

A4.14 ROLE OF DIFFERENT ADIPONECTIN RECEPTORS IN ADIPONECTIN SIGNALING IN RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS

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Objectives Adiponectin levels were found to be increased in the synovial fluid of rheumatoid arthritis (RA) patients suggesting a role in the pathophysiology of the disease. RA synovial fibroblasts (SF) are known to be a key cell in RA and to express adiponectin in vivo. Adiponectin exists in four isoforms, namely the globular, low molecular weight (LMW or trimer), middle molecular weight (MMW) and high molecular weight (HMW) form. These isoforms act by binding to its receptors AdipoR1, AdipoR2, PAQR3 and PAQR10, which are expressed by various cells. Subsequently, different intracellular signalling pathways are activated including AMPK, p38MAPK, and FAK. The purpose of the present study was to determine the adiponectin receptor expression and the specificity of the adiponectin receptors to the respective adiponectin isoforms mediating different signalling pathways in RASF.

Methods AdipoR1, AdipoR2, PAQR3 and PAQR10 mRNA and protein expression were analysed in RASF by Real-time PCR, Western blotting, and immunocytochemistry. AdipoR1 and AdipoR2 down-regulation by siRNA via nucleofection technology and lentiviral transduction were performed followed by signalling analysis via Western blots.

Results Real-time PCR and Western blotting results showed that cultured RASF express AdipoR1, AdipoR2, and PAQR3 but not PAQR10. This was further confirmed by immunocytochemical analysis. Transfection of siRNA specific for AdipoR1, AdipoR2 and PAQR3 via nucleofection showed successful receptor down-regulation at mRNA level but no effect was observed at protein level even after 10 days, most likely due to the long receptor half-life on the cell surface. However, lentiviral transduction showed successful down-regulation of AdipoR2 at mRNA as well as at protein level after 5 weeks (approx. 5 fold). AdipoR2 knockdown in RASF showed a reduction in adiponectin-induced p38MAPK signalling.

Conclusions Our results show that RASF isolated from synovium of RA patients express the adiponectin receptors AdipoR1, AdipoR2 and PAQR3. Down-regulation of these adiponectin receptors by lentiviral transduction was found to be a more efficient technique as compared to siRNA transfection, which will facilitate to study their role in signalling. Moreover, the induction of the p38MAPK pathway by adiponectin seems to be dependent on AdipoR2.

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A4.15 SERUM LEVELS OF VISFATIN AND B-CELL ACTIVATING FACTOR OF THE TNF FAMILY CORRELATE WITH DISEASE ACTIVITY IN PATIENTS WITH MYOSITIS

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Background and Objectives Visfatin is an adipocytokine that supports B-lymphocyte precursor maturation, and also takes part in regulation of inflammation. Anti-histidyl-tRNA synthetase antibodies (anti-Jo-1) are the most frequent myositis specific

autoantibodies. We have shown increased serum levels of B-cell activating factor of the TNF family (BAFF) in dermatomyositis (DM) and anti-Jo-1-positive polymyositis (PM) patients and its association with disease activity. Here we evaluated serum levels of visfatin in anti-Jo-1-positive PM/DM patients, its expression in muscle tissue and investigated potential relations between visfatin, BAFF, disease activity and anti-Jo-1 autoantibody levels.

Material and Methods ELISA was used for detection of visfatin, BAFF and anti-Jo-1 serum samples in patients with PM (n = 27), DM (n = 11) and in 25 age and sex matched healthy controls. Paired samples from two different time points of sixteen patients were available. Disease activity was evaluated by myositis disease activity assessment visual analogue scales (MYOACT) and by serum levels of creatine phosphokinase (CPK), aminotransferases (ALT, AST), lactate dehydrogenase (LDH) and myoglobin. Visfatin was detected by immunohistochemistry in muscle tissues of PM/DM patients (n = 5/5) and compared with non-inflammatory control muscle tissues from patients with myasthenia gravis (n = 5).

Results Serum visfatin and BAFF levels were significantly higher in myositis patients (medians 1.9 and 1.4 pg/l) compared to healthy controls (1.3 and 1.0 pg/l; p < 0.02 and p = 0.003) and were associated with clinical muscle activity (rs = 0.39; p < 0.02 and rs = 0.34; p = 0.04). Trend for correlation of both visfatin and BAFF with the global disease activity was present. Serum levels of visfatin were associated with LDH (rs = 0.39, p < 0.02), whereas BAFF correlated with CPK, myoglobin and AST (rs = 0.51, 0.57 and 0.39; p < 0.05 for all). Positive correlation between visfatin and BAFF serum levels was found in patients with myositis (rs = 0.44; p < 0.01) but was negative in healthy controls (rs = -0.54; p < 0.001). No association between visfatin and anti-Jo-1 autoantibody levels was found while BAFF positively correlated with anti-Jo-1 levels (rs = 0.85; p = 0.001). Visfatin levels decreased significantly over time (p = 0.01), while the decrease of BAFF was not significant. Visfatin expression was present in endomysial and perimysial inflammatory infiltrates of muscle tissues from patients with PM/DM compared with no expression in controls.

Conclusions These results demonstrate that serum levels of visfatin, similarly to BAFF, associate with disease activity in patients with myositis. Increased visfatin levels and expression in inflamed muscle tissues support its potential role in the pathogenesis of idiopathic inflammatory myopathies.

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A4.16 HIGH MOLECULAR WEIGHT ADIPONECTIN AND TUMOR NECROSIS FACTOR- α INFLUENCE RHEUMATOID ADIPOSE-DERIVED MESENCHYMAL STEM CELLS FUNCTION

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Background and Objectives Adipose-derived mesenchymal stem cells (ASCs) are promising target in autoimmune diseases therapy. Adipocytokines are known to influence immune system and their role in rheumatoid arthritis (RA) is intensively studied. Objectives of our work were:

1. to investigate whether leptin, low (LMW) and high (HMW) molecular weight adiponectin isoforms and TNF affect IL-6, IL-8, VEGF and TGF β secretion by RA-ASCs.
2. to determine if conditioned media from RA-ASCs treated with above mentioned adipocytokines, influence function of rheumatoid FLS: IL-6 and MMP-3 secretion, proliferation and apoptosis.

Materials and Methods Articular adipose tissue and synovial membrane were obtained from 18 RA patients during total knee