AB0122

RP53 INDUCES ECTODOMAIN SHEDDING OF TNF RECEPTOR 1 AND THEREBY INHIBITS INFLAMMATORY RESPONSES IN RHEUMATOID FIBROBLAST-LIKE SYNOVIOCYTES

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Background: Rheumatoid arthritis (RA) is a chronic inflammatory disease by autoimmune disorder that primarily affects joints. Usually RA has been treated with disease modifying anti-rheumatic drugs but biological response modifiers has developed the treatment of RA. Among these anti-tumour necrosis factor (TNF) agents were the first to be successfully used in treating RA. Various anti-TNF- α therapy might be lead to substantial functional improvement in RA patients.

Objectives: We studied the isolated two polypeptides (Rp53, Rp54) from *Rubia philippinensis*, traditional medicine plant, about anti-inflammation effects in fibroblast-like synoviocytes (FLS) derived from patients with RA.

Methods: The effects of polypeptides on anti-inflammation were measured by cytokine assay kits (TNF- α , IL-6). The underlying for NF- κ B signalling pathway was examined by western blot and NF- κ B reporter activity. We examined the effect of Rp53 on the formation of the TNFR1 signalling complex, recruitment of TRADD, RIP in response to TNF by immunoprecipitation experiments. To determine whether Rp53 induced TNF receptor 1 shedding were exposed to these compounds for 1 hour, and then culture media and cell lysates were analysed by Western blotting using anti-TNF receptor 1 antibody.

Results: Pretreatment with Rp53 resulted in a remarkable decrease of the secretion of TNF-induced proinflammatory cytokines TNF- α and IL-6 in RA-FLS. Rp53 strongly inhibited the nuclear factor κ B (NF- κ B) signalling pathway induced by TNF- α , but not that induced by IL-1 β . Analysis of the upstream signalling events affected by Rp53 revealed that it strikingly inhibited the TNF-induced recruitment of TNFR1-associated death domain protein (TRADD) and receptor-interacting protein (RIP) to TNFR1. Rp53 reduced the interaction with TNFR1 to TNF cytokines and enhanced the activation of the p38 mitogen-activated protein (MAP) kinase. Rp53, the polypeptides induced the proteolytic cleavage of TNF-R1 and its release into the culture medium by shedding of TNF receptor 1 ectodomain by TNF- α -converting enzyme (TACE). Along with the TACE inhibitor TAPI-2, the p38 kinase inhibitor SB203580 suppressed the ectodomain shedding of TNF receptor 1 induced by Rp53.

Conclusions: Rp53 induces the TACE-dependent ectodomain shedding of TNF receptor 1 through the activation of p38 MAP kinase, and thereby inhibits the TNF- α induced NF- κ B signalling pathway in RA-FLS cells.

Disclosure of Interest: None declared

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AB0123

EXPLORING THE METABOLOMIC PROFILE OF SYNOVIAL FLUID TO REFLECT THE DISEASE ACTIVITY IN RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis (RA) is a chronic, inflammatory disease that mainly affects the synovial joints. Metabolomics, which is defined as the comprehensive analysis of the small-molecule metabolites in a biological system, is a rapidly developing approach in biomarker research.

Objectives: This study was to determine whether there is variation in the synovial fluid (SF) metabolome in RA patients according to the degree of disease activity using gas chromatography/time-of-flight-mass spectrometry (GC/TOF-MS), in order to gain more insight into the pathologic metabolic alterations in RA.

Methods: SF samples were analysed in 47 patients with active RA, divided into moderately active- (2.8<DAS28 ESR<5.1, n=35, male 28.6%, mean age 55.1 years) and highly active group (DAS28-ESR $\geq$ 5.1, n=12, male 41.7%, mean age 59.1 years). SF metabolite profiles of RA based on DAS28-ESR value were performed using GC/TOF MS, with univariate analysis such as student t-test and correlation analysis followed by multivariate analysis such as partial least-squares regression-discriminant analysis (PLS-DA) and pathway analyses such as metabolite set enrichment analysis.

