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THU0074

ANTI-ARTHRITIC EFFECT OF RECOMBINANT CYSTATIN 3 FROM *FASCIOLA HEPATICA* IN COLLAGEN-INDUCED ARTHRITIS

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Background: Cystatins are cysteine proteases-inhibitors secreted by *Fasciola hepatica* in order to modulate the host immune response to promote survival of the parasite. These molecules are able to inhibit different mammal cathepsins, to regulate the immune balance via Th2 and T regulatory responses, to downregulate antigen presentation and the release of pro-inflammatory cytokines (1,2) – mechanisms that are important in the development and maintenance of several immunopathology, as rheumatoid arthritis (RA) (3).

Objectives: To evaluate the therapeutic effect of recombinants cystatin 1 and cystatin 3 from *Fasciola hepatica* in a mice model of collagen-induced arthritis (CIA).

Methods: Twenty-seven DBA/1J mice were induced with CIA by an injection of collagen type-II and Freund's adjuvant at days 0 and 18. Animals were randomly divided into three groups: vehicle (n=9, treated with phosphate-buffered saline), cystatin 1 (n=9, treated with 100 µg/dose of recombinant cystatin 1) and cystatin 3 (n=9, treated with 100 µg/dose of recombinant cystatin 3). Treatment started after day 18 by intraperitoneal injection once a day until the end of the experiment, at day 45 after CIA induction. Clinical arthritis score, nociception, paw edema, body and spleen weight were evaluated. Lymphocytes were isolated from lymph nodes and CD4+CD25+Foxp3+ T regulatory subset was assessed by flow cytometry. Data are expressed as mean ± SEM and were evaluated by one-way or two-way ANOVA followed by Bonferroni post-test.

Results: Treatment with cystatin 1 did not alter any of the analyzed parameters. On the other hand, cystatin 3 was able to reduce clinical arthritis score from day 38 with 32% of reduction at day 45 (9.22±1.22) compared to vehicle (13.56±0.73) (p<0.05). In addition, treatment with cystatin 3 diminished nociception (cystatin 3: 4.0±0.36g, vehicle: 2.7±0.32g) (p<0.05) and paw edema (cystatin 3: 0.051±0.012ml, vehicle: 0.093±0.007ml) (p<0.05). Moreover, the treatment did not alter body weight (cystatin 3: 21.67±0.31g, vehicle: 21.05±0.38g) and spleen weight (cystatin 3: 7.04±0.31, vehicle: 7.16±0.38), as well as the T regulatory population (cystatin 3: 63.38±3.66%; vehicle: 58.31±6.77%).

Conclusion: Treatment with cystatin 3 improved collagen-induced arthritis by attenuating the disease score, nociception and paw edema. Moreover, the treatment did not induce body weight loss or spleen weight alteration. These results suggest that recombinant cystatin 3 from *Fasciola hepatica* has the potential as a treatment for inflammatory and autoimmune diseases such as RA.

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THU0075

MIR-21 IN EXOSOMES DRIVEN FROM DENTAL PULP STEM CELLS AMELIORATE THE TREGS/TH17 IMMUNE RESPONSE VIA TARGETING STAT3 IN COLLAGEN-INDUCED ARTHRITIS MICE

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Background: Rheumatoid arthritis (RA) is a common and systemic autoimmune disease¹. It is vital for deciphering pathogenesis to define the precise CD4⁺Tcell subsets that are expanded or dysregulated in RA patients². An increase in the Th17 cell subset and a decrease in Tregs resulting imbalance of Tregs/Th17 may be responsible for the occurrence and development of RA³. Dental pulp stem cells (DPSCs) appear be a new therapeutic tool for immunological diseases^{4,5}. In RA, the mechanism of the effect of miR-21 driven from DPSCs exosomes (DPSCs-exo) on the balance of Tregs/Th17 is still unclear.

Objectives: We explored the effect of exosomes driven from DPSCs on RA and investigated the correlation between DPSCs-exo and Tregs/Th17 balance.

Methods: Exosomes were isolated through differential centrifugation. Collagen-induced arthritis (CIA) mice were builded to detect histological change and Tregs/Th17 ratio. CD4⁺T cells were isolated from PBMCs. Exosomes and CD4⁺T cells were co-cultured to study the regulation of Tregs/Th17 balance. The relationship between miR-21 and Tregs/Th17 balance was studied by flow cytometry, qRT-PCR and Western blotting etc.

Results: After Dpscs-exo treatment, the clinical scores and paw swelling of CIA mice decreased. At the same time, the expression of Treg cells increased and the expression of Th17 cells decreased in the spleen, changing the ratio of Tregs/Th17. Exosomes and CD4⁺T cells were co-culture, showing that Treg cells increased and Th17 cells decreased. MiR-21 is highly expressed in RA patients. MiR-21 in exosomes were Knockout and overexpressed. The balance of Tregs/Th17 decreased when miR-21 were knockout and increased when miR-21 were overexpressed. In addition, STAT3 in CD4⁺T cells were overexpressed and co-culture with DPSCs-exo, which inhibited Tregs/Th17.

