AB0060

METHOTREXATE DOWNREGULATES P-GLYCOPROTEIN EXPRESSION AND INHIBITS THE ACTIVATION OF JAK2/STAT3 PATHWAY IN RHEUMATOID ARTHRITIS PERIPHERAL BLOOD MONONUCLEAR CELLS

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Background: Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by abnormal synovial hyperplasia, inflammatory cell infiltration, and destruction of cartilage or bone. Early use of disease-modifying anti-rheumatic drugs (DMARDs) can significantly improve the patient's condition. However, many patients had no response after treatment or gradually decreased after treatment for some time. DMARDs Multidrug resistance(MDR) is an important cause of the above phenomenon[1]. How to reverse MDR in RA patients, and then control the disease is a major problem in RA treatment. The mechanism of MDR is complex. ATP binding cassette transporter super family consists of 48 transporters, ABCB1/P-glycoprotein (P-gp) is one of the major proteins associated with MDR[2]. The expression of P-gp is induced not only by genotoxic mechanisms, but also by inflammatory cytokines such as IL-2, IL-4, IL-6, and TNF-α in some inflammatory diseases[3,4].

Objectives: The multiple drug resistance(MDR) to disease modifying anti-rheumatic drugs(DMARDs) is the key factor in Rheumatoid arthritis(RA) treatment field. P-glycoprotein (P-gp) encoded by multidrug resistance gene 1 (MDR1) is involved in the excretion of numerous DMARDs. We investigated the effects of methotrexate(MTX) alone and combined with interleukin 6 (IL-6) on P-gp expression and examined the signaling pathway involved in Peripheral blood mononuclear cells of RA patients.

Methods: The Peripheral blood mononuclear cells were extracted from Fifteen RA patients who were without any DMARDs and biological agents treatment. These cells were treated with IL-6, IL-6+MTX(0.01ug/m), IL-6+MTX (0.1ug/m), IL-6+MTX (1ug/m) for 72h. P-gp expression was measured by flow cytometry and real-time polymerase chain reaction (RT-PCR); JAK2 and STAT3 were measured by RT-PCR.

Results: Methotrexate produce a dose-responsive reduction of both P-gp, JAK2 and STAT3 expression induced by IL-6.

Conclusion: Methotrexate downregulates p-glycoprotein expression and inhibits the activation of JAK2/STAT3 pathway in rheumatoid arthritis peripheral blood mononuclear cells.

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AB0061

INVESTIGATING THE ROLE OF TGF-B AND FATIGUE IN CHRONIC FATIGUE SYNDROME

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Background: Chronic fatigue syndrome (CFS) is estimated to affect up to 5% of people in Europe and is more common in women than men. It is characterised by unexplained fatigue, post-exertional malaise and a range of other symptoms. Recent studies indicate potential immune dysfunction in CFS, specifically regarding cytokines and the adaptive behavioural response.

Objectives: This study aims to investigate serum transforming growth factor-beta (TGF-β) and the expression of the TGF-β Receptor 1 (TGFBR1) and TGF-β Receptor 2 (TGFBR2) genes, in relation to the fatigue associated with CFS.

Methods: Serum active and total TGF-β concentrations were measured in 117 CFS patients and 40 HCs using a TGF-β responsive luciferase bioassay. Expression levels of TGFBR1 and TGFBR2 were analysed using quantitative PCR. Fatigue was measured using the fatigue impact scale (FIS)[1]. FIS was categorised into three groups; 'mild' (0-80), 'moderate' (81-120) and 'severe' (121-160). Linear and ordinal regressions were performed on the continuous FIS and FIS categories respectively.

Results: Serum TGF-β concentrations in the CFS group did not differ significantly compared with the HC group (p=0.58). TGF-β concentrations showed no correlation with disease duration but there was a trend towards decreased TGF-β with increasing symptom duration. There were no significant differences between the levels of TGFBR1 and TGFBR2 in any of the fatigue groups, or between HCs. Active TGF-β concentrations were significantly elevated in the 'severe' FIS group compared to the 'mild' FIS group (p=0.04). Active/total TGF-β levels were significantly higher in the 'severe' FIS group than the 'mild' and 'moderate' FIS groups (p=0.02, p=0.03 respectively).

