

Cartilage injury suppresses endogenous retinoic acid through activation of TGF β -activated kinase 1 (TAK1)

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Background: A recent Genome Wide Association Study (GWAS) in hand OA has identified the association of hypomorphic variants in aldehyde dehydrogenase 1 family member A2 (ALDH1A2) with severe hand OA. This gene encodes the enzyme that irreversibly catalyzes the production of all-trans retinoic acid (atRA) from retinaldehyde. atRA has an essential role in embryonic limb development but its role in adult cartilage and in OA is not clear. Our group has demonstrated that endogenous retinoic acid is strongly suppressed by cartilage injury and this affects the regulation of inflammatory response genes. Maintaining cellular retinoic acid levels at time of injury, with a CYP 26 inhibitor, restrained inflammatory gene regulation. Previous work from our group has found that cartilage injury activates several signaling pathways involving release of transforming growth factor beta (TGF β) and fibroblast growth factor (FGF2), and activation of TGF β -activated kinase 1 (TAK1). Here we investigated the mechanism by which cartilage injury suppresses endogenous retinoic acid signaling.

Methods: Articular cartilage was obtained from porcine metacarpophalangeal joints of 3-6 months old pigs. Cartilage injury was performed by explanting cartilage from the joint surface. To examine the role of specific injury pathways, cartilage injury was also performed on joints that had been injected with selective inhibitors (TGF β R (SB431542), FGF2R (SB402451) and TAK1 ((5Z)-7-oxozeanol) 1 hour prior to injury. RT-PCR was performed for known atRA-dependent genes. To identify injury-induced regulators of retinoic acid, a murine cartilage injury microarray was interrogated for molecules involved in the synthesis and degradation of atRA.

Results: Cartilage injury led to rapid down-regulation of retinoic acid dependent genes (CYP26a, CYP26b and retinoic acid receptors: RAR α , RAR β and RAR γ) between 40 and 80% by 4h. For most genes this was sustained for up to 24h. This apparent fall in atRA dependent genes was not affected by blocking TGF β or FGF2, but was inhibited by the TAK1 inhibitor, indicating that TAK1 controls atRA levels or signaling. Our cartilage injury microarray identified RDH12 as the most strongly regulated gene on the atRA pathway (12.5 fold, $p=1.5 \times 10^{-9}$). RDH12 is an important upstream enzyme, which converts retinaldehyde to retinol, and its overexpression will lead to reduction of cellular atRA level. ALDH1A2 protein was also suppressed 4 hours after porcine cartilage injury, predicting a further reduction of cellular atRA.

Conclusions: Our study demonstrates that atRA dependent genes in chondrocytes are strongly suppressed by mechanical injury through TAK1 activation, indicating a reduction of endogenous atRA level. The strong induction of RDH12 and suppression of ALDH1A2 after injury may be responsible for driving these changes. We conclude that maintaining physiological levels of atRA is anti-inflammatory, and targeting the atRA pathway could reduce the catabolic response of cartilage to injury.