What is the pathogenic meaning of chondrocyte hypertrophy in osteoarthritis? Effect of Evc deletion through Hedgehog signaling

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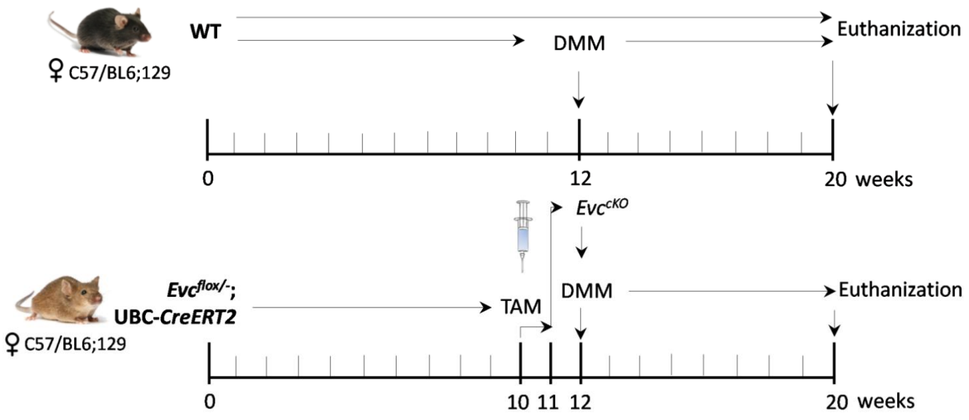
Poster Nº21

INTRODUCTION

Chondrocytes in osteoarthritic (OA) cartilage acquire a hypertrophic-like phenotype, where Hedgehog (Hh) signalling is pivotal. Hh overexpression causes OA-like cartilage lesions, whereas its downregulation prevents articular destruction in mouse models. Mutations in EVC and EVC2 genes disrupt Hh signalling, and are responsible for the Ellis-van Creveld skeletal dysplasia. Ellis-van Creveld syndrome protein (*Evc)* deletion would hamper Hh target gene expression and prevent OA progression avoiding chondrocyte hypertrophy.

Our aim was to study Evc as a new therapeutic target in OA, and whether Evc deletion restrains chondrocyte hypertrophy and prevents joint damage in an Evc tamoxifen induced knockout (Evc^{cKO}) model of OA.

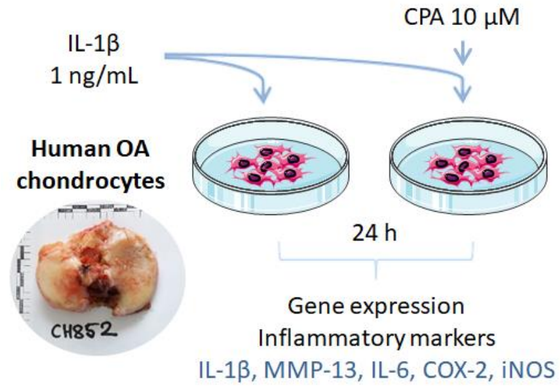
METHODS



NO-WT
non-operated WT mice

DMM-WT
operated OA WT mice

DMM-*Evc*^{cKO}
operated OA *Evc*^{cKO} mice



Statistical analyses: Ordinary one-way ANOVA with Bonferroni post-hoc test for comparisons between groups with normal distribution of the data, based on Shapiro-Wilk normality test. Kruskal-Wallis test for comparisons between multiple groups where data lacked normality, followed by Dunn's post-hoc test, using GraphPad Prism 8.