Conclusion: We elucidated the mechanism of the regulation of Tregs/Th17 balance by DPSCs-exo, that is, miR-21 in exosomes driven from dental pulp stem cells ameliorate the Tregs/Th17 immune response via targeting STAT3 in collagen-induced arthritis mice.

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THU0076

SONIC HEDGEHOG PROMOTES SYNOVIAL HYPERPLASIA AND BONE DAMAGE THROUGH P38 MAPK SIGNALING IN EXPERIMENTAL ARTHRITIS

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Background: Abnormal activation of sonic hedgehog (SHH) signaling has been found in synovium from patients with rheumatoid arthritis (RA). Inhibition of SHH signaling is reported to attenuate inflammation and cartilage damage in adjuvant-induced arthritis (AIA). Previously we have demonstrated that SHH signaling promoted the tumor-like behavior of fibroblast-like synoviocytes (FLSs) through p38 MAPK *in vitro*.

Objectives: In the current study, we aim to further explore the role of SHH-p38 MAPK signaling in regulating synovial hyperplasia and bone erosion in experimental arthritis.

Methods: Collagen-induced arthritis (CIA) mouse model was induced and the mice were injected with adenovirus associated virus (AAV) overexpressing SHH or treated with small molecule inhibitors GDC-0449. SB203580 was administrated for the inhibition of p38 MAPK. The severity of paw inflammation was graded and serum levels of TNFα, IL-6 were detected. The histological features of arthritis were evaluated by H&E staining. The bone erosion was identified by micro-CT assessment and the number and function of osteoclasts were determined.

Results: Blockade of SHH by GDC-0449 significantly alleviated the symptoms and decreased the synovial hyperplasia, inflammatory infiltration, cartilage and bone damage in ankles of CIA. The bone erosions in the area of the metatarsophalangeal and ankle joints and production of TNF α , IL-6 were decreased by SHH inhibition. In addition, the administration of GDC-0449 significantly decreased the number of TRAP positive cells and the expression of NFATc1. On the contrary, SHH overexpression led to increased severity of arthritis and pathological changes. We also observed the accelerated bone injury accompanied with increased number and activity of osteoclasts and increased production of serum IL-6 in mice with upregulation of SHH expression. Of note, the administration of p38 MAPK inhibitor reversed the effects of SHH overexpression, with a reduction of joint swelling and histological scores. Inhibition of p38 MAPK prevented the bone erosion and decreased the number of TRAP positive cells and the expression of NFATc1, which were promoted by SHH overexpression.

Conclusion: The study indicates that SHH promotes the synovial hyperplasia and bone erosion of CIA in a p38 MAPK-dependent manner. SHH-p38 MAPK signaling could be a potential target for the treatment of RA.

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THU0077

CADHERINS GUIDE THE DIRECTIONAL MIGRATION OF THE SYNOVIOCYTES IN RHEUMATOID ARTHRITIS

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Background: Aggressiveness of synoviocytes and collective migration of organized synovial tissues play a key role in the pathogenesis of pannus invasion into adjacent joint structure. Interactions among synovial cells for grouped movement, however, have not been properly elucidated.

Objectives: We hypothesized that cadherins which have functions on the synovial invasion in RA, may play a critical role in collective migration of rheumatoid synoviocytes.

Methods: Cadherins expression patterns on the synoviocytes isolated from patients with RA were evaluated using RT-PCR, flow cytometry, and western blot analysis. Mesenchymal and epithelial phenotypes were examined in cadherin overexpressing cell line by flow cytometry. L-cells with overexpression of CDH2 (CDH2^{hi}), CDH11 (CDH11^{hi}), and combination of CDH2/CDH11 (CDH2^{hi}/CDH11^{hi}) were prepared. Migration of cells was observed by taking time-lapse images with laser confocal microscope. *In vitro* collective migration and directional movement in response to inflammatory mediators and different matrix rigidity were evaluated. *In vivo* homing of CDH2^{hi}/CDH11^{hi}-L-cells into joint tissues was performed in collagen induced arthritis (CIA) mouse. *In vivo* and *ex vivo* migration pattern of CDH11^{hi}-L-cells were investigated in nude mice using optical imaging system.

