KLF15 Deficiency Accelerated Osteoarthritis Via Upregulation Of ATP6v0d2 In Mice

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Disclosures: All authors (N)

INTRODUCTION:

Krüppel-like factors (KLF) families govern transcription, proliferation, differentiation, and tissue homeostasis (1). Atp6v0d2 encodes a subunit of the vacuolar H^+-ATPase (V-ATPase) (2), a key regulator of organelle acidification, implicating lysosomal function in cartilage matrix apoptosis (3). Here, we show that KLF15 deficiency leads to Atp6v0d2 upregulation in cartilage and accelerates OA progression.

METHODS: Tamoxifen-induced chondrospecific KLF15 knockout (KO) mice were generated. KO mice were administered tamoxifen intraperitoneally for five days at six weeks of age. In vitro, fetal-derived chondrocytes from male tamoxifen-treated KO mice and tamoxifen-naive KO (Control) mice, and wild-type mice were used. Cartilage cells were collected from the above three groups, RNA sequencing was performed, and the results were compared with those of KO mice and Control mice to match the genetic background. Gene expression detected by RNA sequencing was examined by RT-PCR. In vivo, At 10 weeks of age, male KLF15 KO mice and control mice underwent medial meniscus destabilization surgery (DMM) to induce OA. Cartilage degeneration was evaluated using safranin-O staining. Immunohistochemical staining for ATP6v0d2, MMP13, and ADAMTS5 was performed. A Mann-Whitney U test and one-way repeated measures analysis of variance (ANOVA) followed by Bonferroni post-hoc tests were performed. The statistical significance level was set at $p < 0.05$.

RESULTS:

1. Based on RNA sequencing results, we focused on 575 genes that were elevated in both KO mice and control mice and examined genes potentially associated with OA. Subsequently, we selected ATP6v0d2, which showed a significant increase in gene expression using RT-PCR. Compared to control mice, KO mice exhibited a significantly higher expression level. (P = 0.03)
2. Histological evaluation using safranin O-fast green staining revealed staining loss, erosion, and reduced chondrocyte density in KO DMM mice. This suggests significant cartilage degradation compared to control mice. According to the OARSI cartilage OA histopathology scoring system, the average total score in KO mice was significantly higher than in control mice at 1 day,1 week and 4 weeks post-surgery (P = 0.003, P = 0.0005 and P=0.03).
3. Immunohistochemical results showed that KO mice exhibited increased susceptibility to OA changes, with elevated expression of ATP6v0d2, MMP13, and ADAMTS5 compared with control mice.

DISCUSSION:

In our study, KO DMM mice exhibited progression of OA compared to WT mice in histological evaluation and immunohistochemical analysis. KLF15 may be involved in the regulation of OA progression. In KLF15-deficient mice, the expression of ATP6v0d2 was increased compared to WT mice. Immunohistochemical analysis revealed increased expression of ATP6v0d2, MMP13, and ADAMTS5. These results suggest that increased expression of ATP6v0d2 may contribute to the progression of OA.

SIGNIFICANCE: The results suggested that testing KLF15 as an osteoarthritis therapeutic should be a focus in further research.

REFERENCES:

1. Dang DT et al. *Int J Biochem cell Biol* 2000 **2.** Ayodele BA et al. *Bone Rep* 2017 **3.** Ansari MY et al. *Osteoarthritis Cartilage* 2020

IMAGES:

Figure 1: Venn diagram of sequences of KLF15 KO mice, control mice, and wild mice and RT-PCR results of ATP6v0d2 in cartilage of KLF15 KO mice and control mice.

Figure 2: Safranin-O/Fast Green staining and OARSI score of KLF15 KO DMM mice and WT mice.

Figure : Immuno-histological staining of KLF15 KO DMM mice and WT chondrocytes for ATP6v0d2, MMP13 and ADAMTS5.

Figure.1

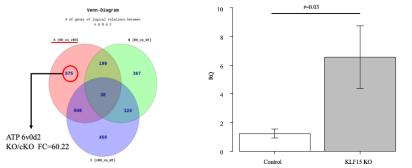

Figure.2

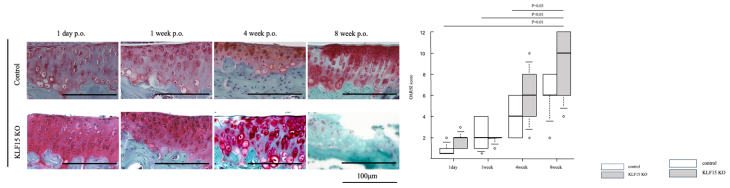

Figure.3