Author’s response to reviews

Title: GIT1 gene deletion delays chondrocyte differentiation and healing of tibial plateau fracture through suppressing proliferation and apoptosis of chondrocyte

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Author’s response to reviews:

Dear Editor-in-Chief,

Thank you very much for having our manuscript entitled “GIT1 gene deletion delays chondrocyte differentiation and healing of tibial plateau fracture through suppressing proliferation and apoptosis of chondrocyte” reviewed in a timely and professional manner and for giving us an opportunity to revise the manuscript. We also deeply appreciate the reviewers for their critical review of the manuscript with thoughtful and constructive comments, based on which we have revised the manuscript and we hope our manuscript can be accepted by “BMC Musculoskeletal Disorders” While the changes made in the revised manuscript are highlighted by colored (blue) text, our point-by-point responses to the reviewers’ comments are detailed below.
Responds to the reviewers’ comments:

Reviewer reports:

Reviewer 1):

1. Understanding the role of GIT1 in healing of tibial fractures, particularly the role in chondrocyte differentiation, proliferation and apoptosis is important in fracture healing and in other joint conditions including osteoarthritis.

The hypothesis that GIT1 deletion results in delayed recovery is only partially supported by the data provided by the authors. The introduction states that "we hypothesize that GIT1 gene expression deletion may decrease the formation of new blood vessels and osteoclasts, which becomes our study focus."

Response: Thanks for your comments. According to your suggestion, we have revised the hypothesis as “we hypothesize that GIT1 gene deletion may delay differentiation of chondrocyte and healing of tibial plateau fracture through suppressing proliferation and apoptosis of chondrocyte.” and have further optimized the methods and results. The results of Rotarod test and neovascularization after fracture have been added in our study, which could fully support our conclusions: The time of mice on the rotating rod in the GIT1 gene deletion group was less than in the control group. Postoperative recovery after tibial plateau fracture of mice in experimental group was slower than in control group. Compared with the control group, expression of mouse type II collagen significantly decreased (referring to delayed differentiation) in the GIT1 gene deletion group, and the proportion of PCNA positive cells significantly decreased (meaning delayed cell proliferation). The TUNEL results indicated that GIT1 gene deletion led to reduction of chondrocyte apoptosis.

2. There is no data showing decreased osteoclast formation or blood vessel formation. In particular, they did not label control or experimental group with markers to show differentiation of chondrocytes to specific lineages. There is no labeling in Fig 1 to show decreased osteoclast or blood vessel invasion or decreased osteoblast formation between control and experimental groups.

Response: Thanks for your suggestion. Considering your comments, the experiment of angiogenesis was added in our study (Figure 3), which showed that GIT1 gene deletion slowed down neovascularization after fracture. Furthermore, in Figure 4, the area of cartilage cells differentiated from progenitor cells for cartilage was presented with an arrow. Meanwhile, the
red circle and arrow were added in Figure 1, which indicated the areas of osteoclast and osteoblast reduction in the experimental and control group, and which showed that compared with the GIT1-WT group, the osteoblast differentiation and angiogenesis were less than the GIT1-KO group according to CT thin layer scanning.

3. The behavioral observations between the two groups are descriptive - there is no quantitative or semi-quantitative documentation. For example, rotarod experiments could have been used to quantify differences in locomotion/pain of the two groups. Were the experimental and control groups matched by age and weight? There is no table or information on the mice. It's also unclear whether the experimental and control groups were housed in same or different cages - there seems to be contradictory information with respect to this in the materials and methods section.

Response: We appreciate your comments. The GIT1 knockout mice with C57/BL6 background were purchased from Nanjing Medical University, and the GIT1 heterozygous mice were obtained after more than seven backcross generations. The GIT1 homozygous knockout mice were obtained by interbreeding GIT1 heterozygous mice with C57/BL6 background. The wild-type littersmates were adopted as the control group. The feeding conditions of mice were the same. According to your suggestion, the basic information of mice was listed in a table. The age and weight of mice between two groups were similar. Moreover, the rotarod test was added in our study, and its results were presented as a figure. The average time of mice on the rotating rod in GIT1-KO group was less than in GIT1-WT group (P < 0.05). Please see our detailed revision in the revised manuscript.

4. Could some sort of semi-quantitative scoring or morphometric measurements have been used to quantify the area of the bone callus in the GIT1 group vs. the control group?

