Altered expression of microRNAs in interleukin-17 producing T cells in rheumatoid arthritis patients

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ABSTRACT
INTRODUCTION:
MicroRNA (miRNA)s are a family of non-coding RNAs identified in organisms ranging from nematodes to humans. Several miRNAs exhibit a tissue-specific or developmental stage-specific expression pattern and have been reported to be associated with human diseases. The discovery of a new lineage of CD4+ effector T helper type 17 cells (Th17 cells) that selectively produce IL-17 has provided exciting new insights into immune regulation, host defense, and pathogenesis of autoimmune and other chronic inflammatory disorders including of rheumatoid arthritis (RA). In several studies, miRNAs play a role in RA pathogenesis. The purpose of this study is to identify miRNAs about differentiation of the IL-17 producing T cells, and to analyze their expression pattern in the RA patients.

METHODS:
Human blood was collected from 5 healthy volunteers (31.8 ±1.1 years of age, mean ± SD). Isolation of human peripheral blood mononuclear cells (PBMC) and CD4+ T cells were isolated from PBMC using auto MACS (CD4+ T cell Isolation kit, Miltenyi Biotec, ). Expansion of IL-17 producing T cells in human PBMC by IL-1β, IL-2, IL-6, IL-23. After confirmation these cells production of IL-17 by real time PCR and ELISA, miRNA microarray was performed. Furthermore, real time PCR for identified miRNAs was performed in PBMC and synovium in RA patients. Moreover, immunohistochemistry of IL-17 in RA and OA synovium demonstrated that RA synovium have abundant Th17 cells.

RESULTS
As a result of microarray in expanded IL-17 producing T cells, six miRNAs were significantly up regulated in the differentiation of the IL-17 producing T cells. Six miRNAs were miR-26a (1.81 fold; p =0.029), 146a (1.43 fold; p =0.040), 146b (1.80 fold; p =0.040), 150 (1.61 fold; p =0.040), 155 (2.03 fold; p =0.007) and let-7a (2.12 fold; p =0.012). These miRNAs expression level in synovium and PBMC was significantly higher in RA patients (Fig.1, 2). Especially, with intensely expression of IL-17 in PBMC and synovium, these miRNAs intensely expressed.

DISCUSSION
Recently, a potential link between microRNA and several human diseases has been examined. In addition, the recent report that expression of microRNA-146 in RA patient's synovial tissue was performed, and the participation of some microRNA was suggested in the cause of the RA appearance of disease. Moreover, the discovery in mice of a new lineage of CD4+ effector T helper cells Th17 cells that selectively produce IL-17 has provided exciting new insights into immune regulation, host defence, and the pathogenesis of autoimmune and other chronic inflammatory disorders. A few studies suggest that IL-17 may have a role in the pathogenesis of human RA as well. We thought whether became it to be a new treatment method to RA being able the control of microRNA related to IL-17. We did not isolate IL-17 producing T cells but expanded. In this point, there might be a limit of this research. However, after it had been concluded IL-17 producing T cells by real time PCR and ELISA, the expanded T cell was analyzed with miRNA microarray. As for the selected miRNAs, expression was intentionally seen from OA by RA in both PBMC and synovium. We are guessing these miRNAs related to the expression of IL-17 producing T cells. If the expression of IL-17 producing T cells were able to be suppressed by using these miRNAs, we thought these mechanisms may be potential targets for the development of new RA therapies.