Results: Compared using the student t-test, the ratios before and after impact were superficial to deep layer OCT signal intensity was obtained and calculated as described above (Fig. 1D). A ratio of the mean signal intensity of the upper cartilage layers was analysis software (VIS). Repeat OCT imaging was also performed for one day and imaged using fluorescent microscopy. The area of putter controlled impact tower. After impact, cores were incubated (0.175 J) or moderate (0.35 J) impact injury using a custom com-

Conclusions: Post-traumatic osteoarthritis following either a fracture or a dislocation of the hip, knee and ankle is an important health problem. The results of this study confirm that POA should be considered as a separate entity from generalized osteoarthritis.

052

OPTICAL COHERENCE TOMOGRAPHY (OCT) SIGNAL CHANGES ARE POTENTIAL BIOMARKERS OF ACUTE CARTILAGE INJURY

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Purpose: Post-traumatic osteoarthritis is a leading cause of disability affecting millions of Americans. Current clinical methods cannot reliably detect cartilage damage prior to breakdown of the articular surface. Optical Coherence Tomography (OCT) is a novel nondestructive imaging technology that show changes to cartilage birefringence predictive of potentially reversible early cartilage metabolic incompetence (Chu et al, J Biomed Opt, 2007). This study tests the hypothesis that OCT signal changes are potential biomarkers of acute cartilage injury following impact at energies sufficient to produce chondrocyte death, but insufficient to produce visible cartilage surface damage.

Methods: Seventeen osteochondral cores were harvested from fresh bovine knees. Cores were imaged using a custom OCT scanner. Matlab imaging software was used to segment and calculate the mean OCT signal intensity from two distinct upper cartilage layers (Fig. 1C). Cores were then divided into three groups and served as either controls or were subjected to a low (0.175 J) or moderate (0.35 J) impact injury using a custom computer controlled impact tower. After impact, cores were incubated for one day and imaged using fluorescent microscopy. The area of dead and living chondrocytes was calculated using custom image analysis software (VIS). Repeat OCT imaging was also performed and the mean signal intensity of the upper cartilage layers was calculated as described above (Fig. 1D). A ratio of the mean superficial to deep layer OCT signal intensity was obtained and using the student t-test, the ratios before and after impact were compared.

Results: For all cores, the articular surface remained intact to visual inspection following low energy impact (0.175 J) (Fig. 1A and 1B). Moderate energy impact (0.35 J) caused a visible disruption to the cartilage surface in 30% of cores. The superficial to deep OCT signal intensity ratio demonstrated a significant 32% increase following low impact and a 39% increase following moderate impact injury (Fig. 1E). No increase in the OCT signal intensity ratio was found in the control group (p=0.35).

Conclusions: This study shows that OCT signal changes are potential biomarkers of acute cartilage injury evidenced by significant chondrocyte necrosis following impact at energies insufficient to cause visible damage to the articular surface. Our findings support the utility of OCT as a nondestructive imaging modality to detect cartilage damage that may not be appreciable by conventional clinical methods. As early identification of impact injury to articular cartilage could reveal a new treatment window to allow for the potential reversal of pathological changes, our findings suggest that OCT could assist in developing new chondroprotective treatments to prevent or delay the onset of post-traumatic osteoarthritis.

053

REACTIVE OXYGEN SPECIES INHIBIT IGF-I INDUCTION OF CHONDROCYTE PROTEOGLYCAN SYNTHESIS THROUGH DIFFERENTIAL REGULATION OF PI-3 KINASE-AKT AND MEK-ERK MAPK SIGNALING PATHWAYS

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Purpose: IGF-I stimulation of chondrocyte proteoglycan synthesis requires activation of the PI-3 Kinase-Akt signaling pathway. With aging and during the development of osteoarthritis (OA), chondrocytes become hyporesponsive to IGF-I. We tested the hypothesis that an increase in reactive oxygen species (ROS) causes IGF-I resistance by altering the activation of the IGF-I signaling pathway. Methods: Human articular chondrocytes were isolated from normal ankle cartilage obtained from adult tissue donors or from OA knee cartilage removed during joint replacement. High density serum-free monolayer cultures were stimulated with 50ng/ml IGF-I

Chondrocyte viability analysis revealed a 230% increase in cell death in the low impact group and a 190% increase in cell death in the moderate impact group.
with or without pre-treatment with hydrogen peroxide (exogenous ROS) or the anti-oxidants MnTBAP or NAC. IGF-I signaling proteins were evaluated by immunoblotting with phosphospecific and control antibodies. Lentiviral constructs were used to overexpress constitutively active (CA)-Akt, CA-MEK and dominant (DN)-Akt. Proteoglycan synthesis was measured by sulfate incorporation correct for cell numbers by DNA and aggregan expression was measured by real-time PCR.

