THE EFFECT OF EXOSOMES FROM BONE MARROW MESENCHYMAL STEM CELLS ON OSTEOARTHRITIS

H. Zhu, J. J. T. Fu, J. Yang, Z. Gu. Department of Rheumatology, Affiliated Hospital of Nantong University, Nantong, China

Background: Mesenchymal stem cells (MSCs) exert chondroprotective effects in clinical models of osteoarthritis (OA), but the exact mechanisms were still unclear. Exosomes that serve as carriers of genetic information have been implicated in many diseases and are known to participate in many physiological processes.

Objectives: Here, we investigate the therapeutic potential of exosomes from human bone marrow MSCs in alleviating OA and explore the mechanism.

Methods: Exosomes were harvested from conditioned culture media of BM-MSCs by a sequential centrifugation process. The anterior cruciate ligament transection and destabilization of the medial meniscus (DDM) surgery were performed on the knee joints of female SD rats as an OA model. After four weeks, the animals were followed by intra-articular injection of either BM-MSCs or their exosomes every week for 4 weeks. Cartilage destruction, matrix degradation and subchondral bone changes were evaluated with histological staining and micro-CT at the post-surgery 8 weeks. Primary human chondrocytes treated with IL-1β were used as an in vitro model to evaluate the effects of exosomes for 24 hours.

Results: We found that intra-articular injection of BM-MSCs and BM-MSCs derived exosomes improve cartilage destruction and subchondral bone remodelling in ACLT+DMM model. BM-MSCs and exosomes equally protected rats from joint damage. These exosomes maintained the chondrocyte matrix by increasing chondronal bone remodelling, which in turn provides a new target for OA drug and drug-delivery system development.

Conclusions: The exosomes from BM-MSCs exert a beneficial therapeutic effect on OA by not only repairing the degenerative cartilage, but also improving subchondral bone remodelling, which in turn provides a new target for OA drug and drug-delivery system development.

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ADALIMUMAB:TNF COMPLEXES ARE CLEARED MORE EFFICIENTLY BY HUMAN OSTEOCLASTS THAN THOSE WITH ETANERCEPT THROUGH FCG-RECEPTOR BINDING AND INTERNALISATION

B.P. Harvey, J. Cohen-Solal, Z. Kaymakcalan. Biologics, Abbvie Bioresearch Center, Worcester, USA

Background: TNF-alpha (TNFa) has been shown to contribute to osteoclastogenesis (OCgen). OCgen is mediated by two key cytokines involved in osteoclast (OC) development. We have previously demonstrated that TNF enhances the kinetics of RANKL-induced human OCgenesis and that its effects are mitigated more effectively by the anti-TNF biologic adalimumab (ADA) as compared to etanercept (ETN).

Objectives: To determine whether Fc-gamma receptor (FcγR)-mediated internalisation of the biologic:TNF complexes is a contributing mechanism responsible for the difference in effectiveness between ADA and ETN in preventing TNF-enhanced OCgenosis.

Methods: TNF biologics [ADA and ETN] alone or in preformed complexes with TNFa at 50:1 molar ratio were tested for FcγR binding by flow cytometry using CHO stably transfected with human FCGRs (FcγRI, FcγRIIA, -RIIB, -RIIC, FcγRIIA and -RIIB). FcγR expression and binding of preformed biologic:TNF complexes at 10:1 ratio ± FcγR blocking antibodies to primary human OC precursors (OCP) was evaluated by flow cytometry. FcγR-mediated internalisation was assessed by monitoring a reduction in OC survival in response to preformed biologic:TNF complexes (25:1 ratio) bound with saporin (ZAP), a ribosome-inactivating protein, as anti-human Fc IgG Fab conjugate sFcγR blocking antibodies.

Results: The binding study to CHO (human FcRgEx) cell lines showed that monomeric ADA and ETN bind similarly to FcγR (highly on high affinity FcγR and loosely on low affinity FcγRs) while preformed biologics: TNF complexes bind different. ADA:TNF complexes bind to low affinity FcγR, whereas ETN:TNF keep a monomeric binding profile with no gain of binding to low affinity FcγR. OCP were found to internalise mostly FcγRI and in development with predominant binding of only ADA:TNF, not ETN:TNF, to this FcγR with additional binding to undefined receptor(s). Despite subsequent increases in FcγRII and IIII later on, ADA:TNF still preferentially bound to FcγRI on the matured OCP with minimal binding to IIII, whereas ETN:TNF binding was observed only to FcγRII. Exposure of OCP to ADA:TNF:ZAP (toxin) complexes led to a significant reduction (4-fold) in mature OC due to complex internalisation as compared to human IgG:ZAP + TNF conditions that was partially rescued only with the addition of FcγRII blocking antibody. Interestingly, a 1.5-fold reduction in mature OC was observed with ETN:TNF: ZAP.