Conclusion: These data suggest no differences in serum concentrations of TGF-β or expression of TGFBR1 and TGFBR2, between the HC and CFS groups. It also suggests no differences in expression levels of TGFBR1/2 between any of the CFS fatigue groups. However, active/total TGF-β levels were increased in more severely fatigued patients based on FIS. This finding could be due to higher levels of circulating TGF-β, or increased amounts of TGF-β activation. Further work is necessary to confirm this finding in a larger cohort of CFS patients, and to explore how this increase in TGF-β relates to fatigue.

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AB0062

P75NTR IN THE MODULATION OF INFLAMMATORY RESPONSE MEDIATED BY SYNOVIAL FIBROBLASTS

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Background: Our previous studies[1] showed high expression levels of p75NTR, the nerve growth factor (NGF) receptor, in mononuclear cells (MNCs) obtained from blood and synovial fluids of patients with juvenile idiopathic arthritis (JIA) and rheumatoid arthritis (RA). p75NTR binds with high affinity proNGF, the immature form of NGF whose concentration, as we recently demonstrated[2], is extremely high in the synovial fluids of arthritis patients. In ex vivo experiments we demonstrated that recombinant proNGF increases inflammatory cytokine production in patient MNCs, an effect that was abolished using p75NTR inhibitors[2].

We also found that synovial fibroblasts (SFs) represent the main source of proNGF in the inflamed synoviae.

At present it is not known whether proNGF can influence the activity of SFs that are key effector cells in synovia inflammation producing inflammatory mediators that regulate chondrocytes and osteoblasts activation.

Objectives: To investigate the mechanisms regulating p75NTR expression in synovial fibroblasts of arthritis patients and to evaluate the effects of its inhibition on the inflammatory response.

Methods: SFs from arthritis patients were used to study the activity of proNGF/p75NTR axis. SFs from osteoarthritis patiens (OA) and skin fibroblasts from healthy donors (HD) were used as controls. p75NTR, NGF, and cytokine transcripts were evaluated by quantitative PCR (qPCR). Protein expression of p75NTR, NGF, and proNGF were analyzed by Western Blot. ELISAs were used to evaluate NGF, proNGF and cytokine concentration in supernatants and synovial fluids. p75NTR was inhibited using LM11A-31, a synthetic inhibitor that blocks the binding between p75NTR and its specific ligand proNGF.

Results: mRNA and protein expression of p75NTR were up-regulated in arthritis SFs compared to OA SFs and skin fibroblasts. *In vitro* stimulation with recombinant cytokines (IL-1β, TNF, IL-6), TLR-ligands (such as LPS), and JIA synovial fluids, containing a mixture of inflammatory mediators, strongly increased p75NTR mRNA expression in arthritis SFs. Interestingly, after stimulation we observed that also OA SFs and HD fibroblasts up-regulated p75NTR transcript, suggesting that p75NTR levels are modulated by the inflammatory mediators.

SFs of arthritis patients spontaneously produced proNGF and its release was further enhanced by all the above-mentioned inflammatory stimuli, albeit with different ability. Thus patient SFs can both produce proNGF and bind it through p75NTR. The inhibition of proNGF binding to p75NTR using LM11A-31 in arthritis SFs, activated with inflammatory cytokines or with synovial fluids, significantly reduced the expression and release of pro-inflammatory cytokine.

Conclusion: In addition to inducing p75NTR up-regulation, inflammatory stimuli increase the release of proNGF in arthritis SFs. Autocrine proNGF binds to p75NTR and further enhances pro-inflammatory cytokine production, creating a vicious circle that amplifies the inflammatory response. Blocking the binding of endogenous proNGF to its receptor p75NTR strongly reduces the production of inflammatory mediators and prospects the use of p75NTR inhibitors as a new therapeutic approach to chronic arthritis.

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AB0063   **A SYSTEMS APPROACH TO INVESTIGATE INFLAMMATION RESOLUTION BY MULTICOMPONENT MEDICINAL PRODUCT TR14**

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Background: Acute inflammation is a nonlinear, spatio-temporal process for the removal of invading pathogens and the repair of damaged tissues to re-establish homeostasis. If not resolved, it leads to chronic inflammatory clinical phenotypes. The current medical paradigm is based on using single-molecule inhibitors aiming to block certain molecular pathways involved in inflammation. Complex diseases, where large numbers of feedback mechanisms play a central role, require multi-target interventions [1]. Dynamics of feedback mechanisms associated with multiple therapeutic checkpoints require systems biology approaches.

Objectives: The objective of our study was to construct a comprehensive molecular interaction map (MIM) of acute inflammation and its resolution. We further aimed to understand the effect of Traumeel (Tr14), a multicomponent drug, on inflammation resolution, by mapping previously published Tr14 transcriptomics data [2] from a wound healing murine model onto the MIM.

