

Peer Review File

Article information: <https://dx.doi.org/10.21037/atm-21-2114>

Response to Reviewer A

Thank you for your detailed comments. The comments point the deficiencies of our paper and provide an important direction for us to revise our paper. The revised parts that correspond to the comments are listed as follows:

Comment 1: The page numbers and lines indicated in the “Narrative Review Checklist”, apparently, are not in accordance with the version of the manuscript that this reviewer received. Please check the information.

Reply 1: We have checked and modified the information in “Narrative Review Checklist”.

Changes in the text: **See “Narrative Review Checklist”.**

Comment 2: I understand that this is a narrative review, but the manuscript would benefit from a sentence indicating some of the criteria used to include or exclude manuscripts in the review. Also, it must be clear what kind of information is present in figures and tables. It is only the interactions experimentally verified or also those inferred with bioinformatic analysis?

Reply 2: We have added the including criteria of references as advised.

Meanwhile, we have thoroughly checked the information presented in Figures and Tables, the interaction only predicted by bioinformatic analysis have been marked.

Changes in the text: **See Page 5-6, line 92-100; see Table 1 and Table 2, Figure 2 and Figure 3).**

Comment 3: The title of the manuscript (Circular RNAs as potential regulators in bone remodeling: a narrative review) indicated that the review will explore the role of circRNAs in bone remodeling. So, I think that the explanation of bone remodeling process and how the osteogenesis and osteoclastogenesis are involved in this process could be more detailed.

Reply 3: We have detailed the processes of bone remodeling as advised.

Changes in the text: **See Page 4-5, line 68-74.**

Comment 4: The authors cite some articles that use transcriptome approaches to identify circRNAs expressed during osteogenesis or osteoclastogenesis. However,

although some studies are described (as reference 37) others that are relevant for the thematic of review (e.g. references 9,10, 27, 28, 29, 54) were underexplored. I believe that the authors could include some more information about these studies, which will contribute to improve the manuscript.

Reply 4: We have included more information of the article mentioned above to describe the involvement of circRNAs during osteogenesis and osteoclastogenesis.

Changes in the text:

See Page 5, line 82-88 (reference 11, Zhang et al. 2019; reference 12, Dou et al. 2016);

Page 8-9, line 152-162 (reference 29, Kang et al. 2020; reference 31, Li et al. 2019; reference 32, Gu et al. 2017);

Page 13, line 264-267 (reference 58, Zhong et al. 2020).

Comment 5: As the topics “BMPs” and “SMADs” seem to be very related, it would be interesting to group them into a single topic. Furthermore, in the citation of Figure 2 (page 6, lines 117-118) or in its legend it would be interesting to specify that is the SMAD-dependent BMP/TGFb pathways.

Reply 5: We have grouped the “BMPs” and “SMADs” into a single topic and modified the citation and legend of Figure 2 as advised.

Changes in the text: **See Page 9, line 168; Page 9-10, line 175-186; Page 44, line 942.**

Comment 6: The circ19142 and circ5846 and its interaction with mir-7067-5p is mentioned in the “BMP” topic (page 6, lines 125-127). The same circRNAs are mentioned in “Wnt” topic (page 10, lines 199-203) and is shown in Figure 3 (Wnt pathway). Then, these circRNAs regulated both pathways? Are there any described mir-7067-5p targets related to these pathways?

Reply 6: The information in this comment is from reference 44 (Qian et al. 2017). In this article, BMP2 was used as osteogenic inducers for MC3T3-E1 cells, subsequent RNA-sequencing showed that circ19142 and circ5846 were both upregulated in BMP2-induced group, so there might be relations between BMP2 and differentially expressed circ19142/circ5846, but there is no evidence shows that these 2 circRNAs can directly target BMP2 to regulate BMP pathway. The downstream mir-7067-5p and Wnt pathway were both predicted by informatics, so more experiments are required to prove that interaction.

Comment 7: Table 1 shows some circRNAs described as participating in the osteogenesis process, and indicating their expression (up or down). But the CDR1as

appear with both expression patterns. So, should CDR1as be up or downregulated during osteogenic differentiation?

Reply 7: Results from 2 researches reported different expression trend of the same circRNA CDR1as during osteogenic differentiation, which is really interesting. Li et al. used PDLSCs for osteogenic induction in reference 47 (Li et al. 2018). However, in reference 63 (Chen et al. 2020), chen et al. isolated hBMSCs from steroid-induced osteonecrosis of the femoral head, which was in pathological condition. The reason might be that these two studies isolated cells from tissues in physiological and pathological condition, respectively. But more evidence is required to prove it.

Comment 8: circRNAs cited in page 18, lines 393-395, seems to regulate the expression of BMP2. It is the case to include them in table 1?

Reply 8: Although BMP2 is a critical factor regulating osteogenic differentiation, authors of reference 105 (Liu et al. 2020) explored the roles of circRNA_0007059/microRNA-378/BMP-2 axis during osteoclastogenesis. So, we list such regulating axis in Table 2.