**Results:** In time course experiments (0,5,15,30 and 60 min) with normal human chondrocytes, IGF-I initiated a strong and sustained phosphorylation of IRS-1 at Tyr-612 and Akt at Ser-473 and Thr-308, and a transient ERK phosphorylation. In contrast, OA chondrocytes possessed an elevated basal ERK and JNK phosphorylation and IGF-I failed to stimulate significant IRS-1 or Akt phosphorylation. In normal human chondrocytes, exogenous ROS triggered strong IRS-1 Ser-312 phosphorylation, which is an inhibitory site, along with ERK and JNK phosphorylation and inhibited IGF-I-induced IRS-1 Tyr-612 and Akt phosphorylation. Lentivirus-mediated overexpression of CA-Akt significantly enhanced proteoglycan synthesis in the absence or presence of IGF-I, while expression of either DN-Akt or CA-MEK (which activates ERK) inhibited both basal and IGF-I-induced proteoglycan synthesis (Fig. 1). ROS treatment and CA-MEK expression also significantly inhibited aggregan mRNA expression in normal chondrocytes. In osteoarthritic chondrocytes, treatment with the antioxidants MnTBAP or NAC induced IRS-1 Tyr-612 and Akt phosphorylation, and promoted IGF-I-mediated proteoglycan synthesis.

**Conclusions:** These results demonstrate for the first time opposing roles for PI-3 Kinase-Akt and MEK-ERK in the IGF-I regulation of chondrocyte proteoglycan synthesis and suggest that excessive ROS in OA chondrocytes inhibit IGF-I mediated proteoglycan synthesis by altering the balance of Akt to ERK activation.

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**EXTRACELLULAR SULFATASES SUPPORT CARTILAGE HOMEOSTASIS BY REGULATING BMP AND FGF SIGNALING PATHWAYS**

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**Purpose:** Cartilage homeostasis is controlled by several major cell signaling pathways. Sulf-1 and Sulf-2 (Sulfs) are the only known extracellular sulfatases and by changing heparan sulfation patterns regulate the activity of several major cell-signaling pathways. This study addressed the role of Sulfs in regulating BMP-7 and FGF2 signaling pathways in articular cartilage.

**Methods:** Sulf-1 and Sulf-2 expression was inhibited in primary cultured human articular chondrocytes using siRNA. Over expression experiments were conducted with an immortalized human chondrocyte cell line (T/C-28a2). Cells were stimulated with BMP-7 (100ng/ml) or FGF2 (100ng/ml), and SMAD and ERK signaling were monitored via western blotting. Knee joints and articular chondrocytes were isolated from Sulf knock out mice, and BMP and FGF signaling was analyzed using western blotting, immunohistochemistry and Tagman PCR.

**Results:** Chondrocyte transfection with siSulf-1 or siSulf-2 reduced Sulf protein levels by 50-70%. This was associated with a reduction in basal SMAD1 protein expression and SMAD1/5 phosphorylation. In contrast, under identical conditions basal ERK1/2 protein expression and phosphorylation increased (Fig. 1a). Sulf siRNA also inhibited the BMP-7 induced increase in SMAD1 protein expression and SMAD1/5 phosphorylation (Fig. 1b). The effects of FGF2 on ERK1/2 protein expression and phosphorylation were dramatically accelerated following Sulf knock down (Fig. 1c). Sulf overexpression enhanced BMP-7-induced SMAD1 protein expression and SMAD1/5 phosphorylation (Fig. 1d and 1e). The FGF2 response was not altered by Sulf overexpression (Fig. 1f). To confirm and extend these in vitro findings, we analyzed Sulf knock out mice. Articular cartilage from Sulf knock out mice showed reduced SMAD1 protein expression and SMAD1/5 phosphorylation, whereas ERK1/2 phosphorylation was increased. mRNA levels of Col2a1, Sox9, Smad1 and Aggrecan were reduced, while increased levels of MMP-13 and Noggin were noted, especially in Sulf-1 knock out chondrocytes.

**Conclusions:** Sulfs regulate BMP-7-induced SMAD1/5 and FGF2 induced ERK1/2 phosphorylation in human chondrocytes.

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**HYPOXIA CONDITIONS DIFFERENTIALLY MODULATE NORMAL AND OSTEOARTHRITIC HUMAN ARTICULAR CHONDROCYTE PROTEOMES**

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**Purpose:** Osteoarthritis (OA) is a degenerative disease characterized by the degradation of articular cartilage. This tissue is avascular, and it is characterized by the low oxygen tension and poor nutrient availability for its cells, the chondrocytes. Hypoxia conditions have been reported to stimulate chondrogenesis and synthesis of extracellular matrix components. Therefore, we pur-