and intracellular level. This inhibition was more pronounced after polyphenol cell pretreatment. In this case cytokine production was completely suppressed both in control and in stimulated cells. Cell pretreatment was extremely effective also in reducing mRNA expression of IL-1 after crystal stimulation while NLRP3 expression was to some extent affected by RES. RES and PD had a slight inhibitory effect on crystal phagocytosis when added along with the stimulus, while pretreated cells did not show any difference in the phagocytosis index. ROS production induced by MSU crystals was more pronounced (4 fold increase) with respect to CPP crystals (2 fold increase). RES was more effective than PD in inhibiting ROS production ($p < 0.05$ crystals vs crystals+RES). The pretreatment showed a more marked decrease of ROS than the simultaneous treatment and the effect reached significance for both PD and RES. Similar inhibitory effects have been obtained when NO production was considered.

Conclusions: Our results demonstrated that RES and PD are effective in inhibiting crystal-induced inflammation affecting IL-1 production and NLRP3 expression and activation. Data obtained after cell pretreatment allow us to hypothesize that these polyphenols act on specific signaling pathway preventing inflammation, and that this action is independent from crystal phagocytosis.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.6830

THU0048 SOLUBLE URIC ACID INCREASED THE EXPRESSION OF ABCG2 AND PDZK1 IN HUMAN INTESTINAL CELL LINES THROUGH THE TLR4/NLRP3/CASPASE-1 AND PI3K/AKT SIGNALING PATHWAYS

M. Chen, X. Lu, H. Wu, Department of Rheumatology, the Second Affiliated Hospital Zhejiang University School of Medicine, Hangzhou, China

Background: Hyperuricemia is the key pathophysiological basis of gout^[1]. The most common reason generating hyperuricemia is verified the obstacle of urate excretion by current researches^[2]. The intestine is known as the most important organ involved the excretion of uric acid besides the kidney^[1]. ABCG2 has an outsized genetic contribution to extra-renal underexcretion and causes a compensatory increase in urinary urate output^[3]. PDZK1 is a kind of an important structural protein regulated ABCG2 function, by potential molecular interaction with ABCG2^[4].

Objectives: The present study was undertaken to explore the effect and its related mechanisms of soluble uric acid on the urate excretion PDZK1 and ABCG2 in HT-29 and Caco-2 cell lines

Methods: HT-29 and Caco-2 cell lines were used as a well-established model of human intestinal epithelial cells. Cells were pretreated with or without inhibitors and then stimulated with soluble uric acid. siRNA transfection was used to assess the interaction between ABCG2 and PDZK1. qRT-PCR and western blotting were used to measure mRNA and protein levels, respectively. Subcellular fractionation methods and immunofluorescence were used to examine the proteins in different subcellular compartments. Flow cytometry experiments examined the function of ABCG2.

Results: Soluble uric acid significantly up regulated the expression of PDZK1 and ABCG2 in human intestinal cell lines. It also activated TLR4/NLRP3/caspase-1 inflammasome and PI3K/AKT signalling pathway through the phosphorylation of the ser473 pAkt. The increased expression of PDZK1 and ABCG2 was suppressed by a block of TLR4 (TAK-242) and caspase-1 inhibitor (acetyl-YVAD-chloromethylketone) and partly reduced by wortmannin, a specific inhibitor of PI3K. Additionally, lipopolysaccharide (LPS), the potent inducer of inflammatory responses mediated through TLR4, activated TLR4/NLRP3/caspase-1 inflammasome and up regulated the expression of ABCG2 and PDZK1. Besides, the stimulation of soluble uric acid facilitated the translocation of ABCG2 from intracellular compartment to plasma membrane and increased the transport activity. Furthermore, PDZK1 knockdown via siRNA significantly inhibited the expression and transport activity of ABCG2 regardless of the activation by soluble uric acid.

