AB0115

SEMAPHORIN 4A PERPETUATE SYNOVIAL FIBROBLAST INTERACTIONS WITH CD4⁺ T CELLS AND OSTEOCLAST DIFFERENTIATION: IMPLICATION IN RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis synovial fibroblast (RASf) and T cells are important contributors in the pathogenesis of RA. Semaphorin 4A has been reported to be elevated in RA patients and play crucial role in promoting inflammation of RA. However, whether semaphorin 4A could facilitate RASf interactions with CD4⁺ T cells and osteoclastogenesis is less known.

Objectives: The present study aims to investigate the role of semaphorin 4A-Plexin B1 axis in RASf interactions with CD4⁺ T cells and osteoclastogenesis in vitro.

Methods: Mouse synovial fibroblasts (SF) were isolated from collagen-induced arthritic mice and cultured in vitro with TNF- α and IL- β for 24h. Small interfering RNA (siRNA) against Semaphorin 4A and Plexin B1 were constructed and transfected into SF via adenovirus. Splenic CD4⁺ T cells isolated from arthritic mice were cocultured with SF under anti-CD3 and anti-CD28. The proportion of CD4⁺IL-17⁺ Th17 cells was determined at day 5. PBMC isolated from blood of healthy donors were incubated with 1 μ g/ml Plexin D1 neutralizing antibody overnight. Cells were washed for monocyte enrichment and cultivated for 14 days in α -MEM supplemented with MCSF and RANKL. Osteoclast differentiation was evaluated by TRAP staining. Total RNA was extracted and expression of osteoclast markers were examined by quantitative real-time PCR.

Results: Adenovirus-Mediated siRNA efficiently inhibited expression of Semaphorin 4A and Plexin B1 in SF. The proportion of Th17 cells was significantly decreased in both Semaphorin 4A and Plexin B1 transfected SF cocultures. The number of Trap positive osteoclasts were significantly decreased in cultures that pretreated with Plexin D1. Consistently, blocking of Plexin D1 signaling dramatically downregulated mRNA expression level of Trap, Cathepsin K and NFATc1.

Conclusion: Semaphorin 4A perpetuate synovial fibroblasts interactions with CD4⁺ T cells by promoting Th17 differentiation. Moreover, Semaphorin 4A-Plexin D1 axis promote osteoclastogenesis and therefore might serve as a potential therapeutic target in the treatment of RA.

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AB0117

HOMOCYSTEINYLATED ALPHA 1 ANTI-TRYPSIN AS A POTENTIAL ANTIGENIC TARGET IN RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune disorder that primarily affects joints. Beside the well-known rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA) have been reported to be a very useful diagnostic and prognostic marker of RA, recently antibodies against carbamylated proteins (anti-CarP) have been described also in ACPA negative RA patients. However, more than 20% of RA cases are still defined as seronegative forms. Therefore, the individuation of new antibody specificities in RA could be helpful for diagnostic and prognostic purposes.

Objectives: The goals of this study were the identification and the immunologic characterization of post-translational modified synovial fluid (SF) autoantigens, specifically targeted by autoantibodies from sera of seronegative RA patients.

Methods: SFs from 5 seronegative RA patients were collected, pooled and treated with hyaluronidase. After removal of both albumin and IgG, the sample was washed and cleansed, concentrated, separated by 2-dimensional

electrophoresis (2-DE) and then transferred by Western blotting to nitrocellulose membrane, for autoantigen detection by immunoassay, using a pool of sera from 5 seronegative RA patients. The antigenic protein spots were identified by peptide mass fingerprint, using a Matrix-Assisted Laser Desorption/Ionization-Time Of Flight (MALDI-TOF) mass spectrometer.

This approach revealed Alpha 1 Anti-Trypsin (A1AT) as a target of RA patients autoantibodies.

Pooled SFs were also analyzed by reverse-phase nanoliquid chromatography and tandem mass spectrometry, to confirm the presence of A1AT and for the identification of A1AT post-translational modifications.

Homocysteinylation A1AT was immunoprecipitated from pooled SFs of RA seronegative patients and, after a diafiltration and concentration process, was used as an antigen to detect anti-homocysteinylation A1AT antibodies by Enzyme-Linked ImmunoSorbent Assay (ELISA). In order to this, consecutive patients with RA, osteoarthritis (OA), psoriatic arthritis (PsA), and healthy donors were enrolled and sera were collected.

Results: Homocysteinylation A1AT was identified as a potential antigenic target not only in ACPA and RF positive RA patients but, more importantly, also in RA seronegative patients.

Antibodies anti-homocysteinylation A1AT were found in 66.7% (44/66) RA patients seronegative for ACPA and RF, 88.6% (39/44) RA patients, 15% (3/20) OA patients, 26.3% (5/19) PsA patients and in none (0/41) of healthy donors.

Conclusion: Homocysteinylation A1AT was identified as a new possible antigenic target of autoantibodies in sera from RA patients, including RA seronegative patients. This tool may be useful in diagnosis and monitoring of the disease and may contribute to understand the immunopathogenic mechanisms of RA in future studies.

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AB0119

MECHANISM OF NEUTROPHIL EXTRACELLULAR TRAP IN PROMOTING SYNOVIAL HYPERPLASIA IN RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis(RA)is an autoimmune disease characterized by chronic inflammation of the synovial membrane and pannus formation. With the discovery of Neutrophil extracellular traps (NETs) in 2004, its role in autoimmune diseases has received much attention^[1]. At present, studies on the pathogenesis of NETs in RA have focused on NETs as a source of citrullinated antigens^[2], producing autoantibodies to citrullinated protein antigens (ACPAs)^[3]. In addition, NETs can activate rheumatoid arthritis fibroblast-like synoviocytes (RA-FLSs) to produce inflammatory cytokines^[2]. Studies have reported that connective tissue growth factor (CTGF) is overexpressed in RA serum^[4], and CTGF plays a key role in the proliferation of RA-FLSs, furthermore leads to joint destruction in RA patients^[5]. Therefore, our study stimulated RA-FLSs with NETs to detect cell proliferation and CTGF mRNA, aiming to explore the pathogenesis of NETs involved in RA.

Objectives: To Explore the potential effects of Neutrophil extracellular traps (NETs) on rheumatoid arthritis synovial fibroblasts (RA-FLSs).

Methods: The synovial tissues of RA patients were isolated and cultured in vitro; peripheral blood neutrophils were extracted from healthy volunteers and stimulated to formation NETs, NETs were extracted as a stimulating agent; MTS proliferation assay was used to evaluate the effect of NETs on the proliferation of RA-FLSs; qRT-PCR was used to determine the expression of connective tissue growth factor (CTGF) mRNA in cells treated with NETs-stimulated RA-FLSs for 60h.

Results: The isolated and purified neutrophils could form NETs by stimulated in vitro. The concentration of extracted NETs -DNA was 58.5ng/ul (1×10⁶ cells). Compared with the control group, NETs could promote the proliferation of RA-FLSs. With the increase of NETs concentration, the proliferation of RA-FLSs was also enhanced (F=99.519, P<0.05); NETs stimulated the up-regulation of CTGF mRNA expression in RA-FLSs (30.696 ± 0.468 vs. 1, p<0.01)

Conclusion: NETs can promote the proliferation of RA-FLSs and stimulate the up-regulation of CTGF mRNA in RA-FLSs in vitro.