SAT0057

THE EFFECT OF EXOSOMES FROM BONE MARROW MESENCHYMAL STEM CELLS ON OSTEOARTHRITISH. Zhu, J. Ji, 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 destabilisation of the medial meniscus (DMM) surgery were performed on the knee joints of SD female rat 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 four 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-1 β 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 rat from joint damage. These exosomes maintained the chondrocyte matrix by increasing collagen type II synthesis and decreasing ADAMTS5, MMP13 and Col II expression in the presence of IL-1 β *in vitro*. In addition, BM-MSCs derived exosomes were also shown to protect chondrocytes from apoptosis and senescence.

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.

REFERENCES:

- [1] Trounoun A, McDonald C. Stem Cell Therapies in Clinical Trials: Progress and Challenges. *Cell stem cell* 2015;17:11–22.
- [2] Stoorvogel W, Kleijmeer MJ, Geuze HJ, Raposo G. The biogenesis and functions of exosomes. *Traffic* 2002;3:321–30.
- [3] Lai RC, Yeo RW, Lim SK. Mesenchymal stem cell exosomes. *Seminars in cell & developmental biology* 2015;40:82–8.
- [4] Zhang S, Chu WC, Lai RC, Lim SK, Hui JH, Toh WS. Exosomes derived from human embryonic mesenchymal stem cells promote osteochondral regeneration. *Osteoarthritis and cartilage* 2016;24:2135–40.
- [5] Wang Y, Yu D, Liu Z, et al. Exosomes from embryonic mesenchymal stem cells alleviate osteoarthritis through balancing synthesis and degradation of cartilage extracellular matrix. *Stem cell research & therapy* 2017;8:189.

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SAT0058

ADALIMUMAB:TNF COMPLEXES ARE CLEARED MORE EFFICIENTLY BY HUMAN OSTEOCLASTS THAN THOSE WITH ETANERCEPT THROUGH FCG-RECEPTOR BINDING AND INTERNALISATIONB.P. Harvey, J. Cohen-Solal, Z. Kaymakalan. *Biologics, Abbvie Bioresearch Center, Worcester, USA*

Background: TNF-alpha (TNFa) has been shown to contribute to osteoclastogenesis (OCgenesis) independently and in conjunction with M-CSF or RANKL, 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 OCgenesis.

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 FcγRs (FcγRI, FcγRIIA, -RIIB, -RIIC, FcγRIIIA and -RIIIB). FcγR expression and binding of preformed biologic:TNF complexes at 10:1 ratio $\pm$ 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 toxin, as anti-human Fc IgG Fab conjugate $\pm$ FcγR blocking antibodies.

Results: The binding study to CHO (human FcγRs) 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 differently. 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 express mostly FcγRII early 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 FcγRI and RIIB later on, ADA:TNF still preferentially bound to FcγRII on the matured OCP with minimal binding to RIIB, whereas ETN:TNF binding was observed only to FcγRI. 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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SAT0059

IMPAIRMENT IN HYDROGEN SULFIDE SYNTHESIS IN OSTEOARTHRITIC CHONDROCYTES FROM DIABETIC PATIENT AND UNDER A HIGH GLUCOSE STRESSC. Vaamonde-Garcia^{1,2}, F. Blanco², R. Meijide-Falide¹. ¹*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.¹

Objectives: In this study we examined the modulation of H₂S levels in osteoarthritic chondrocytes from diabetic (DB) or non-diabetic (non-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. T/C28a2 and primary human chondrocytes were stimulated w/o IL-1 β (5 ng/mL) under a normal (5.5 mM; NG) or a high (25 mM; HG) glucose environment. Gene and protein expression of enzymes involved in H₂S synthesis (cystathionine γ -lyase [CSE], cystathionine β -synthase [CBS], and 3-mercaptopyruvate sulfurtransferase [3-MT]) and HO-1 were assessed by RT-qPCR and WB, respectively. To determine the involvement of H₂S in catabolic pathways activated by HG in chondrocytes, NaSH and GYY 4137 (500 μ M), a fast and slow-releasing H₂S donor respectively, were employed.

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-1 β 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-1 β was significantly higher in chondrocytes under HG than NG condition (6-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=6, p<0.05). GYY 4137 and NaSH co-treatment recovered HO-1 expression and reduced IL-8 levels in chondrocytes under IL-1 β +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:

- [1] Burguera EF, Vela-Anero A, Magalhães J, Meijide-Failde R, Blanco FJ. Effect of hydrogen sulfide sources on inflammation and catabolic markers on interleukin 1 β -stimulated human articular chondrocytes. *Osteoarthritis Cartilage* 2014 Jul;22(7):1026–35.

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SAT0060 PROTEOMIC ANALYSIS OF OSTEOBLASTS SECRETOME PROVIDES NEW INSIGHTS IN MECHANISMS UNDERLYING OSTEOARTHRITIS SUBCHONDRAL BONE SCLEROSIS

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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, periostin, reticulocalbin 1 and osteonectin), and 12 proteins significantly more secreted by SC osteoblasts (CHI3L1, fibulin-3, SERPINE2, IGFBP6, SH3BGRL3, SERPINE1, reticulocalbin3, alpha-2-HS-glycoprotein, TIMP-2, IGFBP3, TIMP-1, SERPINF1). Similar changes in periostin, 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

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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 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 H1–700DX). *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 into 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.

Results: The effect of PDT was optimal at 13.7 J/cm² light exposure to 3T3-FAP cells incubated with 6.67 pM 28 H1–700DX, 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 ($p = 0.16$).