Background: Cystatins are cysteine protease-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 inactivate, and to deactivate antigen presentation and the release of pro-inflammatory cytokines (1,2), mechanisms that are important in the development and maintenance of several immunopathologies, 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 saline, and treated with cystatin 1 (n=9) or 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: 2.7±0.32g) (p<0.05) and paw edema (cystatin 3: 0.05±0.012ml, vehicle: 0.093±0.007ml) (p<0.05), Moreover, the treatment did not alter body weight (cystatin 3: 2.167±0.31g, vehicle: 2.105±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 to be a treatment for inflammatory and autoimmune diseases such as RA.

References:

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THU0075 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 (AA). Previously we have demonstrated that SHH signaling promoted the tumour subset and a decrease in lesional Tregs in CIA mice.

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) mice model was induced and then 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.

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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 pathologic 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 organ- ized 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 syno- vial 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 (CDH2hi), CDH11 (CDH11hi), and combination of CDH2/CDH11 (CDH2/CDH11hi) 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 rigidi- ty were evaluated. In vivo homing of CDH2hi/CDH11hi-L-cells into joint tissues was performed in collagen induced arthritis (CIA) mouse. In vivo and ex vivo migration pattern of CDH11hi-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. Phe- notype analysis of mesenchymal and epithelial cells in CDH11hi-L-cells and CDH2hi/CDH11hi-L-cells showed increased expression of αSMA, CD44s, vimentin, and α-SMA compared with MOCK-L-cells. We then analyzed the pattern of migration of MOCK, CDH2hi, CDH11hi, and CDH2hi/CDH11hi-L-cells using time lapse images. During migration over a hard ECM, CDH2hi and CDH11hi-L cells represented higher aspect ratio compared to a soft ECM. Aspect ratio rela- tively found lower in CDH2hi/CDH11hi-L-cell lines than MOCK cells. CDH2hi/ CDH11hi-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 CDH2hi, CDH11hi, and CDH2hi/CDH11hi-L-cells. CDH2hi/CDH11hi-L-cells collectively migrated with the formation of leader and follower cells. In a chemokine mediated hard stiffness of ECM, durotaxis was observed in CDH11hi-L-cells. After 24 hours of intraarticular knee injection in CIA mouse, higher number of CDH2hi/CDH11hi-L-cells invaded into the carti- lage than MOCK cells. In vivo migration of CDH2hi/CDH11hi-L-cells was also found towards the chemokine and cartilage mixed matrigel plug in the subcutaneous space of the mouse.

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

References:

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