Finally, we evaluated the association between IL-37 gene polymorphisms and the induction of TI in monocytes of healthy donors with *in vitro* functional studies.

Results: The exogenous administration of IL-37 abrogated the pro-inflammatory effects of TI, significantly reducing the production of pro-inflammatory cytokines and the survival of experimental animals subjected to a model of disseminated infection. The inhibitory effects of IL-37 on TI were also associated with reduced recruitment of neutrophils at sites of inflammation. IL-37 and TI programs had differential and opposite effects on the modulation of cellular energy metabolism of monocytes. In humans, polymorphisms in the IL-37 gene were associated with reduced activation of TI programs and reduced production of inflammatory cytokines by healthy donor monocytes.

Conclusion: In conclusion, IL-37 emerges as an endogenous regulator of TI, which makes this cytokine a potential therapeutic target in immune-mediated pathologies.

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THU0011 PEFICITINIB (ASP015K) INHIBITS MONOCYTE CHEMOTACTIC ACTIVITY VIA PROINFLAMMATORY CYTOKINE PRODUCTION IN RHEUMATOID ARTHRITIS FIBROBLAST-LIKE SYNOVIOCYTES

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Background: Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway has been identified as an important signaling pathway in rheumatoid arthritis (RA) of various cytokines (eg, IL-6). Janus kinase (JAK) is a cytoplasmic protein tyrosine kinase associated with various cytokine receptors. Molecules of signaling pathways such as the JAK family are thought to be promising targets for RA treatment. Peficitinib (ASP015K) is a novel JAK inhibitor in development for the treatment of RA. Peficitinib has been suggested for its effectiveness in clinical trials, but clarification of the mechanism of RA for the inflammatory pathology is still inadequate.

Objectives: We clarified the effect of peficitinib on RA fibroblast-like synovocytes (FLS).

Methods: To determine whether JAK1, JAK2 and JAK3 were expressed in RA ST and FLS, immunohistochemistry was performed. In order to confirm if IL-6 and IL-6 receptor (IL-6R) activate JAK-STAT pathway in FLS, western blot analysis was performed. RA FLS were stimulated with IL-6 (100 ng/ml) and IL-6R (100 ng/ml) for 10 minutes or 30 minutes. Next, we investigated effect of peficitinib on IL-6 and IL-6R responses in RA FLS. RA FLS were stimulated with IL-6 (100 ng/ml) and IL-6R (100 ng/ml) after treated peficitinib (0.1, 1, 5 μM) for 24 h. Furthermore, we performed a proliferation assay of FLS and chemotaxis assay using THP-1 (human acute monocyte leukemia cell line) and peripheral blood mononuclear cells (PBMC) to perform functional analysis by peficitinib. In the same procedure as Western blot analysis, peficitinib (5 μM) was added to FLS and stimulate with IL-6 and IL-6R. Finally, we investigated whether peficitinib suppresses the secretion of FLS inflammatory mediator using ELISA. The amounts of RANTES/CCL5, MCP-1/CCL2, MMP-3, fractalkine/CX3CL1, ENA78/CXCL5 and IL-8 in IL-6 and IL-6R stimulated peficitinib treated RA FLS conditioned medium were determined compared with IL-6 and IL-6R stimulated non treated RA FLS conditioned medium.

Results: We found JAK1, JAK2 and JAK3 were expressed in RA STs and FLS. JAK1 and JAK3 were observed in RA ST lining layers. JAK2 was expressed entirely in RA ST cell nucleus. JAK1, JAK2 and JAK3 were detected in RA FLSs. It was confirmed that JAK1, JAK2 and JAK3 is expressed in FLSs of ST. Representative western blotting showing expression of phospho STAT1, phospho STAT3 and phospho STAT5 were increased 10 minutes after stimulation with IL-6 and IL-6R. Phosphorylation of STAT1, STAT3 and STAT5 in RA FLS was suppressed by concentration dependence of peficitinib. It was proved that peficitinib suppress the activation of JAK-STAT pathway. Furthermore, peficitinib treated RA FLS conditioned medium reduced THP-1 migration compared to non-treated RA FLS conditioned medium (number of THP-1 cells migrated ±

