The technique appeared to be especially well suited for newborn mice, since at later intervals, e.g., at the age of three months, details of the tissue structure were no longer as easily discerned by electron microscopy as immediately after birth. Tissue in the newborn mice is probably more loose allowing better penetration of the fixatives and embedding medium into the cartilage.

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PROTEOMIC DIFFERENTIAL EXPRESSION ANALYSIS OF OSTEOARTHRITIS-RELATED PROTEINS IN HUMAN ARTICULAR CHONDROCYTES: ROLE OF HSP90 IN OSTEOARTHRITIS

C. Ruiz-Romero, V. Carreira, M.C. De Andrés, M.J. López-Armada, F.J. Blanco
Osteoarticular and Aging Research Lab, Proteomic Unit. Biomedical Research Center, CH Universitario Juan Canalejo, Coruña, Spain

Purpose: To analyze, by a proteomic approach, the protein changes that are characteristic of OA chondrocytes, and identify new OA-related proteins.

Methods: Chondrocytes were obtained from 10 OA patients undergoing joint replacement, and from 10 cartilages from autopsies without history of joint disease. Whole cell proteins were resolved by means of two-dimensional gel electrophoresis (2-DE) and stained with SYPRORuby. Protein expression patterns of gels from OA and normal chondrocyte proteins were analyzed with PDQuest 7.3.1 software. OA-related proteins were identified by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) or MALDI-TOF/TOF mass spectrometry. Western blot and immunohistochemistry analysis were performed with a polyclonal anti-Hsp90 antibody. Interleukin-1β (5 ng/ml) and Geldanamycin (1-1000 nM) were used to stimulate NO synthesis and inhibit Hsp90, respectively. Normal chondrocytes were treated with these compounds for up to 48 h prior to evaluation of NO production by the Griess method.

Results: We examined an average of 700 protein spots that were present in the different 2-DE gels. Both qualitative and quantitative changes in protein expression patterns between normal and OA chondrocytes were studied. Fifty nine protein spots were found to be statistically increased or present only in OA cells compared to the pattern of normal chondrocytes (ratio OA:N ≥ 2.0, p < 0.05), whereas 50 were decreased in OA chondrocytes or only present in normal cells (ratio OA:N < 0.5, p < 0.05). Many of these proteins were identified, and some of them are shown in Table 1. According to their cellular role, 30% of the over-expressed proteins were involved in cellular metabolism, 11% in cell signalling, 11% in protein targeting, 9% in protein synthesis or turnover, 6% in transport processes, and 23% of them were structural proteins. From the set of under-expressed proteins, 33% of them were implicated in metabolism, 20% in cell signalling, 17% in protein targeting, 8% in transport, 5% in protein synthesis or turnover and 20% were structural. Validation of the results was performed for the NOS positive modulator Hsp90β.

Table 1. Some of the differentially expressed proteins identified between normal and OA chondrocytes

<table>
<thead>
<tr>
<th>Protein name</th>
<th>Ratio</th>
<th>OA:N</th>
<th>Cellular role</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat shock 90kDa protein 1 beta</td>
<td>4.32</td>
<td>Chaperone, NO synthesis</td>
<td></td>
</tr>
<tr>
<td>Gelsolin [precursor]</td>
<td>3.20</td>
<td>Ca-sensitive actin depolymerizer</td>
<td></td>
</tr>
<tr>
<td>Collagen type VI, alpha 1 chain</td>
<td>2.50</td>
<td>ECM structure, cell adhesion</td>
<td></td>
</tr>
<tr>
<td>Glutathione transferase omega-1</td>
<td>2.31</td>
<td>Defence and stress</td>
<td></td>
</tr>
<tr>
<td>78 kDa glucose-regulated protein [precursor]</td>
<td>2.25</td>
<td>Protein targeting</td>
<td></td>
</tr>
<tr>
<td>Glucosidase II, alpha subunit</td>
<td>2.12</td>
<td>Carbohydrate metabolism</td>
<td></td>
</tr>
<tr>
<td>Chloride intracellular channel protein 1</td>
<td>0.62</td>
<td>Transport</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions: This study describes the differences between the protein profiles of normal and OA chondrocytes, pointing out the intracellular protein changes that take part in osteoarthritis. The effect of Hsp90 inhibition in preventing NO synthesis from IL-1β-treated chondrocytes suggests the role of this chaperone in the increase of NO production that occurs in OA cartilage.

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DIFFERENTIAL REGULATION OF UDP-GLUCOSE DEHYDROGENASE EXPRESSION AND ACTIVITY IN ARTICULAR CHONDROCYTES BY CYTOKINES AND STEROIDS

L. Maneix1, A. Servent1, G. Beauchef1, Y. Wegrowski2, F.-X. Maquart2, J.-P. Pujo1, K. Boumediene1, P. Gala1, S. Moslemi1
1Laboratory of Connective Tissue Biochemistry, Caen, France, 2Laboratory of Medical Biochemistry and Molecular Biology, Reims, France

Purpose: To investigate the mechanisms controlling gene expression and activity of uridine diphospho-glucose dehydrogenase (UDGH), a key enzyme involved in the biosynthesis of the GAGs chain precursor, UDP- glucuronic acid, by several cytokines and steroid hormones.

