Results: blockade of SHH by GDC-0449 significantly alleviated the symptoms and decreased the synovial hyperplasia, inflammatory infiltration, cartilage and bone damage in ankles of CIA. The bone erosions in the area of the metatarso-phalangeal joints and ankle joints and production of TNFα, IL-6 were decreased by SHH inhibition. In addition, the administration of GDC-0449 significantly decreased the number of TRAP positive cells and the expression of NFATC1. On the contrary, SHH overexpression led to increased severity of arthritis and pathological changes. We also observed the accelerated bone injury accompanied with increased number and activity of osteoclasts and increased production of serum IL-6 in mice with upregulation of SHH expression. Of note, the administration of p38 MAPK inhibitor reversed the effects of SHH overexpression, with a reduction of joint swelling and histological scores. Inhibition of p38 MAPK prevented the bone erosion and decreased the number of TRAP positive cells and the expression of NFATC1, which were promoted by SHH overexpression.

Conclusions: The study indicates that SHH promotes the synovial hyperplasia and bone erosion of CIA in a p38 MAPK-dependent manner. SHH-p38 MAPK signaling could be a potential target for the treatment of RA.

Acknowledgments: This work was supported by grants from the National Natural Science Foundation of China (81571584, 81701609).

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.6426

THU0077 CADHERINS GUIDE THE DIRECTIONAL MIGRATION OF THE SYNOVIOCYTES IN RHEUMATOID ARTHRITIS

J. H. Kang1, S. Ahamed1, K. H. Sa1, S. W. Han1, Y. M. Kang1, Kyungpook National University School of Medicine, Daegu, Korea, Rep. of (South Korea)

Background: Aggressiveness of synovocytes and collective migration of organized synovial tissues play a key role in the pathogenesis of panus invasion into adjacent joint structure. Interactions among synovial cells for grouped movement, however, have not been properly elucidated.

Objectives: We hypothesized that cadherins which have functions on the synovial invasion in RA, may play a critical role in collective migration of rheumatoid synoviocytes.

Methods: Cadherin expression patterns on the synoviocytes isolated from patients with RA were evaluated using RT-PCR, flow cytometry, and western blot analysis. Mesenchymal and epithelial phenotypes were examined in cadherin overexpressing cell line by flow cytometry. L-cells with overexpression of CDH2 (CDH2hi), CDH11 (CDH11hi), and combination of CDH2/CDH11 (CDH2hi/CDH11hi) were prepared. Migration of cells was observed by taking time-lapse images with laser confocal microscopy. In vitro collective migration and directional movement in response to inflammatory mediators and different matrix rigidity were evaluated. In vivo homing of CDH2hi/CDH11hi L-cells into joint tissues was performed in collagen induced arthritis (CIA) mouse. In vivo and ex vivo migration pattern of CDH11hi-L-cells were investigated in nude mice using optical imaging system.

Results: In rheumatoid synovial tissues, CDH2 and CDH11 were highly expressed compared to synovial tissues from osteoarthritis. CDH2 and CDH11 were also highly expressed on synovial fibroblasts isolated from RA. Phenotype analysis of mesenchymal and epithelial cells in CDH11hi-L-cells and CDH2hi/CDH11hi-L-cells showed increased expression of αSMA, CD44, vimentin, and α-SMA compared with MOCK-L-cells. We then analyzed the pattern of migration of MOCK, CDH2hi, CDH11hi, and CDH2hi/CDH11hi-L-cells using time-lapse images. During migration over a hard ECM, CDH2hi and CDH11hi-L cells represented higher aspect ratio compared to a soft ECM. Aspect ratio relatively found lower in CDH2hi/CDH11hi-L-cell lines than MOCK cells. CDH2hi/CDH11hi-L-cells showed significantly higher migration velocity and Euclidean distance with narrower angle of migratory directions in a cytokine mediated collective migration and directional movement in response to inflammatory mediators and different matrix rigidity.

Conclusion: The expression of CDH2 and CDH11 promotes directional migration of synoviocytes, indicating the potential role of these cadherins on the panus tissues in the invasion into adjacent joint structure in RA.

