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SAT0055 JOINT SPECIFIC TNF RESPONSE OF SYNOVIAL FIBROBLASTS IN RHEUMATOID ARTHRITIS

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Background: Synovial fibroblasts (SF) in rheumatoid arthritis (RA) play a major role in chronic inflammation and joint destruction. We showed previously that their epigenetic and transcriptional profile as well as their function vary significantly between different joints. However, it is unknown whether there is a joint-specific inflammatory response of SF.

Objectives: To compare transcriptional changes between SF from hand, shoulder and knee joints after stimulation with the pro-inflammatory cytokine TNF.

Methods: We cultured SF from synovial tissues of hand (metacarpophalangeal and proximal interphalangeal joint), shoulder and knee joints of RA patients. After stimulation of the SF with 10 ng/ul TNF for 24h (n=2 for each joint location), RNA was sequenced on the Illumina HiSeq4000 platform. We analyzed differential gene expression with R v3.5.2 and CuffDiff and DESeq2 packages.

Results: As shown in Figure 1, principal component analysis showed evident separation of joint locations and condition (unstimulated vs TNF stimulated). In a sample-to-sample distance matrix, hand samples (TNF stimulated and unstimulated) grouped apart from shoulder and knee samples (Figure 2).

Of the regulated genes, 26% appeared in all three joint locations and 56% overlapped between knee and shoulder, but only 30% overlapped between hand and shoulder and between hand and knee SF. Similarly, also enriched pathways differed particularly between hands and the more proximal joints.

'Defense response' ($p=8.11 \times 10^{-23}$) and 'cytokine activity' ($p=1.27 \times 10^{-21}$) were the most significantly enriched gene ontology terms for genes regulated in TNF stimulated hand SF. These processes were less prominently enriched in stimulated knee (1.58×10^{-15} and 8.84×10^{-08}) and shoulder SF (2.02×10^{-11} and 3.20×10^{-07}), where 'cell cycle' ($p=2.26 \times 10^{-30}$ in knee and $p=2.18 \times 10^{-32}$ in shoulder), and 'DNA packaging complex' ($p=4.63 \times 10^{-49}$ in knee and $p=8.91 \times 10^{-36}$ in shoulder) were the most significantly enriched gene ontology terms. These processes were not significantly enriched in stimulated hand SF.

Conclusion: SF from different joints in RA react differently to TNF stimulation. In particular hand SF reacted different to TNF stimulation than shoulder and knee SF, which appeared more similar. These qualitative and quantitative differences of the inflammatory response might translate into joint-specific pathotypes of synovitis with distinct therapeutic responses and disease outcomes.

Figure 1. Principal component analysis of TNF stimulated and unstimulated synovial fibroblasts

Figure 2. Sample-to-sample distance matrix of TNF stimulated and unstimulated synovial fibroblasts

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SAT0056

TAS5315, A NOVEL BRUTON'S TYROSINE KINASE INHIBITOR, DEMONSTRATES POTENT EFFICACY AGAINST BONE DESTRUCTION IN AN ANIMAL MODEL FOR RHEUMATOID ARTHRITIS

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Background: Bone erosions and cartilage damages are a pathological hallmark of rheumatoid arthritis (RA) and are associated with poor functional outcome¹. Aberrant activations of osteoclasts induced by receptor activator of nuclear factor κ B ligand (RANKL)² are involved in the bone erosions of RA. It has also been recently shown that under chronic inflammatory conditions such as RA, inflammatory cytokines in joints induce pathological osteoclast differentiation and cause excessive bone resorption independent of RANKL-RANK signaling³. The Bruton's tyrosine kinase (BTK) signaling pathway plays a pivotal role in inflammatory response and bone resorption⁴. Thus, targeting BTK may be efficacious against not only inflammation but also bone erosion by regulating activation of effector cells such as B cells, macrophages and osteoclasts in RA. We have already shown the inhibitory effects of TAS5315 on RANKL-dependent osteoclast activation. However, it remained uncertain whether TAS5315 inhibits osteoclast activation induced by inflammatory cytokines.

