

## Research Article

# Influence of Periostin on Synovial Fibroblasts in Knee Osteoarthritis

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## Abstract

Osteoarthritis (OA) is a slowly progressive degenerative joint disease characterized by joint space narrowing, osteophyte formation, and subchondral sclerosis. Despite extensive efforts, actual breakthroughs in the identification of biochemical biomarkers of OA have been limited. Therefore, we investigated *in vivo* periostin production in knee synovial fluid of OA patients and assessed its influence on the extracellular matrix using synovial fibroblasts *in vitro*. The study population included 40 OA patients (mean age, 75.3±6.6 years) who were classified according to the Kellgren–Lawrence system. Our results showed that periostin and IL-13 levels were up regulated along with progression of OA. A second round of *in vitro* experiments using human fibroblast-like synoviocytes suggested that elevated periostin mediated an increase in matrix metalloproteinase-9, which is an important molecule in bone turnover. Taken together, these observations indicate that periostin may be a useful diagnostic and/or prognostic marker of OA.

**Keywords:** Periostin; knee osteoarthritis; Synovial fibroblast; Bone turnover

## Abbreviations

HFLSs: Human Fibroblast-Like Synoviocytes; IL: Interleukin; MMP-9: Matrix Metalloproteinase-9; OA: Osteoarthritis; RPMI: Roswell Park Memorial Institute; TGF- $\beta$ : Transforming Growth Factor Beta; TIMP-1: Tissue Inhibitor of Metalloproteinase-1

## Introduction

The number of patients with painful knee Osteoarthritis (OA) continues to dramatically increase with the aging of society: the estimated number of patients exceeds 25 million in the United States [1] and 8 million in Japan [2]. OA refers to the clinical syndrome of joint pain characterized by varying degrees of functional limitation and impaired quality of life. It is the most common form of arthritis and one of the leading causes of pain and disability worldwide, which most commonly affects the peripheral joints, especially the knee. OA is a complex chronic progressive disease attacked by biological and mechanical factors, and as a result from the anabolic and catabolic imbalance in chondrocytes, subchondral bone and extracellular matrix. The degradation and destruction of collagen caused by Matrix Metalloproteinases (MMPs) are considered as the core factor in the occurrence and development of OA for remodeling disorder [3].

Periostin is a member of the fasciclin family of proteins based on its homology to fasciclin I, which was initially identified in insects [4]. Periostin, also termed osteoblast-specific factor 2, is a 93.3-kDa, secreted, vitamin K-dependent, glutamate-containing matricellular protein, originally isolated from a mouse osteoblast cell line [5,6], with known functions in osteology, tissue repair, oncology, cardiovascular and respiratory systems, and various inflammatory settings. Periostin is regulated by Interleukin (IL)-4, IL-13 or Transforming Growth Factor Beta (TGF- $\beta$ ) produced in inflammation, which has a role for remodeling [7].

Periostin is considered an important structural mediator, balancing appropriate versus inappropriate tissue adaption in response to insult/injury. However, the paracrine effect of periostin in OA-synovial fibroblast biology remains poorly understood. Therefore, we investigated the *in vivo* production of periostin in synovial fluid of the knee of OA patients. Furthermore, we evaluated expression levels of Matrix Metalloproteinase-9 (MMP-9) and Tissue Inhibitor of Metalloproteinase-1 (TIMP-1) in synovial fluid from the knees of OA patients [8,9] and examined the influence of these proteins as extracellular matrix modulators of periostin using OA-synovial fibroblasts *in vitro*.

