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SAT0022

CXCL7 PROMOTES OSTEOCLASTOGENESIS IN RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by bone destruction[1]. Chemokine signaling by skeletal cells or by other cells of the bone marrow niche regulates bone formation and resorption[2]. Recent studies have found that CXCL7 enhanced the osteoclast formation in mouse bone marrow cells[3, 4]. Whether CXCL7 plays a role in human osteoclastogenesis especially in RA patients remains unclear.

Objectives: To examine the functional role of CXCL7 in the induction of osteoclastogenesis in RA.

Methods: The level of CXCL7 in CD14⁺ monocyte supernatant was assessed via enzyme-linked immunosorbent assay. Osteoclastogenesis of CD14⁺ monocyte from RA patients and healthy donors were evaluated by TRAP staining and F-actin ring immunofluorescence. Bone resorption pit was observed by scanning electron microscopy. We performed quantitative reverse transcription polymerase chain reaction (RT-PCR) to detect changes in osteoclast markers. RAW264.7 macrophages were also used to investigate specific signaling pathway by which CXCL7 stimulated during osteoclast formation.

Results: CXCL7 level in CD14⁺ monocyte supernatant from RA patients (5690 ± 627.05 pg/ml) was significantly higher than that in healthy controls (2301 ± 535.52 pg/ml) (n=5, P<0.001). In the presence of M-CSF and RANKL, CXCL7 promoted osteoclast formation (Figure 1A and B) and increased bone resorption area (Figure 1C) of CD14⁺ monocyte from healthy donors in the low concentration (10ng/ml) group (n=3, p < 0.05). While in high concentration of CXCL7 (50ng/ml) group, there were no significant changes in the number of osteoclasts. Transcription level of the osteoclast markers RANK, cathepsin K, and MMP-9 was significantly increased in the CXCL7 (10ng/mL) group after 3 days in the presence of M-CSF and sRANKL (n=3, p < 0.05). When using CD14⁺ monocyte from RA patients, the optimal concentration of CXCL7 was 50ng/ml, which significantly increased the number of osteoclasts (Figure 2A and B) and bone resorption area (Figure 2C) (n=3, p < 0.01). Flow cytometry analysis revealed that a higher proportion of CD14⁺ monocytes expressed CXCR2 from healthy donors than those from RA patients (n=6, p < 0.01). Consistent with the results obtained in CD14⁺ monocytes, the effects of exogenous CXCL7 on osteoclast formation were also observed in RAW264.7 cells (p < 0.01). The addition of CXCL7 dramatically promoted phosphorylation ERK1/2 in RAW264.7 cells, but it did not affect the phosphorylation of P65.

Conclusion: CXCL7 level in CD14⁺ monocyte supernatant was higher in RA patients than that of healthy donors. CXCL7 promoted osteoclastogenesis in CD14⁺ monocyte both from RA patients and healthy donors. CXCL7 could be a potential therapeutic target for bone destruction in RA.

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Figure1. CXCL7 induced osteoclast differentiation and bone resorption in healthy donors. CD14⁺ monocytes isolated from healthy donors were cultured in the presence of M-CSF (50 ng/ml) and RANKL (100 ng/ml) with CXCL7 (1, 10, 50ng/ml) during the whole period of osteoclastogenesis.

Figure2. CXCL7 induced osteoclast differentiation and bone resorption in RA patients. CD14⁺ monocytes isolated from RA patients were cultured in the presence of M-CSF (50 ng/ml) and RANKL (100 ng/ml) with CXCL7 (10, 50, 100ng/ml) during the whole period of osteoclastogenesis.

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SAT0023

THE ROLE OF ADAM12 UPREGULATED PROLIFERATION OF SYNOVIAL MEMBRANE IN RHEUMATOID ARTHRITIS

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Background: ADAM12 is a member of a disintegrin and metalloproteinase family and has been reported to participate in the development of a variety of tumors by degrading ECM and shed precursors, thus promoting cell proliferation, invasion, and metastasis¹. Additionally, ADAM12 is involved in chondrocyte differentiation from osteoarthritis (OA) patients by regulation of TGFβ1-induced IGF-1 and RUNX-2 expressions². However, there is no report on the role of ADAM12 for rheumatoid arthritis (RA).

Objectives: To investigate the expression and role of ADAM12 in the synovial tissue of RA.

Methods: (1) The expression of ADAM12 in synovial tissues from RA (18 cases), OA (5 cases) and healthy control (HC) (3 cases) was examined by immunohistochemistry. The synovial tissues of HC were obtained during surgery of hemiarthroplasty for bone tumors. Three researchers evaluated the positive cell ratio. The samples were scored according to the percentage of positive staining: 0 points (weak positive, positive expression was less than 5%), 1 point (moderate positive, positive expression was between 5% and 50%) and 2 points (strong positive, positive expression was greater than 50%). In addition, the samples were scored according to the staining intensity: 0 points (weak intensity), 1 point (moderate intensity) and 2 points (high intensity). (2) The cultured synovial fibroblasts obtained from RA patients at the surgery (RASf) were stimulated by TNFα (1, 5, 10 ng/mL), TGFβ1 (1, 5, 10 ng/mL), PDGF-BB (1, 5, 10 ng/mL) and TNFα+TGFβ1+PDGF-BB (all 10 ng/mL), and the expression levels of ADAM12 relative mRNA was examined by real-time PCR. (3) siADAM12 was transfected in RASf, and the proliferation was examined by WST-1 assay, and the expression of ADAM12 protein was examined by western blotting.

