Results: All specimens with OA Grade III or IV stained for LAMA4, in particular in the clusters of hypertrophic chondrocytes. Lower grades of OA had no intracellular staining for *LAMA4*. The results of the Taqman PCR revealed, that 5-*AZA-deoxycytidine* treatment led to an increase of *LAMA4* expression in grade 0-II OA chondrocytes, whereas this was not the case in grade III and IV chondrocytes.
Conclusions: Our results indicate that the integrine LAMA4 plays a role in the hypertrophy of chondrocytes and that demethylation is the activating process. Further investigations are needed to investigate the function of *LAMA4* in cartilage degeneration.

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POTENTIAL INVOLVEMENT OF GALECTIN-3 ON THE OSTEOARTHRITIC HUMAN CHONDROCYTE PHENOTYPE

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Purpose: Osteoarthritis (OA) accounts for 40 to 60% of degenerative illnesses of the musculoskeletal system. On the whole, approximately 15% of the population suffers from OA. Of this number approximately 65% are 60 years of age and over. The high incidence of this illness is rather disturbing since its frequency increases gradually with the aging of the population.
The end point of OA is cartilage destruction, which impairs joint movement and causes pain. Joint destruction is also associated with joint inflammation, where the synovial membrane plays a key role. Synovitis induces alterations in chondrocyte function by the effects of inflammatory cytokines, the presence of reactive oxygen species. Synovitis also generates cycles of normoxia/hypoxia and enhances angiogenesis. Among other several factors, galectin-3 (gal-3) can be markedly present in OA synovial tissue and secreted in synovial fluid during inflammatory phases. We have recently shown that extracellular gal-3 induced proteoglycan loss as well as MMP3 and ADAMTS5 increase whereas intracellular gal-3 protected chondrocytes from cell death. Pursuing our aim in deciphering the extracellular gal-3 roles on chondrocytes, we investigated whether gal-3 could modify chondrocyte phenotype. Recent works demonstrated that in addition to the classical well known chondrocyte markers, Ankh a PPi transporter, and estrogen receptor-related receptor alpha are also involved in chondrocyte phenotype. In addition, S100A9, a calcium binding protein having inflammatory properties when it is secreted, modified the chondrocyte phenotype. However its intracellular function is not yet known in chondrocyte. Therefore we evaluated these markers as well as type X collagen and gal-3, known as hypertrophic markers.
Methods: Human OA chondrocytes (n=5), were incubated with rh-gal-3 under normoxic or hypoxic conditions for 24 and 72 hours and genes of interest were assessed by real-time RT-PCR.
Results: Our results showed that Ankh and ERR alpha were slightly increased by 135% and 140% respectively, when hypoxic conditions were applied for 72h. Gal-3 (5 microg/ml) stimulated weakly (by 155%) hypoxic induced-Ankh expression whereas ERR alpha expression was not modified by gal-3. S100A9 and type X collagen were down-regulated by 80% and 25% respectively by hypoxia and gal-3 counteracted this effect when the chondrocytes were incubated for 72h. Of note, the stimulation of S100A9 expression is far away from the one induced by interleukin-1 beta, which might suggest in the present case an intracellular role of S100A9 rather than a cytokine role. Finally, gal-3 stimulated its own expression (by 165%) during the 72h hypoxic conditions.
Conclusions: Gal-3 had higher effects during the 72h incubation versus the 24h one. Under the hypoxic conditions, gal-3 tended to stimulate hypertrophic markers which are also induced during OA. Interestingly, S100A9 presented the same pattern of regulation as type X collagen. One hypothesizes that the gal-3-induced S100A9 might regulate intracellular functions involved in the human chondrocyte phenotype changes occurring during OA.

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SIGNALING PATHWAY TO MATRIX METALLOPTROTEINASE-13 EXPRESSION IN IL-1β-TREATED CHONDROCYTES, SW1353 CELLS