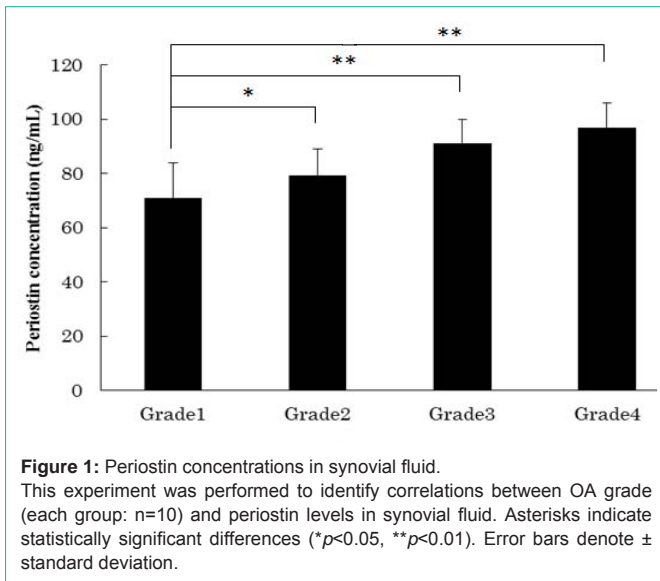
## Patients and Methods

### Patient selection

Synovial fluid was collected from 40 OA patients (mean age, 75.3±6.6 years; age range, 59-82 years) who underwent medical examinations at Showa University Fujigaoka Hospital (Yokohama, Japan). The Institutional Review Board of our teaching hospital approved the study protocol (authorization number: 2013027) and signed informed consent was obtained from all subjects before study participation. Radiographs were reviewed to determine the size and stage of progression of the OA lesions. Radiographic findings were classified according to the Kellgren–Lawrence system [10], as follows: grade 0 = no radiographic features of OA; grade 1 = doubtful Joint Space Narrowing (JSN) and possible osteophytic lipping; grade 2 = the presence of definite osteophyte and possible JSN on anteroposterior weight-bearing radiograph; grade 3 = multiple osteophyte, definite JSN, sclerosis, possible bony deformities; and grade 4 = large osteophyte, marked JSN, severe sclerosis and definite bony deformities.

### Materials

Periostin (recombinant human periostin/OSF-2, CF) was



purchased from R&D systems, Inc. (Minneapolis, MN, USA) and dissolved in Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma-Aldrich Corporation, St. Louis, MO, USA) supplemented with 10% heat-inactivated fetal calf serum (RPMI-FCS; Nihon Bio-Supply Center, Tokyo, Japan), sterilized by passing through 0.2- $\mu$ m pore filters, and stored at 4°C until use.

#### Culture of fibroblasts

Human Fibroblast-Like Synoviocytes (HFLSs) were purchased from Cell Applications, Inc. (San Diego, CA, USA) and resuspended at a density of  $5 \times 10^5$  cells/mL in RPMI-FCS and cultured with different concentration of periostin in 24-well plates in triplicate. After 24 h, culture supernatants were obtained and stored at -80°C until use.

#### Collection of synovial fluid

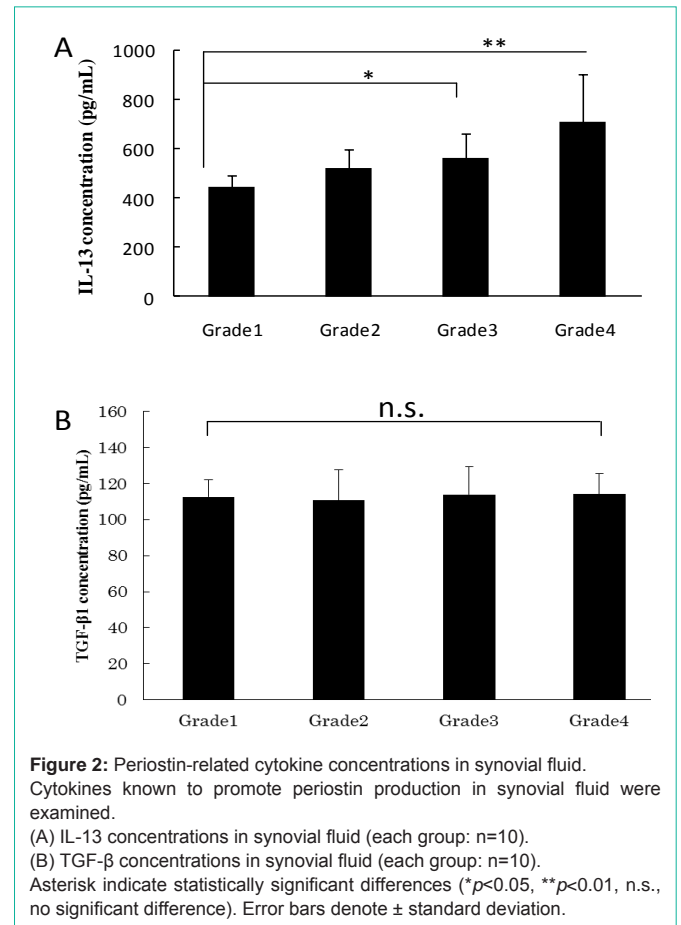
Synovial fluid was collected from the OA patients using an 18-gauge needle and then stored at -80°C until assayed.