Results: There was no differences in demographic data and medications use between two groups. A total of 123 metabolites were identified from samples. These 123 identified metabolites were classified into various chemical classes, such as amino acids (21% of identified metabolites), organic acids (20%), sugar

and sugar alcohols (19%), fatty acids (15%), amines (11%), and phosphates (6%). The SF metabolite profiles obtained from GC/TOF-MS analysis can distinguish moderately active group from highly active group. The variation values of the PLS-DA model are R^2X of 0.171, R^2Y of 0.849, and Q^2 of 0.504, respectively, indicating strong explanation and prediction capabilities of the model. To find potential metabolic biomarkers reflecting the disease activity of RA, variable importance on projection (VIP) values of all the metabolites from the PLS-DA model were determined. Also, other statistical criteria including correlation coefficient and P value were assessed. Twelve potential metabolites reflecting the disease activity of RA are found (2-hydroxyvalerate, fucose, tryptophan, indole-3-lactate, isothreonate, thymine, phenylalanine, lactose, arabitol, and mannose-6-phosphate, citrate, oxoproline). In pathway analysis, we found six unique pathways, namely, fructose and mannose degradation, phenylalanine and tyrosine metabolism, citric acid cycle, galactose metabolism, tryptophan metabolism and pyrimidine metabolism that were significantly associated with disease activity in RA.

Conclusions: Synovial metabolite profiles are robustly altered along the disease activity of RA. Metabolomic approaches based on GC/TOF-MS could provide valuable information on the underlying pathogenic mechanisms of RA activity, and a novel perspective on the search of new biomarkers and drug targets.

Disclosure of Interest: None declared

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AB0124

DETECTION OF PRECURSORS OF RANK-OSTEOCLAST-LIKE CELLS (OLCS) IN PERIPHERAL BLOOD AND OLCS IN BONE TISSUE FROM RHEUMATOID ARTHRITIS PATIENTS

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Background: Previously, we reported that novel osteoclast-like cells (OLCs) were induced, both *in vitro* and *in vivo*, from mouse bone marrow-derived macrophages (BMMs) by addition of a combination of TNF α and IL-6¹. Recently, O'Brien *et al* showed that TNF/IL-6 can drive osteoclastogenesis in BMMs from *RANK*-deficient mice².

Objectives: We aimed to examine the differentiation of OLCs, which were induced by a combination of TNF α and IL-6 from human peripheral blood mononuclear cells (PBMCs) and CD14 +monocytes and to identify differences in molecular expression patterns between OLCs and conventional osteoclasts. Furthermore, we identified OLCs and osteoclasts on the bone tissue of the joint in patients with rheumatoid arthritis (RA).

Methods: PBMCs and CD14 +monocytes from healthy volunteers and/or RA patients were stimulated with TNF α and IL-6 or RANKL. Quantitative RT-PCR was used to measure mRNA expression levels of *RANK*, *cathepsin K*, *calcitonin receptor*, and *dendritic cell-specific transmembrane protein*. Prepared undecalcified tibial bone from 6 RA or osteoarthritis (OA) patients undergoing joint surgery were stained by tartrate-resistant acid phosphatase (TRAP) staining and immunohistochemistry with anti-RANK antibody, expression of which were analysed. Osteoclasts and OLCs were identified as multinucleated TRAP+/RANK+ cells and TRAP+/RANK- cells, respectively, adherent to the bone surface.

Results: The number of OLCs treated with a combination of TNF α and IL-6 from PBMCs or CD14 +monocytes in RA patients was significantly increased compared to that in healthy volunteers. Expression levels of *RANK* mRNA was clearly up-regulated in osteoclasts, and was obviously down-regulated in OLCs compared to that in osteoclast precursors. In cancellous bone, the number of TRAP+/RANK+ osteoclasts was significantly increased in RA patients compared to that in OA patients. Interestingly, numerous TRAP+/RANK- OLCs were present in the cancellous bone of RA patients, while almost none were observed in the cancellous bone of OA patients.

Conclusions: The combination of TNF α and IL-6 strongly induced the differentiation of OLCs from PBMCs or CD14 +monocytes in RA patients. OLCs was characterised with TRAP+/RANK- multinucleated cells, which can distinguish conventional TRAP+/RANK+ multinucleated osteoclasts. TRAP+/RANK- OLCs also were present in the bone tissue of RA patients. These results suggest that conventional osteoclasts and novel OLCs could be involved in the pathogenic mechanisms of inflammatory bone destruction such as RA.

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