Enhancement of Placenta Growth Factor Expression by Oncostatin M in Human Rheumatoid Arthritis Synovial Fibroblasts

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INTRODUCTION:
Rheumatoid arthritis (RA) is a systemic autoimmune disease which causes chronic inflammation of the joints. Although the causes of this disease are still unknown, T cell, B cells, mast cells, and macrophages become activated and contribute to synovial inflammation and joint destruction. In recent studies, evidences show that placenta growth factor (PLGF) might play an important role in RA. PLGF is highly homologous with vascular endothelial growth factor (VEGF) and shares the same receptor Flt-1. In RA, PLGF is highly expressed in synovial tissue, which may trigger the production of proinflammatory cytokines including TNF-α and IL-6. Oncostatin M (OSM) is a multifunctional cytokine that belongs to the IL-6 subfamily, which has been found in many inflammatory diseases, including asthma and RA. In this study, we found that OSM enhanced the production of PLGF, which may contribute to the inflammation and destruction of joints in RA patients.

MATERIALS AND METHODS:

Cell cultures
Primary cultures of human synovial fibroblasts were obtained from patients with rheumatoid arthritis (RA) by total knee replacement surgeries (Taichung Veterans General Hospital, Taichung, Taiwan). Cells were cultured in RPMI1640 supplemented with 10% FCS and 100 IU/ml penicillin at 37°C in a humidified atmosphere with 5% CO2 in air.

RT-PCR
Total RNA was extracted from RA synovial fibroblast cells with the TRIzol kit (MDBio Inc., Taipei, Taiwan). Single-strand cDNA was synthesized using MMLV-RT and Taq polymerase (Promega; Madison, WI) and primers specific for the placenta growth factor (PLGF) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were as follows.

Western blotting
The protein levels of PLGF, Signal transducer and activator of transcription-3 (STAT3) and actin were determined by Western blotting. For the separation of nuclear extracts, cells were cultured onto 10 cm dish. After reaching 80% confluence, cytoplasmic extract and nuclear extracts were separated by NE-PER (Thermo Scientific-Pierce, Rockford, IL, USA). The blots were probed with rabbit antibodies against PLGF (1:1000; Cell Signaling Technology, Beverly, MA), phospho-STAT3 (1:1000; Cell Signaling Technology, Beverly, MA), Akt (1:1000; Santa Cruz Biotechnology, California) and phospho-Akt (1:1000; Santa Cruz Biotechnology, California).

Statistical analysis
The data are given as mean ± S.E.M. The significance of difference between the experimental group and control was assessed by Student’s t test. The difference is significant if the p value is less than 0.05.

RESULTS:
Treatment of synovial fibroblast with oncostatin M (OSM) upregulates the expression of PLGF in a time-dependent and dose-dependent manner. (Fig 1). Different inhibitors were used (Jak3 inhibitor, SB203580, LY294002 and PD98059) and we found that Jak3 inhibitor (3µg/ml) and PI3K inhibitor (LY294002 20µM) could antagonize the production of PLGF induced by oncostatin M (Fig 2A). Immunoblotting analysis showed that oncostatin M activated STAT3 and Akt by phosphorylating STAT3 at Tyr705 and Akt at Ser473 (Fig 2B). It has been reported that after phosphorylation, p-STAT3 could translocate from cytosol into nucleus, so we used nucleus fraction kit to analyze the level of p-STAT3 in the nucleus. The amount of p-STAT3 was increased by oncostatin M in a dose-dependent manner. After treatment for 24 hours (Fig 2C).

DISCUSSION:
Oncostatin M (OSM) is a pro-inflammatory cytokine which was largely secreted in the synovial tissue in RA patients. It has been reported that OSM can induce metalloproteinase-13 (MMP-13) expression and promote cartilage degradation in arthritis. In this study, we found that OSM could enhance the expression of placenta growth factor (PLGF), which may contribute to rheumatoid inflammation.

REFERENCES:
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ACKNOWLEDGEMENT:
This work was supported by research grants from National Science Council, Taiwan.