Methods: Articular chondrocytes (RAC) were isolated from 3-week old rabbits and incubated for 24 hours with TGF-β (3 ng/ml), IGF-I (10 ng/ml), IL-1β (1 ng/ml), IL-6 (25 ng/ml), 17β-estradiol (0.1 nM), 5α-dihydrotestosterone (1 nM) and 17β-estradiol (0.1 nM) plus 5α-dihydrotestosterone (1 nM). Total RNA and proteins were extracted and submitted to real time RT-PCR and Western blotting to determine relative expression of the UGDH gene, respectively. Spectrophotometric analysis was used to assay the activity of the enzyme. In addition, transcriptional activity of several UGDH gene promoter constructs was assayed, using transient co-transfection of RAC with wild type or mutated human estrogen receptor alpha gene (hERα 66 or hERα 46 respectively).

Results: We showed that steroids could exert positive regulatory effects on mRNA, protein and activity levels. In addition, we demonstrated that hERα 46, but not hERα 66, increased the transcriptional activity of UGDH gene. In contrast, no clear correlation between transcriptional, translational and activity of the UGDH was found for the effects of the cytokines studied. However, we observed that TGF-β enhanced the enzyme activity, whereas IL-1β, IL-6 and IGF-I were without significant effect.

Conclusions: 17β-estradiol enhanced UGDH expression in rabbit articular chondrocytes. The effect is associated with an increase of UGDH enzymatic activity. These studies provide insights into the molecular mechanisms involved in the regulation of UGDH expression.
of the UGDH gene and may offer new approaches to investigate its potential alteration in joint diseases, including osteoarthritis and rheumatoid arthritis.

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ARTICULAR CARTILAGE CATABOLISM IN THE PATHOGENESIS OF KASHIN-BECK DISEASE

J. Cao1, S. Li1, Z. Shi1, Y. Yue1, J. Sun1, C.E. Hughes2, B. Caterson2

1 Xi’an Jiaotong University, Xi’an, China, 2 Cardiff University, Cardiff, United Kingdom

Purpose: Kashin-Beck Disease (KBD) is an endemic, chronic, degenerative osteoarthropathy which affects 2.5 million of 30 million people living in the KBD region of China. The symptoms include joint pain, stiffness of the joints after getting up in the morning, disturbances of flexion and extension in the elbows, enlarged phalangeal joints and limited motion in the middle-sized and large joints of the body. It manifests as cartilage degeneration and necrosis, with pathological changes occurring in growth plate and articular cartilage. At present the etiology and pathogenesis of KBD is unclear. One of the most popular hypotheses is that KBD is caused by mycotoxins. The other etiologies include selenium deficiency in soil and water in KBD area, nutrition deficiency and virus infection etc. However, all of the hypotheses lack adequate experimental evidence to support any particular conclusion. This study investigated the extracellular matrix metabolism in cartilage and serum levels of IL-1 beta, TNF-alpha, MMP-1 and CD44 in serum of KBD patients.

Methods: Immunohistochemical analyses of normal and KBD patient cartilage (16 adults and 4 children and normal age-matched patient tissue 4 adults and 3 children) was performed using a monoclonal antibody recognising CD44, the matched patient tissue 4 adults and 3 children) was performed using a monoclonal antibody recognising CD44, the IGD aggrecase-generated neoepitope (monoclonal antibody BC-13 recognising ... EGE373) and monoclonal antibody 3-B-3(-) which recognizes thee nonreducing terminal of CS chains in articular cartilage. At present the presentation the pathogenesis of KBD is unknown. One of the most popular hypotheses is that KBD is caused by mycotoxins. The other etiologies include selenium deficiency in soil and water in KBD area, nutrition deficiency and virus infection etc. However, all of the hypotheses lack adequate experimental evidence to support any particular conclusion. This study investigated the extracellular matrix metabolism in cartilage and serum levels of IL-1 beta, TNF-alpha, MMP-1 and CD44 in serum of KBD patients.

Methods: Immunohistochemical analyses of normal and KBD patient cartilage (16 adults and 4 children and normal age-matched patient tissue 4 adults and 3 children) was performed using a monoclonal antibody recognising CD44, the IGD aggrecase-generated neoepitope (monoclonal antibody BC-13 recognising ... EGE373) and monoclonal antibody 3-B-3(-) which recognizes thee nonreducing terminal of CS chains in articular cartilage. In addition, the serum levels of soluble CD44 (sCD44), IL-1 beta, TNF-alpha and MMP-1 were determined in KBD patients (20 adults and 18 children) and normal patients (20 adults and 18 children) using a competitive ELISA.