References:

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.3632

THU0078 EXPRESSION PROFILE ANALYSIS OF LONG NONCODING RNAs INDUCED BY IL-1β IN RHEUMATOID ARTHRITIS FIBROBLAST-LIKE SYNOVIOCYTES

J. M. Kim1, H. J. Kang2, S. J. Jung3, B. W. Song1, H. J. Jeong1, C. N. Son1, S. H. Kim1, H. Hur1. Keimyung University Dongsan Medical Center, Keimyung University College of Medicine, Division of Rheumatology, Department of Internal Medicine, Daegu, Korea, Rep. of (South Korea); 2Graduate School, Keimyung University, Department of Internal Medicine, Daegu, Korea, Rep. of (South Korea); 3Keimyung University School of Medicine, Department of Anatomy, Daegu, Korea, Rep. of (South Korea); 4School of Medicine Kyungpook National University, Department of Biochemistry and Cell Biology, Cell and Matrix Research Institute, Daegu, Korea, Rep. of (South Korea)

Background: Long noncoding RNAs (lncRNAs) have recently emerged as important transcriptional regulators of various phenotypic and pertinent expressions. lncRNAs are reported in various diseases including cancer, cardiovascular disease, and diabetes mellitus. However, the role of IncRNAs in the pathogenesis of rheumatoid arthritis (RA) remains unknown.

Objectives: Thus, we studied IncRNAs influenced by IL-1, which is one of the key mediators in the pathogenesis of RA, and also investigated whether regulation of NF-κB activation, which is known to be induced by IL-1, could lead to the changes of expression of those IncRNAs.

Methods: Fibroblast-like synoviocytes (FLS) were obtained from the knee joints of the patients with RA. The next-generation sequencing (NGS) data were analyzed to identify differentially expressed IncRNAs between unstimulated RA FLS and IL-1-stimulated RA FLS. The expression levels of the top 5 candidates in NGS data were validated by RT-qPCR using extended number of unstimulated RA FLS and IL-1-stimulated RA FLS. IMD-0560, an inhibitor of IκB kinase (IKK) was used for the regulation of NF-κB activation. Activation and inhibition of NF-κB were confirmed by Western blotting. Changed expressions of the lncRNAs were identified by RT-qPCR.

Results: NGS analysis revealed up-regulated 30 IncRNAs and down-regulated 15 IncRNAs in IL-1-treated RA FLS compared with unstimulated RA FLS. Top 5 IncRNAs were selected among 30 IncRNAs up-regulated by IL-1 in RA FLS based on fold-change with P-value cutoff. The up-regulated IncRNAs including NR_046035, NR_027783, NR_033422, NR_003133, and NR_049759 were validated by RT-qPCR. IMD-0560 inhibited phosphorylation of IκB induced by IL-1 in RA FLS. Overexpression of IncRNAs induced by IL-1 was also inhibited by IMD-0560 in RA FLS.

Conclusion: Our study revealed that IL-1 increased the expression of NR_046035, NR_027783, NR_033422, NR_003133, and NR_049759 in RA FLS. In addition, the expression of these IncRNAs was regulated by inhibition of NF-κB activation. Thus, our data suggest that the IncRNAs might be involved in the pathogenesis of RA through NF-κB signaling pathway.

References:

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2020-eular.3072

THU0079 THE MICROBIOME OF NEW-ONSET RHEUMATOID ARTHRITIS (NORA) PATIENTS DRIVES TLR4-DEPENDENT TH17 RESPONSES

1Radboud University Medical Center, Experimental Rheumatology, Nijmegen, Netherlands; 2New York University School of Medicine, New York, United States of America

Background: Intestinal microbiota plays a prominent role in shaping the T cell immune response. Increasing evidence suggests that the gut microbiota is perturbed in patients with RA, and a variety of animal models demonstrated involvement of (mouse) microbiota in arthritis development. This underlines the necessity of understanding whether and how indigenous human NORA-associated microbiota may trigger RA.