Figure 2. A heatmap shows the correlation between the intestinal microbiota and CD4+T cells in patients with RA, and *Ruminococcus torques* at the genus level was negative related with Treg cells. (Colors indicate the Spearman rank correlation, *** $P < 0.001$).

Disclosure of Interests: None declared

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POS0397 **AGGREGATED SURVIVIN BINDING AROUND HISTONE H3 EPIGENETIC MODIFICATIONS IN RISK LOCI ASSOCIATED WITH RHEUMATOID ARTHRITIS**

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Background: Survivin is an integral part of the Chromosomal Passenger Complex (CPC) which plays a vital role in mitosis. Experiments have demonstrated that survivin can physically bind to DNA. Crystallographic studies show that survivin binds to Threonine-3 of histone H3. In patients with autoimmune diseases, increased survivin expression contributes to an aggravated disease phenotype. Thus, functional, and mechanistic data point to a potential chromatin regulatory role for survivin, possibly in combination with the established gene regulatory function carried out by histone epigenetic modifications (EM).

Objectives: The objective of the study was to analyse the co-localization of chromatin bound survivin with three histone H3 epigenetic modifications – acetylated lysine 27 (K27ac) and trimethylated lysine 4 (K4me3) and lysine-27 (K27me3). The second objective was to analyse if survivin-bound DNA sequences overlapped with sequences in the vicinity of 106 GWAS SNPs that are associated with a risk of developing rheumatoid arthritis (RA).

Methods: Chromatin from CD4 T cells of 14 female subjects was immunoprecipitated with survivin antibodies and each of the histone H3 antibodies, and coupled with sequencing (ChIPseq, Hiseq2000, Illumina). After mapping the annotations of sequenced regions to the human reference genome hg38, enriched peaks were identified through Homer software. The identified survivin ChIP peaks were analysed for colocalization with peaks of the three histone H3 EMs and with RA risk loci, using the Bioconductor package 'ChIP-PeakAnno' through RStudio.

Results: Among the total of ~13,000 individual survivin ChIP-peaks, 33% colocalized with histone H3 EM peaks. The overlapping peaks show a linear increase in average peak size compared with the peaks showing no colocalization with any H3 EM peak. A maximum of 5.5-fold increase in average peak size was observed when survivin bound peaks overlap with peaks of all three H3 EMs. A major proportion (86%) of top RA risk SNPs was associated with either binding of survivin or H3 EMs. In this subset, 63% of RA risk SNPs were found within an area of 100 kilobases from survivin ChIP-peaks, with preferential enrichment of high-scoring peaks when survivin colocalizes with all 3 H3 EMs. Survivin was bound to risk SNPs annotated to, among others, the major immunological genes CD83, IRF4, CD28, ICOS and IL2RA

Conclusion: This study presents experimental evidence that survivin binding to DNA preferentially occurred in regions with high density of histone EMs. The increased aggregation of survivin around histone H3 EMs point to its potential regulatory function in gene transcription. Since regions around RA risk SNPs overlap with survivin peaks, survivin's nuclear function could have immunologically important effects in mechanisms of autoimmune diseases.

Disclosure of Interests: None declared

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POS0398 **ADIPONECTIN INDUCES SYNOVIAL ANGIOGENESIS IN RHEUMATOID ARTHRITIS THROUGH METABOLIC REMODELING**

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Background: Our team have previously reported that Adiponectin correlates well with synovial inflammation and progressive bone erosion in rheumatoid arthritis (RA). Angiogenesis is another important part, which plays a critical role in the pathogenesis of RA.

Objectives: We hypothesized that adiponectin induces synovial angiogenesis in RA.

Methods: Single-cell RNA sequencing (scRNA-Seq) was used to screen cellular changes in local knee joint of collagen-induced arthritis (CIA) after intraarticularly injected of adiponectin. Chimera models of synovium-cartilage-NOD/SCID mice, matrigel plug assay and rat aortic ring assay were performed to demonstrate the pro-angiogenesis role of adiponectin. Cellular experiment, including proliferation, migration, apoptosis, tube formation and angiogenesis related gene expression profile, were detected with Human Umbilical Vein Endothelial Cells (HUVEC) and Mice Lung Microvessel Endothelial Cell (MLMEC) after adiponectin stimulation. Seahorse was performed to clear the influence of adiponectin to cell metabolism.

Results: The synovium and pannus hyperplasia worse in CIA model after intraarticularly injected of adiponectin, along with more serious synovitis and bone erosion. ScRNA-Seq of synovial tissues separated from CIA reminded that endothelial cell barbarically grows via metabolic remodeling after stimulated with adiponectin. Synovial chimera, matrigel plug and rat aortic ring shows adiponectin accelerates angiogenesis significantly in different background conditions. In vitro, endothelial cell proliferation detecting by RCTA and CCK8, migration by wound healing and transwell, apoptosis by FACS, tube formation and angiogenesis related gene expression profile by PCR-ARRAY were promoted by adiponectin in both HUVEC and MLMEC. Seahorse showed HUVEC made more use of glycolysis after co-cultured with adiponectin, a method of cell energy supply that tumor cells possess called warburg effect, that drives endothelial cell hyperplasia in severe environment.

Conclusion: As a classic metabolic regulator, adiponectin exacerbates CIA by promoting angiogenesis through metabolic remodeling. The findings not only provide a novel insight into the pathogenic role of adiponectin, but also reveals a potential therapeutic strategy to attenuate revascularization in RA.

