Activated.

explain the progressive joint damage after inflammatory pathways are
activated. IL-1 decreases the available antioxidant protection in the joint may
damage to structural matrix proteins and alter cell signaling pathways.
Chronic oxidative stress may increase cell senescence, cause
toxic damage. Cartilage tissue generates reactive oxygen species in response
to cytokines and mechanical forces leaving the tissue vulnerable to oxida-
Figure 2. Total nitrate/nitrite measured from OA cartilage treated with and without IL-1beta.

Figure 1. Mean EC-SOD secretion by human OA cartilage

Conclusion: ECSOD scavenges superoxide and prevents oxidative damage in the joint. Cartilage tissue generates reactive oxygen species in response to cytokines and mechanical forces leaving the tissue vulnerable to oxidative damage. Chronic oxidative stress may increase cell senescence, cause damage to structural matrix proteins and alter cell signaling pathways leading to osteoarthritis. The finding that a key inflammatory cytokine (IL-1) decreases the available antioxidant protection in the joint may explain the progressive joint damage after inflammatory pathways are activated.

References

256

BONE MORPHOGENETIC PROTEIN-2 IS A SPECIFIC CHONDROGENIC INDUCER WITH POTENTIAL USE FOR (MATRIX-ASSOCIATED) AUTOLOGOUS CHONDROCYTE IMPLANTATION

S. Claus1, E. Aubert-Foucher2, M. Demoor2, H. Chajra2, N. Mayer2, O. Damour4, P. Galéra2, F. Mallein-Gerin1
1Inst. de Biologie et Chimie des Protéines - CNRS - UMR5086, Lyon, France; 2Matrice Extracellulaire et Pathologie - UMR - INSERM, Caen, France; 3Symatese biomateriaux, Lyon, France; 4Hosp. Edouard Herriot - Laboratoire des Substituts Cutanés, Lyon, France

Purpose: The aim of this study was to investigate if addition of bone morphogenetic protein (BMP)-2 to human articular chondrocytes (HACs) could help to maintain their chondrogenic phenotype in long-term culture conditions necessary for autologous chondrocyte implantation (ACI). We also evaluated the potential of BMP-2 as a repair factor in combination with collagen-based biomaterials, to extend the technique to osteoarthritic lesions.

Methods and Material: In a first step, HACs from 19 donors were cultured independently, according to the procedure used for ACI. Real-time PCR and Western blotting were used to evaluate the chondrocyte phenotype and gel retardation assays were performed to analyze DNA binding of transcription factors under the effect of BMP-2. Next, we evaluated the responsiveness of HAC to BMP-2 when cultured within collagen sponges. This first approach was undertaken with independent cultures of three donors and the cellular phenotype was estimated by using real-time PCR.

Results: Exogenous BMP-2 improved the chondrogenic character of HACs when amplified over two passages in monolayer. The stimulatory effect of BMP-2 on type II collagen expression was observed not only at the mRNA level but also at the protein level, and this is crucial for cartilage matrix reconstruction. Our preliminary data with HACs first amplified in monolayer then cultured in collagen sponges in the presence of BMP-2 have revealed that BMP-2 is able to restore COL2A1 gene expression that was lost during the amplification step.

Conclusions: Adding exogenous BMP-2 to HACs expanded in the conditions generally used for ACI or during Matrix-associated ACI is clearly beneficial to support the chondrocytic phenotype. Importantly, no sign of hypertrophic maturation or osteogenic induction was detected beside the chondrogenic stimulatory effect of BMP-2. This study is the first to reveal the benefit of adding exogenous BMP-2 to HACs as a therapeutic agent for cartilage repair.

257

ELUCIDATING THE MECHANISM OF OSTEOARTHRPATHY IN ALKAPTONURIA: LESSONS FOR OSTEARTHITIS

A.M. Taylor1, A. Boyd2, B. Wlodarski1, J.S. Davidson3, P.J. Wilson1, J.C. Jarvis1, L.R. Ranganath1, J.A. Gallagher1
1Univ. of Liverpool, Liverpool, United Kingdom; 2Barts and The London Sch. of Med. and Dentistry, London, United Kingdom; 3Royal Liverpool and Broadgreen Univ. Hosp. Trust, Liverpool, United Kingdom

Purpose: Alkaptonuria (AKU) is a rare autosomal recessive condition re-

Conclusion: ECSOD scavenges superoxide and prevents oxidative damage in the joint. Cartilage tissue generates reactive oxygen species in response to cytokines and mechanical forces leaving the tissue vulnerable to oxidative damage. Chronic oxidative stress may increase cell senescence, cause damage to structural matrix proteins and alter cell signaling pathways leading to osteoarthritis. The finding that a key inflammatory cytokine (IL-1) decreases the available antioxidant protection in the joint may explain the progressive joint damage after inflammatory pathways are activated.
sulting from lack of homogentisate 1,2 dioxygenase (HGD), the enzyme responsible for the breakdown of homogentisic acid (HGA). HGA accumulates in body tissues resulting in ochronosis, the deposition of pigmented polymers in collagenous tissues, primarily the articular cartilage of the weight bearing joints. Over time, ochronosis leads to severe, early onset joint degeneration presenting clinically as osteoarthritis. The aim of this study was to use light and scanning electron microscopy (SEM) to elucidate the initiation and progression of pigmentation and the consequent osteoarthropathy in AKU.

