Conclusions: The administration of stable (i.e., non-radioactive) strontium at sub-therapeutic doses can serve effectively as a dynamic tracer of bone turnover to assess micro-structural changes associated with the pathogenesis of bone disease including osteoarthritis, at high spatial resolution (micron level) not possible with radionuclide-based scintigraphic imaging. In addition, when co-administered with drug interventions, strontium as a dynamic label could be used to gauge the efficacy of disease modifying drugs upon adaptive bone physiology in osteoarthritis or other adaptive bone pathology, such as osteolytic/sclerotic bone cancer metastases. The KES-SRμCT imaging procedure reported here is the first application of synchrotron micro-CT to segment and visualize regions of active bone turnover in osteoarthritis in 3-D.

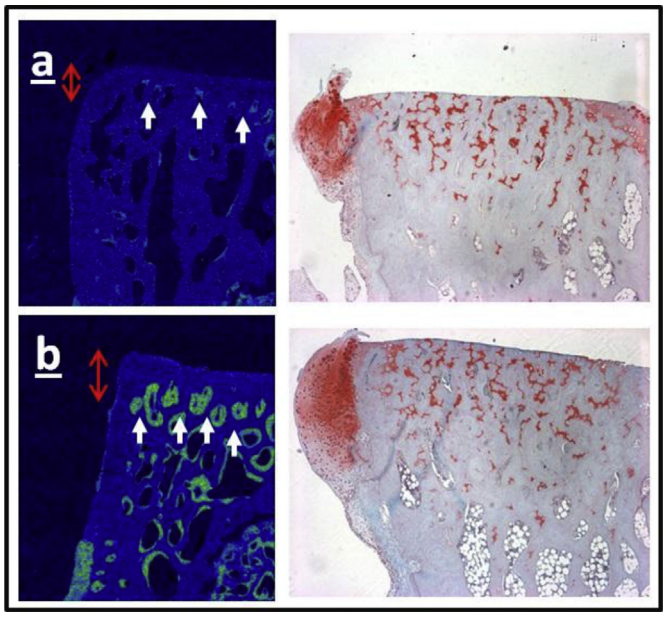

Figure 2. Subchondral sclerosis in end-stage osteoarthritis. Note significant incorporation of strontium in subchondral bone detected by EPMA (light blue color) from week 8 (a) to 12 (b) post-surgery. The corresponding histology (Safranin-O stain) indicates sclerotic subchondral bone (note lack of marrow space) and full thickness loss of articular cartilage.

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ACTIVIN RECEPTOR-LIKE KINASE ALK5 AND ALK1 ARE BOTH REQUIRED FOR TGFβ-INITIATED CHONDROGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS

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Purpose: Cartilage matrix defects, due to osteoarthritis or trauma, are not repaired because chondrocytes have poor regenerative capacity. Bone marrow-derived mesenchymal stem cells (BMSCs) are a promising cell source to treat matrix defects due to their capacity to differentiate into chondrocytes. Transforming Growth Factor-β (TGFβ) potently initiates chondrogenic differentiation of BMSCs and is known to signal either via Activin receptor-Like Kinase (ALK) receptor ALK5 or ALK1, which activate the intracellular SMAD2/3 and SMAD1/5/8 pathway respectively. Since the role of TGFβ receptors in BMSC chondrogenesis is unknown, we investigated whether either ALK5 or ALK1 is crucial to initiate chondrogenic differentiation of BMSCs.
Methods: Human fetal BMSCs (purchased from ScienCell) were transduced with either adenoviral constitutive active (ca)ALK5, caALK1 or LacZ (control) for receptor overexpression. To downregulate receptor expression, BMSCs were transfected with lentiviral ALK5-shRNA, ALK1-shRNA or a vector without shRNA (control). After viral infection, BMSCs were pellet-cultured in serum-free chondrogenic medium for 7 days. All aforementioned conditions were stimulated with 10 ng/mL TGFβ1, which initiates chondrogenic differentiation. Cells overexpressing either caALK5 or caALK1 were cultured without TGFβ1 stimulation in order to determine whether constitutive active receptor signaling initiated BMSC chondrogenesis. To verify chondrogenic differentiation, aggrecan gene expression (ACAN) was measured and proteoglycan deposition was evaluated by Safranin O staining.
Results: Chondrogenesis was observed in LacZ-transfected BMSC pellets (LacZ-BMSCs) stimulated with TGFβ1 as measured by high ACAN expression and positive proteoglycan staining. Overexpressing either caALK5 or caALK1 without stimulating BMSCs with TGFβ1 resulted in activation of the SMAD2/3 or SMAD1/5/8 pathway respectively. However, unstimulated caALK1-BMSCs had ~8-fold and caALK5-BMSCs had ~78-fold lower ACAN expression and proteoglycan staining was absent compared to LacZ-BMSCs that were stimulated with TGFβ1. Downregulating either ALK1 or ALK5 by shRNA in TGFβ1-stimulated BMSCs caused respectively ~18-fold and ~222-fold lower ACAN expression levels than in the control condition and an absence of proteoglycan staining.
Conclusions: Our data suggest that TGFβ needs to activate both its ALK5 and ALK1 receptor to initiate chondrogenic differentiation of BMSCs. Both receptors seem crucial as BMSC chondrogenesis was not initiated when overexpressing either constitutive active ALK5 or ALK1, and chondrogenesis was inhibited when downregulating either ALK1 or ALK5. This study helps to better understand the molecular events induced by TGFβ during chondrogenic differentiation of BMSCs, which is important for improving cartilage matrix formation by mesenchymal stem cells.

