FOXO activators as drugs for osteoarthritis

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INTRODUCTION: Forkhead box O (FOXO) proteins are a family of transcription factors involved in lifespan, aging, and autophagy. FOXO are essential for maintaining cartilage homeostasis as reduced expression of FOXO1 and FOXO3 with aging[1], results in osteoarthritis (OA) due in part to reduction in the expression of autophagy-related genes[2]. FOXO activity is strictly regulated by nuclear-cytoplasmic shuttling. Drugs that promote nuclear FOXO and activity could be a promising therapeutic approach for OA. Here, we discovered candidates of FOXO activators and evaluated their effects on chondrocytes and in a mouse model of OA.

METHODS: We performed in silico drug prioritization of small molecules that enhance nuclear accumulation of FOXO1 or FOXO3 and selected 5 drug candidates, selinexor, dactolisib, cyproheptadine, LOM612, and psammaplysene A from previously identified FOXO activators[3,4] and confirmed their effects on FOXO nuclear translocation in human chondrocytes. Next, chondrocytes were treated with FOXO activators for 24 hours to evaluate their effects on the expression of autophagy-related genes, and the suppression of catabolic factors after IL-1β stimulation (1ng/mL, 6 hours) by qRT-PCR. RNA-seq of chondrocytes treated with cyproheptadine, the most promising drug, for 24 hours with or without IL-1β stimulation (1ng/mL, 6 hours) was performed to clarify the target genes and mechanisms. Histological assessment was performed to evaluate the effects of cyproheptadine treatment (intraperitoneal injection, three times per week) on the structural changes of mouse knee joints 12 weeks after DMM surgery. von Frey test and pressure application measurement (PAM) test were performed to evaluate pain behaviors. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by a post-hoc Tukey-Kramer test. All human tissues were obtained with approval by the Scripps Human Subjects Committee. All animal experiments were performed in compliance with protocols approved by the Institutional Animal Care and Use Committee at Scripps Research.

RESULTS SECTION: Selinexor induced the nuclear accumulation both of FOXO1 and FOXO3, and dactolisib and cyproheptadine induced FOXO3 nuclear accumulation, whereas LOM612 and psammaplysene A did not change FOXO translocation in chondrocytes (Figure 1A). Among the 3 drugs which induced FOXO nuclear accumulation, cyproheptadine most effectively upregulated the expressions of autophagy-related genes and inhibited the induction of catabolic factors under IL-1β stimulation (Figure 1B and C). Metascape enrichment analysis with the upregulated genes in RNA-seq showed that 'Cholesterol metabolism' was the most highly enriched pathway. Cyproheptadine also regulated 'Autophagy' (Figure 2A and B). In the presence of IL-1β, the enrichment analysis with the downregulated genes showed cyproheptadine inhibited 'Cytokine signaling' via NFκB pathway (Figure 2C and D). Cyproheptadine treatment alleviated the severity of osteoarthritis-associated cartilage damage, synovitis and osteophyte maturation (Figure 3A-D). Mice treated with cyproheptadine also showed reduced pain behaviors in von Frey test and PAM test (Figure 3E and F).

DISCUSSION: Cyproheptadine triggered the nuclear accumulation of FOXO3 and influenced at least three pathways associated with OA. Specifically, it impacted inflammation and catabolism through NFκB, promoted cellular homeostasis via the modulation of autophagy-related gene expression, and also influenced cellular cholesterol biosynthesis. Cyproheptadine treatment attenuated OA and pain behavior in mice. Cyproheptadine binds to three classes of receptors, histamine, serotonin, and muscarinic acetylcholine receptors. The receptor(s) and precise signaling mechanisms responsible for the observed activities and their interaction require further investigation.

SIGNIFICANCE/CLINICAL RELEVANCE: Cyproheptadine modulated several OA-relevant mechanisms, including autophagy activation, anti-inflammation, and cholesterol metabolism regulation. Cyproheptadine, which is approved and widely used for the treatment allergies, is a potential candidate for drug-repurposing in OA qualifying it as a candidate for drug-repurposing in OA.

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IMAGES AND TABLES:

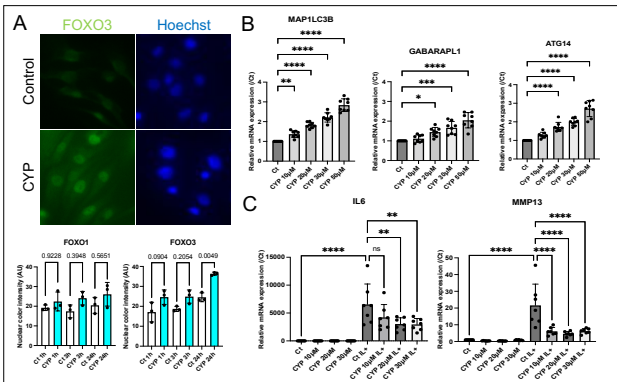

Figure 1. Effects of cyproheptadine (CYP) treatment for 24 hours on FOXO nuclear translocation (A) and the gene expressions of autophagy-related genes (B) and catabolic factors under IL-1β stimulation (C). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

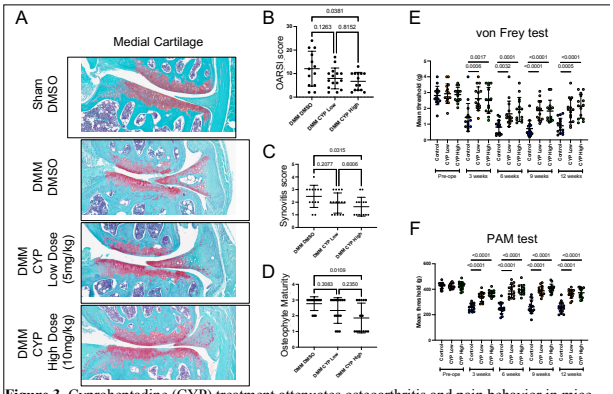

Figure 3. Cyproheptadine (CYP) treatment attenuates osteoarthritis and pain behavior in mice. (A)Representative images of medial cartilage 12 weeks after DMM surgery. (B) OARSI score, (C) Synovitis score, (D) Osteophyte maturity score. Effect of CYP treatment on pain behavior in von Frey test (E) and PAM test (F).

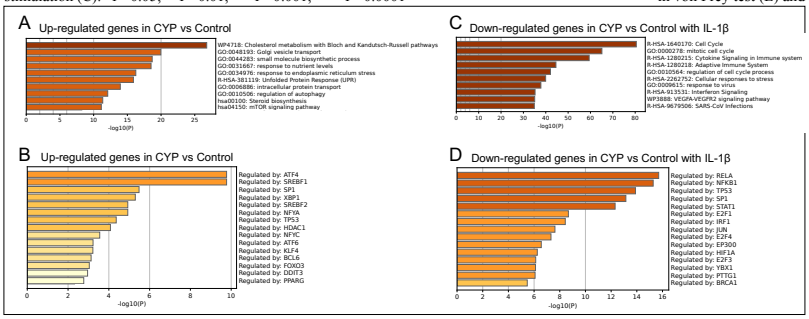

Figure 2. Enrichment analysis in human chondrocytes treated with cyproheptadine (CYP). Pathway (A) and TRRUST (B) analysis for up-regulated genes after cyproheptadine treatment. Pathway (C) and TRRUST (D) analysis for down-regulated genes after cyproheptadine treatment under IL-1β stimulation.