The mean levels of IgG $\alpha\beta$ -GP-1, LA, aOxLDL in SLE patients and mean levels of LA, aOxLDL in RA patients were above standard values but were similar in both groups. Moreover, the mean levels of IgG aCL, IgM $\alpha\beta$ -GP-1, IgG/IgM aAnV and aPT were comparable in patients with SLE and RA. However, the mean levels of IgM aCL in RA patients was higher than in SLE patients. This may indicate an increased autoimmune activity.

Simultaneous elevation in four types of aPL levels was observed in 4.4% patients with RA, in 2.5% - with SLE; simultaneous elevation in three types of aPL was revealed in 11.1% patients with RA, in 17.5% - with SLE; two types of aPL were increased in 35.6% patients with RA and 27.5% - with SLE. Only one type of elevated aPL levels (12.9%) was identified in the control group.

Conclusion: Thus, patients with RA are characterized by a wide range of aPL. Qualitative and quantitative changes in the levels and types of autoantibodies in patients with RA have been established similar to those in SLE patients.

In patients with autoimmune rheumatic pathology, the presence of simultaneously elevated several types of aPL have been proved.

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AB0088

N6-METHYLADENOSINE-MODIFIED CIRC_0088194 PROMOTES MIGRATION AND INVASION OF RHEUMATOID ARTHRITIS FIBROBLAST-LIKE SYNOVIOCYTES THROUGH MIR-766-3P/MMP2 AXIS

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Background: Circular RNAs (circRNAs) participate in the initiation and progression of various diseases by miRNA sponges including postmenopausal osteoporosis[1], bladder cancer[2], and osteoarthritis (OA)[3]. However, the activity of circRNAs as "miRNA sponges" in rheumatoid arthritis (RA) has not been studied.

Objectives: To investigate whether circRNA acts as competing endogenous RNA to regulate pathological processes of RA, and whether the N6-methyladenosine (m⁶A) modification affects Circ_0088194 stability in RA fibroblast-like synoviocytes (RA-FLSs).

Methods: CircRNA microarray analysis was conducted to characterize the expression profiles of circRNAs in 3 RA-FLSs and 3 osteoarthritis fibroblast-like synoviocytes (OA-FLSs). Methylated RNA immunoprecipitation was performed to validate the level of m⁶A modification on Circ_0088194 in RA-FLSs and OA-FLSs. Dual-luciferase reporter assay, bioinformatics analysis, protein array analysis, and fluorescence in situ hybridization were employed to evaluate the interaction between Circ_0088194 and miR-766-3p, and between target miR-766-3p and matrix metalloproteinase 2 (MMP2).

Results: Overexpression of Circ_0088194 promoted the migration and invasion of RA-FLSs and increased MMP2 expression. The expression and function of miR-766-3p were inversely correlated with Circ_0088194, which sponged miR-766-3p to upregulate MMP2 expression. Although m⁶A modification of Circ_0088194 exists in RA-FLSs and OA-FLSs, their level did not differ.

Conclusion: This study presents an important role of this novel circRNA as a sponge of miR-766-3p to promote RA-FLS migration and invasion by targeting MMP2. However, the modification might not affect Circ_0088194 stability in RA-FLSs and OA-FLSs. Therefore, Circ_0088194 may contribute to RA development and represent as an auspicious therapeutic target.

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AB0089

THE ANALYSIS FOR THE INHIBITION OF ANGIOGENESIS BY JAK INHIBITOR

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Background: Many blood vessels are generated in the hyperplastic synovial tissue of patients with rheumatoid arthritis (RA), and lead to chronic tissue inflammation and joint destruction [1]. Janus kinase (JAK) family consisting of JAK1, JAK2, JAK3 and tyrosine kinase 2 (TYK2) are chain receptors which phosphorylate signal transducers and activators of transcription (STAT) and mediate inflammatory diseases including RA [2]. Nowadays, several JAK inhibitors such as Tofacitinib (TOF), Baricitinib (BAR) and Peficitinib (PEF) have been developed and demonstrated to have the inhibitory effects on inflammatory arthritis [3-5]. However, there were few reports concerning their effects on angiogenesis in vitro.

Objectives: The purpose of the present study is to investigate the influence of JAK inhibitors on angiogenesis of human umbilical vein endothelial cell (HUVEC) activated by vascular endothelial growth factor (VEGF).

Methods: The cell line of HUVECs were used for this study. The activity of proliferation and tube formation were analyzed by counting assay and tube formation assay, respectively.