Conclusions: The PI3K/AKT and TLR4/NLRP3/caspase-1 signaling pathways modulate ABCG2 and PDZK1 expression stimulated by soluble uric acid in human intestinal cells. PDZK1 plays a pivotal role in the regulation of ABCG2.

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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.5673

THU0049 29-KDA FIBRONECTIN FRAGMENT SUPPRESSED HMGB1-DEPENDENT AUTOPHAGY PATHWAY IN HUMAN ARTICULAR CHONDROCYTES

M. Choi¹, I.Y. Park¹, K.M. Son², H.S. Hwang¹, H.A. Kim¹. ¹Hallym University Sacred Heart Hospital; ²Hallym University Dongtan Sacred Heart, Gyeonggi-do, Korea, Republic Of

Background: Fibronectin fragments found in synovial fluid induce the catabolic responses in cartilage. High mobility group protein Box 1 (HMGB1), a damage-associated molecular pattern (DAMP), resides in the nucleus, translocates into the cytosol in response to various stimuli, and is subsequently released into the extracellular space.

Objectives: In this study, we investigated the effect of 29-kDa fibronectin fragment (29-kDa FN-f) on HMGB1-mediated autophagy pathway in primary human chondrocytes.

Methods: Human articular chondrocytes were enzymatically isolated from articular cartilage. The mRNA and protein levels of HMGB1 were measured by quantitative real-time PCR (qRT-PCR) and Western blot analysis. The translocation of nuclear HMGB1 into cytoplasm was determined by western blot and immunofluorescence microscopy. Activation of mammalian target of rapamycin (mTOR), protein kinase B (Akt) and 1A/1B-light chain 3 (LC3) was measured by western blot analysis. Interaction of HMGB1 and Beclin-1 were evaluated by immunoprecipitation (IP). Release of HMGB1 into extracellular medium was measured by ELISA.

Results: HMGB1 was significantly reduced in human osteoarthritis (OA) cartilage compared to normal cartilage. qRT-PCR and Immunoblotting assay revealed that 29-kDa FN-f significantly reduced expression of HMGB1 at the mRNA and protein levels and also led to the translocation of the nuclear HMGB1 into the cytoplasm, together with decreased levels of Beclin-1 and phosphorylated Bcl-2. Using IP analysis, we demonstrated that in the presence of 29-kDa FN-f the association of HMGB1 and Beclin-1, which led to HMGB1-dependent autophagy pathway, was decreased, whereas the association of Bcl-2 and Beclin-1 was increased. In addition, prolonged treatment with 29-kDa FN-f significantly increased the release of HMGB1 into the culture medium. A variety of evidence, including down-regulated LC3-II, an autophagy marker, and increased phosphorylated 4EBP1 and p70S6K, substrates of mTOR, revealed that 29-kDa FN-f subsequently suppressed autophagy in primary chondrocytes by activating Akt/mTOR signaling pathway.

Conclusions: These results demonstrated that 29-kDa FN-f has a harmful impact on chondrocytes through suppressing HMGB1-dependent autophagy pathway and releasing HMGB1, DAMP, to extracellular space.

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Acknowledgements: This study was supported by a grant (H114C2248 and H115C2699) from the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea and the Basic Science Research Program through the National Research Foundation (NRF) of Korea funded by the Ministry of Education (2014R1A1A2059823).

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.2812

THU0050 MICRORNA-365 INHIBITS IL-1 β -INDUCED CATABOLIC FACTOR EXPRESSION BY TARGETING HIF-2 α IN HUMAN PRIMARY CHONDROCYTES

M.H. Lee¹, J.-I. Hong¹, H.S. Hwang¹, K.M. Son², H.A. Kim¹. ¹Hallym University Sacred Heart Hospital, Kyunggi; ²Hallym University Dongtan Sacred Heart Hospital, Gyeonggi, Korea, Republic Of

Background: MicroRNAs, small noncoding RNA molecules, are involved in the pathogenesis of various diseases such as cancer and arthritis. Endothelial PAS

domain protein-1/hypoxia-inducible factor-2 α (EPAS-1/ HIF-2 α) is a catabolic transcription factor that regulates osteoarthritis (OA) cartilage destruction.

