Dear reviewer:

Thanks for your efforts and comments. We have revised the manuscript accordingly. Please see our point to point responses below.

Comments of reviewer 1:

1. The reviewer’s concern that the data is not adequately supporting authors’ conclusions. miR-146a-5p can be simply correlating with inflammation in patients and might not be necessarily representative of disease severity only in PsA patients. Authors need to include patients with psoriasis (without arthritis) show the miR-146a-5p is specific for PsA.

Response:

Thanks for the comments. We have incorporated additional 17 patients with psoriasis without arthritis (PsO), along with the original 34 patients of psoriatic arthritis (PsA) and 34 healthy controls (NC), in the study. The data consistently showed that miR-146-5p expression in CD14+ cells from patients with PsA was significantly higher than that from patients with either PsO or NC. Accordingly, title of this manuscript is revised as ‘MiR-146a-5p expression in peripheral CD14+ monocytes from patients with psoriatic arthritis induces osteoclast activation, bone resorption, and correlates with clinical efficacy’.

We have incorporated the demographic data of psoriatic patient without arthritis to Table 1 and we also have incorporated the data of miR-146a-5p expression in patients with PsO into Figure 2. Please refer to the revised Table 1(P14~15) and Figure 2(P17).

2. Based on the presented data authors cannot claim miR-146a-5p can be a diagnostic marker. To do that they need to have samples from other arthritis such as rheumatoid arthritis, spondyloarthritis, osteoarthritis (at least 2-3 types).
Response:
We repeated the experiments in a small scale from 3 patients with rheumatoid arthritis (RA) and 3 patients with ankylosing spondylitis (AS). The results showed that the expression of miR-146a-5p in patients with RA and AS was similar to that in the NC group and significantly lower than that in PsA (as the figure below). Since the numbers of RA and AS were small and osteoarthritis (OA) was not taken into consideration, we did not incorporate these two groups in the study and we replaced the title of this talk as ’MiR-146a-5p expression in peripheral CD14+ monocytes from patients with psoriatic arthritis induces osteoclast activation, bone resorption, and correlates with clinical efficacy’.

3. Authors showed that biologic treatment reduced miR146a expression levels in PsA patients. Authors also need to present what were the expression patterns for the other miRNAs.
In addition to the miR-146a-5p, we also analyzed the expressions of miR-146b and miR-155-5p from CD14+ monocytes in NC, patients with PsA before and after 28 weeks biologic treatment (n=31, 10 and 10). The result showed that only miR-146a-5p, but not miR-146b-5p nor miR-155, was reduced in parallel to the clinical improvement. Please refer to Figure 4C (P21).

It is not clear how the figure 1A and 2A are different and why the differences for same miRNA present in these two figures (figure 1 and Figure 2).
Response: The y-axis is different in Figure 1A and 2A, one is relative expression level and one is the delta Ct representing cycle numbers in PCR. The y-axis as expression level has been unified in all the figures. Please refer to the revised Figure 1 and 2 (P16~18).

The authors did not explain the rationale behind this work and how they decided to study only miR-146a-5p, miR-146b-5p, and miR-155 and no other miRNAs.
Response: More than 40 miRNAs (including miR-21, -29, -31, -124, -133a, -146a, -223, -503, -378, -125a, -148a, -155, and -422a) have been documented to regulate the differentiation of osteoclast precursors into mature osteoclasts. On the other hand, many of the miRNAs also regulated the osteoblast differentiation with known transcription factors and epigenetic regulators, including miR-155, -30, -503, -146a, and -541. Experimental evidences showed that Mir-146a inhibits proliferation and induces apoptosis through bcl-2 in osteoblasts. A study in PNAS also showed that miR-155 is upregulated in CD68+ macrophages in the synovium from RA. Hence, we decided to choose the common miRNAs, miR-155 and miR-146a, in the osteoclastic and osteoblastic...
differentiation to start with since PsA is characterized by both osteoclastic and osteoblastic activation. The other similar form of miR-146a, the miR-146b, was also chosen as an internal control. Please refer to the introduction section.

