In contrast, cytomegalovirus promoter-driven Sp1 overexpression further enhanced Il-1-induced ADAMTS-4 expression. Expression of constitutive ADAMTS-5 was not affected by any of the agents.
Conclusions: These results provide pharmacological and genetic evidence for the importance of Sp1 in ADAMTS-4 gene regulation by Il-1.

232 CHONDROITIN SULFATE MODULATES THE MITOCHONDRIAL ACTIVITY OF HUMAN ARTICULAR CHONDROCYTES

F.J. Blanco[1], M.J. López-Armada[1], B. Cillero-Pastor[1], B. Caramés[1], E. Montell[2], J. Vergés[2], F. Galdo[1]. *[1]Osteoarticular and Aging Research Laboratory. Biomedical Research Center. INIBIC-Hospital Universitario Juan Canalejo, Coruña, SPAIN, [2]Laboratorios Bioiberica Farma, Barcelona, SPAIN*

Purpose: Human articular chondrocytes originating from osteoarthritic cartilage present an altered mitochondrial activity due to an alteration in the mitochondrial membrane potential. This mitochondrial alteration can cause an increment of free radicals which may lead to a rise in the number of dead chondrocytes in the cartilage and a decrease in protoglycans in the extracellular matrix.
Objectives: To analyse the effect of Chondroitin Sulfate (CS) on the mitochondrial activity of osteoarthritic chondrocytes.
Methods: Human articular chondrocytes were isolated from osteoarthritic cartilage obtained by prosthetic surgery. Chondrocytes were cultivated and stimulated with 10 ng/ml TNFα, during different time intervals (6, 12, 24 and 48 hours), which caused depolarisation of the mitochondrial membrane. This depolarization was then measured using flow cytometry. Subsequently chondrocytes were stimulated with different concentrations of CS (10, 50, 100, 150, 200 and 500 μg/ml) at different time intervals (6, 12, 24 and 48 hours), which enables us to analyze the capacity that the drug has in slowing down mitochondrial depolarization induced by TNF. Effects of CS on mitochondrial function were also validated via ATP synthesis.
Results: The concentrations of CS that were used did not alter the membrane potential at 6, 12 and 24 hours. However CS concentrations greater then 50 μg/ml at 48 hours showed a significant increase in the red/green ratio suggesting that CS induces a state of mitochondrial hyperpolarisation in osteoarthritic chondrocytes (50 μg/ml = 135%; 100 μg/ml = 148%; 150 μg/ml = 156%; 200 μg/ml = 164% and 500 μg/ml = 171% vs control 100%). TNFα induced a significant reduction in the red/green ratio at 12 hours = 41%; 24 hours = 41% and 48 hours = 39%. At 6 and 12 hours CS did not decrease the effect of TNFα on the mitochondrial membrane potential. However CS reduced the effect of TNFα on the mitochondrial depolarization at 24 and 48 hours. At 24 hours the effective concentrations used were: CS 150 μg/ml = 58%; 200 μg/ml = 64% and 500 μg/ml = 64% vs. TNFα 41%. At 48 hours the effective concentrations used were: CS 10 μg/ml = 58%; 50 μg/ml = 60%; 100 μg/ml = 60%; 150 μg/ml = 65%; 200 μg/ml = 66% and 500 μg/ml = 73% vs. control 39%). CS at a 200 μg/ml concentration increased the basal synthesis of ATP produced by articular chondrocytes in cultures (Control = 0.55 vs. 200 μl = 0.65).
Conclusions: CS concentrations greater than 50 μg/ml and at 48 hours increases the red/green ratio, reducing the effect of TNFα on the mitochondrial depolarisation and increases the synthesis of ATP. All this data could have a relation to the anti apoptotic effects produced in chondrocytes described by CS.

233 NOTCH PATHWAY: ITS IMPLICATION IN ARTICULAR CHONDROCYTE DEDIFFERENTIATION COULD BE MEDIATED BY MMP13

M. Mahjoub[1], R. Blaise[2], C. Salvat[2], U. Barbe[2], C. Brou[3], M. Corvol[4], J-F. Savouret[4], F. Rannou[4], S. Sellami[1], P. Bausero[2], F. Berenbaum[2]. *[1]La Rabta Hospital, Tunis, TUNISIA, [2]CNRS UMR 7079, Université Pierre et Marie Curie, Paris, FRANCE, [3]CNRS URA 2582, Institut Pasteur, Paris, FRANCE, [4]INSERM UMR-747, Université Paris Descartes, Paris, FRANCE*

