Decoy Receptor 3 Regulates The Expression Of Tryptophan Hydroxylase TPH1 In Rheumatoid Synovial Fibroblasts

Toshihisa Maeda¹, Yasushi Miura¹,², Koji Fukuda¹, Shinya Hayashi¹, Masahiro Kurosaka¹.
¹Kobe University Graduate School of Medicine, Kobe, Japan, ²Kobe University Graduate School of Health Sciences, Kobe, Japan.

Disclosures:

Introduction: Rheumatoid Arthritis (RA) is an auto-immune disease characterized by over proliferation of synovial tissues and following joint destruction [1]. Synovium are composed with two types of synoviocytes, fibroblast-like synoviocytes (FLS) and macrophage-like synoviocytes [2]. Meanwhile, Tryptophan hydroxylase (TPH) which catalyzes the hydroxylation of L-tryptophan is the rate-limiting enzyme involved in the synthesis of serotonin. TPH has two isoforms; TPH1 expresses in peripheral and central nerve system (CNS) tissues expressing serotonin, such as skin, intestine, and pineal gland, in contrast TPH2 expresses exclusively and dominantly in CNS. Recently several studies suggested that serotonergic systems play an important role in modulating inflammatory pain and bone remodeling [3]. We previously reported that decoy receptor 3 (DcR3), a member of TNF receptor superfamily, overexpressed in RA-FLS stimulated with TNFα inhibits Fas-induced apoptosis [4]. We recently reported that DcR3 induced VLA-4 expression in THP-1 macrophages to inhibit cycloheximide-induced apoptosis [5], and that DcR3 inhibited cell proliferation induced by TNFα or IL-1β via TL1A expressed on RA-FLS [6]. We also reported that the concentration of DcR3 in sera and joint fluids of patients with RA was significantly higher than with osteoarthritis (OA) [7]. Further, by using comprehensive genetic analysis using microarrays, we newly identified TPH1 as one of the genes of which expression in RA-FLS was suppressed by DcR3 [8]. Therefore, in this study, we investigated the expression of TPH1 in synovial fibroblasts of RA and OA stimulated with DcR3 and inflammatory cytokines to elucidate the involvement of TPH1 and DcR3 in the pathogenesis of RA.

Methods: Isolation and culture of synovial fibroblasts. Synovial tissues were obtained from 41 patients with RA fulfilling the 1987 criteria of American College of Rheumatology who underwent total knee arthroplasty but had never been treated with biologics, and 40 patients with OA, under a research protocol approved by the ethics committee. Tissue specimens were minced and digested in Dulbecco’s modified Eagle’s medium (DMEM; Gibco BRL, Grand Island, NY) containing 0.2% collagenase (Sigma, St. Louis, MO) for 2 hours at 37°C. Dissociated cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS; BioWhittaker, Walkersville, MD) and 100 units/ml of penicillin/streptomycin. After overnight culture, non-adherent cells were removed, and adherent cells were further incubated in fresh medium. All experiments were conducted using cells from passages 3-7.

Real-time polymerase chain reaction (real-time PCR). Individual lines of primary cultured RA-FLS were seeded in 6-well-plates at a density of 5 to 10 x 10⁵ cells/well in DMEM supplemented with 10% FBS and cultured for 24 hours. The medium was then replaced with serum-free Opti-MEM medium (Gibco-BRL) and the cells were incubated with 1.0 µg/ml recombinant human DcR3-Fc protein (DcR3-Fc; R&D Systems) or 1.0 µg/ml control IgG1 (R&D Systems) for 12 hours, or 1.0 ng/ml recombinant human TNFα (R&D Systems, Minneapolis, MN) or 1.0 ng/ml IL-1β (R&D Systems) for 24 hours. After the incubation, RNA was extracted with QIAshredder and RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacture’s protocol and reverse-transcribed to first-strand cDNA. The relative expression levels of mRNAs were compared using TaqMan® real-time PCR on a StepOne™ real-time PCR system (Applied Biosystems, Foster City, CA). Pre-designed primers and probes for TPH1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as control were obtained from Applied Biosystems.

