Introducing: Rheumatoid arthritis (RA) is one of chronic inflammatory diseases characterized by synovial hyperplasia and invasion into the articular cartilage, leading to progressive joint destruction. Synovial epithelial cells are activated by inflammatory cytokines and immunoantibodies in RA patients, resulting in the activation of multiple intracellular signal pathways mediated by protein phosphorylation. However, it has not been well understood whether these phosphorylation-mediated signalings are further regulated by other intrinsic factors as a post-phosphorylation mechanism.

Peptidyl-prolyl cis/trans isomerase NIMA-interacting 1 (Pin1) is an enzyme that specifically binds phosphorylated serine or threonine, immediately preceding proline (pSer/Thr-Pro), in a subset of proteins, and promotes cis/trans isomerization of the peptide bond, thereby regulating their biological function. Pin1 has been shown to be overexpressed in many human malignancies, autoimmune diseases and chronic inflammatory diseases, and contributes to the constitutive activation of certain intracellular signaling pathways that promote cell proliferation and cell invasion. These previous findings prompted us to study the possible role of Pin1 in the process of cell proliferation and invasion properties of synoviocytes in RA.

Materials and Methods: Synovial tissue was obtained from 11 RA patients and 8 osteoarthritis (OA) patients, who underwent total joint replacement surgery, and informed written consent was obtained from all patients and this study was approved by the ethics committee of Yokohama City University Graduate School of Medicine.

We analyzed Pin1 expression in synovial tissues of RA and OA patients by immunohistochemical staining with Pin1 polyclonal antibodies. To investigate correlation of Pin1 with cell invasion or cell proliferation, we performed immunohistochemical staining with a cell invasion marker MMP-3, and a cell proliferation marker PCNA.

Next, primary fibroblist-like synoviocites (FLS) were established from 11 RA and 8 OA tissues. Cell lines were cultured in DMEM plus 10% FCS in the absence of pro-inflammatory cytokines. Following, expression levels of Pin1, MMP-3 and PCNA were analyzed by western blotting.

To investigate the relevance of Pin1 with cell proliferation and invasive properties in RA-FLS, we constructed a retrovirus-mediated small interfering RNA (siRNA) vectors that targeted Pin1 mRNA. pSUPER-internal ribosome entry site-PURO vector was digested with BglII and HindIII and annealed oligos (Pin1: 5'-gatcCgagTggTaTTggtTTTkAAaTTgTagTTACTACTCGCaTTccggggagAC-3'; control: 5'-gatcCgagTggTaTTggtTTkAAaTTgTagTTACTACTCGCaTTccggggagAC-3') were ligated into this vector. We used high Pin1 expressing cell lines and selected infectants with puromycin for 48 hours, followed by cell proliferation and invasion assays.

Results: Although the steady-state levels of Pin1 expressions were detected in both RA and OA sinovial lining cells, its expression levels were relatively higher in RA-synovial tissues (ST) than in OA-ST, scored by immunohistochemistry grading system (classified into 3 grades according to Pin1 expression levels, i.e. +/-, +, 2+). Pin1 expression was detected in both infiltrating inflammatory cells and synovial epithelium in RA-ST. In RA-ST, approximately 55% RA-ST demonstrates extremely high Pin1 expression (2+), whereas almost all OA-ST show lower levels of Pin1 expression (+/- or +). Both MMP-3 and PCNA expressions were relatively higher in the RA-ST, especially in those with high Pin1 expression (2+). There were significant correlations between Pin1 expression and either MMP-3 or PCNA expression in RA-ST according to Spearman's Rank Correlation test.

To examine the Pin1 expression at cell culture levels under cytokine free condition, we analyzed Pin1 protein expression by Western blotting. The protein expression of Pin1 was relatively higher in RA-FLS than in OA-FLS consistent with the results from tissue staining. The expression levels of Pin1 in RA-FLS were also various, however, there were close correspondences between RA-ST and RA-FLS in terms of Pin1 expression. We selected 2 high Pin1 expressing RA-FLS and 2 low Pin1 expressing RA-FLS as well as 2 OA-FLS for further functional analyses. MMP-3 and PCNA protein expressions were not detected, or very low in low Pin1 expressing RA-FLS and OA-FLS, however, high Pin1 expressing RA-FLS cells still retained high MMP-3 and PCNA expression, suggesting higher cell proliferation and invasive properties even in cytokine-free conditions. These results together indicate that Pin1 might play a role in cell invasion and proliferation in RA-FLS, as an intrinsic regulator dependent from pro-inflammatory cytokines. To investigate cell growth and cell invasion, we knocked-down endogenous Pin1 by specific-siRNA in a high Pin1-RA-FLS and determined the Pin1 protein levels by immunoblot analysis (Fig1B). Both cell growth and invasive properties were significantly inhibited in Pin1-siRNA expressing cells but not in control-siRNA RA-FLS (Fig1A). Concomitantly, both MMP-3 and PCNA protein expression were also suppressed in Pin1-siRNA expressing cells, but not in control-siRNA expressing cells, as revealed by Western blotting. These results suggest that Pin1 contribute to cell invasion and cell proliferation via activating the intrinsic RA-specific signaling irrespective of cytokine treatment.

Discussion: High Pin1 expression was found in certain numbers of RA-ST with enhanced expression of both MMP-3 and PCNA. Furthermore, aberrant high protein expressions of Pin1, MMP-3 and PCNA were maintained in established FLS derived from the RA-ST with high Pin1 expression in regular cell culture conditions without pro-inflammatory cytokines. The targeted inhibition of Pin1 by specific-siRNA in RA-FLS resulted in the suppression of cell proliferation and invasive properties. These results together indicate that Pin1 might play a pivotal role in the maintenance of RA-specific phenotype of RA-FLS in pro-inflammatory cytokine-independent mechanism. Hence, Pin1 could be a new diagnostic marker as well as a therapeutic target in RA.