cells reside in bone marrow cavities and their relative abundance changes between subchondral bone phenotypes. These data suggest that osteoimmunological interactions between these populations might be involved in OA bone remodeling.

180
CHARACTERIZATION OF FOLATE RECEPTOR BETA EXPRESSION ON CELLS OF BONE ORIGIN

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Purpose: Despite being one of the most common types of arthritis and a leading cause of disability worldwide, effective therapeutics for osteoarthritis (OA) are still unavailable. We recently showed in a cross-sectional pilot study (25 patients, 50 knees) the localization of folate receptor beta (FR-β) positive macrophages in joints using 99mTc−EC20 imaging. The presence of activated macrophages, localized to the synovium, was associated with knee OA clinical symptoms; the presence of activated macrophages localized to the knee joint capsule of the tibiofemoral joint was associated with severity of knee joint space narrowing. However, 16% of the patients revealed positive 99mTc-EC20 uptake in the subchondral bone of osteoarthritic knees. The potential for expression of this receptor on cells of the bone has not been previously evaluated but is possible based on the knowledge that osteoclasts are multinucleated cells that derive from hematopoietic progenitors in the bone marrow, which also give rise to monocytes in peripheral blood, and to various types of tissue macrophages2. To better understand the cell types identified and targeted by folate conjugates and thereby assist with interpretation of 99mTc-EC20 images in patients with arthritis, the goal of this study was to evaluate the expression of folate receptors on cells of bone origin under resting and activated conditions.
Methods: The quantitation of FR-β, macrophage markers (CD14, CD163, CD64, CD80, TGF-B, and iNOS), osteoclast markers (cathepsin K and calcitonin receptor), and osteoblast markers (collagen type I and osteocalcin) mRNA was evaluated by reverse transcriptase polymerase chain reaction (RT-PCR) on human macrophage, osteoclast, and osteoblast cell lines. The expression of these proteins was also evaluated by multi-color confocal fluorescence microscopy using Alexa Fluor® 568, Alexa Fluor® 647, and fluorescein isothiocyanate conjugates.
Results: Gene expression analysis indicated the presence of low levels of FR-β in resting macrophages and precursor osteoclasts, and the absence of expression in osteoblasts, in comparison to the housekeeping genes ACTB and RPL37A. FR-β expression decreased 3.6 fold after osteoclast differentiation and maturation. Cell staining indicated similar results, suggesting that FR-β is primarily expressed on synovial macrophages.
Conclusions: These results suggest that synovial macrophages are the primary cell type expressing FR-β in the synovium of patients with OA. It is possible that targeting and inhibiting FR-β+ macrophages may be used to reduce the inflammation observed in OA.

181
USING EPIPHYSEAL VOLUME TO SCREEN FOR GENES INFLUENCING OSTEOPHYTE FORMATION IN MUTAGENIZED MICE

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Purpose: Genetic modification in mice is a powerful tool for investigating pathways that influence the course of experimental osteoarthritis. For the most part these involve testing candidate pathways that have been identified from in vitro studies. The majority of published studies focus on the influence of such pathways on degeneration of the articular cartilage. Changes occurring in other tissues of the joint such as the bone, synovium and ligaments are relatively neglected. Mutagenesis programmes, in which random mutations are induced in populations of mice, provide an opportunity to discover novel genes in disease in an unbiased fashion. Here we report our preliminary data in which we use epiphyseal volume measurements, an indicator of osteophyte volume, after induction of OA, to screen for aberrant bone responses in mutagenized mice.
Methods: We are analyzing mice from the Harwell ENU mutagenesis programme, a large-scale phenotype-driven screen. Such screens make no a priori assumptions on the genetic basis of a phenotype and hence act as a discovery tool for the identification of new genes and pathways associated with a particular phenotype. Mice bearing random point mutations throughout their genome are analysed phenotypically and, once confirmed, outliers undergo SNP mapping and whole genome sequencing to identify the underlying mutation. In addition to the modifier screen, cohorts of the same mice are aged to 18 months as part of the Harwell Ageing screen. This provides the ability to screen the same mutations in induced as well as spontaneous (age-related) OA.
To date, 50 ten week old male mice from this on-going mutagenesis programme have undergone surgical destabilistion of the medial meniscus (DMM). At 4 weeks post surgery joints were scanned by microCT using CTan (2012-13 Bruker microCT) imaging software, and epiphyseal volume calculated by manually outlining the tibial epiphysis from the right (operated) and left (non-operated) knees (as described by us recently, Das Neves Borges et al, 2014). Selected joints were sent for serial sectioning and histological scoring to validate the bone phenotype and to look for associated joint changes in other tissues.
Results: We observed that at 4 weeks post DMM the majority of mice gained epiphyseal volume in the destabilized joint compared with the non-operated joint. By comparing the epiphyseal volumes in operated versus non-operated knees of individual mice, two pedigrees immediately stood out as outliers. One of these had higher non-operated epiphyseal volume but had an appropriate increase in volume upon joint destabilization. The second outlier had normal epiphyseal volume in the non-operated joint but lost substantial volume after joint destabilisation. 3 mice that were related to the latter pedigree showed a modest reduction in epiphyseal volume following DMM. Tissue samples from outlier mice have been sent for mapping and the lines have been re-derived for further in vivo studies.
Conclusions: Mutagenesis studies combined with rapid validated screening tests provide a realistic approach for discovering novel genes in OA pathogenesis.

