horses slaughtered for human consumption (grade 0, n=6 mean age 8.8±4.7 yrs).

Expression of IGF-1R was determined using immunohistochemistry on 7 μm cryostat sections. Mean percentage of cells staining positively for IGF-1R in the superficial zone (%pos.S) and middle+deep zones (%pos.MD), and microscopic (modified Mankin) score were determined for each site. All data were analysed using two-way ANOVA and linear regression.

Results: The %pos.S was significantly greater than %pos.MD at four of the six sites investigated (P<0.01), however although there was wide variation there were no significant differences between sites for either variable. The mean (SD) %pos.S and %pos.MD across the joint sites were 45.03 (±21.71) and 14.87 (±11.28), respectively, while the microscopic score ranged from 1.81−7.38 (mean=4.52). There was a significant positive correlation between both %pos.S and %pos.MD and microscopic score at sites 1 and 2 (Figure 2).

Conclusions: The findings of this study indicate that considerable, though statistically insignificant, variations in IGF-1R and microscopic score exist in normal joints. The wide range of microscopic score seen in the normal cartilage used in this study could suggest that some of the scoring criteria of the microscopic scoring system used are transient measures of cartilage condition, or that initial gross grading of cartilage should include additional measures such as Indian Ink stains or the cartilage degeneration index. The unexpected finding of a strong positive correlation between IGF-1R expression and microscopic score in two regions of the joint may indicate that potentially, IGF-1R plays a role in cartilage degradation.

165 MELANOCYTE-STIMULATING-HORMONE AFFECTS GENE EXPRESSION IN HUMAN ARTICULAR CHONDROCYTES

S. Grässel1, S. Anders2, M. Böhm3. 1University of Regensburg, Regensburg, GERMANY, 2University of Regensburg, Bad Abbach, GERMANY, 3University of Muenster, Muenster, GERMANY

Purpose: Melancortins (MCs) are related peptides and are involved in food intake, energy homeostasis, sexual behaviour, endocrine gland function and inflammatory responses. The natural MCs, adrenocorticotropic hormone (ACTH) and the melanocyte-stimulating hormones (α-, β-, γ-MSH) derive from proopiomelanocortin (POMC), which is processed by members of the prohormone convertase family. The effects of MCs are transduced by melanocortin receptors (MC-R), a family of five transmembrane G-protein coupled proteins. The MC-system is well described in brain and skin. The additional presence of MC-Rs in rodent and murine osteoblasts and chondrocytes suggests a role for the melanocortin system in cartilage and bone formation. α-MSH inhibits TNF-α induced MMP-13 expression in human chondrosarcoma cells and, thus may be an inhibitor of MMP-13 mediated collagen degradation. ACTH mediated activation of MC-Rs on knee joint macrophages, reduced II-1β and II-6.
release in experimental arthritis and inhibits neutrophil accumulation in the inflamed joint, thereby revealing anti-inflammatory potential. Apart from these data, little is known about localisation, regulation, signal transduction and function of melanocortins in cartilage.

**Methods:** Human articular chondrocytes isolated from OA-cartilage were proliferated in monolayer until passage one and cultured in 3D-micromass pellets for either 1 or 7 days. After stimulation for 48 h with 10^{-6} M α-MSH, RNA was isolated and the cDNA copy number of Sox9, collagen I, II and X, MMP2, -7, -9 and -13, II-1, TNFα and II-6 was assayed with qRT-PCR. MC-1R expression was determined by RT-PCR and immunohistochemistry. Signal transduction of α-MSH was evaluated by cAMP ELISA and Ca^{2+} assay using Fura2-AM.

**Results:** We have detected protein and gene expression of the MC-1R in articular chondrocytes derived from osteoarthritic cartilage. The MC-1R detected in human chondrocytes appears functional as stimulation with α-MSH resulted in a moderate but significant increase in intracellular cAMP levels but not in changes of the intracellular Ca^{2+} level. In vitro application of α-MSH altered gene expression of collagens, MMPs, cytokines and Sox9 in a culture time dependent pattern in articular chondrocytes. In general, at an early time point α-MSH induced a downregulation of the majority of these genes while at a later time point they mostly were upregulated.

**Conclusions:** The time-dependent gene expression induction/suppression pattern indicates a differentiation specific involvement of α-MSH in processes like re-differentiation and endochondral ossification as a factor with immunomodulatory functions. The α-MSH dependent regulation of cytokines and MMPs points towards a role in modulation of inflammatory or degenerative processes in diseases such as RA and OA.

**166 REGULATION AND FUNCTION OF MATRIX METALLOPROTEINASE 28**

U.R. Rodgers¹, T.E. Swingleler¹, L. Kevorkian¹, A.K. Surridge¹, A.E. Parker², I.M. Clark³, ¹University of East Anglia, Norwich, UNITED KINGDOM, ²AstraZeneca Pharmaceuticals, Cheshire, UNITED KINGDOM

**Purpose:** MMP-28 (epilysin) is a recently discovered member of the matrix metalloproteinase family. We have previously reported expression of MMP28 in normal human articular cartilage and synovium with significant induction of expression in osteoarthritis. The regulation of the MMP28 gene and the physiological function and substrates of the MMP-28 protein are currently unknown.