RESULTS

Hh signalling is effectively blocked in DMM-*Evc*^{cKO} mice

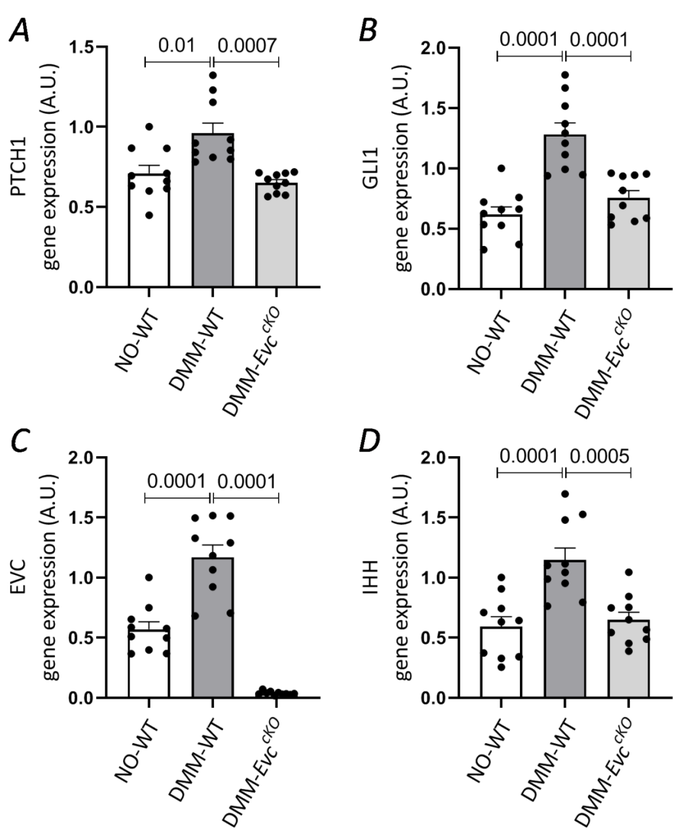


Figure 1. Gene expression of Hedgehog (Hh) mediators in the OA *Evc*^{cKO} model and cartilage structure. Gene expression of PTCH1 (A), GLI1 (B), EVC (C) and IHH (D) in the knees of NO-WT, DMM-WT and DMM-*Evc*^{cKO} mice. Individual measurements, mean ± S.E.M. (NO-WT n≥7; DMM-WT n≥7; DMM-*Evc*^{cKO} n≥7).

Evc deletion does not prevent cartilage catabolism in DMM-*Evc*^{cKO} mice

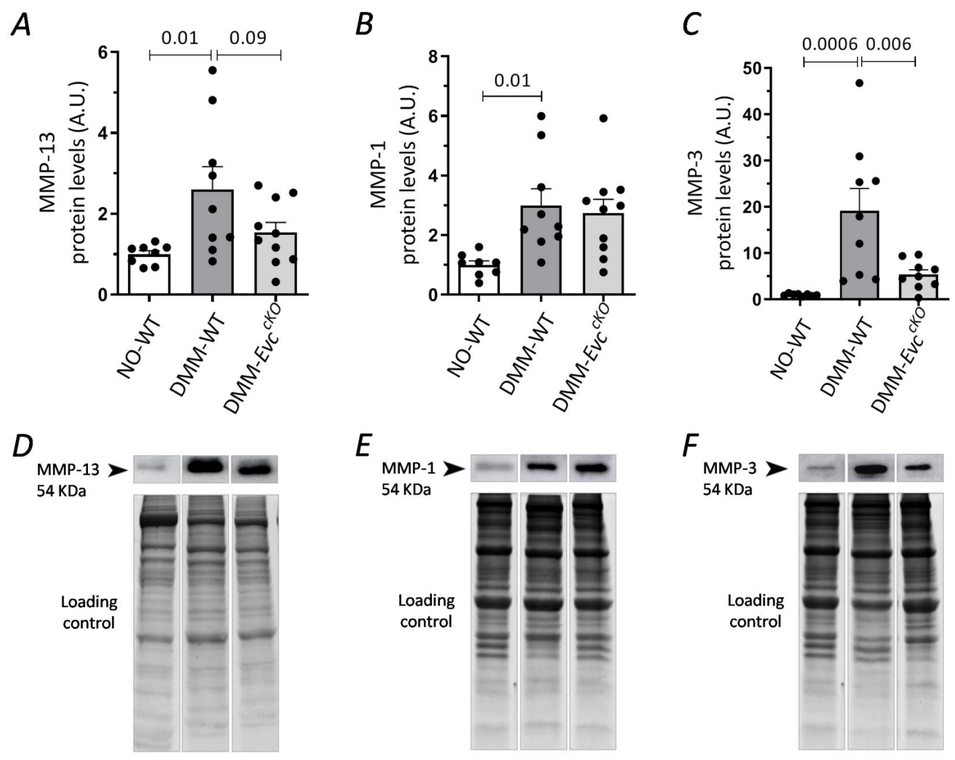


Figure 2. Metalloproteinases (MMP) protein levels in mouse knee joints. Protein levels of MMP-13 (A), MMP-1 (B) and MMP-3 (C) in the knees of NO-WT, DMM-WT and DMM-*Evc*^{cKO} mice and their representative western blots (D,E,F). Individual measurements, mean ± S.E.M. (NO-WT n≥7; DMM-WT n≥7; DMM-*Evc*^{cKO} n≥7).