Comment 9: Some of the circRNAs mentioned in Table 2, of reference [10], were not cited or discussed in the manuscript.

Reply 9: We have added information as advised.

Changes in the context: See Page19, line 386-391 (reference 12, Dou et al. 2016).

Comment 10: Is it possible to affirm, with only the components shown, many being predicted interactions, that these are the "regulatory networks" during osteogenic/osteoclastogenic differentiation?

Reply 10: We have replaced the description "regulatory networks" by "circRNAs-miRNAs-mRNAs axis" in Table 1 and Table 2.

Changes in the text: See page 46, line 975; and Page 48, line 984.

Comment 11: In the conclusion section, authors affirm that "The imbalance of these two parts brings about diseases such as osteoporosis and osteopetrosis, which are also tightly correlated to the aberrant presence of circRNAs". But, actually, for osteopetrosis, based on the review, it was no clear evidences of the involvement of circRNA. Please verify.

Reply 11: Currently, there was indeed no evidence showing the involvement of circRNAs in osteopetrosis, we have modified the related description as advised. Meanwhile, we have searched other circRNA-related bone remodeling disorder and found that the occurrence of osteonecrosis of femoral head was correlated

with circRNAs, so we have added information about the roles of circRNAs in osteonecrosis of femoral head.

Changes in the text: **See Page 25-27, line 521-563; and Page 28, line 591.**

Comment 12: *Since many of the papers use in vitro differentiation models, results may vary depending on the cell type (cell source, whether human or not) and on the differentiation method (culture medium, time of differentiation). It would be interesting to include some discussion about these points throughout the text or in the conclusion, where the authors already bring a series of problems and challenges that must be overcome to improve understanding of the circRNAs in osteogenesis and osteoclastogenesis.*

Reply 12: **We have added discussions concerning this question in the part “conclusion and perspective”.**

Changes in the text: **See Page 28-29, line 595-606.**

Comment 13: *The manuscript is well referenced, but I would suggest that the authors consider the inclusion of some others papers, as:*

-Ji H, Cui X, Yang Y, Zhou X (2021). CircRNA hsa_circ_0006215 promotes osteogenic differentiation of BMSCs and enhances osteogenesis–angiogenesis coupling by competitively binding to miR-942-5p and regulating RUNX2 and VEGF. Aging (Albany NY) 13:10275–10288. doi: 10.18632/aging.202791

-Ouyang Z, Tan T, Zhang X, et al (2019). CircRNA hsa_circ_0074834 promotes the osteogenesis-angiogenesis coupling process in bone mesenchymal stem cells (BMSCs) by acting as a ceRNA for miR-942-5p. Cell Death Dis 10:. doi: 10.1038/s41419-019-2161-5

-Long T, Guo Z, Han L, et al (2018). Differential Expression Profiles of Circular RNAs During Osteogenic Differentiation of Mouse Adipose-Derived Stromal Cells. Calcif Tissue Int 103:338–352. doi: 10.1007/s00223-018-0426-0

Reply 13: **We have included the relevant references as advised.**

Changes in the text: **See Page 8, line 154-157 (reference 30, Long et al. 2018); and Page 16, line 324-332 (reference 71, Ji et al. 2021; reference 72, Ouyang et al. 2019).**

Comment 14: *Page 3, lines 62, 65 and page 4, line 67. Do sentences start with (a), (b), (c)? Please verify.*

Reply 14: **We have replaced the (a), (b), (c) by 1), 2), 3).**

Changes in the text: **See page 6, line 110, 112, 114.**

Comment 15: *Page 4, line 70. Is it 3 or 4 types of circRNAs?*

Reply 15: **There are 3 types of circRNAs, we have modified our text as advised.**
Changes in the text: **See Page 7, line 118.**

Comment 16: *Page 4, line 83. Lowercase "a".*

Reply 16: **We have modified our text as advised.**

Changes in the text: **See Page 7, line 131.**

Comment 17: *Page 5, lines 94-96. Are there some references for the sentence which indicates the potential (or use) of circRNA to be biomarkers and treatment targets?*

Reply 17: **We have included relevant reference in our text as advised.**

Changes in the text: **See Page 8, line 146.**

Comment 18: *Page 6, line 127; Page 7, line 148; Page 12, lines 249; Page 16, line 350; Page 18, line 380. Please include the references to each phrase.*

Reply 18: **We have modified our text as advised.**

Changes in the text: **See Page 10, line 196 (reference 44, Qian et al. 2017); Page 11, line 218 (reference 46, Wu et al. 2018); Page 16, line 323 (reference 70, Guo et al. 2021); Page 21, line 446 (reference 96, Nakashima et al. 2012); and Page 23, line 475 (reference 7, Feng et al. 2011).**