#### Assay for biologically active substance

Periostin content in synovial fluid was measured using a commercially available Enzyme-Linked Immuno Sorbent Assay (ELISA) kit (catalog no.: EK-074-41; Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA). Concentration of the inflammatory cytokines IL-13 and TGF- $\beta$  in synovial fluid were measured using commercially available ELISA test kits (catalog nos.: D1300B and DLAP00; R&D Systems, Inc.) according to the manufacturer's recommendations. Additionally, MMP-9 and TIMP-1 concentrations in culture supernatants were measured with commercially available ELISA test kits (catalog nos.: RPN2614 and RPN2611; GE Healthcare, Ltd., Chalfont St Giles, Buckinghamshire, UK) according to the manufacturer's recommendations. The minimum detectable level of these ELISA kits was 0.14 ng/mL for human periostin, 57.4 pg/mL for human IL-13, 3.4 pg/mL for human TGF- $\beta$ , 0.6 ng/mL for human MMP-9, and 1.25 ng/mL for human TIMP-1.

#### Statistical analysis

Data are expressed as means  $\pm$  standard deviations. All assays were repeated three times to ensure reproducibility. Statistical significance



between the control and experimental groups was analyzed by one-way analysis of variance followed by the Scheffe test. A probability ( $p$ ) value <0.05 was considered statistically significant.

## Results

### Analysis of biologically active substances in synovial fluid

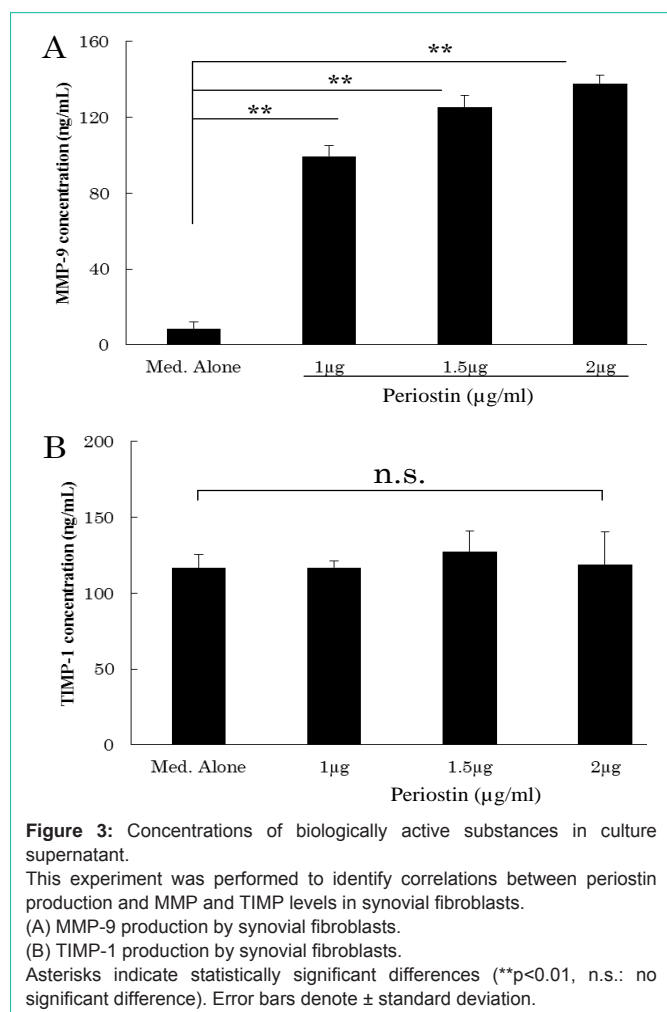
We conducted analysis of synovial fluid samples to identify correlations between OA grade and periostin concentration. As shown in Figure 1, periostin concentration significantly increased with OA. Next, we examined levels of cytokines and periostin production in synovial fluid samples. As shown in Figure 2A, IL-13 levels were significantly increased in the samples along with progression of knee OA. As shown in Figure 2B, no significant difference was found in TGF- $\beta$  content between each stage of OA.

### Analysis of biologically active substances in culture supernatant

Next, we evaluated the influence of periostin on MMP-9 and TIMP-1 levels, which are OA-associated synovial fibroblast products. As shown in Figure 3, MMP-9 levels increased in a concentration-dependent manner according to periostin levels. However, there were no significant differences in TIMP-1 levels among groups.

