Author’s response to reviews

Title: Identification of differentially expressed miRNAs and mRNAs in synovial of osteoarthritis via RNA-sequencing

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Version: 1 Date: 12 Dec 2019

Author’s response to reviews:

Dear editors and reviewers,

Many thanks for your high efficiency of work and good suggestions. We have revised the manuscript according to the comments and the recommendations suggested by the editors and reviewers. We use ‘Track changes’ model to show the part that we revised in the manuscript.

The responses to the reviews’ comments are as follows:

Reviewer reports:

Reviewer 1: The manuscript by Zhou and colleagues represent a bioinformatics approach to identify differentially expressed miRNAs and mRNAs in synovial of osteoarthritis (OA) via RNA-sequencing. The authors performed next-generation based RNA sequencing and identified significant differences at mRNA and miRNA levels in OA patients compared to controls. The data presented in this article is interesting. However, the authors need to address some major concerns before publication in BMC Medical Genetics.

Major concerns:
1. The main weakness of the study is the number of samples used in the RNA-Seq experiment. The authors used only 5 affected and three control individuals for the current study. The current number of participants is not sufficient for such kind of studies. The author needs to add more samples for the
successful replication of study findings and to confirm the top regulated transcripts identified in this study.

Response: Thanks for your comment. We have to admit that the small sample size was a limitation of our study, and we have added the limitation in the last paragraph of discussion. To validate the results of RNA-sequencing, qRT-PCR and western blot analysis were performed. Except for TLR7, the expression of the others in the qRT-PCR and western blot analysis was consistent with the results of RNA-sequencing. These findings suggested the reliability of our RNA-sequencing results. Currently, we are collecting OA samples, and the key genes and miRNAs identified in this study will be validated in our following research with larger sample size.

2. In addition, qRT-PCR or immunohistochemistry should be performed to confirm some target transcripts in patients' samples.

Response: Thanks for your good suggestion. According for your suggestion, qRT-PCR was performed to confirm the expression levels of three DEmRNAs (including TLR7, CTSS and TIMP3) and two DEmiRNAs (including hsa-miR-17-5p and hsa-miR-20b-5p). The results revealed that except for TLR7, expression of the others in the qRT-PCR results was consistent with the RNA-sequencing results, generally.

3. The bioinformatics-based prediction of miRNA targeting mRNA through RNA-Seq data provides initial information but not conclusive evidence of regulation. The author should at least confirm few target mRNA through luciferase assay (to confirm the miRNA binding with target mRNA) or use the western blot to identify differentially expressed gene at the protein level.

Response: Thanks for your good suggestion. According for your suggestion, western blot was performed to identify three DEmRNAs (including TLR7, CTSS and TIMP3) at the protein level. The results revealed that except for TLR7, expression of the others in the western blot analysis was consistent with the RNA-sequencing results, generally.

4. The author reported that a large number of studies have been done to explore miRNAs and genes associated with OA. What was the purpose of the current study is missing in the introduction?

Response: Thanks for your comment. This current study aimed to represent a new avenue to understand the pathogenesis and develop potential biomarkers for OA which we have indicated in the introduction section.

5. Link is missing between previously identified OA genes and differentially expressed mRNA/miRNA identified in current studies. The author should discuss it in detail and explain the differences and similarities between current and previous studies, such as samples ethnicity, etc.

Response: Thanks for your comment. Three DEmRNAs (including TLR7, CTSS and TIMP3) we discussed in this study have been reported in previous studies, while two DEmiRNAs (including hsa-miR-17-5p and hsa-miR-20b-5p) were the first report to link them with OA. Due to the complexity of studies on OA-related genes identification, it is difficult to comprehensively compare the similarities and differences between current and all previous studies in multiple aspects including sample ethnicity in a paper. Hence, we discussed the sample used in partial previous studies, such as, “Appleton et al. identified increased expression of CTSS in the OA model”, “A case-control study in a Chinese Han population linked TIMP3 polymorphism with severe knee OA”.
6. They performed protein-protein interaction (PPI) network analysis and there is no information on the method, results, and discussion sections that what is identified in this analysis and how it could be correlated with the current study.
Response: Thanks for your comment. We’re very sorry to make readers confused. After deeply consideration, the protein-protein interaction (PPI) network-related part was indeed a superfluous context. Hence, according to your suggestion, we deleted this part in our manuscript.

