Reviewer's report

Title:(5R)-5-hydroxytriptolide Inhibits Osteoclastogenesis through RANKL/RANK/OPG Signaling Pathway

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Reviewer:Kyung-Hyun Park-Min

Reviewer's report:

This study examines the role of LDDT-8 on RANKL and OPG expression in CD3+ T cells isolated from PBMCs and SFMCs of rheumatoid arthritis patients. Authors also test the effect of LDDT-8 on RANKL-induced osteoclastogenesis of RAW cells. LDDT-8 is a compound isolated from the extracts of traditional Chinese medicine and has shown efficacy on various diseases. Author shows that LDDT-8 treatment on patients' PBMC and SFMCs increases OPG expression and OPG/RANKL ratio in CD3+ T cells. LDDT-8 treatment also regulates cytokine profiles in patients' PBMC and SFMCs and suppresses RANKL-induced osteoclastogenesis of RAW cells by inhibiting NF-KB activation. Data shown in the proposed manuscript are interesting but three parts of the data are not well linked in the manuscript.

- Major compulsory Revisions

1. Fig 1 and 2: The data in figure 1 and 2 are important points of the manuscript. The author should show the representative graph (either density plot or contour diagram) and mark the gate settings.

2. Fig 3 and 4: It would be useful to know the baseline of each cytokine in unstimulated samples and author should include the results from unstimulated samples. In addition, baseline cytokine profiles would determine the contribution of activated T cells on cytokine production.

3. Fig 5: Osteoclasts are defined as TRAP-positive multinuclear (more than three nuclei) cells. It is difficult to determine the effect of LDDT-8 on osteoclastogenesis by current images. Improved photographic images are required. Either 10X or 20X magnification is better to determine the effect of LDDT-8 on osteoclast differentiation.

4. Fig 7: Data will be more convincing if the author shows the graph of densitometry of pIκB/GAPDH.

- Minor Essential Revision

1. Fig. 5: Labels are missing.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable

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

Declaration of competing interests:
I declare no competing financial interests