Conclusions: Our in vitro findings demonstrate that human OCP can bind and internalise ADA:TNF complexes more efficiently than ETN:TNF complexes. In addition, this process is partially mediated through FcγRII. Clearance of the ADA:TNF complexes may help reduce exposure of the OCP to localised TNF by removing TNF more effectively in the joint environment. Additional in vivo analysis need to be done to verify these in vitro findings.

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IMPAIRMENT IN HYDROGEN SULFIDE SYNTHESIS IN OSTEOARTHRITIC CHONDROCYTES FROM DIABETIC PATIENT AND UNDER A HIGH GLUCOSE STRESS

C. Vázquez-Garcia1, 2, F. Blanco1, R. Meijide-Falade1, 3, Biological science, Medicine and Physiotherapy, University of A Coruña, 2 Rheumatology, INIBIC, CHUAC, A Coruña, Spain

Background: A growing number of findings support the hypothesis that type 2 diabetes is an independent risk factor of osteoarthritis (OA). However, the mechanisms underlying the connexion between both diseases remain unclear. Hydrogen sulfide (H2S) plays an important role in the pathogenesis of diabetes and its complications. In relation, we and other authors have observed a protective impact of H2S induction on activation of pathological pathways in the chondrocyte.

Objectives: In this study we examined the modulation of H2S levels in osteoarticular chondrocytes from diabetic (DB) or non-diabetic (DB) patients subjected or under glucose stress, in order to elucidate whether impairment in H2S-mediated signalling could participate in the establishment of diabetes-related OA.

Methods: Chondrocytes were isolated from OA cartilage of diabetic (DB) or non diabetic (non-DB) patients. TC28a2 and primary human chondrocytes were stimulated w/o IL-1β stimulated w/o IL-1β diabetic (non-DB) patients. T/C28a2 and primary human chondrocytes were stimulated w/o IL-1β and under glucose stress, in order to elucidate whether impairment in H2S-mediated signalling could participate in the establishment of diabetes-related OA.

Results: Fresh isolated chondrocytes from OA cartilage of diabetic patients showed lower levels of H2S synthesising enzymes (CSE, CBS and 3-MT) than those of non-DB patients (figure 1). In relation, chondrocytes T/C28a2 exposed to HG stress expressed lower mRNA and protein levels of these 3 enzymes after 3 days of incubation compared to those incubated in NG conditions (0.41-fold and 0.83-fold [CSE], 0.42-fold and 0.66-fold [CBS], and 0.52-fold and 0.79-fold [3-MT] for mRNA and protein expression, respectively), n=6, p<0.05. IL-1β also attenuated the gene and protein expression of CBS elicited by chondrocytes incubated in NG (0.47-fold and 0.86-fold, respectively; n=6, p<0.05). Additionally, the expression of pro-inflammatory chemokine IL-8 induced by IL-1β was significantly higher in chondrocytes under HG than NG condition (8-fold; n=5, p<0.05); whereas protein levels of heme oxygenase 1, an anti-inflammatory enzyme, were reduced in HG exposed chondrocytes (0.77-fold; n=4, p<0.05). 3YV 4137 and NaSh co-treatment recovered HO-1 expression and reduced IL-8 levels in chondrocytes under IL-1β+HG conditions. Furthermore, similar results were registered in primary human chondrocytes from OA cartilage.
Conclusions: The results indicate a reduction of H2S synthesis as a critical feature involved in hyperglucidic-mediated dysregulation of articular chondrocytes. The impairment of H2S signalling could participate in the mechanisms underlying the predisposition to OA development in diabetic individuals and may open new opportunities for treating patients with a diabetes-related OA phenotype.

REFERENCE:

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SAT0060

PROTEOMIC ANALYSIS OF OSTEOBLASTS SECRETOME PROVIDES NEW INSIGHTS IN MECHANISMS UNDERLYING OSTEOARTHRITIS SUBCONDONAL BONE SCLEROSIS


BACKGROUND: Osteoarthritis (OA) is characterised by cartilage degradation but also by other joint tissues modification like subchondral bone sclerosis.

