Inhibition of YAP and LATS Prevents Post-Traumatic Osteoarthritis in A Murine Model

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INTRODUCTION: Traumatic injury to the knee, such as ACL rupture, typically leads to post-traumatic osteoarthritis (PTOA). However, the pathogenesis of osteoarthritis continues to evolve, recently emphasizing the role of inflammation and the immune system. Our previous *in vitro* studies evaluated excessive mechanical loading in a 3D chondrocyte model, specifically investigating the Hippo mechanotransduction pathway and the significance of intracellular kinases, LATS1/2 and YAP, in the mechanotransduction cascade. Findings suggested that activation of Hippo signaling is necessary for the induction of inflammatory events and subsequent matrix degradation, both stimulated by an initial mechanical overloading event (**Figure 1**). Additionally, a positive association was discovered relating the active Hippo pathway and the Protein Kinase C (PKC) / Nuclear factor-kappaB (NFκB) signaling axis, which demonstrated the functional interplay between PKC and Hippo signaling and its pertinent role in NFκB-activated inflammation. Building upon these insights, we utilized an *in vivo* mouse model of PTOA to further investigate the Hippo pathway and its relationship to the NFκB inflammatory pathway. We modeled PTOA by traumatically rupturing the ACL of the mouse. Once the model was confirmed to induce osteoarthritis, experimental groups were administered various Hippo pathway inhibitors to evaluate inhibition of osteoarthritis progression.

METHODS: PTOA was induced in mice via a mechanical compression device to rupture the ACL. Experimental groups received an intra-articular injection (50 uL), immediately following the injury, of either PKC, LATS, or YAP inhibitor (10 μM) or medical-grade saline. Mice received adequate pain control, were monitored daily, and then sacrificed 3 weeks later. Legs were dissected, fixed in formalin for 48 hours, and decalcified for 10 days in 15% EDTA. Knees were bifurcated into medial and lateral components, embedded and sectioned, stained using H&E and Toluidine Blue (TB), and semi-quantitatively analyzed using the OARSI scale. Histological photomicrographs were analyzed using a one-way ANOVA test. Significance was defined as $p<0.05$.

RESULTS: ACL rupture was confirmed in all mice for that underwent mechanical compression ($n = 30$). Cartilage degradation was confirmed in injured mice, which were sacrificed 3 weeks after injury. Mice that underwent injury had significantly higher OARSI scores compared to non-injured controls ($p<0.05$) (**Figure 2A**). Mice that received injury and intra-articular injection with the PKC, LATS, or YAP inhibitor had significantly improved OARSI scores than those with injury plus saline injection ($p < 0.001$, $p<0.05$, $p<0.001$, respectively) (**Figure 2B**).

DISCUSSION AND CONCLUSION: From the semi-quantitative analysis of histological samples (**Figure 3A/B**), it is evident that the presence of mechanical injury significantly worsened OARSI scores with focal loss of TB staining, indicating decreased proteoglycan concentration. Other notable findings were minor fissuring of articular cartilage and frequent loss of the articular surface zone. H&E staining demonstrated an increased inflammatory response and diminished cellular proliferation. In addition, we theorize there was minimal activation of repair mechanisms within the articular cartilage, demonstrated by lowered nucleotide expression on TB staining, likely associated with disruption or inhibition of these repair processes by inflammatory cascades. However, when compared to saline injections post-mechanical injury, injections with PKC, LATS, or YAP inhibitor led to statistically significant improvements in each of these osteoarthritic pathogenesis markers. Treatment groups exhibited increased chondrocyte proliferation on TB staining, likely due to disrupted inflammatory signal transduction induced by the Hippo pathway inhibition. Limitations of this study include the relatively small sample size and lack of protein quantification. Current studies are underway to quantify protein and RNA expression. Regardless, these results demonstrate the clinical potential of Hippo inhibition in reducing activation of the inflammatory cascade, thus hindering subsequent progression to PTOA following traumatic injury. Larger animal models will be necessary to further support the clinical applicability of these inhibitors.

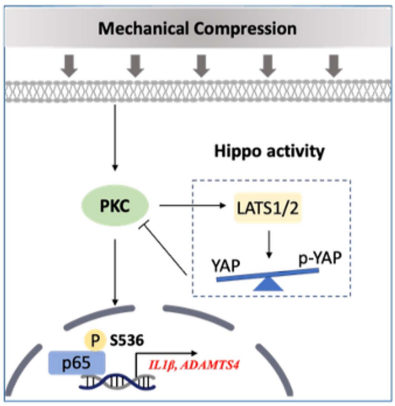

Figure 1: Schematic depicting the proposed mechanism of action for the Hippo chondrocyte pathway following mechanical compression.

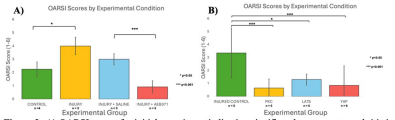

Figure 2: A) OARSI scores for initial experiment indicating significantly worse osteoarthritis in the injured group relative to control and significant improvement of osteoarthritis using PKC inhibitor relative to saline. **B)** OARSI scores for secondary experiment indicating significantly less OA in treatment groups relative to saline control.

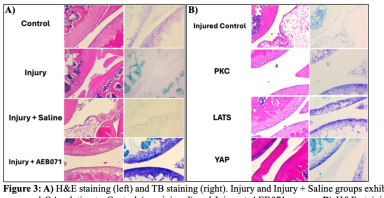

Figure 3: A) H&E staining (left) and TB staining (right). Injury and Injury + Saline groups exhibit worsened OA relative to Control (non-injured) and Injury + AEB071 groups. **B)** H&E staining (left) and TB staining (right). Injured Control (Saline) group exhibits worsened OA relative to PKC, LATS, and YAP inhibitor groups. In both figures, the femoral condyle is depicted on the top half of the images and the tibial plateau is depicted on the bottom half of the images.