CS 200 µg/mL at 24 hours significantly increased ATP production compared to basal condition (0.05 µM vs 0.65 µM). After TNFα stimulation, ATP production fell to 0.40 µM; but also in this case, CS was able to inhibit TNFα-induced decrease in the ATP synthesis up to 62%. Furthermore, we have evaluated the effect of CS on NO and ROS production. CS was able to reduce NO synthesis induced by IL-1β, TNFα or LPS. The NO levels were reduced after cytokine stimulation up to 21%, 32% and 31% respectively. On the other hand, in presence of IL-1β, ROS production and SOD2 activity were increased. In this case, intracellular ROS production as well as SOD activity decreased in CS treated chondrocytes at an average of 80%. The decrease was more marked (up to 62%) when we specifically analysed the mitochondrial isomorph of this important antioxidant enzyme (SOD2).

Conclusions: In summary, CS improves mitochondrial activity in human OA chondrocytes by affecting several mitochondrial processes. The mitochondrial membrane hyperpolarization and the increased ATP production could correlate with a greater resistance of CS treated chondrocytes to apoptosis. Moreover, the reduction of NO and ROS levels, as well as the reduction in SOD2 activity, provide evidence of the effect of CS on oxidative stress regulation.

246 EFFECT ON THE CHONDROCYTE PHENOTYPE BY SPECIFICALLY INHIBITING THE β-CATENIN DEPENDENT WNT SIGNALING USING SMALL PEPTIDES

A. Held, A. Glas, T. Grossmann, T. Pap, J. Bertrand. Inst. of Experimental Musculoskeletal Med., Munster, Germany; 1Chemical Genomics Ctr. (CGC) of the Max Planck Society, Dortmund, Germany

Purpose: In osteoarthritis (OA), chondrocytes undergo hypertrophic differentiation. In course of phenotypic change of chondrocytes, the matrix is remodelled, including downregulation of genes such as ACAN, COL2A1 and MMP13. One of the signaling pathways involved in this chondrocyte differentiation is the canonical β-catenin dependent WNT signaling pathway. SAH-BCL9 (stabilized alpha-helix of Bcl9) and StAx-35R (stapled axin β-catenin binding domains), two small peptides have been developed to inhibit the oncogenic canonical WNT signaling by directly targeting β-catenin. We hypothesized that SAH-BCL9 and StAx-35R may inhibit the dedifferentiation of chondrocytes towards hypertrophy and may reduce OA-like changes in the cartilage.

Methods: In vitro analyses were performed using primary chondrocytes isolated from neonatal mice. The peptides SAH-BCL9 and StAx-35R were synthesized and provided by the Grossmann Lab at the Chemical Genomics Centre (CGC) of the Max Planck Society in Dortmund, Germany. Micromass cultures were stained with alcam blue to examine the proteoglycan production. Expression of marker genes (ACAN, COL2A1, MMP13 and AXIN2) was measured by quantitative RT-PCR. Effects of the inhibitors on the phosphorylation of LRP6 and on total β-catenin were analyzed using Western Blot. With dual luciferase assays the efficacy of the inhibitors on the phosphorylation of LRP6 and on total β-catenin were analyzed using Western Blot. Dual luciferase assays the efficacy of the inhibitors was investigated by using confoal laser microscopy.

Results: Based on first results, confocal laser microscopy demonstrated intracellular but not intranuclear localization of FITC-labeled SAH-BCL9 and StAx-35R peptides in primary chondrocytes and C28 cells, chondrocyte-like immortalized cells. Treatment of hip caps with inhibitors showed better penetration into cartilage for SAH-BCL9, but not for StAx-35R, independent of previous IL-1 treatment. TCF/LEF promoter activity was reduced by application of increasing concentrations of SAH-BCL9, but not with StAx-35R, upon simultaneous induction of the canonical WNT signaling with WNT3A. Western blot analysis revealed that StAx-35R did not affect pLRP6 and β-catenin. However first results indicated less phosphorylation of LRP6 and less stabilization of β-catenin when cells were treated with SAH-BCL9 and WNT3A compared to cells which were only treated with WNT3A alone. Quantitative RT-PCR showed decreased expression of aggrecan, collagen type II and MMP13 upon stimulation with WNT3A, which was not rescued to baseline by treatment with TNFα, but inhibition of the ATP synthase activity via StAx-35R and still enhanced when cells were treated simultaneously with WNT3A and SAH-BCL9 or StAx-35R. With regard to the phenotypic stability of chondrocytes, alcin blue staining of micromass cultures showed that WNT3A treatment led to proteoglycan loss, which could not be reduced by using the small peptides.

