microRNA-146 inhibit osteoclast differentiation

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Introduction
MicroRNAs (miRNAs) are a class of non-coding RNAs that regulate gene expression by translational inhibition and messenger RNAs degradation. Several microRNAs exhibit a tissue-specific or developmental stage-specific expression pattern and have been reported to be associated with human diseases. miRNA (miR)-146 was reported to highly express in rheumatoid arthritis (RA) synovium and peripheral blood mononuclear cell (PBMC), and inhibits the expression of IRAK1 and TRAF6 by binding to the 3' UTR of their mRNAs. TRAF6 is the one of the important mediator in osteoclastogenesis, therefore, the aim of this study was to confirm whether over expression of miR-146 inhibit osteoclastogenesis mediated tumor necrosis factor (TNF) α from human PBMC in vitro, and to investigate intravenous administration of ds miR-146a could prevent bone destruction in collagen induced arthritis (CIA) mice.

Methods
Human blood was collected from healthy volunteers, and PBMCs were isolated, and seeded in 96-well culture plates. The following day, double strand (ds) miR-146a was transfected into the cells in various concentrations and cultured for 3 weeks in the presence of macrophage colony stimulating factor (M-CSF) and TNFα. After 3 weeks, TRAP positive and multinuclear cells were counted, and pit formation assay was performed. At 3 days after transfection, the expression level of PU-1, c-Jun, NFATc1 and TRAP were evaluated by real time PCR.

CIA mice were induced in male DBA/1 mice. After the onset of clinically distinct arthritis, the experimental group (n = 5) was given an intravenous injection of ds miR-146a (20µg/50µl). In control group (n = 5), scrambled siRNA was injected. Radiographic and arthritis scores were evaluated at 3 weeks after injection.

Results
The number of TRAP positive large multinucleated cell was significantly decreased by over expression of miR-146 in a dose dependent manner. Bone resorption on dentin slice could not be observed with ds miR-146 of concentration of 50nM (Figure 1). The expression of PU-1, c-Jun, NFATc1 and TRAP was significantly down regulated at 3 days after the transfection of miR-146a (Figure 2).

At 3 weeks after the injection, there is no significant difference of arthritis score between 2 groups. However, bone destruction tended to be prevented in radiographic examination (Figure 3).

Discussion
Quite recently, miRNA was attracted attention because it plays a crucial role in human disease and can be the new therapeutic target. Several miRNAs were reported to play a role in the pathogenesis of RA, therapeutic trials which regulate the endogeneous miRNAs in vivo were reported. Our result indicated that miR-146 could inhibit TNFα mediated osteoclastogenesis from human PBMC in vitro. However, intravenous injection of ds miR-146a could not improve arthritis score. In radiographic evaluation, bone destruction tended to prevent by the administration of ds miR-146. There is the possibility that increasing the dose of ds miR-146 might lead to improve the arthritis. Further study must be needed to prevent bone destruction completely by examine the condition of ds miR-146a injection. Administration of miR-146 might be a novel therapeutic target for bone destruction of RA.