Has1 were regulated upon explantation but their expression appeared to be independent of FGF2.

**Conclusions:** Murine cartilage injury mirrors that seen in porcine tissue and reveals distinct cellular responses to explantation and re-cutting. The re-cutting response is almost exclusively due to FGF2. Explantation elicits activation of inflammatory signalling and leads to the induction of genes; both FGF2-dependent, and FGF2-independent. The upstream pathway through which non-FGF dependent signals are initiated upon explantation is currently unknown.

IL-1BETA TRANSLOCATES THE PROTEIN DIMETHYLA RGINASE 2 (DDAH2) TO THE MITOCHONDRION OF HUMAN NORMAL CHONDROCYTES

B. Cilleró-Pastor, J. Mateos, C. Ruiz-Romero, F.J. Blanco

**Osteoarticular and Aging Res. Lab. INIBIC-Complejo Hosp. Univ. A Coruña, A Coruña, Spain**

**Purpose:** Mitochondria is acquiring an important role in the osteoarthritis pathology (OA). IL-1beta is one of the main cytokines related to the inflammation and the destruction of the cartilage which is known to regulate mitochondrial functionality and to produce nitric oxide (NO). Previously, we have described the total proteome of chondrocytes regulated by IL-1beta identifying some mitochondrial proteins. Nevertheless, the exact role that IL-1beta has in the regulation of mitochondrial protein expression and their implication in the OA process, is not well understood. For this reason, we analyzed the mitochondrial protein expression profile by the action of IL-1beta, in order to understand the development of different rheumatic pathologies.

**Methods:** Human normal chondrocytes were isolated from the cartilage of knees of autopsies from donors without previous history of joint disease. The cells were incubated for 48 hours in basal conditions or with IL-1beta (5 ng/ml) and mitochondrial proteins were purified. A pool of these mitochondrial proteins of 4 different donors in each condition, was resolved by bidimensional electrophoresis (2-DE). Proteins were visualized with Sypro stain. The qualitative and quantitative analysis were carried out with PD-QUEST software. After that, proteins were identified by mass spectrometry (MS) using the MALDI-TOF/TOF technology. The validation of the results and the study of interesting proteins were made by real-time PCR, western blot and microscopy. Total NO quantification was evaluated with Griess reagent assays.

**Results:** The comparative analysis of mitochondrial proteome of chondrocytes stimulated with IL-1beta for 48 hours with respect to the basal situation revealed a differential expression of signal transduction proteins, regulators of cytoskeleton, transcription, metabolic and stress related pathways. IL-1beta increased with respect to the basal situation different proteins like annexin A2 (ANXA2) or mitochondrial superoxide dismutase (SOD2). Another protein that increased its expression 2.59 times after the stimulation with IL-1beta, with respect to basal condition was dimethylarginase 2 (DDAH2) [basal-1, n=4; *P*<0.05]. This protein has an important role as regulator of NO synthesis when it hydrolyzes the inhibitor of NO synthase, ADMA. DDAH2 did not show any regulation at mRNA nor total protein expression after the stimulation with IL-1beta, nevertheless the study of mitochondrial extracts showed a clear increase of DDAH2 in the condition of IL-1beta with respect to basal condition (4.18±1.41 vs. basal-1, n=4; *P*<0.05). By means of techniques of conventional immunofluorescence and confocal microscopy, we observed that DDAH2 was located again in the mitochondria of IL-1beta-stimulated chondrocytes. These results were also reproducible in cartilage slices treated with IL-1beta. In addition to this we demonstrated that DDAH2, which hadn’t been before described in chondrocytes nor cartilage, regulated the NO production induced by IL-1beta.

**Conclusions:** Our studies indicate that IL-1beta increased the expression of certain proteins with a mitochondrial localization such as DDAH2 that was identified for the first time in human normal chondrocytes and cartilage. It seemed to have an important role in the IL-1beta-NO production. Its specific localization in the mitochondria could help to understand the role of this organell and NO in rheumatic pathologies.

