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.

REFERENCES:

- [1] Cretu D, Diamandis E P, Chandran V. Delineating the synovial fluid proteome: recent advancements and ongoing challenges in biomarker research. [J]. Critical reviews in clinical laboratory sciences, 2013,50(2):51-63.
- [2] McArdle A J, Menikou S. What is proteomics? [J]. Archives of disease in childhood. Education and practice edition, 2020.

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

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

K. Sakuraba¹, A. Krishnamurthy², A. Circiumaru², V. Joshua², H. Wähämaa², M. Engström², M. Sun², X. Zheng³, C. Xu³, K. Amara², V. Malmström², S. B. Catrina³, C. Grönwall², B. Réthi², A. Catrina². ¹National Hospital Organization Kyushu Medical Center, Department of Rheumatology, Fukuoka, Japan; ²Karolinska Institutet & Karolinska University Hospital, Department of Medicine, Solna, Stockholm, Sweden; ³Karolinska Institutet & Karolinska University Hospital, Molecular Medicine and Surgery, Stockholm, Sweden

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.

REFERENCES:

- [1] Grönwall C. et al. J. Autoimmunity 84 (2017): 29-45.

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