In counting assay, HUVECs (5×10^4 cells/ml) were seeded onto 96-well cell culture plate with 20ng/ml VEGF including various doses (0.1 μ M, 1 μ M, 5 μ M) of TOF, BAR or PEF. After 48 hours incubation at 37°C in a humidified atmosphere containing 5% CO₂, cell proliferation of each groups was assessed using cell counting kit. In tube formation assay, HUVECs (5×10^4 cells/ml) were treated with 20ng/ml VEGF including various dose (0.1 μ M, 1 μ M, 5 μ M) of TOF, BAR or PEF for 00 hours, then seeded onto 48-well plate applied with Matrigel. After 24 hours incubation on Matrigel, the capillary-like tube formation of each well was photographed using phase contrast microscopy. Tube formation were quantitated by measurement of the length of branch.

Results: HUVECs were activated in proliferation and tube formation by VEGF treatment. And, the proliferation and tube formation of HUVECs activated by VEGF were suppressed by All of TOF, BAR and PEF. In particular, TOF and PEF could suppress them highly.

Conclusion: This study showed the inhibitory effect of JAK inhibitors on proliferation and tube formation of HUVECs activated by VEGF. In particular, the angiogenesis of HUVECs activated by VEGF was highly suppressed by TOF and PEF. VEGF is reported to regulate the angiogenesis through multi JAK-STAT signaling pathways [6]. The inhibitory effects on angiogenesis of TOF, BAR and PEF might depend on the differences in their affinity for JAKs. VEGF has been shown to have a central involvement in the angiogenic process in RA [7]. JAK inhibitors might suppress the angiogenesis in RA synovial tissues by inhibiting VEGF signaling.

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AB0090

DEATH RECEPTOR 3 REGULATES THE GENE EXPRESSIONS OF VARIOUS KEY MOLECULES IN RHEUMATOID SYNOVIAL FIBROBLASTS

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Background: Rheumatoid arthritis (RA) is a chronic inflammatory disease that causes hyperplasia of synovial tissue. Death receptor 3 (DR3) is a tumor necrosis factor receptor and binds to TL1A, a member of the TNF family. DR3 is involved in the mechanism of cell proliferation and apoptosis through NF- κ B signaling. Suppression of DR3 in rheumatoid synovial fibroblasts (RA-FLS) is associated with hyperplasia of rheumatoid synovial tissue [1]. We previously revealed the expression profiles regulated by TL1A, suggesting that TL1A might affect the pathogenesis of RA, including proliferation, regulation of B cells and T cells, inflammation, and cytokine processing [2].

Objectives: In this study, we investigated the gene expression profiles regulated by DR3 in RA-FLS to reveal how DR3 is involved in the pathogenesis of RA.

Methods: RA-FLS were from patients with RA. Four individual lines of primary cultured RA-FLS were incubated either with 1000ng/ml of human DR3-Fc

protein or 1000ng/ml of human IgG1 as a control for 12h. Gene expressions were detected by microarray assay.

Results: Microarray data analysis revealed that DR3 up-regulated or down-regulated the expression of various genes in RA-FLS (Figure). The function of regulated genes included protein-L-isoaspartate (D-aspartate) O-methyltransferase activity, carboxyl-O-methyltransferase activity, protein carboxyl O-methyltransferase activity, regulation of cilium assembly, O-methyltransferase activity, regulation of plasma membrane bounded cell projection assembly, regulation of cell projection assembly, regulation of organelle assembly, protein methyltransferase activity, and S-adenosylmethionine-dependent methyltransferase activity. The most up-regulated 2 genes by DR3 were KIAA1109 (KIAA1109), and adhesion G protein-coupled receptor A3 (ADGRA3). The most down-regulated 2 genes by DR3 were RNA exonuclease 2 (REXO2), and family with sequence similarity 120A (FAM120A).

Conclusion: In this study, we first revealed the expression profiles of genes regulated by DR3 in RA-FLS. KIAA1109/TENR/IL2/IL21 gene is strongly associated with RA in European descent populations [3]. ADGRA3 is a member of G protein-coupled receptors (GPCRs). GPCRs associates with the regulation of cytoskeletal organization, the cell adhesion and migration, cell proliferation and apoptosis, and cell differentiation [4]. Loss of REXO2 affects cell growth and morphology [5], and REXO2 was identified as a target gene for inflammatory bowel disease-associated variants [6]. FAM120A regulates activity of Src kinase to protect cells from oxidative stress-induced apoptosis [7]. DR3 regulates the gene expressions of various key molecules in RA-FLS and may affect the pathogenesis of RA by regulating gene expression of RA-FLS.

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AB0091

PD-1 AND GAL3 REGULATE OSTEOCLAST DEVELOPMENT IN RHEUMATOID ARTHRITIS

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Background: Bone erosions in rheumatoid arthritis (RA) is a major complication. Despite improved treatment, erosions still occur and progress. Therefore, a continuous investigation of the interplay between bone regulation and immune activity is needed.

