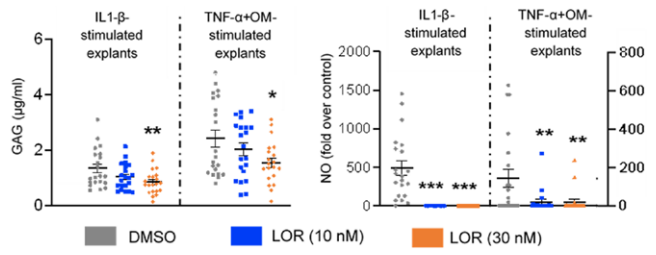

Figure 1. Effects of LOR on the quantity of the cartilage catabolism end products glycosaminoglycan (GAG) and nitric oxide (NO) in supernatants. Knee cartilage explant cultures stimulated with pro-inflammatory cytokines were subsequently treated with DMSO (control) or LOR as shown. N=22; *P<0.05, **P<0.01, ***P<0.001 vs. DMSO by one-way ANOVA.

Disclosure of Interests: Vishal Deshmukh Shareholder of: Samumed, LLC, Employee of: Samumed, LLC, Shawn Grogan: None declared, Tim Seo Shareholder of: Samumed, LLC, Employee of: Samumed, LLC, Deepti Bhat Shareholder of: Samumed, LLC, Employee of: Samumed, LLC, William Bugbee: None declared, Darryl D'Lima: None declared, Yusuf Yazici Shareholder of: Samumed, LLC, Grant/research support from: Bristol-Myers Squibb, Celgene, and Genentech, Consultant of: Celgene and Sanofi, Employee of: Samumed, LLC
DOI: 10.1136/annrheumdis-2020-eular.6346

AB0071 THERAPEUTIC EFFECTS OF BONE MARROW MESENCHYMAL STEM CELLS-DERIVED EXOSOMES ON OSTEOARTHRITIS

C. Dong[1], Y. Liu[1], A. Deng[1], J. Ji[1], W. Zheng[1], Z. Gu[1]. [1]*Affiliated Hospital of Nantong University, Nantong, China*

Background: Mesenchymal stem cells (MSCs) have shown chondroprotective effects in clinical models of osteoarthritis (OA)[1].
Objectives: The study aimed to investigate the therapeutic potential of exosomes from human bone marrow MSCs (BM-MSCs) in alleviating OA.
Methods: The anterior cruciate ligament transection (ACLT) anddestabilization of the medial meniscus (DMM) surgery were performed on the knee joints of a rat OA model, followed by intra-articular injection of BM-MSCs or their exosomes. The beneficial effects were evaluated by histological staining, OARSI scores and micro-CT. Furthermore, BM-MSCs-derived exosomes were administrated to primary human chondrocytes to observe the functional and molecular alterations. In addition, lncRNA MEG3 were investigated in chondrocytes to explore the biological contents accounting for anti-OA effects of BM-MSCs-derived exosomes.
Results: Based on the observation in the rat OA model, both of BM-MSCs and BM-MSCs-derived exosomes alleviated cartilage destruction, reduced joint damage and restored the trabecular bone of OA rats. In addition, *in vitro* assays showed that BM-MSCs- exosomes could maintain the chondrocyte phenotype by increasing collagen type II synthesis and inhibiting IL-1β- induced senescence and apoptosis. Furthermore, exosomal lncRNA MEG3 also reduced the senescence and apoptosis of chondrocytes induced by IL-1β, indicating that lncRNA MEG3 might partially account for the anti-OA effects of BM-MSC exosomes.
Conclusion: The exosomes from BM-MSCs exerted beneficial therapeutic effects on OA by reducing the senescence and apoptosis of chondrocytes, suggesting that MSCs-derived exosomes might provide a candidate therapy for OA treatment.
References:
[1] Mckinney J M, Doan T N, Wang L, et al. Therapeutic efficacy of intra-articular delivery of encapsulated human mesenchymal stem cells on early stage osteoarthritis[J]. Eur Cell Mater, 2019, 37: 42-59.
Disclosure of Interests: None declared
DOI: 10.1136/annrheumdis-2020-eular.6040

AB0072 A MULTICOMPONENT MEDICATION PROMOTES CHONDROGENESIS AND REDUCES MMP-13 IN PRIMARY ARTICULAR CHONDROCYTES FROM KNEE OSTEOARTHRITIS PATIENTS IN VITRO

C. Sanchez[1], K. Hemmer[2], N. Kroemmelbein[2], B. Seilheimer[2], J. E. Dubuc[3], C. Antoine[4], Y. Henrotin[1,4]. [1]*University of Liège, Bone and Cartilage Research Unit, Liège, Belgium;* [2]*Biologische Heilmittel Heel GmbH, Baden-Baden, Germany;* [3]*Cliniques Universitaires Saint-Luc, Orthopedic Department, Brussels, Belgium;* [4]*Artialis, Liège, Belgium*