Objectives: In this study, we evaluated the effects of TAS5315 on osteoclast activation induced by inflammatory cytokines (*in vitro*) and the infiltration of tartrate-resistant acid phosphatase (TRAP)-positive osteoclasts in the joint of a mouse CIA model.

Methods: *In vitro* biochemical assay was performed using available kinase assay panels. The BioMAP[®] Diversity PLUS panels were used to determine the profile of TAS5315 in primary human cell systems. The effects of TAS5315 on osteoclasts were assessed by examining osteoclast-mediated bone resorption. TAS5315 was orally administrated once a day in an established mouse CIA model. TRAP-positive osteoclasts were counted manually. Bone mineral density (BMD) and bone erosion were assessed using micro-CT analysis. The mechanical properties of the tibia were evaluated by a compression test of proximal metaphysis using a material-testing machine.

Results: TAS5315 selectively inhibited the enzyme activity of BTK and had less off target inhibition against other kinases. In BioMAP[®] systems, TAS5315 decreased the production of IgG and the expression of cytokines (TNF- α , IL-6, IL-17A). TAS5315 also inhibited osteoclast-mediated bone resorption induced by inflammatory cytokines. On the other hand, anti-RANKL antibody did not inhibit bone resorption induced by inflammatory cytokines. Furthermore, in the mouse CIA model, TAS5315 significantly ameliorated paw swelling and pathological changes. TAS5315 significantly decreased the infiltration of TRAP-positive osteoclasts in the joint and also showed improvement of BMD and bone erosion by time-dependent micro-CT analysis. In vehicle-treated mice, the mechanical strength of tibia was decreased compared with normal mice. TAS5315-treated mice recovered the decreased parameters of the mechanical strength compared with vehicle-treated mice.

These data suggests that TAS5315 improves bone erosion in murine model for RA through direct inhibition of osteoclast activation induced by inflammatory cytokines as well as RANKL.

Conclusion: Our study demonstrates that TAS5315 would be an attractive RA therapeutic drug that could improve bone destruction as well as inflammation.

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SAT0057

INCREASED MICRORNA-155 IS ASSOCIATED WITH A SPECIFIC DEFECT OF ANTI-INFLAMMATORY M2 MACROPHAGES POLARIZATION BOTH IN HUMAN RHEUMATOID ARTHRITIS AND IN COLLAGEN-INDUCED-ARTHRITIS MICE

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Background: Monocytes/macrophages are key players in the pathogenesis of Rheumatoid arthritis (RA). Monocytes can differentiate into pro-inflammatory macrophages (M1-like) or into anti-inflammatory macrophages (M2-like). We previously found a defect of monocytes polarization into M2-like macrophages, in RA patients and not in healthy donors (HD) or in patients with other inflammatory diseases (such as connective tissue diseases or spondyloarthritis), and a propensity for preferential maturation towards M1-like pro-inflammatory macrophages that could contribute to synovial inflammation.

MiR-155 expression has been shown elevated in RA synovial macrophages and in blood monocytes. MiR-155 inhibits mRNA of 2 important proteins involved in programming of M2 macrophages (SOCS-1 and C/EBP- β).

Objectives: We assessed if miR-155 could play a role in the defect of monocytes polarization into M2-like macrophages both in human RA and in a mouse model of collagen-induced-arthritis (CIA) mice.

Methods: Monocytes were isolated from PBMCs for HD and RA by negative selection. Purified monocytes were transfected using **AMAXA technologies** with miR-155 mimic (for HD) or antagomiR155 (for RA) or controls miRs. Then monocytes were incubated in the presence of human serum (SAB) (M2-like) for 6 days. Expressions of total macrophages markers (CD11b and CD71), M2-like macrophage markers (CD163, CD206 and Arg-1), M1-like macrophage marker (iNOS) were evaluated by flow cytometry, ELISA or immunofluorescence.

A. Monocytes from healthy donors

B. Monocytes from RA patients

C. Monocytes from CIA or control mice