>fl/fl</sup> mice and *Gfpt2*<sup>fl/fl</sup> mice were bred by our laboratory. *Agc1CreERT2*; *Gfpt1*<sup>fl/fl</sup> mice and *Agc1CreERT2*; *Gfpt2*<sup>fl/fl</sup> mice were viable and produced in Mendelian ratios. Articular chondrocytes were isolated from the femoral head of 2-week-old pups from *Gfpt1*<sup>fl/fl</sup> and *Gfpt2*<sup>fl/fl</sup> pups. The day after plating, cells were transduced with adenoviruses expressing GFP or Cre at a multiplicity of 50 in the presence of polybrene (10 µg/ml). After recovery, cells were treated with vehicle or TGFβ. Real-time qPCR and RNAseq analyses were employed to assess the relative gene expression levels and elucidate the underlying signaling pathways. Mitochondrial stress test by Seahorse Analyzers was performed to measure oxygen consumption rates (OCR) and extracellular acidification (ECAR). To induce gene recombination, 1mg of tamoxifen per 10g of body weight was administered for 5 days via intraperitoneal injection to 2-month-old male mice from control *Gfpt1*<sup>fl/fl</sup> and *Gfpt2*<sup>fl/fl</sup> mice, and to experimental *Agc1CreERT2*; *Gfpt1*<sup>fl/fl</sup> and *Agc1CreERT2*; *Gfpt2*<sup>fl/fl</sup> mice. Following tamoxifen injection, we performed medial meniscal ligament injury (MLI) surgery on the right knee joints of male mice at 3 months of age. The right knee joints were then collected for Micro-CT analysis and histological staining at 4 and 12 weeks post-MLI surgery. All data were expressed as means±SD and were analyzed using GraphPad. A p value < 0.05 was considered statistically significant.

**RESULTS and DISCUSSION:** In micromass cultures, both basal and TGFβ-induced GAG accumulation was reduced with the knockdown of either of *Gfpt1* or *Gfpt2* (Fig. 1), demonstrating that both GFPT isoforms contribute to GAG synthesis. In isolated articular chondrocyte cultures, TGFβ induced the expression of both *Gfpt1* (22%) and *Gfpt2* (82%), indicating that the HBP is regulated by anabolic growth factors in chondrocytes. RNAseq analysis confirmed a key role for GFPT in chondrocyte metabolism (Fig. 2) showing significant upregulation in key energetic pathways with deletion of *Gfpt1* or *Gfpt2*, including glycolysis, oxidative phosphorylation, pyruvate metabolism, and the TCA cycle. This suggests that deleting *Gfpt1* or *Gfpt2* prompts a shift in glucose metabolism, directing a greater portion of glucose towards glycolysis and the TCA cycle. This finding is confirmed by Seahorse mitochondrial stress test which showed enhanced mitochondrial respiration with a significant increase in both basal OCR and maximum OCR, as well as an increase in ECAR with the deletions of *Gfpt1* and *Gfpt2*. While, independent knockdown of either *Gfpt1* or *Gfpt2* in isolated chondrocytes did not yield significant changes in aggrecan gene expression, RNAseq demonstrated an increase in GAG degradation pathways in articular chondrocytes with the *Gfpt* gene deletions. Thus, *in vitro* data suggest that deletion of either *Gfpt1* or *Gfpt2* leads to increased energy metabolism, but with a negative impact on GAG accumulation and chondrocyte anabolism. To further assess the role of *Gfpt1* and *Gfpt2* in regulating cartilage homeostasis, we used a murine meniscal ligament injury (MLI) model in mice with conditional *Gfpt1* and *Gfpt2* loss-of-function (LOF) in articular chondrocytes. Mice with *Gfpt1* or *Gfpt2* conditional gene deletion had accelerated OA following MLI injury. While control mice still had normal appearing cartilage 4 weeks after MLI, mice with *Gfpt1* or *Gfpt2* gene deletions had reduced GAG staining and the evidence of cartilage degeneration (Fig 3). At 12 weeks more severe OA was observed in gene deletion mice with significantly increased cartilage degradation and increased subchondral bone sclerosis.

Overall, our findings define important roles for both *Gfpt1* and *Gfpt2* in maintaining the HBP and chondrocyte homeostasis. Impairing the HBP pathway in chondrocytes disturbs the balance of glucose metabolism and accelerates the development of OA. In the future, we will generate transgenic mice with a dual knockout of *Gfpt1* and *Gfpt2*. This will allow us to delve deeper into the mechanisms governing the HBP pathway in maintaining cartilage homeostasis and regulating the progression of OA.

**SIGNIFICANCE/CLINICAL RELEVANCE:** (1) GFPT plays a crucial role in the HBP to maintain articular chondrocyte homeostasis by providing UDP-GlcNAc for proteoglycans and GAG synthesis. (2) Modulation of the HBP in chondrocytes could provide a novel approach to restore metabolic balance and mitigate OA-associated changes in cartilage.

## REFERENCES:

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