Background: HE-1100 is a multicomponent medicinal product. Initial preclinical data potentially suggest a preventive effect on cartilage degradation.
Objectives: This study aims to understand the mode of action of HE-1100 on OA chondrocytes *in vitro*.
Methods: Primary chondrocytes were obtained from 10 knee osteoarthritis (OA) patients undergoing knee replacement surgery. The cultures were treated with 20% (v/v) HE-1100 or placebo. Samples were collected for subsequent RNA extraction using standard methods. The reads were generated with Illumina NextSeq5000 sequencer and aligned to the human reference genome (UCSC hg19) to generate the transcriptome. Differential expression analysis between HE-1100 and placebo was made in R using the DESeq2 package to identify the differentially expressed genes in the OA-associated regulatory pathways. The protein production of the selected genes was quantified by ELISA in 10 independent human OA chondrocytes cultures.
Results: According to the DESeq2 analysis, HE-1100 significantly modified the expression of 13 genes in OA chondrocytes by at least 10% with an adjusted p-value < 0.05: EGR1 (+93%), FOS (+87%), NR4A1 (+43%), DUSP1 (+18%), ZFP36 (+18%), ZFP36L1 (+14%), NFKBIZ (+16%) and CYR61 (+14%) were upregulated and ATF7IP (-10%), TXNIP (-11%), C10orf10 (-12%), CLEC3A (-12%) and MMP13 (-18%) were downregulated after 24h HE-1100 treatment. HE-1100 significantly increased (2.3 fold +/-1.2 after 24h, p=0.0444 and 2.3-fold +/-1.0 after 72h, p=0.0239) the CYR61 protein production by human OA chondrocytes. After 72h, HE-1100 slightly but not significantly increased aggrecan production by 14 ± 19 % (p=0.1117) and significantly increased type II collagen pro-peptide production by 27 ± 20 % (p=0.0147). For both time points CYR61 production by OA chondrocytes was positively and significantly correlated with aggrecan (r=0.66, p=0.0004) and type II collagen pro-peptide (r=0.64, p=0.0008) production. In alginate beads culture, pro-MMP-13 was significantly decreased by HE-1100 treated cultures from day 7 to day 14 (from -16 to -25 %, p<0.05) and from day 17 to 21 (-22 %, p=0.0331) in comparison to controls.
Conclusion: HE-1100 significantly modified the expression of DUSP1, C10orf10, ZFP36/L1 and CLEC3A, which are pathway mediators involved in MMP-13 expression and activation. Further, long-term (28 days) treatment with HE-1100 significantly reduced the production of pro-MMP-13, the inactive precursor of the metalloproteinase MMP-13 involved in type II collagen degradation. HE-1100 also promoted extracellular matrix formation probably through CYR61 production, a growth factor well correlated with type II collagen and aggrecan production.
References: /
Acknowledgments: We would like to thank the staff of the GIGA ULiège Genomic Next Generation Sequencing platform for performing the RNA sequencing and Benoit Charloteaux for his help in RNAseq data analysis.
Disclosure of Interests: christelle sanchez: None declared, Kathrin Hemmer Employee of: Heel, Natascha Kroemmelbein Employee of: Heel, Bernd Seilheimer Employee of: Heel, Jean-Emile Dubuc: None declared, Christophe Antoine Employee of: Artialis, Yves Henrotin Grant/research support from: HEEL, TILMAN
DOI: 10.1136/annrheumdis-2020-eular.2106

AB0073 BOSWELLIA SERRATA EXTRACT AND CURCUMIN INCREASE GDF15 PRODUCTION BY HUMAN PRIMARY OSTEOARTHRITIS CHONDROCYTES: A NEW MECHANISM OF ACTION

C. Sanchez[1], J. Zappia[1], Y. Dierckxsens[2], J. P. Delcour[3], Y. Henrotin[1,4]. [1]*University of Liège, Bone and Cartilage Research Unit, Liège, Belgium;* [2]*Tilman, Baillonville, Belgium;* [3]*Centre Hospitalier du Bois de l'Abbaye, Seraing, Belgium;* [4]*Princess Paola Hospital, Marche-en-Famenne, Belgium*

