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Targeting Rac1 via microparticle-based drug delivery system protects OA cartilage *in vivo*

The small GTPase Rac1, also known as Ras-related C3 botulinum toxin substrate 1, is a positive regulator of chondrocyte hypertrophy and endochondral ossification during bone formation, processes that are recapitulated in osteoarthritis (OA). New research led by Professor Hong Wei Ouyang shows that inhibition of the abberant activation of Rac1 in OA, using a novel drug delivery approach, could have therapeutic potential in this disease.

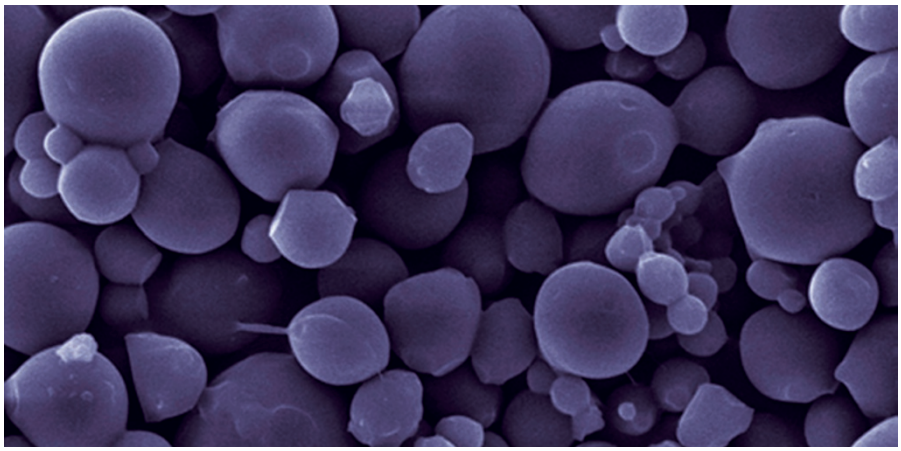
In the study published in *Annals of the Rheumatic Diseases*, the authors demonstrated *in vitro* that, although Rac1 is abundantly expressed in both normal and OA cartilage, its activated form is overexpressed in the latter. Furthermore, treatment of chondrocytes with IL-1β upregulated expression of this form of Rac1.

Tellingly, the introduction of active Rac1 (CA-Rac1) into chondrocytes led to increased expression of genes related to matrix degradation and hypertrophy. Further experiments demonstrated that Rac1 upregulates the expression of MMP13, ADAMTS5 and COLX, in part via the β-catenin pathway, adding to the evidence implicating Wnt signalling in cartilage destruction in OA.

In vitro activation of Rac1 also resulted in increased chondrocyte calcification; by contrast, both expression of dominant-negative mutant Rac1 (DN-Rac1) and pharmacological inhibition of Rac1 with NSC23766 decreased calcification.

In a mouse model, intra-articular injection of DN-Rac1 lentivirus into OA joints downregulated Rac1 and had a protective effect on cartilage, whereas CA-Rac1 lentivirus elevated Rac1 activity and accelerated OA progression and cartilage degradation. Intra-articular injection of NSC23766 inhibited the activity of Rac1 and prevented cartilage degeneration in the mice.

Having shown the therapeutic effect of Rac1 inhibition *in vivo*, the investigators set about solving the problem of maintaining an effective intra-articular concentration of the Rac1 inhibitor, by exploring the potential of a novel drug delivery approach. "We developed a strategy of controlled release of the Rac1 inhibitor NSC23766 from chitosan microparticles for local delivery to OA joints," says Ouyang. The drug was encapsulated in polymeric chitosan microspheres of ~100 μm in size. In mice, weekly intra-articular injection of hyaluronic acid containing these microspheres into OA joints led to decreased cartilage destruction and delayed OA development. "This finding suggests that new approaches integrating biology and bioengineering technology might revolutionize OA treatment," enthuses Ouyang, although he points out that the chitosan delivery system is limited by its 3-day drug-release time.



Scanning electron micrograph of chitosan microspheres. Image courtesy of Hong Wei Ouyang.

Professor Christopher Evans of Harvard Medical School (who was not involved in the study) finds the work interesting and innovative, but expresses surprise that gene transfer to chondrocytes was achieved with lentivirus. "The virus is ~120 nm in diameter, which is normally too big to penetrate the matrix of articular cartilage. Transduction of synovial cells, however, is very efficient ... It is possible that some of the effects on cartilage were secondarily mediated via the synovium." He adds, "It would also be interesting to know where the chitosan particles reside within the joint. At 100 μm, they would be quickly cleared from the joint space via the lymphatics. Presumably they lodge in the synovium, where they should be readily visible on histology." Notably, however, no tissues other than cartilage were examined either *in vitro* or *in vivo*.

Professor Chris Little of the University of Sydney, Australia, also suggests that further study is needed of the effect of targeting Rac1 on OA as a whole-joint disease. "From the data presented," he contends, "it is not clear if the effects of Rac1 inhibition on cartilage breakdown in the mouse models are a direct effect on chondrocytes or might be secondary to regulating other aspects of OA pathology such as synovial inflammation." Such insights could have implications for the utility of this therapy for different subtypes of OA or for the timing of its application.

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