PROTEASE ACTIVATED RECEPTOR-2 (PAR-2) AND ACTIVATED PROTEIN C (APC) IN CARTILAGE DEGRADATION

M.T. Jackson, M.M. Smith, S. Smith, C. Jackson, C.B. Little

**Kolling Inst. of Med. Res., St Leonards, Australia**

Introduction: PAR-2 has been implicated in cartilage breakdown in arthritis because: (i) it is expressed by chondrocytes and upregulated in osteoarthritis (OA) and by IL-1 and TNF; (ii) its activation increases MMP expression and activity in cartilage in vitro; and (iii) PAR-2 KO mice have reduced inflammatory arthritis. PAR-2 is unique in being activated by APC but not thrombin. APC is synthesized by chondrocytes and can activate cartilage MMPs causing cartilage breakdown. In this study we investigated the role and relationship of PAR-2 and APC in MMP activation and cartilage degradation.

**Methods:** Femoral head cartilage from wild-type (WT; C57/B16) and PAR-2 knock-out (KO) mice was cultured ± 10ng/ml IL-1±20ng/ml APC for 4 days in serum-free media. Glycosaminoglycan (GAG) and collagen (hydroyprylene) release, and MMP-2, -9 and -13 activity in conditioned media were analysed. Chondrocyte expression of matrix proteins and enzymes was measured by qRT-PCR.

The onset and progression of medial-meniscal destabilization (DMM)-induced OA in knees of WT and PAR-2 KO mice was quantified historically and by means of techniques of conventional immunofluorescence and confocal microscopy. We observed that DDAH2 was located again in the mitochondria of IL-1beta-stimulated chondrocytes. As previously described in other species, APC alone did not induce GAG or collagen release from WT mouse cartilage. IL-1-induced GAG release was augmented by APC, and collagen release was induced in IL-1+APC cultures. IL-1 increased pro-MMP-9 and active MMP-13 release from WT mouse cartilage, while IL-1+APC increased active MMP-2, -9 and -13. IL-1 increased mRNA expression for endothelial protein C receptor, ADAMTS-4, TIMP-1, MMP-9, and this was unaltered by APC. There was no difference in GAG release, MMP activation or the response to IL-1+ APC in PAR-2 KO compared with WT cartilage. PAR-2 KO chondrocytes expressed increased IL-1-induced ADAMTS-5 mRNA and lacked PAR-2 mRNA compared with WT. There was a decrease in tibial cartilage aggrecan loss and structural damage 4 but not 8 weeks after DMM in PAR-2KO mice. There was no difference in osteophytes between genotypes, but PAR-2 KO mice had reduced subchondral bone sclerosis at 4 and 8 weeks after DMM.

**Discussion:** APC activates mouse cartilage MMPs similar to our previous studies in sheep, with subtle differences associated with articular versus epiphyseal cartilage. Actual release of collagen from cartilage only occurs with co-activation of MMP-2, -9 and -13. APC-induced activation of MMPs and cartilage degradation does not require PAR-2. Furthermore, PAR-2 does not appear to be critical for the induced cartilage degradation. The increase in IL-1-induced ADAMTS-5 mRNA in PAR-2 KO chondrocytes suggests that activation of PAR-2 normally acts as a negative regulator of this enzyme. The early inhibition of DMM-induced cartilage damage in PAR-2 KO mice could be associated with a reduction in the post-surgical inflammation. The early and sustained inhibition in subchondral bone change in PAR-2 KO mice is consistent with the role of PAR-2 in osteoblast activity and osteoclast differentiation/activation but suggests that cartilage and bone change in this OA model are not co-dependent.

IMPAIRED EXPRESSION OF GENES INVOLVED IN REVERSE CHOLESTEROL TRANSPORT IN HUMAN OSTEOARTHRITIC CHONDROCYTES: BENEFICIAL EFFECT OF LXR AGONIST TREATMENT

T. Simopoulou, K.N. Malizos, F. Kostopoulou, A. Tseou

**Univ. of Thessalia, Larissa, Greece**

**Purpose:** Osteoarthritis (OA) is a common age-related joint disease. As in other age-related degenerative diseases, such as those of the cardiovascular system, altered lipid metabolism has been implicated as a critical player in disease development. Increased serum cholesterol has been considered a risk factor for OA independent of obesity and also articular manifestations have been demonstrated in familial hypercholesterolemia patients. As excessive accumulation of free cholesterol is toxic for the cells, intracellular cholesterol accumulation is prevented by tight regulation of influx and efflux pathways. We have previously shown that chondrocytes are