Results: Toluidine Blue and Saffron Q staining indicated there was a proteoglycan loss in both of KBD children and adult cartilage. Strong immunohistochemical staining for CD44 occurred in 14 of 16 adult KBD patients with moderate staining present in the remaining two patients. Similarly, strong CD44 immunostaining occurred in 3 of the 4 KBD children with moderate staining in the fourth KBD child. In contrast, weak CD44 immunostaining was only found in two of the four normal adult cartilage and none of the three normal child samples. Immunohistochemical staining with MAb BC-13 and 3-B-3(-) showed an intensive staining in both of KBD children and adult cartilage when compared with the normal cartilage samples. Furthermore, statistically significant elevated levels of sCD44, IL-1 beta and TNF-alpha were found in the sera of both adult and child KBD patients when compared to serum levels of normal adult and child controls. However, there was no difference in MMP-1 level between KBD children and adult cartilage when compared with the three normal child samples. Immunohistochemical staining for CD44 was found in 13 of 16 adult KBD patients with moderate staining present in the remaining two patients. Similarly, strong CD44 immunostaining occurred in 3 of the 4 KBD children with moderate staining in the fourth KBD child. In contrast, weak CD44 immunostaining was only found in two of the four normal adult cartilage and none of the three normal child samples. Immunohistochemical staining with MAb BC-13 and 3-B-3(-) showed an intensive staining in both of KBD children and adult cartilage when compared with the normal cartilage samples. Furthermore, statistically significant elevated levels of sCD44, IL-1 beta and TNF-alpha were found in the sera of both adult and child KBD patients when compared to serum levels of normal adult and child controls. However, there was no difference in MMP-1 level between KBD and normal children.

Conclusions: Our results demonstrate that altered IL-1 beta, TNF-alpha, aggrecan and CD44 metabolism occurs in the pathogenesis of KBD and there is an increased aggrecanase-generated proteoglycan loss from KBD adult and children cartilage. These primary metabolic changes are a contributing factor causing pathology in joint formation and instability which in turn leads to the onset of secondary osteoarthropathy in the major load-bearing joints of KBD patients.

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PROTECTIVE ABILITIES OF INTERLEUKIN-10 IN BLOOD-INDUCED CARTILAGE DAMAGE

N.W. Jansen, J.A. Van Roon, G. Roosendaal, J.W. Bijlsma, F.P. Lafeber

UMC Utrecht, Utrecht, The Netherlands

Purpose: Joint bleeds occur frequently in patients suffering from haemophilia or can occur as a consequence of a trauma. It has been demonstrated that even a limited number of bleeds in time leads to severe joint damage. With respect to the mechanisms of blood induced joint damage, we have demonstrated that monocytes/macrophages within the mononuclear cell population together with the red blood cells as present in blood are responsible for the irreversible inhibition of cartilage matrix synthesis. Small amounts of IL-1 that are produced by activated monocytes/macrophages increase the production of hydrogen peroxide (H2O2) by chondrocytes. The hydrogen peroxide reacts with haemoglobin-derived iron from damaged and phagocytosed red blood cells, which results in the formation of hydroxyl radicals in the vicinity of chondrocytes. This leads to chondrocyte apoptosis and with that the irreversible inhibition of cartilage matrix synthesis. In search of possible interventions to prevent or limit the cartilage damaging effects of joint bleeds, we tested interleukin-10 (IL-10) as an inflammation-controlling cytokine on blood-induced cartilage damage.

Methods: Healthy human articular cartilage tissue explants were cultured in the presence or absence of 50% v/v blood for 4 days, followed by a recovery period of 12 days. IL-10 was added in 0.1, 1 or 10 ng/ml. The effect on cartilage matrix proteoglycan synthesis, release, and content were determined.

Results: IL-10 was able to prevent the decrease in proteoglycan synthesis and the increase in proteoglycan release of cartilage exposed to blood dose dependently. As a consequence, the decrease in proteoglycan content after blood exposure could be prevented dose dependently. With 10 ng/ml IL-10, which is still a low dose for local therapeutic treatment, at least 50% reduction in adverse effects was observed.

Conclusions: The present results show that interleukin-10 prevents the direct harmful effects of blood on articular cartilage. This effect of IL-10, in addition to its anti-inflammatory properties, may add to prevention of irreversible degenerative joint damage as a consequence in time of joint haemorrhages. Although treatment studies in addition to prevention studies have to be performed, IL-10 might be of use for local treatment of joint.

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VENOUS ENDOTHELIAL GROWTH FACTOR PRODUCTION BY CHONDROCYTES CULTURED IN MONOLAYER AND IN MICROMASS

M.C. Honorati1, L. Cattini1, A. Facchini2

1 Laboratorio di Immunologia e Genetica, Istituti Ortopedici Rizzoli, Bologna, Italy, 2 Dipartimento di Medicina Interna e Gastroenterologia, University of Bologna, Bologna, Italy

Purpose: To verify the involvement of proinflammatory cytokines IL-17, IL-1β and TNF-α in cartilage vascularization on the production of vascular endothelial growth factor (VEGF) by chondrocytes isolated from patients with osteoarthritis (OA), rheumatoid arthritis (RA) and patients with femoral neck fracture (FP), and to evaluate the production of VEGF by chondrocytes freshly isolated from patients with OA, in comparison with chondrocytes differentiating in micromass.

Methods: Chondrocytes isolated from patients with OA were maintained in monolayer culture for several passages. Chondro-