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THE DYSFUNCTION OF THE MITOCOCHONDRIAL RESPIRATORY CHAIN REGULATES THE METALLOPROTEINASES EXPRESSION IN HUMAN NORMAL CHONDROCYTES IN CULTURE

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Purpose: Mitochondria is acquiring an important role in the progression of osteoarthritis (OA). Previously we have demonstrated that the alteration of the mitochondrial respiratory complexes III and V contributes to the inflammatory answer of the chondrocyte. Nevertheless, the possible implication of this organel in the process of destruction of the cartilage is not yet defined. In this study, we have investigated the relationship between the dysfunction of the mitochondria and the possible modulation of extracellular matrix components in human normal chondrocytes in culture.

Methods: Human normal chondrocytes were isolated from cartilage obtained from autopsies without history of joint disease. Rotenone, NPA, antimycin A (AA), azide and oligomycin were employed to inhibit the mitochondrial complexes I, II, III, IV and V, respectively. MMPs –1, -3 and -13 mRNA expression was studied by real time PCR. Intracellular protein expression was evaluated by western blot as well as by immunohistochemistry. Protein production was evaluated by ELISA. Proteoglycan presence was analyzed by alcian blue and safranin O studies.

Results: We treated cells with all the MRC inhibitors observing an up-regulation of MMP-1 and -3 mRNA expression at 24 hours of treatment with oligomycin 5 μg/ml (MMP-1: 68.10±39.9 vs. basal=-1; MMP-3: 60.13±28.7 vs. basal=-1). mRNA expression of MMP-13 decreased after treatment with AA 60 μg/ml and oligomycin to 0.34±0.1 and 0.67±0.3 vs. basal=1, respectively. Also, we observed an increase in intracellular protein levels of MMP-1 and -3 after treatment with oligomycin 25 μg/ml. At 24 hours: 15.20±8.46 and 4.59±0.83 times vs. basal=1, respectively (n=4; P<0.05). However, AA and oligomycin decreased the intracellular protein levels of MMP-13 (0.70±0.16 and 0.33±0.24, respectively vs. basal=1). In addition to this, levels of MMPs in the supernatants were evaluated. At 36 hours, MMP-1: 18.06±10.35 for oligomycin 25 μg/ml vs. basal=1, and MMP-3: 8.49±4.32 for oligomycin 5 μg/ml vs. basal=1 (n=5; P<0.05). MMP-13 levels in the supernatants decreased after treatment of chondrocytes with AA 60 μg/ml (0.50±0.13 vs. basal=1) and oligomycin 25 μg/ml (0.41±0.14 vs. basal=1). In addition to this, levels of MMPs in the supernatants were evaluated. At 36 hours, MMP-1: 18.06±10.35 for oligomycin 25 μg/ml vs. basal=1, and MMP-3: 8.49±4.32 for oligomycin 5 μg/ml vs. basal=1 (n=5; P<0.05). The stimulation of tissue explants with the MRC inhibitors, showed an increase in the positive cells for MMP-1 and -3 after oligomycin treatment. By the same manner, stimulated punchs with AA or oligomycin revealed a decrease in the MMP-13 expression. Alcian blue and safranin O stain, showed a loose of proteoglycans in tissues that were incubated with oligomycin.

Conclusions: These results show that the dysfunction of MRC modulates the MMPs expression in human normal chondrocytes.

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NEUROTRANSMITTER FROM THE SYMPATHETIC AND SENSORY NERVOUS SYSTEM MODULATE METABOLIC ACTIVITY OF CHONDROCYTES IN VITRO

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Purpose: Fracture repair constitutes a sequential event following bone injury and recapitulates the steps of endochondral ossification observed during embryonic skeletal development and growth. Neurotransmitter and neuropeptide containing nerve fibres of sympathetic and sensory origin are known to innervate bone and fracture callus, however, little is known about their role in fracture healing and their influence on callus maturation and bone formation. Therefore, we employed a 3D micromass pellet culture model of murine primary chondrocytes which focuses on the role of neuropeptides and neurotransmitters from the sympathetic and sensory nervous system for the organization and differentiation of the cartilaginous callus.

