Author's response to reviews

Title: The expression of p-ATF2 involved in the chondeocytes apoptosis of an endemic osteoarthritis, Kashin-Beck disease

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Author's response to reviews: see over
Kashin-Beck disease (KBD) is a chronic, endemic, degenerative osteoarthropathy with severe skeletal deformation and dwarfism. This manuscript mainly investigated the changes of p38, JNK and ATF2 in apoptosis in chondrocytes of patients with KBD, using cultured chondrocytes samples. They showed the involvement of JNK signal pathway involved in pathogenesis of KBD, and further suggested that ATF2 may serve as a marker for KBD pathology. Overall it is an interesting and potential useful study.

1. In introduction: Line 63 to 65, “however…..KBD”, the sentence could be deleted.
Response: The sentence was deleted.

2. In materials and methods: Line 119, provide some detail information on, how and why the normal articular was obtained from total knee replacement surgery.
Response: The normal donors were suffered from accidents. Line 102-107.

3. Line 123, the word “genetic” is inappropriate for OA and RA.
Response: We revised the sentence.

4. In Figures 2 and 4 showing western blot analyses, how much protein was loaded per lane, and also provides more information on antibodies used.
Response: Thanks for your advices. We showed the concentration and antibody information. Line 159, 167-169.

5. What about the power of the RT-PCR testing? In the text, it seems that 1.5 is the power, what’s the criteria and reference?
Response: The PCR results were first normalized to GAPDH, then to control groups, finally we could read the ratio of the two groups from the ordinate axis. We added the reference about the statistical method, number 23.

6. The result of the RT-PCR in this manuscript is relevant fold change. Please provide the methods for statistical analysis and detailed P value. Especially, line249, what about the power? 1.5? How did you get this number?
Response: We gave more description about the method, and the p value is showed as well.

7. Please describe the statistical analysis in more detail. I would suggest having a statistical analysis on the western blot results.
Response: We provide the statistical analytic results for the western blot in Fig 3.

8. The levels of p-p38 were shown in Figure 2D and E. While in the manuscript, it was not described.

Response: Thanks. The results of p-p38 were described in details.

9. In figure 3B, by H&E staining, Line 304, authors described that “cells to became round, with condensed chromatin and a shrunken cytoplasmic”. Please indicate these phenomena in the figure.

Response: As we rewrite the paper, these results were deleted.

10. In figure 4A, what did “fold change” in figure 4A mean? Where is the control?

Response: As we rewrite the paper, these results were changed. More details about the method in reference 23.

11. In discussion: the discussion part should be well organized to summarize the major findings of this study.

Response: Thank you for your suggestion. We have reorganized the discussion part to focus on the major findings.

12. In references: could be more updated, considering the author’s lab has published a lot recently in this field.

Response: For your helpful suggestion, we updated the references and provided some latest findings.

13. Figure 5 didn’t add any valuable information, it could be deleted.

Response: Thank you for your advice and the paper was rewritten.

Reviewer 2:

In this paper Jing Han et al. study the function of JNK and p38 signal pathways in the pathogenesis of Kashin-Beck disease (KBD). To carry out this Project authors have compared the expression of ATF2, JNK and p38 mRNAs and proteins. Authors have also used and chondrocytic apoptotic model with T-2 toxin. Authors suggest that expression of p-ATF2 by JNK and p38 signal pathways are involved in the chondrocyte apoptosis of KBD cartilage. Paper is interesting because it is necessary to understand the pathogenesis of KBD and their hypothesis is well justified. Authors suggest that T-2 toxin is a mechanism responsible of the apoptotic chondrocytes in KBD because the toxin activates the JNK and p38 pathways. According these results a potential treatment for KBD could be p38 inhibitor drugs.

However the paper has important limitations and the results showed by the authors cannot support the hypothesis. Authors must improve the scientific
quality of the paper, some suggestions to delete the limitations are:

1. To improve the quality of the WB figures.
   
   **Response:** Thanks for your advice and we improved the figures.

2. To quantify the WB results with densitometry graphics.
   
   **Response:** For your helpful advice, we quantified the WB results with densitometry graphics.

3. To calculate the “p value” of all WB experiments and to show the significant “p values”.
   
   **Response:** We calculated the “p value” and showed the significance.

4. To calculate the “p value” of cytometry experiments. It is necessary to know whether the reduction of apoptosis by inhibitors is statistically significant.
   
   **Response:** We calculated the “p value” and showed the significance.

5. A graphic showing the effect of different concentrations of T2 toxin in cell apoptosis must be included, as well as the effect of different concentrations of the p38 and ATF2 inhibitors.
   
   **Response:** Thank you for your suggestions, actually, we used p38 and JNK inhibitors and this part was rewritten.

6. Figure 1 is not necessary to include in the paper.
   
   **Response:** Thank you for your advice and we added pathological staining of cartilage tissues from KBD and normal in Fig 1.

7. Figure 2 B is not necessary, authors must to show morphologic changes characteristic of apoptosis. Then DAPI stain or Electronic Microscopy can show the apoptotic nuclear and cytoplasmic changes.
   
   **Response:** Thank you and we have revised the manuscript.

8. I suggest studying with deep detail the apoptotic results. This is a very critical point, then morphologic characterization (as previously mentioned) and study of the role of caspases in this model is crucial.
   
   **Response:** For your helpful advices, we rewrite the paper to provide more details.

9. Figure 5 must explain only the pathways involving to KBD using the model of T2 toxin as inductor.
   
   **Response:** We reorganized the manuscript according to your suggestions.