182
OSTEOSCLEROTIC BONE PHENOTYPE IS STABLY IMPRINTED IN SUBCHONDRAL MESENCHYMAL STROMAL CELLS IN HIP AND KNEE OSTEOARTHRITIS

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Purpose: The subchondral bone tissue plays a key role in the onset and progression of osteoarthritis (OA), since alterations of the bony bed precede cartilage lesions. Subchondral bone sclerosis in OA is characterized by an increase of bone material that is hypomineralized, caused by a dysregulated osteoblast phenotype. This increased bone anabolism suggests the involvement of mesenchymal stromal cell (MSC) recruitment and their differentiation into osteoblasts. In this study, we investigated whether the properties of these osteoprogenitors have changed in nonsclerotic and sclerotic OA subchondral bone.
Methods: Five knee tibial plateaus and four femoral heads were obtained from patients (mean age 65) undergoing total knee and hip arthroplasty, respectively. Subchondral bone tissues (± 1.5 g wet weight) from nonsclerotic and sclerotic regions were digested in αMEM containing 0.6 mg/ml collagenase IA for three hours and nucleated bone marrow cells were isolated by plastic adherence. First passage cells were seeded at colony forming densities and cultured for 2 weeks in control or osteogenic medium. Osteogenic (CFU-O) and total number of colonies (CFU-f) were counted after alkaline phosphatase (ALP) and methylene blue staining, respectively. Confluent cultures were subjected to osteogenic differentiation for three weeks and evaluated using ALP staining, quantitative ALP assays and qualitative analysis of mineralization by Alizarin Red staining.
Results: MSC clonogenicity, assessed by CFU-f counts, was 20.1±2.2% in nonsclerotic and 17.0±3.5% in the sclerotic subchondral tissue,

showing no statistical differences between regions. In vitro osteogenic potential of MSCs from knee joints, expressed as CFU-O efficiency, was very high without significant differences between nonsclerotic (91.8±1.6%) and sclerotic (91.6±3.4%) tissues. For the hip we also did not find significant statistical differences comparing nonsclerotic (69.8±5.6%) and sclerotic (56.0±19.1%) tissues; but their osteogenic potential was significantly reduced ($p<0.05$) compared with MSCs from knee joints. Efficient induction of osteogenic differentiation was corroborated by quantitative assays, demonstrating a 5.4- and 4.2-fold increase of ALP activity in nonsclerotic and sclerotic MSCs, respectively. Interestingly, in five out of six donors significant differences were found in ALP activity between nonsclerotic and sclerotic MSCs. Unexpectedly, in vitro mineralization was absent in nonsclerotic MSCs of all donors. Sclerotic MSCs demonstrated a blunted mineralization phenotype, evidenced by absence of Alizarin Red staining in six donors, mild staining in two donors and strong mineralization in a single donor.

Conclusions: Our findings suggest that MSCs derived from OA subchondral tissues adopt an aberrant osteoblastic phenotype upon osteogenic induction. Differential ALP activity and hypomineralization, characteristics of OA subchondral bone sclerosis, appear stably imprinted in tissue-resident osteoprogenitors.

Cartilage Biology & Biochemistry

183
LOSS OF ANP32A INCREASES SEVERITY OF OSTEOARTHRITIS IN DIFFERENT MOUSE MODELS

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Purpose: Osteoarthritis is one of the most common chronic musculoskeletal disorders and a cause of serious morbidity and disablement, particularly in the elderly population. The disease can be confined to one or more joints, but can also be generalized. Progressive damage to the articular cartilage and bone leads to pain and loss of joint function. The current therapeutic strategies are limited: changes in lifestyle and appropriate exercise and use of painkillers and anti-inflammatory medication. Eventually, in many cases, joint replacement surgery may be required, especially for the larger joints. The development of osteoarthritis is very complex, due to the activation of several signaling pathways in the different tissues composing the joint, and is influenced by both genetic and acquired or environmental risk factors. We have recently described an association between polymorphisms in the ANP32a gene and osteoarthritis. Anp32a (acidic leucine-rich nuclear phosphoprotein 32 family member a) functions as a tumor suppressor gene and a regulator of gene transcription. It is also implicated in the stabilization of RNA, intracellular transport and apoptosis. Moreover, Anp32a associates with Axin-1 and Phosphatase 2A, molecules that exert a regulating role in the Wingless-type signaling (Wnt) pathway. Wnts are key players in embryonic development with effects on cell differentiation, proliferation and migration. Wnt signaling is also critical for the induction as well as the maintenance of skeletal tissues, is involved in the very early stages of joint formation and appears to be re-activated during adult joint homeostasis.