In fact, more than 40 miRNAs (including miR-21, -29, -31, -124, -133a, -146a, -223, -503, -378, -125a, -148a, -155, and -422a) have been documented to regulate the differentiation of osteoclast precursors into mature osteoclasts(23). On the other hand, many of the miRNAs also regulated the osteoblast differentiation with known transcription factors and epigenetic regulators, including miR-155, -30, -503, -146a, and -541(24). Experimental evidences showed that Mir-146a inhibits proliferation and induces apoptosis through bcl-2 in osteoblasts(25). A study published in PNAS also showed that miR-155 is upregulated in CD68+ macrophages in the synovium from RA(26). Hence, we decided to choose the two common miRNAs, miR-155 and miR-146a, in the osteoclastic and osteoblastic differentiation to start with since PsA is characterized by both osteoclastic and osteoblastic activation. The other similar form of miR-146a, the miR-146b, was also chosen as an internal control.

4. The authors did not establish well what is the correlation between miR-146a-5p and osteoclast precursors (OCP) numbers. It is already known that PsA patients have higher amounts of OCP, so it comes as no surprise. Moreover, authors did not clearly show how miR-146a-5p and OC number significantly different between HC and PsA patients. They even did not present the standard deviation or standard error clearly in Figure 3D.

Response:
We asked whether the number of osteoclast differentiation would be associated with the expression of miR-146a-5p in patients with PsA. The R Square of PsA and NC group are 0.4821 (p=0.08) and 0.2425(p=0.41),
respectively, indicating a small but significant association between miR-146a-5p expression and the number of osteoclasts in PsA. Please refer to Figure 3E (P20).

Authors also need to present the correlation between miR-146a-5p and any inflammation marker such as CRP.

Response:
We asked whether the expression of miR-146a in CD14+ monocytes was associated with the blood CRP level in 22 patients with PsA. The result showed that R Square is 0.3645 (p=0.0029) (Figure 5A), indicating a small but significant association of miR-146a-5p in CD14+ monocytes with blood CRP level in patients with PsA. Please refer to Figure 5A (P23).
Minor concerns
It is not clear why the authors used TNF with RANKL for OC and how these experiments are helping their conclusion (data do not add little knowledge due to the limited experiment was done).
Response:
In 1998, two different research groups reported that reception activation of NF-kB ligand (RANKL) was essential for osteoclast differentiation (Proceedings of the National Academy of Sciences of the United States of America, vol. 95, no. 7, pp. 3597–3602, 1998; Cell, vol. 93, no. 2, pp. 165–176, 1998). RANKL induces osteoclast differentiation by binding to RANK in myeloid cells and monocytes. TNF-a was reported to induce the formation of osteoclasts from bone marrow macrophages in vitro. TNF-a was shown to permit the osteoclast activation by RANKL as reported in J Clin Invest in 2000 (Journal of Clinical Investigation, vol. 106, no. 12, pp. 1481-1488, 2000). Hence, to induce osteoclast differentiation from circulating monocytes in this study, RANKL and TNF-a were used to induce osteoclastogenesis as a model.
Authors need to present the data from each patient with individual measures for their data presented as bar graphs.
Response:
Thanks for the comments. The bar graph has been replaced by the dotted plots. Please refer to the revised Figures.

What is delta delta Cq (or authors meant delta delta Ct)?
Response:
Actually, the delta Cq was a typo of delta Ct. However, for standardization and consistence, the relative expression levels are used throughout the figures to avoid confusion between delta Ct and expression levels.

**Overall comments**
The presented work is interesting but not the presented data are not adequately convincing and do not support the author's major conclusions. Need more experimentations and better controls.
Response:
As pointed out earlier in the reply, the title has been replaced to avoid the use of diagnostic biomarker. In addition, patients with psoriasis without arthritis (n=17), two more small groups from RA and AS (n=3 and 3, respectively) were used to compare their expression levels of miR-146a-5p in PsA and in NCs. There was an association of miR-146a-5p expression and CRP level from patients with PsA.
We hope that the revised manuscript in its current form is of general interest for the readers and is appropriate for publication in Journal of Clinical Medicine.

With best regards,

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