Background: Boswellia serrata extract (BSE) and curcumin are used to relief symptoms in osteoarthritis (OA).
Objectives: This study aims to better understand the mode of action of these compounds on OA chondrocytes *in vitro*.
Methods: Therapeutic plasmatic concentrations of the different components of BSE correspond to an *in vitro* range from 25 to 100 µg/ml of total BSE (100 µg/ml of BSE corresponds to 9.2 µM of 11-keto-β-boswellic acid (KBA), 5.2 µM of acetylKBA, 22 µM de αBA, 34 µM de βBA, 4.4 µM de acetylαBA and 13.2 acetyl βBA), and between 2 to 10 µM for bioavaibility-increased curcumin. BSE (5-100 µg/ml) and curcumin (0.04 to 4 µg/ml corresponding to 0.1 to 10 µM) were tested separately on primary chondrocytes from 3 OA patients. Lactate Deshydrogenase LDH, nitrite (NO_2), interleukin (IL)-6 and Growth Differentiation Factor (GDF)15 were quantified in 72h-treated supernatant using enzyme activity, Griess reaction and ELISAs, respectively.
Results: No mortality was observed at the tested concentrations. BSE and curcumin both decreased concentration-dependently NO_2 and IL-6 production, and increased GDF15 production. For NO_2 production, the decrease was observed from 0.2 µg/ml of curcumin and 10 µg/ml of BSE. For IL-6 production,

the decrease was observed from 1 µg/ml for curcumin and 10 µg/ml for BSE. For GDF-15, the increase was observed from 2 µg/ml for curcumin and 50 µg/ml for BSE. Maximal effect was observed at 4 µg/ml for curcumin: -67% NO_2 ($p<0.0001$), -71% IL-6 ($p=0.0001$) and +80% GDF15 ($p<0.0001$) and at 100 µg/ml for BSE: -40% NO_2 ($p=0.0003$), -70% IL-6 ($p=0.0003$) and +73% for GDF15 ($p=0.0017$).

Conclusion: At therapeutic plasmatic concentrations, BSE and curcumin decreased the production of NO_2 and IL-6, two inflammatory mediators. Furthermore, BSE and curcumin enhanced GDF-15 production, an anti-inflammatory growth factor. GDF15 was first identified as Macrophage inhibitory cytokine-1 or NSAID-activated gene-1 (by a prostanoid-independent manner), and is known as a regulator of inflammatory, cell repair and apoptosis pathways. GDF-15 has pro-apoptotic and anti-tumorigenic activity in vitro and in vivo. It could represent a new pathway explaining the beneficial effects of BSE and the curcumin on synovium inflammation and cartilage degradation.

Disclosure of Interests: christelle sanchez: None declared, Jérémie Zappia: None declared, Yvan Dierckxsens Shareholder of: Tilman SA, Employee of: Tilman SA, Jean-Pierre Delcour: None declared, Yves Henrotin Grant/research support from: HEEL, TILMAN

DOI: 10.1136/annrheumdis-2020-eular.3457

AB0074 KRÜPPEL-LIKE FACTOR 10 IS A IMPORTANT MODULATORY FACTOR OF CHONDROCYTE HYPERTROPHY IN DEVELOPING SKELETON

J. Y. Ko[1], E. Lee[1], J. Kim[1], G. I. Im[1]. [1]*Research Institute for Integrative Regenerative Biomedical Engineering, Dongguk University, Goyang, Korea, Rep. of (South Korea)*

Background: To investigate the functional role of KLF10 as a modulator of chondrocyte hypertrophy in developmental skeleton, the developmental characteristics in the long bone of KLF10 knockout mice and characteristics of MSCs from KLF10 KO mice were investigated regarding chondrogenesis and osteogenesis. Delayed long bone growth and delayed formation of primary ossification center were observed in an early embryonic stage in KLF10 KO mouse along with very low Ihh expression in epiphyseal plate. While the chondrogenic potential of mMSCs appeared normal or slight decrerased in KLF10 KO mice, osteogenesis and hypertrophy were extensively suppressed. KLF10 was found to be a mediator of chondrocyte hypertrophy in developing skeleton. Suppression of KLF10 may be considered as a new strategy for preventing hypertrophy in cartilage regeneration using MSCs.

Objectives: Investigated the functional role of KLF10 to present new insights into the transcriptional network regulating skeletal development and provide a novel strategy for preventing aberrant hypertrophic differentiation in cartilage regeneration strategies using MSCs.

Methods: Generation of KLF10 KO mice and genotyping / Skeletal preparations, embryo heights, and mineralized bone length measurements / Histological and Fluorescent Immunohistochemical Analysis / ALP staining and activity / Alizarin red staining / Von Kossa staining and calcium salts quantification / Isolation and Establishment of Mouse Clonal MSC Lines / Chondrogenic pellet culture and differentiation of mMSCs / DNA Quantification and GAG Contents Analysis / Rq-PCR Analysis / Statistics

Results: The overall results showed that mMSCs from KLF10 KO mice have significantly decreased osteogenic potential with very low Ihh expression while an increase in chondrogenic potential was not significant. In addition to Ihh promotor demonstrated in our previous study, KLF10 can activate Runx2 expression through its proximal-promoter region. Thus, KLF10 may indirectly stimulate Ihh expression upstream of Runx2 or directly bind to Ihh promoter and activate Ihh expression. As shown in this and out previous study, KLF10 also enhances Wnt/β-catenin signalling in MSCs. KLF10 modulates β-catenin sub-cellular localization and enhances Wnt signalling in osteoblasts.