Methods: All experiments were carried out with expanded (1 passage) murine costal chondrocytes. To determine the influence of neurotransmitters on matrix differentiation, proliferation, and apoptosis, monolayer cultures and micromass pellets were stimulated daily for 7 days with substance P (SP; 10−6, 10−7, 10−8 M) and norepinephrine (NE; 10−6, 10−7, 10−8 M). Micromass pellets were cultured in minimal medium containing DMEM/F12, 1mM cysteine, 1mM pyruvate, 50g/ml ascorbate, 50ng/ml L-thyroxine, and 1% penicillin/streptomycin. Cells and pellets were harvested after 7, 14, and 21 days and gene and protein expression was analyzed histologically and by quantitative PCR.

Results: RT-PCR analysis of mRNA expression showed that both, primary chondrocytes and cultured chondrocyte pellets, express SP, its receptor NK1-R, and most of the adrenoceptors. After 7 days of stimulation with SP and NE, Col1α1 and Col1α1 gene expression in micromass pellets was enhanced compared to non-stimulated controls. Additionally, gene expression of MMPs and cytokines was altered by SP and NE, resulting in lower MMP13 levels at day 21 and lower TNF-α levels after 14 days compared to the controls. However, histological (alcan blue) and immunohistochemical (collagens I, IX, and X) stainings revealed a regular cartilage-like extracellular matrix development in the proliferation phase, unaffected by substance P and norepinephrine.

After stimulation with SP, proliferation in monolayer cultures increased in a dose-dependent manner while apoptosis was unaffected. Stimulation with NE on the other hand, showed an unaffected proliferation rate and decreased apoptosis.

Conclusions: Sympathetic and sensory nerve fibres invading the fracture callus release neurotransmitters and neuropeptides which in turn modulate the metabolic activity of chondrocytes. Chondrocytes originated from callus tissue express SP and its receptor SP1-R. This implicates yet unknown, possible trophic functions of neuropeptides during cartilage differentiation and endochondral ossification in adults. Preliminary results of our chondrocyte monolayer system suggest contrary effects of SP and NE on chondrocyte proliferation and apoptosis.

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MECHANICAL STRESS INCREASED P21(CIP1) EXPRESSION IN CHONDROCYTES

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Purpose: The cyclin-dependent kinase inhibitor p21(Cip1) was initially identified as a potent inhibitor of cell cycle progression. Subsequent studies further identified that p21(Cip1) has an important role in controlling cytoisrosis and cell death. p21(Cip1) transcription is activated by p53, and P21(Cip1) is part of a negative feedback mechanism that controls p53 activity during apoptosis. We have shown that mechanical stress induced chondrocytes apoptosis, and inhibition of p53 activation prevented chondrocyte from apoptosis induced by mechanical stress. Recently, Olive et al. reported that p21(Cip1) activity was essential for the regulation of cell proliferation and inflammation after arterial injury in local vascular cells. Further, p21(Cip1) regulated the expression of SDF-1 and MMP-13. These molecules are believed to be onset of osteoarthritis (OA) in articular cartilage. In this study, we analyzed the expression of p21(Cip1) in OA and normal chondrocytes. Furthermore, we evaluated the expression levels of p21(Cip1) in response to shear stress.

Methods: Normal cartilage samples were obtained from femoral head of patients (n=5) undergoing joint replacement surgery for the neck fracture of the femur. OA cartilage samples were collected from patients (n=8) during total knee joint replacement surgery. Endogenous p21(Cip1), p53, SDF-1 and MMP-13 mRNA were quantified by quantitative PCR and normalized to levels of 18S RNA. Values were average mRNA levels in OA chondrocytes/normal chondrocytes. Mechanical stress was introduced to NHAC-kn (cell line derived from human normal chondrocyte). Values were mRNA levels after loading 5%, 10% shear stress for 12h, 0.25Hz in comparison with control [non-stress].

Results: The expression levels of p21(Cip1), p53, SDF-1, MMP-13 were much higher in OA chondrocytes than in normal chondrocytes (Table 1).
average expression levels of p21^{Cip1} in OA chondrocytes were almost 300 folds against normal chondrocytes.

In order to evaluate the p21^{Cip1} expression in response to shear stress, we analyzed the expression levels of p21^{Cip1}, p53, SDF-1, MMP-13 after stress. Expression levels of p53 and MMP-13 were increased in a dose dependent manner (Figure). Expression levels of p21^{Cip1} and SDF-1 were increased when 5% shear stress were introduced (Figure). However, those expression levels were decreased when 10% shear stress were introduced (Figure).