OBJECTIVES: In this study, we used a proteomic approach to compare secretome of osteoblasts isolated from sclerotic (SC) or non sclerotic (NSC) area of OA subchondral bone.

METHODS: Secretome was analysed using differential quantitative and relative label free analysis on nanoUPLC G2 HDMS system. mRNA of the more differentially secreted proteins were then quantified by RT-PCR and the most relevant proteins identified using western-blotting and immunoassays.

RESULTS: 175 proteins were identified in NSC osteoblasts secretome. Compared to NSC osteoblasts secretome, 13 proteins were significantly less secreted (Osteomodulin, CSF-1, IGFBP5, VCAM-1, IGF2, 78 kDa glucose-regulated protein, versican, calumenin, IGFBP2, thrombospondin-4, peristin, reticulocbin 1 and osteonectin), and 12 proteins significantly more secreted by SC osteoblasts (CHI3L1, fibulin-3, SERPINE2, IGFBP6, SHBG, SERPINE1, reticulocbin3, alpha-2-HS-glycoprotein, TIMP-2, IGFBP3, TIMP-1, SERPINF), Similar changes in peristin, osteomodulin, SERPINE1, IGFBP6, fibulin-3 and CHI3L1 mRNA levels were observed. Finally, osteomodulin and fibulin-3 specific sequences were quantified by western blot and immunoassays in serum and osteoblasts conditioned culture supernatants.

CONCLUSIONS: We highlighted some proteins differentially secreted by NSC and SC osteoblasts of OA subchondral bone sclerosis. These changes contribute to explain some features observed in OA subchondral bone, like the increase of bone remodelling or abnormalities in bone matrix mineralization. Among identified proteins, osteomodulin was found decreased and fibulin-3 increased in serum of OA patients. These findings suggest that osteomodulin and fibulin-3 fragments could be biomarkers to monitor early changes in subchondral bone metabolism in OA.

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SAT0061

TARGETING ACTIVATED SYNOVIAL FIBROBLASTS USING PHOTODYNAMIC THERAPY IN RHEUMATOID ARTHRITIS

D.N. Dorst1, M. Rlijpema1, M. Butlinga2, M. Bron1, D.L. Bos1, A. Freimoser3, C. Klein3, B. Walgreen1, P. van der Kraan 1, M. Gotthardt2, M.I. Koenders1

1RADBOUDUMC, Nijmegen, Netherlands; 2KU Leuven, Leuven, Belgium; 3Roche innovation center, Zurich, Switzerland

BACKGROUND: Activated synovial fibroblasts (SF) play an important role in the pathogenesis of rheumatoid arthritis (RA). They contribute to the pro-inflammatory environment in the joint as well as to the degradation of cartilage. Depleting SF could ameliorate both the symptoms of joint inflammation and degradation in RA. SF are characterised by the expression of Fibroblast Activation Protein (FAP). Here, we investigated the potential of photodynamic therapy (PDT) targeting FAP to selectively induce cell death in these cells as well as in synovial tissue from RA patients. In PDT, a light-sensitive molecule is delivered to a target cell and activated with a light of a specific wavelength. This causes cell death through the production of reactive oxygen species.

METHODS: The anti-FAP antibody 28 H1 was conjugated with the photosensitizer IRDye700DX (28 H1–IRDye700DX). In vitro PDT assays were performed with 3 T3 fibroblasts stably transfected with FAP. 3T3-FAP cells were incubated with 28 H1–700DX or a control conjugate for 4 hours, and exposed to varying 690 nm light exposures. Subsequently, cell viability was measured using the CellTiter-Glo assay. For ex vivo evaluation of PDT efficiency, human RA synovial tissue obtained after joint replacement surgery was processed in standardised 6 mm biopsies and used for FAP-based PDT. The biopsies were incubated with 28 H1–700DX for 4 hours, subjected to 52 J/cm² light exposure and fixed in formalin after 1 hour. Tissue was then embedded in paraffin and stained for the presence of gH2AX and caspase 3 as indicators of DNA double-strand breaks and early apoptosis on sequential slides. The presence of FAP was also determined on subsequent slides.

Conclusions: The effect of PDT was optimal at 13.7 J/cm² light exposure to 3T3-FAP cells incubated with 6.67 pM 28 H1–IRDye700DX for 4 hours, which dramatically reduced cell viability with 89.27%±2.48 compared to control (p<0.001). No cell death was observed with the control 700DX-conjugate (p=0.16).

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