Results: (1) ADAM12 positive cells were found in synovial lining cells, plasma cells, and vascular endothelial cells. ADAM12 was highly expressed in RA synovial tissues. The immunostaining scores of RA, OA, and HC were 3.9±0.01, 1.9±0.27, and 0.8±0.18, respectively. (2) Stimulation by TNFα, TGFβ1, and PDGF-BB resulted in the upregulation of the expression of ADAM12 relative mRNA in RASf, and TGFβ1 stimulation notably tended to increase the expression by about 5 to 6 times. (3) siADAM12 successfully suppressed the expression of ADAM12 protein and simultaneously suppressed the proliferation of RASf.

Conclusion: ADAM12 might be involved in the pathogenesis of RA, promoting the cell proliferation of RASf.

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Fig. 1: Immunohistological staining of ADAM12 for synovial tissues

Fig. 2: ADAM12 relative mRNA expression and the proliferation of RASF after siADAM12 transfection

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SAT0024

JAK-INHIBITION WITH BARICTINIB INHIBITS ACTIVATION OF NLRP1/CASPASE1/GSDMD PYROPTOSIS PATHWAY IN RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS

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Background: Synovial fibroblasts (SFs) play a major role in the pathogenesis of rheumatoid arthritis (RA) and develop an aggressive phenotype destroying cartilage and bone, thus termed RASFs.

Objectives: We aim to examine the presence of GSDMD-mediated pyroptosis and its role in activated RASFs.

Methods: Fibroblasts were isolated from RA synovium obtained from knee replacement surgeries. NLRP1, Caspase-1, GSDMD expression in synovial tissue and TNF-treated RASFs were assessed by qPCR and Western blot. Interleukin (IL)-1 was measured by ELISA in supernatant after pretreated with TNF and baricitinib. LDH release was measured using the CytoTox 96 Non-Radioactive

Cytotoxicity Assay Kit. Endogenous NLRP1, Caspase-1, and GSDMD was knocked down using small interfering RNA.

Results: The expressions of NLRP1, pro-Caspase-1, Caspase-1 p10, GSDMD and its pyroptosis-inducing fragment GSDMD-N were greater in RA synovium than OA synovium. TNF-induced NLRP1, pro-Caspase-1, Caspase-1 p10, GSDMD, and GSDMD-N expression at the transcript and protein level in a time-dependent manner ($P < 0.05$). Meanwhile, the release of LDH and IL-1 were significantly increased in RASFs after treated with TNF. We also confirmed the presence of pyroptosis in electron microscopy. Furthermore, blocking the JAK pathway with baricitinib significantly reduced TNF-induced pyroptosis at the transcriptional, protein and activity levels ($P < 0.05$). Finally, blocking the JAK pathway, we observed a reduction of IL-1 bioactivity in RASFs ($P < 0.05$).

Conclusion: Our results demonstrate an important role of GSDMD-mediated pyroptosis and shed lights on a potential pyroptosis-targeted treatment. Meanwhile, JAK inhibition alleviates inflammasome-induced pyroptosis by blocking pyroptosis pathway in RASFs.

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SAT0025

NEUROMEDIN U SUPPRESSES COLLAGEN-INDUCED ARTHRITIS THROUGH ACTIVATION OF ILC2

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Background: Reduction and dysregulation of ILC2 was linked to delayed resolution of arthritis. The neuropeptide Neuromedin U (NMU) has been reported to rapidly activate ILC2 and initiate a Th2 type immune response through NMUR1 expressed on the surface of ILC2. However, one previous study reported that NMU promoted autoantibody-mediated arthritis.

Objectives: The aim of this work was to investigate the effect of NMU on collagen-induced arthritis (CIA) mice and the potential mechanisms.

Methods: CIA was induced in C57BL/6 WT and C57BL/6^{Nmu} deficient mice on day 1. WT mice were treated i.p. daily by NMU-23 (20ug/mice) or by PBS for 10 days from day 1 to 5 and day 21 to 25. The clinical scores of CIA mice were assessed every two days from day 22 and determined on a scale of 0–4 for each paw. The proportion of ILC2 as well as Th1, Th2, Th17 and Treg in spleen, mesenteric lymph node (mLN) and joints of arthritic mice were analyzed by flow cytometry on day 42.

Results: NMU-23 dramatically inhibited clinical onset and severity of arthritis in treated WT mice compared with control mice. Interestingly, NMU-deficient mice also developed significantly less severe arthritis compared with WT control (Fig 1). Flow cytometry analyses showed that the proportion of ILC2, which defined as Lin-CD45+CD127+KLRG1+ICOS+ST2+, was elevated in the joint but not in the spleen and mLN of arthritic mice treated with NMU-23. In contrast, the proportion of ILC2 was significantly lower in the spleen of NMU-deficient mice than WT control. The percentage of Th2 cells in the spleen and mLN tend to be higher in NMU-23 treated mice, but there is no statistical significance. Surprisingly, Th1 cells were increased in the mLN of NMU-23 treated and NMU-deficient mice compared with control whereas Th17 was comparable among groups. In addition, the proportion of Treg was decreased in the joint of NMU-23 treated and NMU-deficient mice compared with control mice.

Conclusion: Our preliminary results show that repeated injection of NMU-23 during induction (early) and development (late) stage of CIA strongly suppressed clinical onset and severity of arthritis, which might be ascribed to activation of ILC2 in the joint. Further study is needed to explore other cellular and molecular mechanisms in the effect.

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