**Methods:** Expression constructs for wild-type proteinases and inactive mutants were cloned into pcDNA4-FLAG. Protein expression after transfection into a variety of cell types was assessed by western blotting and immunocytochemistry. Stable cell lines were generated using SW1353 chondrosarcoma cells and clonal lines were then generated. Functional analyses included cell proliferation, adhesion and migration. Promoter-reporter constructs were made in pGL3 and transiently transfected using FuGene6. DNA-protein interaction was assessed by gel mobility shift assay. Acetylation of Sp1 and Sp3 was probed using immunoprecipitation with a specific antibody and western blot with an anti-(pan acetyl lysine) antibody.

**Results:** Lysates from SW1353 cells over-expressing the full-length MMP28 cDNA contained pro-MMP28, conditioned medium contained pro-MMP-28 and a cleaved C-terminal domain, and ECM fractions contained some pro-MMP-28 and also the active form. Using a furin inhibitor, we found that the activation of MMP-28 was furin dependent. Overexpression of MMP-28 leads to increased adhesion on both fibronectin and type II collagen, though migration and proliferation are not altered. An inactive MMP28 expression and activity is also seen. MMP28 expression is induced by histone deacetylase (HDAC) inhibitors and this effect is mediated via Sp1 at a promoter proximal G-box. Immunoprecipitation experiments have shown that the acetylation of both Sp1 and Sp3 is increased by HDAC inhibitor treatment, however no evidence for DNA binding was observed. Histone acetyltransferases such as p300 and P/CAF increased induction of the MMP28 promoter by Sp1. Knockdown of HDAC1 using siRNA also induces the MMP28 promoter. Oligonucleotide pulldown and ChIP assays identified other proteins recruited to the MMP28 promoter including c-Jun and serine threonine receptor-associated protein (STRAF).

**Conclusions:** In initial studies to characterise the regulation and function of MMP-28, we observed that the activation of pro-MMP-28 is furin dependent. Interestingly, the active enzyme preferentially associates with the ECM. This may suggest a matrix substrate for MMP-28. We have successfully generated SW1353 cell lines stably transfected with MMP28 (full length, EA mutant and pro-cat). Phenotypic changes to adhesion and proliferation have been measured, along with altered expression of other metalloproteinase genes. A more complete analysis including candidate protein and proteomic screens of potential substrates is currently underway. We also present evidence that MMP28 is regulated by HDAC inhibitors and that this is via alterations in transcription factor acetylation. Since HDAC inhibitors are known to be chondroprotective, this is important both to potential function of MMP-28 and the design of therapeutics which target HDACs.

**167 EFFECTS OF VA441, A NEW SELECTIVE COX-2 INHIBITOR, ON HUMAN CHONDROCYTE CULTURES: A BIOCHEMICAL AND MORPHOLOGICAL STUDY**

T. Laura¹, T. Serchi¹, A. Fioravanti¹, G. Collodel², E. Moretti², M. Galeazzi¹, M. Rovini³, G. Vomero³, A. Cappelli³, M. Anzini³, ¹University of Siena – Dept. Clinic Medicine and Immunology Sciences, Siena, ITALY, ²University of Siena – Dept. General Biology, Siena, ITALY, ³University of Siena – Dept. of Pure and Applied Medicinal Chemistry, Siena, ITALY

**Purpose:** Non-steroidal anti-inflammatory drugs and selective COX-2 inhibitors are commonly used for the symptomatic treatment of osteoarthritis (OA). Several conflicting reports about the effects of these drugs on articular cartilage have been reported. VA441 is a novel compound, strictly related to coxibs, which in vitro proved to be a potent and highly selective COX-2 inhibitor being equipotent with rofecoxib and in the HJB test demonstrated to be as protective as valdecoxib. Furthermore, VA441 showed, in vivo, a very good activity against both carrageenan induced hyperalgesia and carrageenan induced oedema in the rat paw, with a complete remission 1 hour after the administration. In the abdominal constriction VA441 was able to reduce the number of writhes in a statistically significant manner.

The aim of the present study was to investigate the in vitro effect of VA441 in comparison with Indomethacin and Celecoxib on chondrocyte proliferation and ultrastructure and on some biochemical parameters in human chondrocyte cultures in presence or in absence of II-1.

**Methods:** Human articular cartilage was obtained from 5 subjects with OA undergoing surgery for total hip replacement. Chondrocytes were seeded at 4·10^{4} cells/well. VA441, Indomethacin and Celecoxib were added at concentration of 0.1, 1 and 10 mM with and without II-1 (5 ng/mL) for 3 days. Proliferative response of chondrocyte was examined by a colorimetric assay (MTT). Proteoglycans and MMP-3 release in the culture medium and Prostaglandin E2 levels were determined by an ELISA assay. Aggrecan gene expression was evaluated by RT-PCR. Morphological analyses were performed by transmission electron microscopy (TEM) and scanning electron microscopy (SEM) and at least 100 cells from each group were evaluated.

**Results:** In comparison with control cultures, Indomethacin and Celecoxib on chondrocyte proliferation and ultrastructure and on some biochemical parameters in human chondrocyte cultures in presence or absence of II-1.

**Conclusions:** This in vitro study shows the beneficial effects of VA441 on chondrocyte metabolism and morphology.