SEMAPHORIN 4A PERPETUATE SYNOVIAL MECHANISM OF NEUTROPHIL EXTRACELLULAR TRAP

Zhuxchun: Yuanyuan Zhang, Xu Zheng, Li Xiaomei. University of Science and Technology of China, Hefei, China

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 Th17 cells was determined at day 5. PBMC isolated from blood of healthy donors were incubated with 1 μg/ml Plexin D1 neutralizing antibody overnight. Cells were washed for monocyte enrichment and cultured for 14 days in αMEM 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, C blush 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.

REFERENCES


SEMOPHORIN 4A PERPETUATE SYNOVIAL MECHANISM OF NEUTROPHIL EXTRACELLULAR TRAP

AB0117 HOMOCYSTEINYLATED ALPH A 1 ANTI-TRYPSIN AS A POTENTIAL ANTIGENIC TARGET IN RHEUMATOID ARTHRITIS


1Sapienza University of Rome, Department of Internal Medicine and Medical Specialties, Roma, Italy; 21University Hospital “Shechat Nioqpo”, Department of Internal Medicine, Tirana, Albania.

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 individualization of new antibody specificities in RA could be helpful for diagnostic and prognostic purposes.

Objectives: The goal of this study was 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 immunobayoas, 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. But of these protein spot, only Alpha 1 Anti-Trypsin (A1AT) was identified as a target antigen of autoantibodies. Antibodies anti-homocysteinylated A1AT were found in 66.7% (44/66) RA patients serum positive for ACPA and RF, 86.6% (39/44) RA patients, 15% (3/20) OA patients, 26.3% (5/19) PSA patients and in none (0/14) of healthy donors.

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

Disclosure of Interests: Tania Colasanl: None declared, Danilo Sabatelli: None declared, Carmine Mancone: None declared, Arbi Pecani: None declared, Mariangela Spazi: None declared, Marta Vornero: None declared, cristiana barbati: None declared, Alessandra Ida Celia: None declared, Annarica Faraci: None declared, Carlo Perricone: Speakers bureau: BMS; Lilly, Celgene, Sanofi, Fulvia Cecaere: None declared, Francesca Spinelli: None declared, Vincenzo Barnaba: None declared, fabrizio conti: None declared, Guido Valesini: None declared, cristiano alessandri: None declared

REFERENCES


Disclosure of Interests: None declared

AB0120 THE MECHANISM OF TRADITIONAL CHINESE MEDICINE PRESCRIPTION ER-MIAO-SAN IN THE TREATMENT OF RHEUMATOID ARTHRITIS BASED ON CHOLINERGIC ANTI-INFLAMMATORY PATHWAY

Z. Wang, H. Hao, L. Zhen, Y. Gao, J. Guan. SHANXI UNIVERSITY OF CHINESE MEDICINE, Basic Laboratory of Integrated Traditional Chinese and Western Medicine, Shan Xi, China

Background: Rheumatoid arthritis (RA) is a progressive autoimmune disease. The traditional Chinese herbal formula Er-miao-san (EMS) has been used to treat RA demonstrating significant clinical efficacy; however, the mechanism of action remains unclear. In view of the important role of α7 nicotinic acetylcholine receptor (α7nACHR) in the cholinergic anti-inflammatory pathway (CAP), for the regulation of inflammation and cytokines. Indeed, we previously found a correlation between CHRNA7 (encoding α7nACHR) expression and EMS, we hypothesized that it may play a role in the anti-inflammatory effects of EMS.

Objectives: The mechanism of EMS in the treatment of RA based on CHRNA7 involved in the regulation of CAP.

Methods: We established a CIA model with female Wistar, and the effects of intragastric administration of EMS on the expression of CHRNA7, arthritis score, inflammatory, and articular cartilage changes, was examined in the joints. The serum levels of TNF-α, IL-6, and IL-1β were determined using commercial ELISA kit.

Results: The CIA model was successfully established. Macroscopic changes of arthritis, such as redness and swelling, were clearly observed in the CIA rats, but were attenuated by the treatment of EMS. The mean arthritis score was markedly lower in the EMS-treated group (EG, P < 0.05). The serum level of TNF-α was significantly lower in EG compared with CIA group (P < 0.05). The same results were found in the serum levels of IL-6 and IL-1β. Synovial edema and extensive infiltration of inflammatory cells occurred in the CIA rats, but were repaired by the treatment of EMS. Cartilage tissue was thinning, dissolution and disappearance, as well as extensive inflammatory cell infiltration with plasma cells and lymphocytes, was observed in the articular cartilage of the ankles in CIA group. In contrast, EMS treatment prevented cartilage degeneration and markedly reduced inflammation. Immunohistochemistry (IHC) analysis showed positive signals of CHRNA7 was expressed on fibroblast-like synoviocytes, macrophages, and endothelial cells in the joints. Effect of EMS on the expression of CHRNA7 protein in the joint by Western blot (WB) analysis. IHC and WB relative optical density values of CHRNA7 was significantly higher in EG compared with CIA group (P < 0.05).

Conclusion: EMS can significantly alleviate the symptoms of arthritis in CIA rats by regulating the expression of CHRNA7 in CAP. It provides a scientific research foundation for the further development of EMS and explores more ways to treat RA.

REFERENCES


Disclosure of Interests: None declared

AB0121 EXPRESSION OF CHEMOKINES AND CHEMOKINE RECEPTORS IN DIFFERENT TISSUES AND THEIR LOCALIZATION IN THE JOINTS OF RATS WITH RHEUMATOID ARTHRITIS

Li Zhen1, Huiqin Hao1,2, Ze Wang1, Wenjing Lu1, Yang Li1, on behalf of Innovation Team of Chinese and Western Medicine Combined with Inflammatory Joint Disease Research at Shanxi University of Chinese Medicine. 1Shanxi University of Chinese Medicine, College of Basic Medical Sciences, Jinzhong, China. 2Shanxi University of Chinese Medicine, Basic Laboratory of Integrated Traditional Chinese and Western Medicine, Jinzhong, China

Background: Rheumatoid arthritis (RA) is a complex, chronic, multisystem autoimmune disease characterized by a sustained immune response that leads to inflammation in the body and destruction of joints. While not completely understood, immune cells, as well as soluble factors such as cytokines and chemokines, are believed to be involved in the pathogenesis of RA. Chemokines play an important role in the development of inflammation and the regulation of cytokines. However, studies of chemokines have focused mainly on their role in cancer, and...