

Sodium, sequestered by aggrecan, is responsible for displacement of pericellular growth factors upon cartilage injury; a mechanism that fails when aggrecan is lost in osteoarthritis.

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Introduction

The pericellular matrix (PCM) of cartilage contains the basement membrane proteins laminin, collagen VI, nidogen and perlecan. We have identified fibroblast growth factor (FGF2), connective tissue growth factor (CTGF/CCN2) and hepatoma-derived growth factor (HDGF) that are bound to the heparan sulfate chains of perlecan in the PCM and are released rapidly upon cartilage injury. These growth factors have either confirmed or putative roles in cartilage repair. Here, we tested the hypothesis that cartilage injury causes a flux of sodium from the charged, (aggrecan-rich) territorial matrix, which displaces the growth factors from the PCM.

Materials and Methods

Injury was performed in rested (48h in serum-free DMEM) porcine articular cartilage explants. Some porcine explants were freeze-thawed (dead), incubated with protease inhibitors, NaCl (0.137–0.822 M), or treated with IL-1 for 7 days to deplete aggrecan, prior to injury. Porcine knee osteochondral explants, upon load (20% static strain) and free unloading, were imaged for sodium dynamics on a Bruker 9.4T Ultra-high field microimaging system. Human osteoarthritic cartilage, graded according to disease severity, was injured (cut) then extracted with proteinase K or 2.5 M NaCl. Growth factors were measured in the injury conditioned medium by Western blot or ELISA.

Results

FGF2, CTGF and HDGF were rapidly released upon cartilage injury. Rapid release upon injury was unaffected by cell viability or protease inhibition. NaCl addition to cartilage, displaced the growth factors, and increased the amount of injury-released growth factor. Aggrecan depletion by IL-1 abrogated growth factor release upon injury without affecting total tissue growth factor. By Na-MRI a flux of free tissue sodium was measured upon compression, which recovered during relaxation. More severely osteoarthritic human cartilage released less growth factor upon injury, despite being extractable by exogenous NaCl.

Discussion

We have demonstrated a Na-dependent mechanism by which heparan sulfate-bound growth factors of the cartilage PCM are released upon injury. Our results suggest a novel role of aggrecan in cartilage; maintaining a pool of sodium that can be mobilised to alter growth factor bioavailability. This is relevant for osteoarthritis, where aggrecan loss prevents this growth factor injury response.