Disclosure of Interests: None declared

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POS0399 **TMT-BASED QUANTITATIVE PROTEOMICS ANALYSIS OF SYNOVIAL FLUID-DERIVED EXOSOMES IN RHEUMATOID ARTHRITIS, AXIAL SPONDYLOARTHRITIS, GOUT AND OSTEOARTHRITIS**

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Background: The pathogenesis of the joint diseases rheumatoid arthritis (RA), axial spondyloarthritis (axSpA), gout, and osteoarthritis (OA) are still not fully elucidated. Exosomes in synovial fluid (SF) has a critical role in the pathogenesis of arthritis. None of study has compared the proteomics of SF-derived exosomes in RA, axSpA, gout and OA.

Objectives: To compare the proteomics of SF-derived exosomes in RA, axSpA, gout and OA based on tandem mass tags (TMT) labeled quantitative proteomics technique.

Methods: SF-derived exosomes was isolated from RA, axSpA, gout and OA patients by the Exoquick kit combined ultracentrifugation method. TMT labeled quantitative proteomics technique was used to compare the proteomics of SF-derived exosomes. Volcano plot, hierarchical cluster, Gene Ontologies (GO), Encyclopedia of Genes and Genomes (KEGG) pathway analysis were conducted.

Results: A total of 1678 credible proteins were detected. With the cut off criteria of $|\log_2(\text{fold-change})| \geq 1.2$ and $p\text{-value} < 0.05$, 267 (140 up-regulated and 127 down-regulated) differential proteins were found in OA vs gout, 291 (179 and 112) in axSpA vs OA, 515 (109 and 406) in RA vs axSpA, 298 (191 and 107) in axSpA vs gout, 462 (160 and 302) in RA vs gout, 536 (170 and 366) in RA vs OA. GO analysis showed that the biological progress of differential proteins were mainly enriched in the "immune response". Regarding the molecular function, the differential proteins mainly mediated "antigen binding". GO analysis of the cellular components indicated that most proteins were annotated as "extracellular exosomes". KEGG pathway analysis demonstrated differential proteins were significantly enriched in "complement and coagulation cascades". The hierarchical cluster analysis of the differential proteins in the four groups showed that Lysozyme C and Keratin were more abundant in gout, Hemoglobin and Actin-related protein 2/3 complex subunit 3 in OA, Sodium/potassium-transporting ATPase subunit alpha-1 and Immunoglobulin heavy constant delta in axSpA, Pregnancy zone protein and Stromelysin-1 in RA.

Conclusion: The protein profiles of SF-derived exosomes in RA, axSpA, gout and OA patients were different. The differential proteins were the potential biomarkers of RA, axSpA, gout and OA.

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Figure 1. The hierarchical cluster analysis of differential proteins in axSpA, OA, Gout and RA.

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POS0400

METABOLIC CHANGES INDUCED BY ANTI-MALONDIALDEHYDE/MALINDIALDEHYDE-ACETALDEHYDE ANTIBODIES PROMOTE OSTEOCLAST DEVELOPMENT

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Background: Malondialdehyde (MDA) is a highly reactive compound generated during lipid-peroxidation in conditions associated with oxidative stress. MDA can irreversibly modify proteins (e.g. lysine, arginine and histidine residues). In addition, acetaldehyde can further react with MDA adducts to form malondialdehyde-acetaldehyde (MAA) modification. Such protein modifications can lead to immunogenic neo-epitopes that are recognized by autoantibodies. In fact, anti-MDA/MAA IgG antibodies are significantly increased in the serum of patients with autoimmune diseases, such as rheumatoid arthritis (RA) (1). Interestingly, anti-MDA/MAA antibodies have been shown to promote osteoclast (OC) differentiation *in vitro* suggesting a potential role for these autoantibodies in bone damage associated with RA (1).

Objectives: Little is known about the molecular mechanisms activated by autoantibodies in RA. Here, we elucidate the pathways specifically triggered by anti-MDA/MAA autoantibodies in developing osteoclasts.

Methods: Recombinant human monoclonal anti-MDA/MAA antibodies, which were previously cloned from single synovial B cells of RA patients, were added to different OC assays. OCs were generated from monocyte-derived macrophages in the presence of the cytokines RANK-L and M-CSF. OC development was monitored by light microscopy following tartrate-resistant acid phosphatase staining and by erosion assays using calcium phosphate-coated plates. Bone morphometrics were studied in anti-MDA/MAA-injected mice using X-ray microscopy. Cellular metabolism was analyzed by mass spectrometry, Seahorse XF Analyzer and a colorimetric L-Lactate assay.

Results: Anti-MDA/MAA antibodies induced a robust OC differentiation *in vitro* and bone loss *in vivo*. The anti-MDA/MAA antibodies acted on developing OCs by increasing glycolysis via an Fcγ receptor I-mediated pathway and the upregulation of the transcription factors HIF-1α, Myc and CHREBP. Such regulation of cellular metabolism was exclusively observed in the presence of the osteoclastogenic anti-MDA/MAA clones, whereas other RA-associated autoantibodies (anti-MDA/MAA or anti-citrullinated protein antibodies) had no effect on metabolism. The anti-MDA/MAA treatment induced a shift in the tricarboxylic acid (TCA) cycle activity in developing OCs, leading to the accumulation of citrate and aconitate.

Conclusion: We described a novel type of autoantibody-induced pathway in RA, which might contribute to increased OC activation and a consequent bone loss. Anti-MDA/MAA antibodies promoted osteoclast development by increasing glycolysis and by modulating the TCA cycle through a signaling pathway that included Fcγ receptor I and a network of transcription factors acting on glycolysis. A TCA cycle bias towards citrate production suggests that the anti-MDA/MAA antibodies might stimulate OCs via increasing lipid biosynthesis in the cells.

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