Methods: Different established mouse models for osteoarthritis (OA) were introduced in Anp32a knockout mice. These include papain-induced arthritis, which is characterized by direct cartilage proteoglycan loss, and DMM-induced arthritis, which is a surgical model of meniscus destabilization, leading to uni-compartimental arthritis, caused by mild instability of the knee. Severe instability of the knee was caused by affecting collagen-rich structures in a collagenase-induced arthritis model. A last model mimics Il1-driven inflammatory arthritis. A transcriptome analysis to study molecular changes in the articular cartilage of mice deficient in Anp32a was also performed. RNA was isolated from articular cartilage of the tibial plateau of knees and used in a microarray study to compare gene expression with cartilage from normal wildtype C57Bl/6 mice. Four C57Bl/6 wild type (WT) and 4 Anp32a-/- mice RNA samples were analyzed on Affymetrix Mouse 430 2.0 chips.

Results: In the DMM- and collagenase-induced OA models, absence of Anp32a resulted in increased cartilage damage, contributing to the development of osteoarthritis. In the papain-induced OA model, a trend for increased cartilage damage was observed in the Anp32a-/- mice. Induction of the mBSA OA model in the Anp32a-/- mice lead to increased inflammation and bone erosion. Several genes related to chondrogenesis, to the development of OA and to the Wnt signaling pathway were identified in our transcriptome analysis.

Conclusions: Our analysis provides evidence for an important role for Anp32a in joint homeostasis and highlights the complex biology of Wnt signaling in the joint.

184
HIF1α/β-CATENIN COMPLEX IS A TRANSCRIPTIONAL REPRESSOR OF MMP13

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Purpose: Activation of Wnt/β-catenin pathway triggers chondrocyte catabolism and MMP13 activation that contribute to osteoarthritis (OA). The mechanism of down-regulation of Wnt/β-catenin pathway in cartilage homeostasis is unknown. Because chondrocytes are in a hypoxic environment that is lost in OA, we speculated that Hypoxia Inducible Factor 1α (HIF1α) inhibits Wnt signaling and chondrocyte catabolism. Therefore, we here investigated the interaction of β-catenin/TCF4 and the molecular regulation of Mmp-13 in articular chondrocytes and in OA mice

Methods: Primary murine chondrocytes from WT and ΔHIF1α were cultured with Wnt3a in normoxic (21% O2) and hypoxic (1% O2) conditions and analyzed the expression of catabolic markers. The binding of TCF4 to Mmp-13 regulatory regions was assessed in Chip assay in both conditions. To determine the impact of the interaction of Wnt and HIF1α in healthy cartilage and in OA, ΔHIF1αchon and fl/fl HIF1α underwent DMM and received articular injection of PKF 118-310, an inhibitor of β-catenin and TCF4 interaction.

Results: Hypoxia prevented the increase in Mmp-13 and the decrease in Col2 and ACAN expression induced by Wnt. Blocking HIF1α by siRNA or cre-recombinase enhanced the expression of Mmp-13, but not Col2 expression suggesting that HIF1α regulated catabolic rather than anabolic genes. In hypoxic chondrocytes, Chip assay revealed that HIF1α lowered β-catenin/TCF4 transcriptional activity and the expression of Mmp13. Indeed, HIF1α interacted with β-catenin which displaced TCF4 from Mmp13 regulatory sequences. Moreover, DMM resulted in decreased HIF1α expression in articular cartilage of control mice. Furthermore, cartilage lesions were higher in ΔHIF1αchon mice submitted to DMM along with higher expression of β-catenin and Mmp13. Local administration of PKF 118-310 in ΔHIF1αchon reduced Mmp13 expression and prevented cartilage lesions. These results showed that HIF1α limited cartilage breakdown by blocking the binding of β-catenin to TCF4 subsequently leading to a lower Mmp-13 activation.

Conclusions: We show here that HIF1α forms a complex with β-catenin that acts as a negative regulator of Wnt signaling and Mmp13 transcription. Therefore, targeting the interaction is a new approach to reduce chondrocyte catabolism and prevent from osteoarthritis.

185
CARTILAGE PERMEABILITY ASSESSMENT BASED ON PROTEOMIC ANALYSIS OF PLASMA PROTEIN PENETRATION

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