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Purpose: Matrix metalloproteinase-13 (MMP-13, mammalian collagenase) is one of pivotal enzymes to degrade cartilage matrix on certain pathological condition such as osteoarthritis. MMP-13 inhibitors and/or down-regulators may show some beneficial effects on arthritic diseases. Here, to establish the signaling pathway to MMP-13 induction, effects of MAPK pathway and the possibility of some other signaling pathways involved are investigated in human chondrosarcoma cell line, SW1353 cells.
Methods: SW1353 cells were cultured and treated with IL-1β. MMP-13 and signaling molecules including MAPKs were detected with Western blotting and RT-PCR analysis. Activation of transcription factors such as NF-κB and AP-1 was determined by EMSA. siRNA transfection study for STAT involvement was also carried out.
Results: When IL-1β (10 ng/ml) was treated, SW1353 cells strongly induced MMP-13 with concomitant activation of NF-κB, AP-1 and MAPKs including ERK, p38 MAPK and JNK. Among these MAPKs, only p38 MAPK inhibitor (SB203580) blocked MMP-13 induction and AP-1 activation in IL-1β-treated SW1353 cells. SB203580 also inhibited c-Fos translocation to the nucleus (but not c-Jun). Most importantly, it was also found that IL-1β treatment provoked JAK2 activation as well as STAT1/2/3 activation. JAK2 inhibitor (AG-490) blocked STAT1/2 activation as well as MMP-13 induction in IL-1β-treated SW1353 cells.
Conclusions: Through the concerted action of these transcription factors, NF-κB, AP-1 and STATs, MMP-13 may be induced in IL-1β-treated chondrocytes. An interference of these crucial signaling pathways may possibly show chondroprotective effect in cartilage degenerative diseases.

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INHIBITION OF HYDROGEN PEROXIDE-INDUCED OXIDATIVE STRESS TO CHONDROCYTES BY THE COMBINATION OF AVOCADO/SOYBEAN UNSAPONIFIABLES AND EPIGALLOCATECHIN GALLATE

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Purpose: The present study determined whether hydrogen peroxide (H_2O_2) induction of inflammation and oxidative stress markers can be inhibited by the combination of avocado/soybean unsaponifiables (ASU) and the green tea component epigallocatechin gallate (EGCG). ASU is reported to exert anti-inflammatory activity whereas EGCG is both anti-inflammatory and antioxidant.
Oxidative stress has been proposed to play a role in aging as well as in the pathogenesis of osteoarthritis (OA). It is associated with excess production of reactive oxygen species (ROS) by tissue cells. There is also ineffective detoxification of ROS. Oxidative stress is thought to be coupled with inflammation suggested by generation of ROS by pro-inflammatory mediators. The generation of ROS can be triggered in articular cartilage chondrocytes by cytokines, prostaglandins, and nitric oxide (NO). Elevated concentrations of ROS inflict damage to DNA, proteins, and subcellular structures resulting in cell death by apoptosis. Hydrogen peroxide (H_2O_2) is an ROS known to induce oxidative stress in cultured tissue cells. H_2O_2 also inhibits proteoglycan synthesis, causes chondrocyte apoptosis and facilitates cartilage degradation.
Methods: Equine chondrocyte cultures were preincubated at 37°C, 5% CO_2 with: (i) control media alone, (ii) known antioxidant N-acetyl cysteine (NAC, 10 mM), or (iii) combination of ASU (ASU®-NMX1000, 8.3 μg/ml) and EGCG (4 or 40 μg/ml) for 24 hrs. Chondrocytes were next exposed to 100-4000 μM H_2O_2 for 1-24 hrs. Production of PGE_2 and NO were measured by ELISA and the nitrite Griess reagent respectively. Apoptosis was assessed by microscopic analysis of: (a) annexin V/propidium iodide (PI) staining using the Vybrant® Apoptosis Assay Kit and (b) caspase 3 activity using Apo-ONE® Homogeneous Capase-3/7 Assay (Promega). Data was analyzed using one-way ANOVA, Tukey post-hoc at $p<0.05$ level of significance.
Results: PGE_2 production in chondrocyte cultures significantly increased after exposure to ≥300 μM H_2O_2 for 24 hrs. In contrast, NO production was detectable only after exposure to 4000 μM H_2O_2. Dose- and time-dependent cytotoxicity induced by H_2O_2 was identified by PI nuclear staining. Approximately 40-50% of chondrocytes were dead after 3 hrs of exposure to 500 μM H_2O_2. Chondrocytes displayed a rounded, granular appearance and eventually detached. Annexin staining in the plasma membrane was observed following 1-3 hrs of incubation with 300-4000 μM of H_2O_2, suggesting apoptosis. Caspase 3 activity was also induced by at least 50% with 300 μM of H_2O_2. Pre-incubation of chondrocytes