Conclusion: In conclusion, primary ossification in KLF10 KO mice was critically delayed during early endochondral bone development. KLF10 KO inhibited hypertrophy via reduced Ihh expression in developing skeleton. TGF-β-induced hypertrophy was inhibited during chondrogenesis of KLF10 KO mMSCs. Our findings present new insights into the transcriptional-network system of skeletal development and provide a novel strategy for suppressing hypertrophy in cartilage tissue engineering.

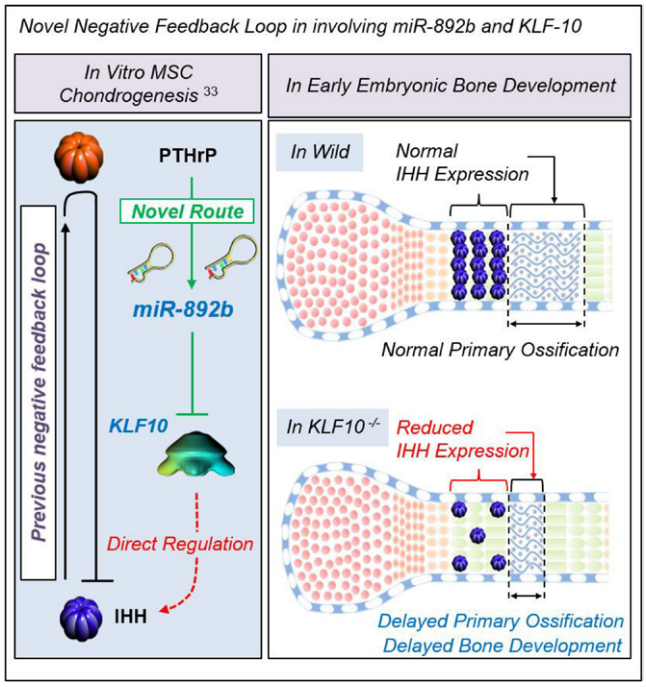

Figure.

Acknowledgments: This research was supported by the National Research Foundation of Korea (NRF-2019R1H1A2039685 and 2019R1I1A1A01043778).

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.2065

AB0075 SYNOVIAL TISSUE MACROPHAGES ARE DOMINANTLY ALTERNATIVELY ACTIVATED IN PATIENTS WITH MATURE OSTEOARTHRITIS

G. Laskarin[1,2], T. Kehler[2,3], D. Legović[4], V. Šantić[4], B. Ćurko-Cofek[1], V. Drvar[5], M. Rogoznica[6], D. Rukavina[1,7]. [1]*Faculty of Medicine, University of Rijeka, Department of Physiology and Immunology, Rijeka, Croatia;* [2]*Hospital for Medical Rehabilitation of Hearth and Lung Diseases and Rheumatism "Thalassotherapia-Opatija", Opatija, Croatia;* [3]*Faculty of Medicine, University of Rijeka, Department of Medical Rehabilitation, Opatija, Croatia;* [4]*Orthopaedic University Hospital - Lovran, Lovran, Croatia;* [5]*Clinical Hospital Centre Rijeka, Clinical Department of Laboratory Diagnostics, Rijeka, Croatia;* [6]*Hospital for Medical Rehabilitation of Hearth and Lung Diseases and Rheumatism "Thalassotherapia-Opatija", Rijeka, Croatia;* [7]*Croatian Academy of Sciences and Arts, Department of Biomedical Sciences in Rijeka, Rijeka, Croatia*

Background: Macrophages are abundant inflammatory cell type in the synovial membrane of knee osteoarthritis (OA) (1). Their quantity is associated with radiographic severity of knee OA and joint symptoms (2), while their functions are set in response to micro-environmental signals (3). Classically activated macrophages M1 support T helper 1 (Th1) driven pro-inflammatory reactions, while alternatively activated macrophages M2 strengthen Th2 inflammatory processes (3).

Objectives: To investigate activation status of synovial tissue macrophages in patients with mature OA in terms of M1 / M2 polarization.

Methods: Synovial tissue samples (6) with abundant lymphocyte infiltration were obtained during aloarthroplasty. Double immunofluorescence labeling was performed on paraffin-embedded synovial tissue sections using primary rabbit anti-macrophage CD68 mAb in combination with mouse anti-human antibodies directed toward CD3, arginase-1, TNF-alpha and IL-15. CD206 and CD163 were single labelled.

Results: CD68+ macrophages mostly co-expressed arginase-1 (4/6 samples), indicating their M2 orientation. Macrophages were placed in lining synovial tissue and nearby tissue-resident CD3+ cells. M2 markers CD206 and CD163 were