Author's response to reviews

Title: MicroRNA-146a expresses in interleukin-17 producing T cells in rheumatoid arthritis patients

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Version: 4 Date: 20 August 2010

Author's response to reviews: see over
August, 20, 2010

Dr. Melissa Norton, MD
BMC Musculoskeletal Disorders Editor in chief
Dear Dr. Melissa Norton

Re: “MicroRNA-146a expresses in interleukin-17 producing T cells in rheumatoid arthritis patients” by Takuya Niimoto, Tomoyuki Nakasa.
(submitted on May 24 2010.MS: 4970360233971936)

Here is a manuscript (Full – Length Article) we have just written entitled, “MicroRNA-146a expresses in interleukin-17 producing T cells in rheumatoid arthritis patients”. Now we have addressed criticisms raised by the reviewers, and modified a manuscript.

On behalf of my co-authors, all of whom have approved the paper, I am re-submitting the manuscript for possible publication in “BMC Musculoskeletal Disorders”. No portion of the paper has been published, and the manuscript is not under consideration of any other journal.

Please re-consider this paper for publication.

The following are responses to all of the reviewers’ comments:

Reviewer’s report
Title: MicroRNA-146a expresses in interleukin-17 producing T cells in rheumatoid arthritis patients
Version: 3 Date: 2 July 2010
Reviewer: Annette van der Helm-van Mil

Reviewer’s report:
Major revisions.
When I read the article I found several inconsistencies. For example in the methods they describe to have 6 PA and 6 OA patients. In the results section there appear to be 11 RA patients.
Ans). In patient RA6, we obtained both PBMC and synovial tissue. Therefore, RA patients are 11 people in total.

In the method section they describe that pt RA 2 en 4 have erosions on small
joints, in the result they mention that the larsen score is low, which does not seem to fit.

Ans). We corrected and added the following sentence, p6, line 6-7, "soft tissue swelling, juxta-articular osteopenia, and loss of joint space were observed."

I do not understand the larsen scoring that is used (table 1). Generally this is a number between 0 and 200.

Ans). We corrected Table 1 in this article.

minor revions
There are multiple typos and grammatical errors throughout the manuscript.

Ans). We took a check of native speaker, and we revised this article.

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Reviewer’s report**

**Title:** MicroRNA-146a expresses in interleukin-17 producing T cells in rheumatoid arthritis patients

**Version:** 3 **Date:** 6 July 2010

**Reviewer:** DIMITRIOS ILIPOULOS

**Reviewer’s report:**

Niimoto et al., identified that six microRNAs are significantly up-regulated in IL-17 T cells. Also, it is shown that miR-146a is highly expressed in RA synovium. The authors very nicely have shown miR-146a and IL-17 staining by immunohistochemistry. However, there are some important points that need to be addressed.

**Major Compulsory Revisions**

1) The authors have identified that miR-155 and SOCS1 are both up-regulated in
IL-17 T cells. Previous studies have shown that miR-155 targets directly SOCS1 expression, thus it is expected that miR-155 up-regulation would decrease SOCS1 expression levels. What is the explanation for this discrepancy?

Ans). SOCS1 was reported to be up regulated during Th17 differentiation. Indeed, SOCS1 is the direct target as miR-155, therefore, there is the possibility that up regulation of miR-155 can decrease the expression of SOCS1 during the differentiation of IL-17 T cells. However, the target genes of miRNAs are estimated to range between one and several hundred, based on target predictions using the bioinformatics approach. Therefore, many molecular networks via miR-155 might interact in the differentiation of IL-17 T cells. Moreover, miR-155 might not function dominantly as regulator of SOCS1 in the differentiation of IL-17 T cells.

2) The main finding of this manuscript is that miR-146a is highly expressed in RA. One important direct target of miR-146a is IRAK1. The authors should check IRAK1 protein levels during differentiation of IL-17 T cells.

Ans). As the reviewer mentioned, it is important to confirm the protein of IRAK1. Therefore, we performed western blotting of IRAK1 protein during differentiation of IL-17 T cells. We added the following sentence, p9, line 12-20,

"Western Blotting

Ten µg of the protein were separated on NuPAGE® Novex® Bis-Tris Mini Gels (Invitrogen, Carlsband, CA) and transferred onto a nitrocellulose membrane (Invitrogen, Carlsband, CA). Mouse monoclonal antibody against a partial recombinant IRAK1 (Abnova, Taiwan) and rabbit polyclonal anti-actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA) were used as primary antibodies. Anti-mouse goat IgG (MP Biomedicals, LLC, Santa Ana, CA) for IRAK1 and anti-rabbit goat IgG (MP Biomedical, LLC, Santa Ana, CA) for actin were used for secondary antibodies. Band detection was performed using the enhanced chemiluminescence reagent, ECL Western Blotting Detection Reagents (GE Healthcare UK Ltd, Little Chalfont, Buckinghamshire).", and p12, line 13-14, “However western blotting revealed that IRAK1 was down regulated in expanded IL-17 producing T cells in protein level (Figure 1C).", and p15,
line 18-20, “However IRAK1 protein was decreased in the expanded cells, which suggested miR-146a/b might inhibit the translation to IRAK1 protein from mRNA.”, and modified Figure1.

3) MicroRNAs exert their function through regulation of their down-stream targets. The authors should identify by using any microRNA prediction program (for example TargetScan) the down-stream targets of the six important microRNAs that are expressed in T cells. These genes should be presented in a table.

Ans). According to the reviewer’s comment, we summarized the validated target genes of 6 miRNAs and presented as Table 3.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests:
I declare that I have no competing interests

Sincerely yours,

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