Objectives: In this study, we examined whether microRNA-365 (miR-365) affects interleukin (IL)-1 β -induced expression of catabolic factors in chondrocytes via regulation of HIF-2 α .

Methods: Total RNA was isolated from normal and OA cartilage tissues and human chondrocytes. miR-365 expression was quantified by TaqMan assay. The effects of miR-365 on HIF-2 α and HIF-2 α -modulated genes were assessed by quantitative real-time reverse polymerase chain reaction (qRT-PCR), Western blot analysis, and enzyme-linked immunosorbent assay (ELISA). Direct interaction of miR-365 with the 3' untranslated region (UTR) of HIF-2 α mRNA was examined using a luciferase reporter assay. Nitrite concentration was measured in culture medium using a Griess assay.

Results: miR-365 levels were significantly decreased in human OA cartilage relative to normal cartilage. Overexpression of miR-365 significantly suppressed IL-1 β -induced expression of HIF-2 α in human articular chondrocytes. Pharmacological inhibition of various IL-1 β -associated signaling pathways revealed mitogen-activated protein kinase and nuclear factor- κ B as the primary pathways driving IL-1 β -mediated decreases in miR-365 and subsequent increase in HIF-2 α expression. Using a luciferase reporter assay encoding the 3'UTR of human HIF-2 α mRNA, we showed that overexpression of miR-365 significantly suppressed IL-1 β -induced up-regulation of HIF-2 α . Furthermore, miR-365 overexpression significantly suppressed IL-1 β -induced expression of catabolic factors, including cyclooxygenase-2, inducible nitric oxide synthase, and matrix metalloproteinase-1, -3 and -13, and nitric oxide (NO) production in chondrocytes.

Conclusions: MiR-365 regulates IL-1 β -stimulated catabolic effects in human chondrocytes by modulating HIF-2 α expression.

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Acknowledgements: This study was supported by the Basic Science Research Program through the National Research Foundation (NRF) of Korea funded by the Ministry of Education (2014R1A1A2059823 and 2016R1D1A1B03932259).

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.2818

THU0051 ASSOCIATION BETWEEN BIOACTIVE TNF AND EULAR RESPONSE TO TNF INHIBITORS IN RHEUMATOID ARTHRITIS

J. Poudroux¹, H. Marotte¹, J.-E. Gottenberg², S. Paul³. ¹Rheumatology, CHU Saint Etienne, Saint Priest en Jarez; ²Rheumatology, CHU Strasbourg, Strasbourg; ³Immunology, CHU Saint Etienne, Saint Priest en Jarez, France

Background: ROC study provided data to manage rheumatoid arthritis (RA) treatment after a failure to a first TNF blocker (1). We previously reported that high circulating TNF bioactivity was associated with good clinical response (2).

Objectives: To explore ability of TNF bioactivity to predict response to second TNF blocker.

Methods: Here, we assessed TNF bioactivity in 130 RA patients from the group rotation to a second TNF blocker at the time of randomisation and after 6 months. Clinical and biological data were recorded at baseline and 6 month with Eular response assessed at 6 month.

TNF bioactivity was assessed with HEK-Dual TNF cells adapted from our previous work (2). Due to non-normalised distribution, non parametrical analysis were performed.

Results: Baseline circulating TNF bioactivity was similar according to Eular response. However, circulating TNF bioactivity at 6 month was associated

with clinical response with low TNF bioactivity in responders. A ROC analysis suggested a cut-off at 0.320 with a sensitivity at 92% and a specificity at 41%.

Conclusions: After a failure of a first TNF blocker, circulating TNF bioactivity is not able to predict response to a second TNF blocker despite a lower level in responders compared to non-responders.