Immunohistochemistry. Mouse anti-human serotonin monoclonal antibody (AnaSpec, San Jose, CA) diluted 1:20 was applied to 9 µm frozen sections of rheumatoid synovial tissues cut on a cryostat for overnight. HistoFine Simple Stain Kit (Nichirei, Tokyo, Japan) with peroxidase was used as a secondary antibody, and the sections were developed with DAB, followed by counterstaining with hematoxylin. Stained sections were photographed with Axioskop 2 plus (Carl Zeiss Microscopy, LLC, USA).

Results: TPH1 mRNA was expressed in both RA and OA-FLS (Fig. 1). TPH1 mRNA expression was decreased significantly, 0.71-fold, by DcR3-Fc in RA-FLS, but was not influenced in OA-FLS (Fig. 2). Meanwhile, TPH1 mRNA expression was significantly decreased by TNFα or IL-1β both in RA and OA-FLS (Fig. 3). TPH1 mRNA expression in RA-FLS was suppressed to 0.45-fold by TNFα and 0.27-fold by IL-1β, and that in OA-FLS was suppressed to 0.52-fold by TNFα and 0.34-fold by IL-1β respectively. Immunohistochemistry confirmed that serotonin was present in RA-FLS (Fig. 4).

Discussion: In this study, we first revealed that the expression of TPH1 in FLS was down-regulated by inflammatory cytokines and that TPH1 in RA-FLS was suppressed by DcR3 in a disease-specific fashion. Previous studies suggested that serotonin has pleiotropic effects other than physiological functions. For example, serotonin synthetized by TPH1 in the periphery has functions in immune system and vasoconstriction [9]. Especially, serotonergic pathways play an important role in modulating...
inflammatory pain, compared with mechanistic pain [10]. Further, serotonin decreases osteoblast proliferation and bone formation [10] and increases the total number of differentiated human osteoclasts as well as osteoclast activity [11]. Although the inflammatory cytokines suppress TPH1 expression both in RA-FLS and OA-FLS, DcR3 suppresses only in RA-FLS. Therefore, TPH1 expression in RA-FLS regulated by DcR3 in a disease specific manner may affect serotonin expression to be involved in the pathogenesis of RA, such as modulating inflammatory pain and bone remodeling. Both DcR3 and TPH1 could be a possible therapeutic target of RA.

**Significance:** The correlation between DcR3 and TPH1 in RA-FLS was newly revealed.

**Acknowledgments:** This study was supported by a grant-in-aid from the health science research grant of the Japanese Ministry of Education, Science, and Culture (No. 24592261).

**References:**
1. Alamanos Y. et al., Autoimmunity Reviews, 2005
5. Tateishi K. et al., Biochem Biophys Res Commun, 389, 593-598, 2009
11. Gustafsson et al., J Cell Biochem, 98, 139-151, 2006
Figure legends:

Figure 1. Real-time PCR analysis of TPH1 mRNA expression in RA-FLS and OA-FLS. Values were normalized to GAPDH mRNA expression. The relative expression levels of TPH1 mRNA were compared to GAPDH mRNA, which was assigned a value of 1.

Figure 2. RA-FLS (left panel) and OA-FLS (right panel) were stimulated with 1000 ng/ml IgG1 or DcR3-Fc for 12 hours. The relative expression levels of TPH1 mRNA were determined by real-time PCR. Cells stimulated with IgG1 as a control were assigned a value of 1. *P<0.05.

Figure 3. RA-FLS (left panel) and OA-FLS (right panel) were incubated with 1 ng/ml TNFα or IL-1β. After 24 hours, the relative expression levels of TPH1 mRNA were determined by real-time PCR. Untreated cells were assigned a value of 1. *P<0.05.

Figure 4. Photographs illustrate; (A) brown immunoperoxidase staining of serotonin positive cells in representative synovial tissue sections from patients with RA counterstained with haematoxylin, (B) negative control Ig. The length of each bar is 50 µm.

ORS 2014 Annual Meeting
Poster No: 1257