Methods: Tissues were collected from patients undergoing joint replacement surgery for alkaporionic osteoarthritis (n=14). Samples were processed as follows:: histology to identify pigment deposition, topographical 3D SEM to observe the trabecular bone network and quantitative back scattered electron SEM (qBSE-SEM) to determine the mineral content of cartilage and bone.

Results: Histological examination of articular cartilage revealed significant variation in the extent of pigment deposition between samples, and regionally within samples. Initial pigmentation was associated with single chondrocytes near the tidemark and was present intracellularly, and in the lacunae and territorial matrix. From there, deposition of pigment progressed to the interterritorial matrix in the deep zones and then towards the articular surface. Once the hyaline cartilage became extensively pigmented, there was aggressive remodeling of the calcified cartilage and underlying bone. Eventually this led to complete loss of the subchondral plate leaving non-calcified, pigmented hyaline cartilage in contact with the trabecular bone network. In addition, poorly or non-mineralised bone was present in the trabecular network in closest proximity to the pigmented hyaline articular cartilage. Pigmentation was not detected in mineralised bone matrix, however osteocytes, osteoclasts and osteoblasts all displayed intracellular pigmentation, along with the canalicular network and osteocyte lacunae. Osteoclasts were also seen phagocytosing pigmented osteocytes.

Conclusions: Calcified cartilage and subchondral bone appear to play key roles in the pathogenesis of osteoarthropathy in AKU. Our findings indicate that cartilage matrix is initially protected from ochronosis. Early pigmentation occurs deep in the cartilage, possibly in response to mechanical factors and biochemical injury. HGA thus appears to be an endogenous marker of osteoarthritic changes. Once the initial pigment is deposited, the biomechanical and functional properties of cartilage are further altered leading to additional damage, more pigmentation and a downward spiral of tissue destruction producing a more rapid, severe and earlier onset of osteoarthropathy than is seen in typical OA. Extreme phenotypes in monogenic diseases can help elucidate the molecular pathogenesis of more common disorders. We believe that the initiation and rapid progression of ochronosis and in osteoarthropathy in AKU may also help elucidate the molecular pathology of OA.

258

EXPRESSION OF TRANSIENT RECEPTOR POTENTIAL VANILLOID (TRPV) CHANNELS IN DIFFERENT PASSAGES OF EQUINE ARTICULAR CHONDROCYTES

I. Hedid1, A. El-Shafei2, P. Loughna1, R. Barrett-Jolley3, A. Mobasheri1
1Univ. of Nottingham, Sutton Bonington, United Kingdom; 2Univ. of Al-Azhar, Cairo, Egypt; 3Univ. of Liverpool, Liverpool, United Kingdom

Purpose: Chondrocytes are highly mechanosensitive cells responsible for the production and maintenance of the extracellular matrix (ECM) in articular cartilage. ECM turnover is influenced by the mechanical and osmotic factors and calcium signalling is a key component of mechanical responsiveness in these cells. The mammalian Transient Receptor Potential Vanilloid (TRPV) subfamily consists of six members. TRPV1-4 are temperature sensitive calcium-permeable, non-selective cation channels whereas TRPV5 and TRPV6 show high selectivity for calcium over other cations. Recent studies have demonstrated the presence of functional TRPV channels in porcine articular cartilage. The purpose of this study was to investigate the effect of time in culture and passage on expression of TRPV4, TRPV5 and TRPV6 in equine articular chondrocytes.

Methods: Articular cartilage was obtained from weight-bearing regions of equine metacarpophalangeal. Chondrocytes were enzymatically isolated and cells were cultivated in low glucose Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 2% penicillin-streptomycin and 10% Fetal Calf Serum (FCS). Polyclonal antibodies raised against TRPV4, TRPV5 and TRPV6 were used to compare the expression of these channels in lysates from first expansion cells (P0) and cells from passages 1-3 (P1, P2 and P3) by western blotting. Densitometry was carried out using Image J (Image Processing and Analysis in Java; http://rsb.info.nih.gov/ij/).

Results: Western blotting confirmed TRPV4, TRPV5 and TRPV6 expression in all passages of equine chondrocytes. The corresponding immuno-reactive bands were calculated to be approximately 98 kDa for TRPV4 and 83 kDa for both TRPV5 and TRPV6. TRPV5 and TRPV6 were upregulated with time and cell passage in culture (Figure 1).

259

PREDNSISONOLE INDUCES A CATABOLIC EFFECT ON BONE FORMATION; BUT INHIBITS CYTOKINE-INDUCED CARTILAGE DEGRADATION AND SUBCHONDRAL BONE RESORPTION, INDICATING ANTI-CATABOLIC EFFECTS

S.H. Madsen, A.S. Goettrup, K. Henriksen, K. Andreassen, M.A. Karsdal, A.-C. Bay-Jensen
NORDIC BIOSCI, Herlev, Denmark

Purpose: Glucocorticoids are beneficial in the treatment of inflammatory and immune disorders, but have also been shown to protect cartilage when treating for osteoarthritis. Unfortunately, prolonged glucocorticoid therapy, in high doses, has been associated with bone loss, resulting in severe osteoporosis. These conflicting effects by glucocorticoids on bone and cartilage may result from a miscommunication between the bone and cartilage cells. We investigated the effect of the glucocorticoid, prednisolone, in an ex vivo murine femoral head model, which allowed the interactions between osteoblasts, osteoclasts and chondrocytes.

Methods: Femoral heads from 12-week-old female mice were isolated and cultured for 21 days in serum-free media in the absence or presence of prednisolone [10 uM], OSM [10 ng/ml] + TNFα [20 ng/ml]