Chondrocyte hypertrophy is partially inhibited in DMM-*Evc*^{cKO} mice

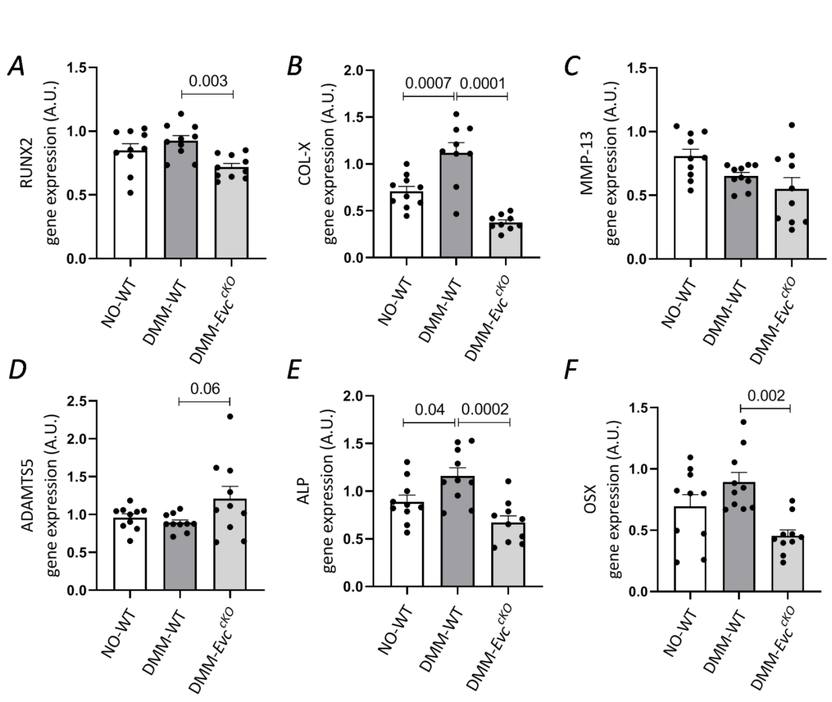


Figure 3. Effect of *Evc* deletion on OA-associated chondrocyte hypertrophy *in vivo*. Gene expression of chondrocyte hypertrophic markers RUNX2 (A), COL-X (B), MMP-13 (C) and ADAMTS5 (D), ALP (E) and OSX (F) in the knees of NO-WT, DMM-WT and DMM-*Evc*^{cKO} mice. Individual measurements, mean ± S.E.M. (NO-WT n≥7; DMM-WT n≥7; DMM-*Evc*^{cKO} n≥7).

Evc deletion in DMM-*Evc*^{cKO} mice does not prevent OA-associated cartilage damage

Table 1. Histopathological cartilage score in the knee joints. OARSI score in NO-WT, DMM-WT and DMM-*Evc*^{cKO} mouse knee joints. Mean ± S.E.M. (NO-WT n=10; DMM-WT n=11; DMM-*Evc*^{cKO} n=11). *p<0.05 vs NO-WT

Table 1 Histopathological score in the knee joints

Group	NO-WT	DMM-WT	DMM-*Evc*^{cKO}
OARSI Score (S.E.M.)	1.15 (0.198)	2.818 (0.615)*	2.727 (0.718)

Human OA cartilage co-express hypertrophic and inflammatory phenotypes

Figure 4. Co-localization of hypertrophic and inflammatory markers in human OA cartilage. Immunofluorescence of IHH (A) and COX-2 (B), chondrocyte nuclei staining with DAPI (C) and merge (D) in human OA cartilage samples.

Human OA chondrocytes inflammatory response is not modified by Hh inhibition

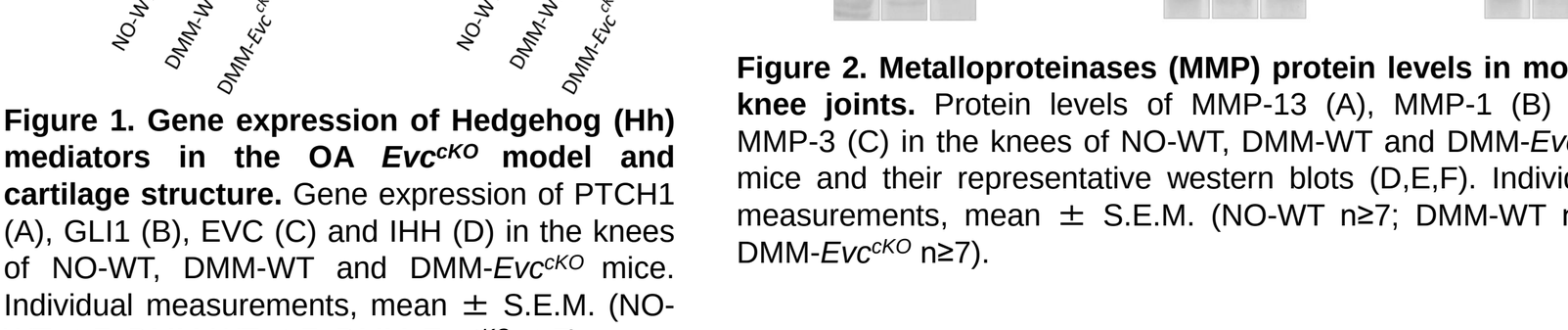


Figure 5. Inflammatory effect of IL-1β on human OA chondrocytes *in vitro*. Gene expression of proinflammatory mediators IL-1β (A), MMP-13 (B), COX-2 (C), IL-6 (D), and inducible nitric oxide synthase (iNOS) (E) in human OA chondrocytes treated with IL-1β and cyclopamine (CPA), or vehicle (DMSO), for 24 hours. Mean ± S.E.M (n=5).

CONCLUSION

Our results show that Evc-mediated Hh inactivation partially prevented chondrocyte hypertrophy but did not ameliorate OA cartilage damage in DMM-*Evc*^{cKO} mice. Our data suggest that chondrocyte hypertrophy could be a frustrated regenerative mechanism that correlates with OA progression, but not a leading cause of cartilage degeneration per se.

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