Soluble lectin-like oxidized low-density lipoprotein receptor-1 is elevated in the plasma and joint fluids of rheumatoid arthritis patients

Introduction
Rheumatoid arthritis (RA) is one of the immune-mediated inflammatory diseases. LOX-1 (Lectin-like oxidized low-density lipoprotein receptor 1), one of functional receptors for oxidized LDL (ox-LDL), is expressed in various cells, including endothelial cells and chondrocytes, and its expression is enhanced by oxidative stress, inflammatory cytokines (1). Several studies have reported that the ox-LDL/LOX-1 axis modulates cartilage degradation in RA (2) (3). LOX-1 can be cleaved by proteases and released into the circulation as soluble form (sLOX-1) (4). Elevated sLOX-1 levels may reflect increased membrane expression and diseases activities of inflammatory diseases. Although the importance of LOX-1 in RA is expectable, little is known whether LOX-1 is expressed on the synovial cells, and sLOX-1 is present in the plasma and synovial fluids of patients with RA. In this study, we investigated the diagnostic values of sLOX-1 for RA, and the functional roles of LOX-1 and sLOX-1 in the pathogenesis of RA.

Methods
Plasma and synovial fluid samples with RA and osteoarthritis (OA) patients were prepared at the total knee replacement. Ethical approval was granted by the institutional ethics committees, and the written consent of every patient was obtained. Soluble LOX-1 levels in plasma and synovial fluids were measured by Enzyme-Linked Immunosorbent Assay (ELISA). Human fibroblast like synoviocytes (FLSs) were prepared from synovium of RA joints. At confluent, the FLSs were stimulated with TNF-α, IL-1β or ox-LDL for 24 hours. The supernatants or the cell lysates were then collected for further analyses. The supernatants or cell lysates were subjected to 10% SDS-PAGE and then transferred onto nitrocellulose membranes. The membranes were incubated with the first antibody at 4°C overnight, with alkaline phosphatase conjugated second antibody (dilution 1:1000) at room temperature for 1 hour, and immunoreactive bands were visualized with ECL plus kit. Total RNA was isolated from cell lysate for RT-PCR.

Chinese hamster ovary-K1 (CHO-K1) cells stably expressing human LOX-1 (hLOX-1-CHO) were established by transfection with a pME vector containing the full-length human LOX-1. After 24 hours incubation, the supernatants were collected as sLOX-1 conditioned medium. FLSs viability was assessed by the WST-1 assay. Cells were stimulated with ox-LDL at various concentrations for 48 hours. To elucidate the function of sLOX-1, cells were cultured with conditioned medium, and stimulated with ox-LDL (100 μg/ml) for 48 hours. Cells were then incubated with 100 ul DEMEM supplemented with 10 ul of WST-1 for another 5 hours. All data were reported as the mean ± SEM. Student’s t test was used for statistical analyses, unless indicated otherwise. P < 0.05 was considered significant.

Results
Soluble LOX-1 levels in the plasma and synovial fluid of RA patients were significantly higher than those of OA patients (Fig. 1A). In RA patients, sLOX-1 levels were increased in the synovial fluid compared with plasma. In addition, sLOX-1 levels in the plasma and synovial fluid were found to correlate with the levels of plasma C-reactive protein (CRP), as well as with the erythrocyte sedimentation rate (ESR) (Fig. 1B).

FLSs were treated with TNF-α, IL-1β or ox-LDL, and RT-PCR and immunoblotting analysis was performed to evaluate expression of LOX-1 and sLOX-1. We found that ox-LDL dose-dependently increased the amount of LOX-1 mRNA (Fig. 2A). In a non-stimulated condition, the expression of LOX-1 protein was barely detectable, and treatment with TNF-α, IL-1β or ox-LDL markedly increased levels of LOX-1 expression. Treatment with TNF-α increased sLOX-1 expression levels but not with IL-1β or ox-LDL (Fig. 2B).

Discussions
Inflammation is one of hallmarks in RA. Although the evidence has shown that LOX-1 is involved in inflammation, but, to date, the functional role of sLOX-1 that is cleaved from LOX-1 is not fully understood, especially in RA. Results of this study indicate that sLOX-1 levels are predictive of diseases activity in patients with RA. This study shows that sLOX-1 levels of RA patients in the plasma and synovial fluid were significantly higher than those of OA patients and production of sLOX-1 in the RA patients was related to the level of plasma CRP and ESR. Moreover, in RA, there was a significant concentration difference of sLOX-1 between the joints and the peripheral blood circulation. Therefore, this result suggests that the local production of sLOX-1 within joints is greatly increased in proportion to the extent of inflammatory diseases activity, and sLOX-1 levels in the plasma and synovial fluid reflect of disease activity of RA. From present in vitro studies, LOX-1 protein was presented, and TNF-α, IL-1β, or ox-LDL enhanced the expression of LOX-1 in FLSs. ox-LDL at 50, 100 μg/ml significantly reduced FLS viability, whereas sLOX-1 reversed the cell viability. Taken together, sLOX-1 modulates inflammatory condition to inhibit the cell viability of ox-LDL. In conclusion, sLOX-1 levels of plasma and synovial fluid are elevated in RA patients. The present study suggests that sLOX-1 may work for antagonizing the inflammation of RA.

References