Methods: We constructed a comprehensive MIM using damage-, and pathogen-associated molecular patterns (DAMPs and PAMPs) and established disease genes from selected acute inflammatory clinical phenotypes as seed molecules. From the MIM, we identified feedback loops (FBLs) with the NetDS Cytoscape plugin using our previously published methodology [1]. All FBLs were merged to create a core regulatory network responsible for the overall dynamics of acute inflammation initiation, transition and resolution.

Using Tr14 transcriptomics data to identify acute inflammatory processes enriched at various time points, we combined RPKM values of each individual splice variant into the parent gene using the RSEM method [3]. The expression profile of whole genes was favored due to the lack of splice variant-specific ontology data. Further, the fold change expression profile for each gene was calculated by comparing Tr14-treated vs untreated mice. These data were mapped to the core regulatory network, and time point-specific sub-networks were identified by keeping nodes with absolute fold change expression of ≥ 1.5 and a p-value ≤ 0.05. Furthermore, we identified enriched immune-related processes regulated by Tr14 at each time point using the Cytoscape ClueGo app.

Results: The MIM has, as of January 2019, 3300 interactions, which are divided into 24 functional modules based on acute inflammation-related gene ontology terms. From the MIM, we identified 435 FBLs which were merged to extract a core regulatory network. After mapping of Tr14 gene expression fold change data at time points 12h, 24h, 36h, 72h, 96h, 120h and 192h, we identified that large numbers of FBLs were differentially regulated at early time points. This number decreased substantially at later time points. At time point 192h, we could identify only 6 differentially regulated nodes with no specific role in immune regulation, indicating that the inflammation was resolved mostly before 192h after injury. Combined expression profile of all the genes associated with the "increase acute inflammation" ontology showed an approximately 24hrs shift towards faster resolution in Tr14-treated mice.

Conclusion: Molecular interaction maps facilitate gene expression data analysis to identify enriched molecular processes and cell/tissue specific-phenotypes. Tr14-induced gene expression changes can be linked to inflammation-related biological functions as defined by enriched GO terms. Our analyses suggest that Tr14 modulates multiple network components at early time points, which can be linked to inflammation resolution.

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AB0064   **SERUM TENASCIN-C LEVELS ARE ELEVATED IN PATIENTS WITH AXIAL SPONDYLOARTHRITIS**

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Background: Tenascin-C (TNC) is a pro-inflammatory extracellular matrix glyco-protein that is synthesized in various pathological conditions. TNC induces inflammatory activity and promotes damage of joints. Its expression in adults is restricted to sites of tissue injury, particularly during phases of inflammation and active tissue remodelling. Only a few studies have described elevated serum TNC levels in ankylosing spondylitis (AS) compared to healthy controls (HC).

Objectives: The aim of this study was to examine the levels of serum TNC among different axial spondyloarthritis (axSpA) subsets and whether TNC levels are related to disease activity measures or other clinical features.

Methods: Sixty-one patients who fulfilled the Assessment of SpondyloArthritis international Society (ASAS) classification criteria for axSpA and twenty age and sex-matched HC were included in this study. Based on imaging, patients were further classified as AS (n = 45) and as nr-axSpA (n = 16). Patients with AS were further divided into two subsets based on the absence (n=22) or presence of syndesmophytes (n=23).

TNC serum levels were determined using ELISA. The following data were collected: clinical and laboratory disease activity measures; demographic status; disease-related factors such as the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and CRP levels. Statistical analyses were performed with GraphPad Prism 5.1. The data are presented as the median and interquartile range.

Results: TNC serum levels were elevated in axSpA patients (535.3 (457.7-677.2) ng/mL) compared to HC (432.1 (329.1-565.9) ng/mL, p= 0.007). Dividing axSpA into nr-axSpA and AS subsets the difference was observed only between AS patients and HC (535.3 (434.5-677.2) vs. 432.1 (329.1-565.9) ng/mL, p=0.022). TNC serum levels did not correlate with disease activity biomarkers (serum CRP or BASDAI) in patients with axSpA. Although we have not observed correlation between TNC and mSASSS radiographic score, weak correlation with disease subsets was found (r=0.25, p=0.025).

Conclusion: We demonstrated here elevated serum TNC levels in patients with axSpA, particularly in those with syndesmophytes, which may suggest its role in bone formation during radiographic stage of the disease.