Peer review file

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Reviewer A

Tian and colleagues propose here a short review about the role of sphingosine 1-phosphate (S1P) and osteoporosis. S1P in bone and joint diseases is a "hot topic", and several reviews have been published the last 3 years, so this one is somehow redundant concerning to available literature. Authors should strongly improve the manuscript in order to make it more "attractive".

Major points:

Comment 1: Abstract is not clear enough, please explain better the concept "the transfer of osteoclasts precursors". Keywords should be reduced and organized according to importance.

Reply 1: Modified in the manuscript (shown in track change format).

Change in the text: It is clarified and re-written "S1P also controls the migration of osteoclast precursors between blood and bone….."

Comment 2: Introduction about S1P metabolism is poor. For example, S1P can be produced in nucleus, and several intracellular targets of S1P have been discovered the last ten years related to nuclear S1P. S1P signaling 'inside out" has to be better described. The release of S1P to the extracellular medium has to be better explained. Several receptors (ATP-binding cassette receptors, spinster 2, Mfsd2b…) are implicated in S1P sorting and could be important for bone and joint diseases including osteoporosis.

Reply 2: More information has been added in the text (shown in track change format).

Changes in the text: The indicated section in Introduction has been re-written "The cell surface receptors regulate the activities of S1P, and S1P signaling has been regarded as the model of inside-out signaling. S1P is produced inside the cells and exported outside of cells. Endothelial cells release S1P in extracellular milieu through specific type of transporter including ATP-binding cassette (ABC) receptors, SPNS2 (Spinster Homologue 2) or major facilitator superfamily transporter 2b (Mfsd2b); attaches and transfer signals through one of the five specific G protein-coupled receptors on cell

surface (named S1PR1, S1PR2, S1PR3, S1PR4, S1PR5) to promote various types of cellular functions".

Comment 3: The part "role of S1P in bone injury and repair" has to be renamed. This title is not very accurate. Authors should describe better the role in bone homeostasis, especially osteoblast-osteoclast coupling and specific functions in each cell type. Some bonafide articles are missing (Grey2002 and 2004, Pederson, 2008…). Authors state that "Recently, S1P has been shown to control the transfer of osteoclast precursors…" the first article was published in 2009, son it's not very "recently".

Reply 3: The suggested subheading has been modified, and more information has been added in the text (shown in track change format).

Changes in the text: The modified heading is now "Role of sphingosine 1 -phosphate in bone metabolism". This section has been improved with more clear description, as follows: "S1P exerts receptor-mediated effects on cell proliferation, differentiation and migration of both osteoclasts and osteoblasts. S1P stimulates bone formation and plays an essential role in bone homeostasis by targeting both osteoclastogenesis and osteogenesis. S1P released by cells in extracellular environment binds with S1P receptors in osteoblast leading to an increase in RANKL expression through the induction of COX2 level. The enhanced expression of RANKL in osteoblasts then binds with more RANK and mediates osteoclast differentiation. Extracellular S1P also regulates bone homeostasis by coupling osteoclast and osteoblast activity". And the word 'recently' has been removed from suggested place.

Comment 4: The part "therapeutic aspect of S1P in osteoporosis" has to be better organized. Authors should organize the different studies in "in vitro", "in vivo" and "human studies", everything is mixed.

Reply 4: Thanks reviewer. Most of the studies discussed here are on in vivo studies, as very few studies on human subject (not reported here as human studies not yet confirmed with clear findings). We have organized this section on mechanism based actions. If we separate in vitro studies from these in vivo studies, mechanism of therapeutic strategies become messed up. We request editor and reviewer to reconsider this. If they still think in vivo and in vitro studies should be completely separated, we may do. But we believe that will not be better to present this section

Changes in the text: We have done some rearrangements and also made several paragraphs in this section for better understanding.

Comment 5: FTY720 is an analog of sphingosine not S1P. Its mechanism of action and possible role in osteoporosis has to be better discussed. Several articles have been published about FTY720 and osteoclasts or osteoblasts, please discuss them.

Reply 5: More information has been added in the text (shown in track change format).

Changes in the text: We made some addition about FTY720. For example, "treatment with FTY720 inhibited osteoporosis in in murine bone marrow cells by inhibiting proinflammatory cytokine production and suppressing osteoclastogenesis. It is reported that FTY720 could prevent bone loss in animal model via inhibiting RANKL induced osteoclastogenesis"

Comment 6: English level is really poor; please check it and correct it with an editing service.

Reply 6: Corrected in the manuscript.

Changes in text: The whole article has been carefully read and language has been corrected.

Minor points:

Comment 7: The correct way to write S1P is sphingosine 1-phosphate, please correct.

Reply 7: Corrected in the manuscript.

Changes in text: Corrected in whole manuscript.

Comment 8: Line 74-76, please include ceramide and correct the phrase.

Reply 8: Corrected in the manuscript.

Changes in text: Ceramide has been included and phrase corrected (now line 81-83).

Comment 9: Line 97, please change "homeostatis" with "homeostasis" and "resorpstion" with "resorption".

Reply 9: Corrected in the manuscript.

Changes in manuscript: Corrected to 'homeostasis' and 'resorption' (in revised text, line 112-113).

Comment 10: Line 179, please change "markers" with "marker".

Reply 10: Corrected in the manuscript.

Changes in manuscript: Corrected to ‘marker’ (in revised text, line 201).

Reviewer B

Comment 1: Editing errors should be corrected (lines 74-77).

Reply 1: Corrected in the manuscript.

Changes in manuscript: Corrected the linguistic problems in text.

Comment 2: The word “bone metabolism” is more appropriate than “bone injury and repair” for osteoporosis.

Reply 2: Corrected in the manuscript.

Changes in manuscript: Changes made in different places in text and headings (“bone metabolism” has been used instead of “bone injury and repair”).

Comment 3: In the sections titled “Role pf S1P in bone injury and repair”, more comprehensive roles of S1P in bone metabolism, including a direct role of S1P on osteoclasts and bone formation, should be described.

Reply 3: More information has been added in the text.

Changes in text: This section has been modified a lot with more description and clarity.