Results: In rheumatoid synovial tissues, CDH2 and CDH11 were highly expressed compared to synovial tissues from osteoarthritis. CDH2 and CDH11 were also highly expressed on synovial fibroblasts isolated from RA. Phenotype analysis of mesenchymal and epithelial cells in CDH11^{hi}-L-cells and CDH2^{hi}/CDH11^{hi}-L-cells showed increased expression of α 5 β 1, CD44s, vimentin, and α -SMA compared with MOCK-L-cells. We then analyzed the pattern of migration of MOCK, CDH2^{hi}, CDH11^{hi}, and CDH2^{hi}/CDH11^{hi}-L-cells using time lapse images. During migration over a hard ECM, CDH2^{hi} and CDH11^{hi}-L-cells represented higher aspect ratio compare to a soft ECM. Aspect ratio relatively found lower in CDH2^{hi}/CDH11^{hi}-L-cell lines than MOCK cells. CDH2^{hi}/CDH11^{hi}-L-cells showed significantly higher migration velocity and Euclidean distance with narrower angle of migratory directions in a cytokine mediated migration. Compared to the MOCK cells, persistence ratio and aspect ratio of migration were also higher in CDH2^{hi}, CDH11^{hi}, and CDH2^{hi}/CDH11^{hi}-L-cells. CDH2^{hi}/CDH11^{hi}-L-cells collectively migrated with the formation of leader and follower cells. In a chemokine mediated hard stiffness of ECM, durotaxis was observed in CDH11^{hi}-L-cells. After 24 hours of intraarticular knee injection in CIA mouse, higher number of CDH2^{hi}/CDH11^{hi}-L-cells invaded into the cartilage than MOCK cells. *In vivo* migration of CDH2^{hi}/CDH11^{hi}-L-cells was also found towards the chemokine and cartilage mixed matrigel plug in the subcutaneous space of mice.

Conclusion: The expression of CDH2 and CDH11 promotes directional migration of synoviocytes, indicating the potential role of these cadherins on the pannus tissues in the invasion into adjacent joint structure in RA.

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THU0078

EXPRESSION PROFILE ANALYSIS OF LONG NONCODING RNAs INDUCED BY IL-1SS IN RHEUMATOID ARTHRITIS FIBROBLAST-LIKE SYNOVIOCYTES

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Background: Long noncoding RNAs (lncRNAs) have recently emerged as important biological regulators and the aberrant expression of lncRNAs has been reported in various diseases including cancer, cardiovascular disease, and diabetes mellitus. However, the role of lncRNAs in the pathogenesis of rheumatoid arthritis (RA) remains unknown.

Objectives: Thus, we studied lncRNAs influenced by IL-1, which is one of the key mediators in the pathogenesis of RA, and also investigated whether regulation of NF- κ B activation, which is known to be induced by IL-1, could lead to the changes of expression of those lncRNAs.

Methods: Fibroblast-like synoviocytes (FLS) were obtained from the knee joints of the patients with RA. The next-generation sequencing (NGS) data were analyzed to identify differentially expressed lncRNAs between unstimulated RA FLS and IL-1-stimulated RA FLS. The expression levels of the top 5 candidates in NGS data were validated by RT-qPCR using extended number of unstimulated RA FLS and IL-1-stimulated RA FLS. IMD-0560, an inhibitor of I κ B kinase (IKK) was used for the regulation of NF- κ B activation. Activation and inhibition of NF- κ B were confirmed by Western blotting. Changed expressions of the lncRNAs were identified by RT-qPCR.

Results: NGS analysis revealed up-regulated 30 lncRNAs and down-regulated 15 lncRNAs in IL-1-treated RA FLS compared with unstimulated RA FLS. Top 5 lncRNAs were selected among 30 lncRNAs up-regulated by IL-1 in RA FLS based on fold-change with P-value cutoff. The up-regulated lncRNAs including NR_046035, NR_027783, NR_033422, NR_003133, and NR_049759 were validated by RT-qPCR. IMD-0560 inhibited phosphorylation of I κ B α induced by IL-1 in RA FLS. Overexpression of lncRNAs induced by IL-1 was also inhibited by IMD-0560 in RA FLS.

Conclusion: Our study revealed that IL-1 increased the expression of NR_046035, NR_027783, NR_033422, NR_003133, and NR_049759 in RA FLS. In addition, the expression of these lncRNAs was regulated by inhibition of NF- κ B activation. Thus, our data suggest that the lncRNAs might be involved in the pathogenesis of RA through NF- κ B signaling pathway.

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THU0079

THE MICROBIOME OF NEW-ONSET RHEUMATOID ARTHRITIS (NORA) PATIENTS DRIVES TLR4-DEPENDENT TH17 RESPONSES

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Background: Intestinal microbiota plays a prominent role in shaping the T cell immune response. Increasing evidence suggests that the gut microbiota is perturbed in patients with RA, and a variety of animal models demonstrated involvement of (mouse) microbiota in arthritis development. This underlines the necessity of understanding whether and how indigenous human NORA-associated microbiota may trigger RA.