SEM; 42 ± 3 and 66 ± 6 cells migrated, respectively, $p < 0.05$). Peficitinib treated RA FLS conditioned medium also reduced PBMC migration compared to nontreated RA FLS conditioned medium (number of PBMC migrated ± SEM; 36 ± 5 and 63 ± 9 cells migrated, respectively, $p < 0.05$). In addition, peficitinib treated RA FLS showed a 14 ± 2% decrease in proliferation of RA FLS compared with nontreated RA FLS. Finally, we found peficitinib suppress the secretion of inflammatory mediators in RA FLS. MCP-1/CCL2 in RA FLS supernatant was suppressed in peficitinib compared to nontreated (mean ± SEM; 160.1 ± 65.6 and 846.0 ± 107.1 pg/ml, respectively, $p < 0.05$). From these results, suppression of chemotaxis of THP-1 and PBMC was also observed through suppression of MCP-1/CCL2 in RA FLS supernatant.

Conclusion: Peficitinib suppressed the JAK-STAT pathway of RA FLS and was involved in the suppress of monocyte chemotaxis and the proliferation of FLS through suppress of inflammatory cytokines. These results suggest that peficitinib acts on FLS and suppresses inflammatory pathology.

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THU0012 ESTROGEN REGULATES MICRO RNA BIOPROCESSING AND PRODUCTION OF IL9 CYTOKINE WITHIN LEUKOCYTES IN RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis (RA) is more prevalent in females. It is reported to be alleviated during pregnancy, and increase in severity during menopause, which implies estrogen as an important contributor in RA pathogenesis. Micro-RNA (miR) are short, non-coding RNAs, that act within a RNA-induced silencing complex. miRs have recently emerged as important epigenetic controls of leukocyte maturation and function.

Objectives: To study the epigenetic effect of estrogen on the transcription of microRNA and their bio-processor enzymes in RA patients.

Methods: The leukocytes of 145 female RA patients split for estrogen receptor alpha (ERα) (dCT 9.57) were analyzed for the expression of Dicer, Drosha and DGCR8 mRNA by RT-PCR and serum levels of TGFβ-dependent cytokines IL4 and IL9. Micro-RNA transcription array was performed by 3D-Gene™ microarray measuring >2560 miRs (TATAA Bio-center, Gothenburg) in human primary leukocytes, fat tissue and plasma. The samples were split by expression of estrogen receptor alpha (ERα, dCT 9.57), a proxy of an active estrogen signaling, and used to identify miRs of interest. Bioinformatic analysis was performed using DIANA, miRDB, miRTarBase and Ensembl, using microRNA nucleotide sequences obtained from miRBase, to predict signaling pathways and gene targets of miR. To confirm estrogens effect on miR processing, leukocyte cultures of RA patients were exposed to estrogen and subjected to miR, gene and protein analysis.

Results: Comparing leukocytes with different ERα, we identified 214 miRs with high and 7 miRs with low expression when ERα was high. Cross-analysis in the fat and serum sample miR array identified most of those miRs. Bioinformatic analysis of the upregulated miRs confirmed that 16miRs were involved in the estrogen signaling pathway ($p = 0.0063$) and 15 TGFβ signaling pathway ($p < 0.0001$), where these miRs had 61 common predicted gene targets.

To study if transcription of these predicted targets was dependent on estrogen signaling, we took advantage of the clinical RA material. Patients with high ERα were significantly younger (51y vs 61y, $p < 0.00001$), which confirmed active estrogen signaling. High ERα had significantly higher expression of miR bioprocessing enzymes Dicer ($p = 0.0197$), Drosha ($p = 0.0454$), DGCR8 ($p = 0.0192$) and lower disease activity (DAS28, $p = 0.0023$; ESR $p = 0.005$, IL6, $p = 0.0129$).

Exposure of leukocytes to estrogen in culture induced a significant reduction in IL9 ($p = 0.0078$), which suggests a suppression of TGFβ signaling.

Conclusion: High ERα expression can be considered a significant regulator of miR transcription in leukocytes. miR and bio-processors regulated by estrogen could be of importance in the pathogenesis on RA by targeting genes responsible for regulation of inflammation and the non-protective elements of RA development; explaining the ameliorating effects of estrogen.

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