KLF15 deficiency activates β-catenin and is involved in the development of osteoarthritis

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Disclosures: All authors (N)

INTRODUCTION:

Krüppel-like factor 15 (KLF15) is a metabolism-related transcription factor that has been implicated in the regulation of Wnt/β-catenin signaling. This study aimed to elucidate KLF15 function in cartilage, particularly its interaction with β-catenin, using inducible cartilage-specific KLF15 knockout (KO) mice.

METHODS:

Tamoxifen-induced cartilage-specific KLF15 knockout (KO) mice were generated. KLF15 KO mice were administrated tamoxifen intraperitoneally for five days at six weeks. 10-week-old male control mice and KLF15 KO mice was performed destabilization of the medial meniscus (DMM) surgery to induce OA. Histological assessment of the cartilage degeneration was performed using Safranin-O staining. Immunohistochemistry were performed regarding beta-catenin, SOX9, MMP13, ADAMTS5. In vitro, we performed co-immunoprecipitation, double immunofluorescence and RT-PCR in control mouse chondrocytes. Immunoprecipitation was performed for β-catenin, and KLF15 was detected by Western blot. Double immunofluorescence was performed with staining for KLF15, β-catenin, and DAPI. RT-PCR was performed on four groups, divided into groups with and without Wnt3A stimulation/XAV939 (β catenin inhibitor) treatment. We performed the Mann-Whitney U test and one-way repeated analysis of variance followed by a Bonferroni post-hoc test. The thresholds for statistical significance were set at $p < 0.05$.

RESULTS:

1. Histological evaluation revealed increased OA changes in KLF15 KO DMM mice. According to the OARSI cartilage OA histopathology scoring system, KLF15 KO mice showed significantly higher OA scores compared to control mice at 4 and 8 weeks postoperatively ($P < 0.001$ and $P < 0.001$, respectively).
2. Immunohistochemical staining revealed that KLF15 KO mice exhibited altered OA susceptibility, with increased expression of β-catenin, MMP13, and ADAMTS5 and decreased expression of SOX9.
3. Coimmunoprecipitation revealed that β-catenin and KLF15 form a protein complex, and immunofluorescence staining demonstrated nuclear colocalization of KLF15 and β-catenin.
4. RT-PCR revealed increased expression of β-catenin, Axin2, MMP13, and ADAMTS5, and decreased expression of Col2A1 upon Wnt stimulation, and these changes were reversed by XAV939.

DISCUSSION:

In this study, KLF15 KO DMM mice showed histological progression of OA compared with control mice. KLF15 may be involved in OA progression. Furthermore, immunohistochemical staining showed increased expression of β-catenin, MMP13, and ADAMTS5 and decreased expression of SOX9 in KLF15 KO DMM mice. In vitro, KLF15 and β-catenin were shown to colocalize in the nucleus of chondrocytes and interact through a protein complex. Wnt stimulation induced OA-like changes, and these changes were reversed by XAV939 treatment. These results suggest that KLF15 deficiency activates Wnt/β-catenin signaling via increased β-catenin expression, contributing to OA progression.

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

REFERENCES:

1. Noack C et al. *EMBO Mol Med* 2012. **2.** Li Y et al. *J Interferon Cytokine Res* 2018. **3.** Caressa L et al. *JCI Insight* 2018. **4.** Wang J et al. *Front. Mol. Biosci* 2023. **5.** Mei Z et al. *JBMR* 2009.

IMAGES:

Figure 1: Safranin-O/Fast Green staining and OARSI score of KLF15 KO DMM mice and control mice.

Figure 2: Immuno-histological staining of KLF15 KO DMM and control mice chondrocytes for β -catenin, MMP13, ADAMTS5 and SOX9.

Figure 3: Co-immunoprecipitation and double immunofluorescence of chondrocytes from control mice.

Figure 4: RT-PCR of chondrocytes with and without Wnt3A stimulation/XAV939 treatment.

Figure.1

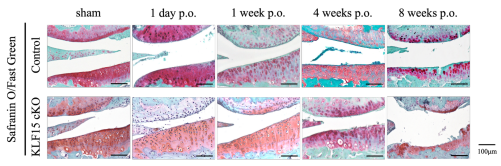

Figure.2

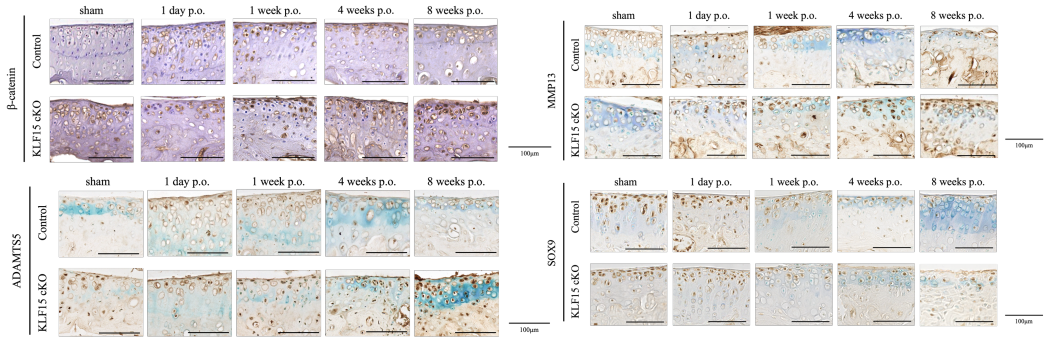

Figure.3

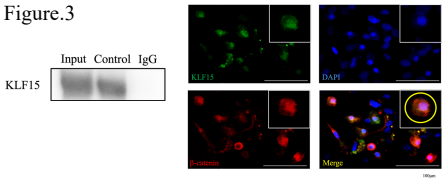

Figure.4