Co-inhibitory receptors, like CTLA-4, participate in modulating osteoclast activity¹, and blocking these receptors in cancer treatment results in autoimmune disease². Programmed death 1 (PD-1) is a central co-inhibitory receptor, also present in a soluble (s) form. We have previously shown that sPD-1 is associated with disease activity and reduced bone erosions in RA³. PD-1 and its ligands are glycosylated and the glycosylation affects signaling through the PD-1 pathway^{4,5}. Galectins (GAL) bind to specific glycosylation patterns on glycoproteins by their carbon recognition domain. Gal-3 can bind to multiple immune receptors shaping the immunological response⁶.

Objectives: We aimed to investigate if PD-1 and GAL-3 modulate osteoclastogenesis in RA.

Methods: Plasma and synovial fluid (SF) samples were collected from patients with chronic (c) RA (>8 years of disease, n=14) SF and blood samples were obtained when patients presented with disease flare. Soluble PD-1 and GAL-3 were investigated by ELISA. GAL-3:PD-1 complexes were captured in an optimized ELISA using both GAL-3 and PD-1 antibodies.

Surface plasmon resonance was used to evaluate on the binding properties between GAL-3 and PD-1, on a Biacore 3000. Lactose was used to block the potential binding. Cells from the synovial fluid (SFMC) were differentiated into osteoclasts with M-CSF and RANKL, recombinant human (rh) PD-1 and rhGAL-3 were added and osteoclast formation evaluated by TRAP in the supernatant.

Results: Soluble PD-1 and GAL-3 were present in both plasma and SF from cRA patient, and the ratio (PD-1/GAL-3) was increased in SF. PD-1:GAL-3 complexes were detected in both plasma and SF, with the highest concentration in SF. The binding between PD-1 and GAL-3 was confirmed by surface plasmon resonance analyses, with an estimated K_d of 5uM. Binding could be blocked by addition of lactose, confirming the binding to be glycan dependent. In SFMC osteoclast cultures, rhPD-1 and rhGAL-3 slightly decreased osteoclast formation evaluated by TRAP. However, combining rhPD-1 and rhGAL3 further potentiated the reduction in osteoclast formation by 37%.

Conclusion: We confirm glycan dependent binding between the co-inhibitory receptor PD-1 and GAL-3. Both sPD-1 and Gal-3, and the PD-1:GAL-3 complexes, are upregulated in the inflamed joint at site of erosions. *In vitro* RA culture demonstrates that GAL-3 potentiate the functions of PD-1 and reduces osteoclastogenesis. These findings indicate that the binding between Gal-3 and PD-1 could provide a novel target to control erosions in RA. Future *in vivo* studies on this interaction is needed.

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AB0092

FIBRIN ADHESION IS A PANNUS-INDEPENDENT MECHANISM OF CARTILAGE DEGENERATION IN RHEUMATOID ARTHRITIS

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Background: Current concepts of cartilage destruction in inflammatory arthritis include pannus infiltration by inflamed synovial tissue as well as direct detrimental effects of inflammatory cytokines and proteinases. Fibrin maintains chronic inflammatory processes in arthritis but has never been shown to be directly involved in cartilage damage occurring in rheumatoid arthritis (RA).

Objectives: To investigate fibrin-mediated cartilage degradation and the possible underlying mechanisms in arthritis.

Methods: Human cartilage samples were obtained from patients with RA undergoing joint replacement and investigated by H.E. and immunohistochemistry for cartilage damage and fibrin deposition. Cartilage explants from RA patients were incubated *in vitro* with autologous synoviocytes and assessed by immunohistochemistry for cell-adhesion and colocalization with fibrin. Experimental RA was studied in the RA murine model of adjuvant-induced arthritis (AIA), in wildtype (WT) and fibrinogen deficient (Fg^{-/-}) mice. Cartilage damage and chondro-synovial adhesion were analyzed by safranin-O staining and fibrin deposition by immunohistochemistry. Fibrinogen expression (*Fga*, *Fgb*, *Fgy*) was studied in murine primary chondrocytes by qRT-PCR. Cartilage explants were stained with alizarin-red staining and assessed for colocalization of calcific deposits and fibrin. Calcification of murine primary chondrocytes stimulated with secondary calciprotein particles (CPP) and treated with purified human plasma fibrinogen (100 µg/ml) was assessed by alizarin red staining and gene expression for chondrocytic differentiation (*Agg*, *Col2*, *Col10*, *Sox9*, *Runx2*), calcification (*Alpl*, *Ank*, *Anx5*, *Pc1*, *Pit1*, *Pit2*), and extracellular matrix remodeling (*Adams4*, *Adams5*, *Mmp3*, *Mmp13*, *Comp*) by qRT-PCR.

Results: Abundant fibrin deposition on cartilage co-localized and positively correlated with cartilage damage in knee joints of patients with RA. In the AIA model,