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.

Objective: The purpose of the present study is to investigate the influence of JAK inhibitors on angiogenesis of human umbilical vein endothelial cells (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 × 10^5 cells/ml) were seeded onto 96-well culture plate with 20ng/ml VEGF including various doses (0.1µM, 1µM, 5µM) 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 × 10^5 cells/ml) were treated with 20ng/ml VEGF including various dose (0.1µM, 1µM, 5µM) 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 structures were photographed using phase contrast microscopy. Tube formation was 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.

References:

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Scientific Abstracts

PD-1 and GAL-3 were investigated by ELISA. GAL-3:PD-1 complexes were detected in patients with chronic (c) RA (>8 years of disease, n=14) SF and blood samples. Methods: Patients with disease activity and reduced bone erosions in RA. Objectives: To investigate if PD-1 and GAL-3 modulate osteoclastogenesis in RA. 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.

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 cytokoskeletal 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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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 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 (Fgα, Fgβ, Fgγ) was studied in murine primary chondrocytes by qRT-PCR. Cartilage explants were stained with alkaline-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 safranin-O staining and gene expression for chondrocytic differentiation (Agg, Coll2, Col10, Sox9, Runx2), calcification (Alp, Ank, Anx5, Ptc1, Pit1, Pit2), and extracellular matrix remodeling (Adamts4, Adamts5, 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, surface plasm resononance was used to evaluate 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 and 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 plasm resononance analyses, with an estimated Kd of 5μM. 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 potentiates the function 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.

References:

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