## Discussion

OA is a chronic disease that causes cartilaginous degeneration and bone tissue destruction [11]. Symptomatic knee OA is highly



prevalent among people aged 50 years and over in most parts of the world and has become a serious healthcare burden worldwide [12,13]. Periostin is transiently up regulated during physiological or pathological changes in OA [14]. Extensive research has helped to elucidate the mechanism of periostin action, yet like OA, its mechanism of action remains unresolved. Therefore, the aim of the present study was to examine the influence of periostin in synovial fluid and OA-associated synovial fibroblasts.

First, periostin levels in synovial fluid obtained from the knees of OA patients was measured, which indicated that periostin levels in synovial fluid increased with progression of osteoclasia in OA. Results of preliminary research indicated high expression levels of periostin in osteoblasts and osteocytes in OA [15]. Based on these findings, we investigated periostin levels according to disease stage based on osteoclastic images, which showed that periostin production increased with progression of OA. Additionally, concentration of the inflammatory cytokine IL-13 increased in synovial fluid along with increasing periostin levels with progression of OA. Tsuchida et al. [16] stated that IL-13 is a potentially useful biomarker to monitor the efficacy of therapeutic treatments for OA. Because IL-13 was found to be associated with periostin concentration, IL-13-associated up regulation of periostin may contribute to progression of OA. Periostin is a matricellular protein with known functions in

osteology, tissue repair, oncology, cardiovascular and respiratory systems, and in various inflammatory settings [14]. The protein is mainly expressed by periosteal osteoblasts and osteocytes [17], and its signaling pathways appear to enhance osteoblast differentiation and bone formation via Wnt/ $\beta$ -catenin signaling [18]. Periostin deficiency alters bone material properties and may adversely impact bone metabolism, which occurs in osteoporosis [19]. Matricellular proteins, such as periostin, interact with cell surface receptors, such as integrins, and are able to bind growth factors as well as structural components of the cellular collagen matrix. In fact, periostin is not only involved in the regulation of bone formation and BMD, but plays a role in the regulation of collagen cross linking [19]. However, the paracrine effect of periostin as a matricellular protein remains poorly understood in OA of the knee. Therefore, we secondly investigated the influence of periostin on MMP-9 expression by fibroblasts *in vitro*. The MMP-9 concentration increased in a periostin-dependent manner, but TIMP-1 concentration did not change in response to periostin. *In vitro* studies on HELSs suggested that elevated periostin mediated an increase in MMP-9 concentration. Several studies have reported increased MMP-9 expression levels in knee synovial fluid with progression of OA [20,21]. When MMP-9 coexists with collagenase, it cleaves type I collagen in the bone matrix in concert with other proteases. MMP-9 has been recognized as an important molecule in bone turnover [22-24]. Our results support a correlation between expression of periostin and MMP-9 in synovial fluid.

TIMPs and MMPs activities are mainly regulated through MAP kinases cascade including AP-1 transcription factors [25]. TIMPs are specific inhibitors that bind MMPs in a 1:1 stoichiometric ratio [26,27]. Tissue homeostasis is achieved by a balance of MMP proteolysis to TIMP expression. However MMPs including MMP-9 are activated by multiple factors, including E-26 (Ets) transcription factors, NF- $\kappa$ B, Polyomavirus Enhancer A-binding Protein-3 (PEA3), Specificity Protein 1 (Sp-1), and Serum Amyloid A-Activating Factor (SAF)-1 as well as AP-1 [28].

In addition, it was reported that periostin may activate the NF- $\kappa$ B pathway in fibroblasts synergistically [29]. These reports and our results support that periostin coordinates the MMPs expression by cascade except the MAP kinase. Elevated MMP/TIMP expression ratios are often viewed with arthritis including OA. In the context of disease pathogenesis, MMP and TIMP expression are interpreted with respect to the proteolytic consequences of increased MMP/TIMP ratios [26,27].

Thus, we speculate that up regulation of periostin in synovial fluid of the knee in OA may confer a cytoprotective effect to promote tissue repair, and propose periostin as a novel biomarker of knee-OA progression. The results in this study showed that periostin had an important role in development of OA. Our findings provide evidence that periostin in OA may reflect tissue changes in this chronic degenerative disease. We showed that periostin affects OA-associated synovial fibroblast, while the articular tissue still contains chondrocytes. It is possible that the chondrocytes contribute to MMP secretion under inflammation. Therefore, we think it is necessary to investigate the influence of periostin towards MMP secretion of either synovial tissue or chondrocytes.

## Conclusion

In summary, although it should be noted that the predictive value of periostin in OA has yet to be confirmed in large clinical trials, our findings provide evidence that periostin in OA may reflect tissue changes in this chronic degenerative disease.

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