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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.4227

THU0052 LEVELS OF INFLAMMATORY SEROLOGIC BIOMARKERS IN HLA-B27 AND HLA-B15 POSITIVE PATIENTS WITH SPONDYLOARTHRITIS

J. Londono¹, A.M. Santos², J.C. Rueda², P. Peña², I. Briceño³, E.-L. Saldarriaga², J.-I. Angarita², E. Calvo⁴, M. Avila², N. Martinez-Rodriguez⁵, H. Cubides², V. Parra², J.F. Medina⁶. ¹Rheumatología, Universidad de la Sabana-Hospital Militar Central, Bogotá; ²Rheumatología; ³Facultad de Medicina, Grupo Genética Humana, Universidad de la Sabana, Chia; ⁴Departamento de Imágenes diagnósticas, Universidad Nacional, Bogotá, Colombia; ⁵Departamento de Investigación de Salud Comunitaria, Hospital Infantil de México Federico Gomez, Ciudad de Mexico, Mexico; ⁶Unidad de Entrenamiento Clínico, Facultad de Medicina, Universidad de Navarra, Pamplona, Spain

Background: A main challenge in spondyloarthritis (SpA) management was the availability of reliable biomarkers related with disease activity, or predicting joint damage and the response to treatment. Although erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are currently used as biomarkers for disease activity, they lack sensitivity, specificity and reproducibility. With the understanding of SpA pathogenesis, additional biomarkers like metalloproteinase 3 (MMP-3), interleukin (IL)-1 α , IL-6, lipopolysaccharide-binding protein (LBP), tumour necrosis factor α (TNF α), macrophage colony stimulating factor (M-CSF), interferon gamma (INF-g), IL-17 and IL-23, had been proposed

Objectives: We aimed to evaluate the associations of MMP-3, IL-1 α , IL-6, M-CSF, LBP, IL-17 and IL-23 levels in SpA patients positive for HLA-B27 or HLA-B15

Methods: 178 patients (100 men and 78 women) with SpA according to ASAS criteria were included in the study. HLA typing was performed by PCR using the Biorad[®] HLA-GSP ABDR plates. The levels of TNF α , IL-1 α , IL-6, INF-g and IL-17 were measured by a cytometric bead-array (CBA Flex Set) using a FACS Canto II Flow Cytometer. Enzyme-linked immunosorbent assay (ELISA) was used to determine serum levels of IL-23, M-CSF and MMP-3. CRP and LBP levels were measured by chemiluminescence. Statistical analysis was made with SPSS v19. For comparison of quantitative variables with a normal distribution we used the Student's *t*-test. Categorical variables were presented in frequency charts and percentages, and the Chi-squared test and Fisher's exact test were used when necessary, for comparing groups. Two-tailed P-value <0.05 was considered statistically significant

Results: Of the 178 patients, 70 were positive for HLA-B27, 34 for HLA-B15 and 74 had other HLA-B. According to ASAS classification criteria, 152 patients had axial SpA (axSpA) manifestations, 161 had peripheral SpA (pSpA) manifestations, and 148 patients had mixed axial and peripheral manifestations. Figure 1 shows the mean levels of inflammatory serologic biomarkers in these subgroups of patients

Figure 1. A. Mean activity biomarkers (CRP, C-reactive protein; LBP, lipopolysaccharide-binding protein) in axial spondyloarthritis (axSpA) and peripheral spondyloarthritis (pSpA). B. Cytokines (INF-g, interferon gamma; IL-1 α , interleukin; IL-6, IL-6; INF-g, interferon gamma; IL-17, IL-17; IL-23, IL-23; TNF α , tumour necrosis factor α) in axSpA and pSpA. C. Enzyme activity biomarkers (MMP-3, metalloproteinase 3; ESR, erythrocyte sedimentation rate) in HLA-B27 and HLA-B15 positive SpA patients. D. Cytokines in HLA-B27 and HLA-B15 positive SpA patients.

Conclusions: High levels of IL-17 and IL-23 were associated with the presence of HLA-B27, which mainly correlates with an axial presentation of the disease as