Conclusions: Our data indicate that SAH-BCL9 inhibits the β-catenin dependent WNT signaling pathway on protein level and functionally with regard to the TCF/LEF reporter activity at high concentrations. Despite both – SAH-BCL9 and StAx-35R – appear to be not able to reverse WNT3A induced changes on the chondrocyte phenotype.

247 EFFECT OF GROWTH HORMONE AND HYALURONAN AMIDE DERIVATIVE ON HUMAN OSTEOARTHRITIC CHONDROCYTES

F. Paololla, E. Gabusi, C. Manferdini, L. Gambabi, A. Schiavinito, B. Girigolo, G. Ligni. Istituto Ortopedico Rizzoli, Bologna, Italy; 1Fidia Farmaceutici s.p.a., Abano Terme, Padova, Italy

Purpose: Knee osteoarthritis (OA) is one of the main causes of pain and disability and significantly affects the patients’ quality of life. The homeostasis of articular cartilage is mediated by a complex network of interactions mainly due to locally produced growth factors, ECM components and circulating hormones. The development of pharmacological treatments with the potential for structure-modifying activity in OA joint treatment has become a major focus in the field of OA research. Aim of this study was to evaluate the effects of hyaluronamide derivative (HYAADD)4-4G, HAD alone or in combination with human growth hormone (hGH) on OA chondrocytes.

Methods: Chondrocytes were isolated from cartilage of OA patients undergoing knee arthroplasty. Chondrocytes were treated with different concentrations of hGH (from 0.01µg/mL to 1µg/mL) and with or without HAD (1mg/mL) and analyzed at different time points (24, 48, 72 hours and after 7 days). They were analyzed for: 1. the presence of GHR and CD44 by immunocytchemistry, 2. cell viability by vital dye, 3. metabolic activity by alamar blue assay, 4. the release of IGF-1, FGF2 and IL6 by immunoassay tests, 5. gene expression of collagen type 1, collagen type 2, collagen type 10 and SOCS2 by qRT-PCR.

Results: OA chondrocytes express GH receptor and all doses of hGH tested did not affected cell viability and metabolic activity, as well as, the expression of collagen type 2, 1, or 10 and the release of insulin like growth factor-1 (IGF-1) or fibroblast growth factor-2 (FGF-2) or IL6. HAD treatment increased the expression of hyaluronan receptor CD44. HAD alone or combined with hGH reduced metabolic activity, the release of IL6 and the gene expression of collagen type 1, 2 and 10, except SOCS2, that was increased. Interestingly, all the parameters analyzed proportionally decreased with increasing age of the patients.

Conclusions: hGH did not induce human chondrocyte metabolic activity and did not affect typical chondrocytic (collagen type 2) or fibrotic or hypertrophic (collagen type 1 and 10) marker expression or IGF1 or FGF2 or IL6 release but induced CD44 receptor. HAD reduced all the effects induced by hGH on human OA chondrocytes partially through a significant induction of SOCS2 expression. It has been demonstrated that SOCS2 is significantly reduced in OA chondrocytes and our preliminary results demonstrate an unknown interplays between hGH and hyaluronic acid probably due to SOCS2.

248 SANGUIS DRACONIS RESIN STIMULATES THE OSTEOSTEGESIS IN OSTEOBLASTIC MC3T3-E1 CELLS IN VITRO

W. Wang, P. Yuan, H. Guo. The Second Affiliated Hosp., Xian Jiaotong University, Xian, China; 1Shaanxi Traditional Chinese Med. Coll., Xianyang, China; 3Dept. of Publ. Hth., Med. Coll., Key Lab. of Environment and Genes Related to Diseases, Xian Jiaotong University, Xian, China

Purpose: Sanguis Draconis (SD) is a resin that is obtained from Daenmonoropos draco (Palmae). Used in traditional medicine, it has shown activity in the prevention of osteoporosis as well as promoting the healing of bone fractures. The purpose of this study was to investigate the effect of SD on the MC3T3-E1 cells in vitro.

Methods: In this study, the effects of Sanguis Draconis ethanol extract on b-glycerolphosphate and ascorbic acid induced differentiation using mouse calvaria origin MC3T3-E1 osteoblastic cells was examined. We looked at osteoblast differentiation, proliferation, and mineralization by measuring alkaline phosphatase (ALP) and specific bone marker activities. Osteoblast-like MC3T3-E1 cells were cultured in various concentrations of SD ethanol extract (0.005-1 mg/mL) during the osteoblast differentiation and period of 24, 48, 72 hours and 25 days.

Results: As measured by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay, SD extracts increased cell proliferation as compared to control. The most pronounced effect was observed at the concentration range between 0.01 and 0.1 mg/mL (P<0.01). This SD stimulatory effect for cell proliferation was observed during the whole