Comment 19: *Please include a sentence indicating if the canonical or non-canonical Wnt pathways must be activated or not to induce osteogenesis. This will facilitate the understanding of discussion.*

Reply 19: **We have included the relationship between canonical and non-canonical Wnt pathways during osteogenesis as advises.**

Changes in the text: **See Page 15, line 300-302.**

Comment 20: *Page 17, line 358. It would be interesting to cite some of the miRNAs and circRNAs identified with potential to modulate the RANKL/RANKL pathway.*

Reply 20: **In reference 90 (Lin et al. 2020), the circRNA–miRNA–mRNA network was constructed by bioinformatic prediction, so there was no circRNAs and miRNAs identified to target the RANKL/RANK pathway.**

Comment 21: *Page 19, line 405. Is it “key effectors”?*

Reply 21: **We have modified as our text as advised.**

Changes in the text: **See Page 24, line 502.**

Comment 22: *Page 19, line 417. “In vivo, drugs were injected intramedullary into the femur of mice”. What are the drugs?*

Reply 22: **We have verified the drugs and included in the paper as advised.**

Changes in the text: **See Page 25, line 514.**

Comment 23: *Page 35, line 756 (legend from figure 1). There is a typo: "reverse complementary sequences!". Please check.*

Reply 23: **We have modified as our text as advised.**

Changes in the text: **See Page 44, line 937.**

Comment 24: *Page 40, line 793. Two "in" in the title of table 3.*

Reply 24: **We have modified as our text as advised.**

Changes in the text: **See Page 49, line 996.**

Response to Reviewer B

Thank you for your detailed comments. The comments point the deficiencies of our paper and provide an important direction for us to revise our paper. The revised parts that correspond to the comments are listed as follows:

Comment 1: *Line 83 – Upper case at the first word in the sentence “a subset of circRNAs which present with internal ribosome entry site”.*

Reply 1: **We have modified our text as advised.**

Changes in the text: **See Page 7, line 131.**

Comment 2: *Line 98 - CircRNAs modulating osteoblasts and bone forming topic. I missed some kind of introduction to the discussion of each factor that comes next. In the end of the paragraph put something like: In addition, we will discuss some examples of circRNAs involved in the regulation of crucial osteogenic signaling pathway factors such as BMPs, SMADS, Wnts, RUNX and FOXs. (This is a suggestion)*

Reply 2: **We have modified our text as advised.**

Changes in the text: **See Page 9, line 164-166.**

Comment 3: *Line 145 – “Meanwhile, miR-22-3p, downregulated in CGRP-induced group, was found to contain binding sites both with mm9_circ_009056 and BMP7.” – I suggest write BMP7 mRNA to make clear that the miR binding site is at the mRNA.*

Reply 3: **We have modified our text as advised.**

Changes in the text: **See Page 11, line 216.**

Comment 4: *Line 296 – “can be block by osteoprotegerin” – should be “can be blocked by osteoprotegerin”*

Reply 4: **We have modified our text as advised.**

Changes in the text: **See Page 18, line 380.**

Comment 5: *Line 285 - CircRNAs modulating osteoclasts and bone resorbing topic. Same as above. Maybe something like this in the end: (Line 300) “The regulatory networks have been listed in Table 2 and some examples discussed below.”*

Reply 5: **We have modified our text as advised.**

Changes in the text: **See Page 19, line 391-393.**

Comment 6: *Line 431 – “There were several researches reported the correlation” - There were several researches reporting the correlation OR There were several researches that reported the correlation.*

Reply 6: **We have modified the sentence “There were several researches reported the correlation” as “Several studies have found a correlation”.**

Changes in the text: **See Page 27, line 572.**

Comment 7: *Line 451 – “However, there remains deficiency to be solved.” – I’d suggest change to: However, still remain deficiencies to be solved – since there are more than one that the authors have mentioned.*

Reply 7: **We have modified our text as advised.**

Changes in the text: **See page 28, line 595.**

Response to Reviewer C

Thank you for your detailed comments. The comments point the deficiencies of our paper and provide an important direction for us to revise our paper. The revised parts that correspond to the comments are listed as follows:

Comment 1: *This review summarizes the latest findings in bone metabolism of circRNA and provides useful information to the reader. However, this journal aims to extend the reader's vision and horizon from bench to bed and from bed to bench. Much of what is described in this review is based on basic research, and there is little information on the association of circRNA with bone metabolic diseases. We need information on the molecular mechanisms of circRNA on other bone metabolic disorders, not just osteoporosis*

Reply 1: **We have thoroughly searched the researches that focus on the roles of circRNAs in bone remodeling disorders, and discovered that the expression of circRNAs is related to the occurrence of osteonecrosis of the femoral head (ONFH). Hence, we included information about the roles of circRNAs (i.e. circPVT1, circUSP45, circRNA_25487) in the ONFH.**

Changes in the text: **See Page 25-27, line 521-563.**