Preventing post-traumatic arthritis

Can blocking ion channels preserve cartilage after ACL, rotator cuff surgeries?

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A look at a novel molecular approach to improve surgical outcomes

Rotator cuff tears and anterior cruciate ligament (ACL) tears can result in post-traumatic arthritis and limited function, even after successful surgery and rehabilitation. Physicians and scientists at the University of Rochester Medical Center are investigating the potential benefit of blocking the mechanically sensitive ion channel Piezo1 to protect injured cartilage, and calibrating the precise amount of activity that can promote homeostasis, thereby preserving cartilage and preventing arthritis.

Current therapies only mitigate the effects of cartilage damage after it happens; UR researchers Sandeep Mannava, MD, PhD, and Whasil Lee, PhD, aim to find a preventive treatment for arthritis that could be a game-changer for millions of patients.

Your research investigates the causes and potential medical interventions in post-injury arthritis. How can this work impact patient care?

Mannava: As a sports medicine surgeon at Strong Memorial Hospital, I treat many patients who suffer from athletic injuries in their teens and young adulthood. I also treat many older adults who suffer from injuries but want to remain active. I'm very interested in better understanding the processes involved in post-traumatic arthritis and how we can best intervene to prevent the kind of arthritic cartilage damage we see following ACL injuries and rotator cuff tears (RCTs). An example I use often is of a teenage patient we treat with an ACL reconstruction; if they end up with end-stage arthritis in their late 30s or early 40s, that can have a profound impact on their quality of life, employment options, and ability to remain active.

Understanding the role of the mechanically activated ion channel Piezo1 in the regulation of articular cartilage homeostasis after injury may give us a target to decrease chondrocyte vulnerability after injury and protect the joint after restoration of physiologic biomechanical loading via ACL reconstruction or rotator cuff repair.

This research could benefit patients who suffer from non-traumatic osteoarthritis as well. Approximately 32 million Americans suffer from OA, characterized by the progressive degeneration of cartilage. Disease-modifying therapies to prevent cartilage degeneration are unavailable and urgently needed.

Why are you focusing on Piezo1? Why is this pathway a promising target for the treatment of osteoarthritis?

Lee: Mechanical factors heavily influence chondrocyte metabolic activities and play a critical role in cartilage homeostasis and degeneration. Chondrocytes are the unique post-mitotic cells expressing both Piezo1 and Piezo2 mechanosensitive channels robustly. Since the discovery of Piezo1 and Piezo2 in 2010, research has found that they exhibit distinct gating properties, expression patterns, and mechanotransduction-signaling mechanisms in human physiology and pathology.

We and others have reported that in chondrocytes, Piezo1 and Piezo2 sense injurious loading, and pre-treatment with GsMTx4, a pan Piezo1/Piezo2 blocking peptide, significantly reduced the injury-induced chondrocyte death *in vitro*. Our *ex vivo* study shows the critical roles of Piezo1 and Piezo2 in mechanical trauma or inflammation-associated chondrocyte death and cartilage degeneration. These study findings have given us insights into osteoarthritis (OA) pathology and therapeutic strategies targeting Piezo1- and Piezo2-mediated mechanotransduction for cartilage degeneration and regeneration.

What conclusions regarding OA prevention can you draw from the experiments you've conducted?

Mannava: Chondrocyte death causes more rapid cartilage degeneration, a hallmark of OA. Murine femoral and humeral head chondrocytes respond to injurious mechanical loading, in part, through the mechanically gated calcium ion channel Piezo1. Piezo1 may be blocked to reduce chondrocyte death induced by trauma. Yet, our preliminary data suggest differential Piezo1-mediated chondrocyte mechanotransduction mechanisms in knee versus shoulder OA. Although further study is needed, we are hopeful that our work will provide therapeutic Piezo1-targets for osteoarthritis prevention.

What's the next step in this research?

Mannava: In future studies, we plan to evaluate the hypothesis that blockade of Piezo1 attenuates chondrocyte vulnerability to protect the cartilage after traumatic injury and inflammation. Surgical treatment that subsequently restores the biomechanics of the joint such as rotator cuff repair or ACL reconstruction may increase the longevity of the joint through our proposed molecular pathway. We recognize that we are studying just one protein-mediated pathway for cell vulnerability and there are additional mechanically gated channels that respond to trauma and inflammation, which are also the topic of ongoing studies in our laboratory.

How did you conduct the research?

Lee: Our group established two mouse models: a non-invasive ACL-injury induced mouse knee OA model and a surgical rotator cuff tear-induced mouse shoulder OA model. We have a great team helping with these studies, and our success is based on the hard work of our trainees Dr. Alexander Kotelsky, Dr. Devon Anderson and Katherine Broun.

Briefly, we quantified Piezo1-dependent chondrocyte viability against injurious impact using a confocal microscopy and a custom-built apparatus which was invented by the Buckley Laboratory in UR/BME. The femoral knee cartilage or glenohumeral cartilage of mice were treated with or without a Piezo1-specific agonist - yoda1, and tissues were subjected to a 1 mJ impact. Next, we simulated ACL-injury, rotator cuff tears (RCTs) or sham surgery and examined the effect of these injuries on Piezo1-mediated chondrocyte mechanotransduction and chondrocyte mechano-vulnerability.

What were the results?

Lee: There was a significant increase in femoral or humeral head chondrocyte cell death by impact in Yoda1-treated groups relative to controls, indicating that Piezo1, in part, drives chondrocyte vulnerability to injurious loading. Strikingly, we observed distinct Piezo1 gene regulation between ACL-injured femoral chondrocytes and RCT-induced humeral chondrocytes. Gradually, augmented Piezo1

expression was observed in knee chondrocytes status post-ACL-injury; in contrast, decreased functional expression of Piezo1 was observed in humeral chondrocytes status post-RCT. We are excited for these differential injury responses in chondrocytes at the lower versus the upper extremity joints that warrants further investigation. We plan to investigate the role of Piezo1 and Piezo2 channels in both joints using cartilage-specific Piezo1- or Piezo2-knockout mice, also test the Piezo1/2 inhibitors post-injury *in vivo*. We anticipate differential mechanisms and therapeutic strategies for light or heavy load-bearing joints.