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AGRIN: A NEW PLAYER IN JOINT BIOLOGY AND A POTENT GROWTH FACTOR FOR CARTILAGE REGENERATION

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Purpose: Osteoarthritis is a chronic disabling disease characterized by cartilage breakdown for which there is no cure. Disruption of the gene encoding for the heparan sulphate proteoglycan Agrin results in embryonic skeletal dysplasia suggesting a role for Agrin in cartilage biology and prompting us to study its role in articular cartilage biology and osteoarthritis. The aims of this study are to establish the expression pattern of Agrin in healthy/normal cartilage and compare it to the expression pattern in osteoarthritic or injured cartilage; determine the effects of knockdown and over-expression of Agrin within cartilage in vitro and in vivo and to determine the epistasis of Agrin in articular chondrocytes.
Methods: Agrin expression was determined by immunohistochemistry and qPCR. Osteoathritis was induced in 8 week old 129sv mice by destabilisation of the medial meniscus (DMM) and Agrin expression was evaluated by immunofluorescence 8 weeks post-surgery. Paired human samples of preserved cartilage vs severely osteoarthritic cartilage were compared by immunofluorescence. Gain and loss of function experiments were performed using an expression plasmid encoding mammalian Agrin and siRNAs in C28/I2 and bovine chondrocytes in micromass culture. In vivo cartilage formation was assessed using an ectopic implantation model in nude mice; growth-arrested COS7 cells overexpressing Agrin or GFP were combined with bovine chondrocytes (ratio 1:10) and implanted ectopically into nude mice for two weeks. Retrieved implants were characterised by histology and qPCR.
Results: Agrin and its known receptors were expressed in healthy adult human articular cartilage and downregulated in human and experimental murine osteoarthritis. Silencing of Agrin by siRNA resulted in reduced GAG production in C28/I2 and in chondrocyte de-differentiation characterised by decreased expression of SOX9, COL2A1 and ACAN mRNA. Overexpression of Agrin in the human chondrocyte cell line C28/I2 and bovine primary articular chondrocytes resulted in enhanced GAG production and SOX9 upregulation. Importantly, in contrast to BMP-2, Agrin over-expression did not induce markers of cartilage hypertrophy including COL10A1 and MMP-13. Delivering Agrin to primary bovine chondrocytes transplanted in the muscle of nude mice resulted in enhanced formation of ectopic cartilage, which did not display signs of hypertrophy, vascular invasion, or endochondral bone formation.
Conclusions: Our data show that Agrin is essential for the maintenance of the chondrocytic phenotype and extracellular matrix production whilst exogenous Agrin enhances chondrocyte differentiation and cartilage formation in vitro and in vivo; and therefore may be a valuable chondrogenic molecule in tissue engineering technologies.