Purpose: Members of Notch family receptors act as membrane-tethered transcription factors that are tightly associated with cell fate decisions while controling differentiation, proliferation and apoptosis.
Upon ligand binding, Notch molecules undergo proteolytic cleavages by the γ-secretase complex that lead to the activation of the transcription of Notch target genes. Although, the molecular mechanism of Notch activation is well characterized, further analyses in an appropriate cellular context will provide new insight into several diseases such as osteoarthritis (O.A). We therefore examined correlations between Notch pathway activation and regulation of metalloproteases (MMPs) as well as chondrocyte markers expressions, during phenotypic modulation process of chondrocytes, as it occurs in O.A.
Methods: We used serial monolayer primary cultures of immature murine articular chondrocytes (MACs) as an in-vitro model of the events which occur during phenotypic modulation. MACs were cultured with or without a Notch inhibitor, and transfected with different notch expressing vectors. Cells were used for quantitative RT-PCR and immunoblotting analyses to characterize Notch molecules and to determine their impact on chondrocytes markers and MMPs expressions.
Results: Differentiation chondrocyte markers (Type II collagen, Aggrecan) are down regulated during passages. Inversely, the dedifferentiation marker, type I collagen is up regulated during MACs culture.
Concomitantly, Notch ligands and target genes are up regulated. In the presence of a γ-secretase inhibitor, DAPT, we observed a diminution of type II collagen degradation, but without variation of *Col2a1* gene expression. In order to elucidate type II collagen protection mechanisms, we studied the expression of MMPs with or without Notch inhibitor. We demonstrated that expression of MMP13 is regulated in a Notch-dependant manner while MMP2 expression is not modified. Using constitutively active forms of Notch1 receptor or a dominant negative form of the Notch1 transcriptional co-activator (CSL), we demonstrate a direct effect of Notch in MMP13-dependent Type II collagen degradation.
Conclusions: These data suggest that Notch signalling could be critical for the MMP13-dependent degradation of type II collagen during phenotypic modulation of chondrocytes occurring during O.A.

234 CARTILAGE CALCIFICATION AND BMP SIGNALING IN EARLY HAND OSTEOARTHRITIS

M.H. Stradner[1], M. Asslaber[2], H. Angerer[1], D. Setznagl[1], F.C. Fürst[1], H. Denk[2], W.B. Graninger[1]. *[1]Department of Rheumatology, Medical University of Graz, Graz, AUSTRIA, [2]Institute of Pathology, Medical University of Graz, Graz, AUSTRIA*

Purpose: The aim of this project was to reveal basic morphological and biochemical changes in early hand osteoarthritis of the proximal interphalangeal (PIP) joint.
Methods: We explanted affected and normal PIP joints of human dissecting room cadavers with Bouchard's nodes according to the rules of our local ethic committee. The joint specimens were fixed in formalin, decalcified in EDTA and embedded in paraffin. Sections were stained Safranin O and H&E. Furthermore immunohistochemistry for bone morphogenetic protein (BMP)-2, -4, and -6 was performed.
Results: Histological examination of macroscopically normal PIP joints from patients with manifest Bouchard's nodes of other PIP joints revealed tidemark duplication proteoglycan loss and cartilage calcification. This finding was especially prominent at the dorso-lateral edges of both the caput of the proximal phalanx and the basis of the phalanx media. At these sites the remaining cartilage lining in some sections was reduced to less than 80 μm. Using immunohistochemistry we found marked BMP-6 expression in both articular cartilage and adjacent ligament tissue. BMP-2 was expressed to a lesser content in cartilage, whereas BMP-4 was not detected at all.
Conclusions: We suggest that cartilage calcification of the dorso-lateral edges of the PIP joints is the first morphological step in the pathogenesis of Bouchard's nodes. As BMP signalling is associated with cartilage ossification, the finding of BMP expression in the vicinity of these sites may imply a role of BMP signalling in the development of hand osteoarthritis.

235 PROLONGED EXPOSURE TO LEPTIN INDUCES A CATABOLIC EFFECT IN NORMAL AND OSTEOARTHRITIC CARTILAGE

R. Dryer-Minnerly, V. Stojanovic-Susulic. *Centocor, Radnor, PA, USA*

Purpose: Obesity is a major risk factor in development and progression of Osteoarthritis (OA). Although there is an established link between OA and the biomechanical factors associated with obesity, there undoubtedly exists a metabolic component that influences the disease in the non-weight bearing joints, such as hands. The correlation of leptin levels with body mass index (BMI), coupled with findings of increased leptin in synovial fluid of OA patients, suggests a role of this cytokine-like, pleiotropic hormone in the progression of OA. In this study, we investigated the