7. All figures are blurry and difficult to read, and the author should provide detail information in the legend to clearly understand the figures.
Response: Thanks for your comment. We have uploaded figures with higher resolution in the submission system, and provided detail information of the figures in the legend.

Reviewer 2: In the current study, authors have identified differentially expressed miRNAs and mRNAs associated with osteoarthritis through RNA sequencing and bioinformatics analyses. In my point of view, Authors need to address following points:

1- The sample size of the study is too small. Authors must include significant number of samples (both patients as well as controls) in order to draw some solid conclusion from the current study.
Response: Thanks for your comment. We have to admit that the small sample size was a limitation of our study, and we have added the limitation in the last paragraph of discussion. To validate the results of RNA-sequencing, qRT-PCR and western blot analysis were performed. Except for TLR7, the expression of the others in the qRT-PCR and western blot analysis was consistent with RNA-sequencing results. These findings suggested the reliability of our RNA-sequencing results. Currently, we are collecting OA samples, and the key genes and miRNAs identified in this study will be validated in our following research with larger sample size.

2- In order to make the current study understandable, authors need to add some flow chart of the analyses they performed in this study or otherwise they need to pay more attention in the results and discussion section as it needs a thorough explanation. As for example, authors didn't elucidate/mention the results of protein-protein interaction network construction that they performed. Manuscript also lacks in explaining the exclusion/inclusion criteria properly which led to the identification of DEmRNA/DEmiRNA as authors did not provide the basis on which the final transcripts were selected which they included in the discussion section.
Response: Thanks for your comment. A flow chart of the analyses was showed in Figure S1. After careful consideration and to avoid confusing the readers, the protein-protein interaction (PPI) network-related part was deleted. The two DEmiRNAs (including hsa-miR-17-5p and hsa-miR-20b-5p) included in the discussion section were the top two DEmiRNAs that covered most DEmRNAs which we have indicated in the results and discussion sections. The three DEmRNAs (including TLR7, CTSS and TIMP3) included in the discussion section were target genes of the two DEmiRNAs mentioned above.

3- In discussion section authors did support their results with the previous findings but they did not explain how the findings of their study are different from the previous work? Did authors find any DEmRNA and DEmiRNA other than the previous findings which can add novel aspect in current knowledge as authors stated in abstract that current study can provide new clues for the roles of DEmRNA and DEmiRNA in osteoarthritis?
Response: Thanks for your comment. Three DEmRNAs (including TLR7, CTSS and TIMP3) we
discussed in this study have been reported in previous studies, while two DEmiRNAs (including hsa-miR-17-5p and hsa-miR-20b-5p) were the first report to link them with OA. Importantly, TLR7, CTSS and TIMP3 were target genes of hsa-miR-17-5p, which suggested that hsa-miR-17-5p may participate in OA by regulating TLR7, CTSS and TIMP3. Similarly, TLR7 and CTSS were targeted by hsa-miR-20b-5p which indicated that miR-20b-5p may involve in OA by regulating TLR7 and CTSS. We first reported these DEmRNA-DEmiRNA pairs may involve in OA, which may add novel aspect in current knowledge of OA-related.

4- Authors must upload figures with higher/better resolution as the figures are not clear and it's very difficult to understand.
Response: Thanks for your comment. We have uploaded figures with higher resolution in the submission system.

5- Authors need to provide the references of following:

(i) page 7, lines 40-48

(ii) page 8, lines 13-19 & 32-36

Response: Thanks for your comment. We are sorry for our mistakes, and we have provided the corresponding references in our manuscript.

Thank you again for your great help and attention. I am looking forward to hearing from you about the final decision.

Best regards and wishes!

Yours sincerely,
Yuehong Liu