Response: Thank you very much for your comments. In accordance with your suggestion, quantitative statistical results of callus area was added and presented in Figure 2. Please see our detailed revision and addition across the whole text.

5. The authors use the word delay indicating that at some time point the GIT1 group does differentiate towards bone - but presumably this is after the 14 and 21 day data shown? If the
word delay is being used, then some kinetic data indicating resumption of differentiation towards osteoblast lineages, and invasion by osteoclasts and blood vessels, and apoptosis of chondrocytes should be demonstrated. Otherwise, it's hard to see if it's the absence of this vs. a temporal delay.

Response: Thank you for pointing this out. The relevant experiment of angiogenesis was added in our study (Figure 3), and kinetic analysis was applied in statistical analysis. In the curve of mathematical model, coefficient of GIT1-WT group was more than twice the coefficient of GIT1-KO group, which showed that the speed of vascular invasion in the experimental group was slower than in the control group. According to the immunohistochemistry results of type II collagen and PCNA and TUNEL results, the speeds of vascular invasion, chondrocyte proliferation and apoptosis (chondrocyte differentiation) of mice in the GIT1-KO group were slower than in the GIT1-WT group after 14 days (P < 0.05). Therefore, compared with postoperative recovery of the normal mice, the postoperative recovery of mice in the GIT1-KO group delayed. There may a time point that cartilage cells of mice in the GIT1-KO group just began to differentiate, while cartilage cells of mice in the GIT1-WT group had been differentiated. According to our present data, if there was a time point, it should be between the 7th day and the 14th day, which need a further experiment to prove.

6. In the discussion section, I'm unclear why there is an increase in bone mass of GIT1 KO mice if they result in decreased bone callus? Perhaps there's some point here that I missed, but the explanation was not very clear here.

Response: Much obliged for your carefully reading and kindly reminding. Actually, the increase in bone mass of GIT1-WT mice was more than of GIT1-KO mice.

7. For stats, can they show that the use of the tests is valid and the data is normally distributed with low residuals?

Response: Thank you for your reminding. We found that chi-square test was adopted imprecisely in the statistical analysis, and a two-tailed unpaired t test has been applied in comparison between two groups. Furthermore, we have made a quantile-quantile plot basing on statistical data. Data regression in the quantile-quantile plot was presented as a straight line, and scatters were evenly distributed besides the line, both of which showed that the data was in accordance with normal distribution of small residuals.
8. Overall, I thought the study method and experimental results could be further developed to support the original hypothesis.

Response: Special thanks for your suggestion. We have further optimized the methods and results, and have revised the inaccurate expression in our study. We greatly appreciate your warm work and hope that the corrections can meet expectations.

Reviewer 2:

1. The manuscript would benefit from eliminating typos (for example page 3 line 3 from the bottom "neutral growth")

Response: Really appreciated for your reminding. We have reviewed and revised all the typos in our manuscript.

2. The manuscript would benefit from citing:

   “Menzdorf et al. BMC Musculoskeletal Disorders 2016; 17: 255” (PMID: 27283180)

   “Klüter et al. BMC Musculoskeletal Disorders 2015; 16: 79 concerning mechanisms in fracture healing” (PMID: 25886252)

   “Pufe et al. Bone 2003; 33: 869-76 concerning role of growth factors in bone remodeling” (PMID: 14678846)

Response: Thanks very much for your kindness advice. We have cited the references you suggested. Please see the revisions in our manuscript: Some previous studies investigated that some medical treatments such as rivaroxaban and bisphosphonates, exert positive effects on fracture healing [6, 7]. It is well-known fact that vascular endothelial growth factor (VEGF) plays a crucial role during endochondral bone formation in hypertrophic cartilage remodeling, ossification, and angiogenesis [23].
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Declarations - Ethics approval and consent to participate

- Consent to publish

- Availability of data and materials

- Competing interests

- Funding

- Authors' Contributions

- Acknowledgements

- Authors' Information

Response: Thank you very much for consideration of our paper. We have carefully revised our manuscript according to the requirements of the journal. Please check it again.

We tried our best to improve the manuscript and have made some changes in the manuscript. These changes will not influence the content and framework of the paper. Here we did not list all the specific changes but they are marked in red in the revised paper. We greatly appreciate your warm work and hope that the corrections can meet expectations.

Once again, thank you very much for your comments and suggestions.

Thank you and best regards.

Yours sincerely,

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