Author's response to reviews

Title: BRG1 overexpression in smooth muscle cells promotes the development of thoracic aortic dissection

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Author's response to reviews: see over
Dear editor,

We would like to resubmit our manuscript entitled “BRG1 overexpression in smooth muscle cells promotes the development of thoracic aortic dissection” for consideration as an original article in BMC Cardiovascular Disorders. We appreciate the helpful comments of the reviewers and have tried to respond to their concerns on a point-by-point basis. Specifically, we have prepared and included a detailed description of all changes made to the revised manuscript and believe that we have mounted sufficient evidence to conclude that BRG1 is a critical modulator in the development of thoracic aortic dissection.

All authors have read and approved submission to BMC Cardiovascular Disorders and that the manuscript, or part of it, has neither been published nor is currently under consideration for publication by any other journal. In addition, there is no financial or professional conflict of interest to disclose from any of the authors relating to the subject of this manuscript. Thank you again for your time in considering our manuscript.

Sincerely yours,

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Point-by-point response to reviewers' comments

Reviewer: LI JIA
Reviewer's report:
The authors demonstrated that BRG1 is upregulated in the aortic SMCs of TAD. BRG1 overexpression may enhance MMP2 and MMP9 expression, promote apoptosis and SMCs phenotype change. These results were taken by the authors as evidence that BRG1 overexpression in aortic SMC may play a role in the development of TAD. This experiment is carefully designed and the various steps of the demonstration are well described. However, some points need to be addressed.

We thank the reviewer for the positive comments.

Minor Essential Revisions

1. Abbreviations should be spelled out first in abstract.
   Thank you for reviewing our manuscript so carefully. We have spelled out the abbreviations in abstract (page 1, paragraph 1, lines 1-3, paragraph 2, line 1).

2. N number for each experiment should be indicated.
   Thanks for your suggestion. We have added the information of number for each experiment (page 7, paragraph 5, line 3).

3. There should be a brief methods description (or reference) regarding adenovirus mediated cell transfection.
   Thanks for your suggestion. We have added the information of adenovirus mediated cell infection in the methods section (page 5, paragraph 4).

Discretionary Revisions

4. In the Figure 1, the authors stained the expression of BRG1 in TAD and normal aortic tissue section. According to the BRG1 staining, the authors indicated that BRG1 was mainly localized in the nucleus of aortic SMCs. It is more convincing if there is one more staining to indicate smooth muscle layer in aortic tissue, such as using #-actin staining.
   This is a good suggestion and we agree that it is more convincing to indicate smooth muscle layer in aortic tissue with one more staining than just using hematoxylin stain.

5. In Figure 6A, the representative blots are not entirely convincing and should be replaced with better quality images.
Thanks for your suggestion. We have replaced those images with new higher quality ones (Figure 6A).

6. The authors suggest that BRG1 promotes the development of TAD by increasing MMP2 and MMP9 expression, inducing SMC apoptosis and the transition from contractile to synthetic phenotype. Does BRG1 induce these responses respectively? Do they have interaction? Is ECM degradation due to MMP2 and MMP9 overexpression involved in the process of apoptosis and phenotype switch? The author should discuss it.

Thanks for your comments. It was reported that BRG1 is required in the transcriptional regulation of specific gene expression and plays an important role in the regulation of some cell physiological activities. In the present study, we found that overexpression of BRG1 in SMCs could increasing MMP2 and MMP9 expression, inducing cell apoptosis and the transition from contractile to synthetic phenotype. However, here we focused on the role of BRG1 expression in the development of TAD and did not exam the interactions among MMPs expression, cell phenotypes transition and apoptosis. Further experiments using SMCs and appropriate animal models are warranted to investigate the interaction. We have discussed this point in the revised manuscript (page 14, paragraph 2, lines 12-13).

Level of interest: An article whose findings are important to those with closely related research interests.

Quality of written English: Acceptable
Is the difference in BRG1 the cause or the result of dissection? In other words, did this state exist prior to dissection” Could the inflammatory state after aortic dissection produce the findings you noted? Does BRG1 increase in inflammatory states?

Thanks for your comments. Considering that the samples used in our study were obtained from developed TAD patients and difference may be existed between the in vitro and in vivo studies, further experiments using appropriate animal models (e.g. BRG1 knock-out mice) are warranted to establish the role of the BRG1 in the development of TAD.

It was reported that inflammatory cytokines such as IL-1β could regulated phenotype transition and induce expression of proinflammatory genes in SMCs. However, here we focused on the role of BRG1 expression in the regulation of SMCs activity. Further experiments may be handled to investigate the interaction between BRG1 expression and inflammatory states. We have discussed this point in the revised manuscript (page 14, paragraph 2, lines 4-7).

Are aortic SMCs really “responsible for the tensile strength and elasticity of the aortic wall”? Do you mean directly responsible via their intrinsic mechanical properties, or rather through their synthetic products?

Thanks for the comments. It was reported that SMCs are the contractile cells and the main source of extracellular matrix in the aortic media. The interplay between SMCs and extracellular matrix plays an important role for the structural and functional integrity of the aortic wall. We have added the information in the section of background (page 3, paragraph 1, lines 5-7)

How could the “age, gender, smoking status, hypertension or diabetes” be the same between organ donors and the dissection group? This would be very surprising. Please provide the exact numbers in tabular form.

Thanks for the comments. Control specimens were obtained from the patients undergoing aortic valves replacement without aortic disease. Statistical analysis results showed that there is no significantly difference between organ donors and the dissection group in clinical features including age, gender, smoking status, hypertension or diabetes. We have added the information in the section of methods (page 5, paragraph 1, lines 5-8)

RNAs are notoriously unstable. What was the interval from onset of dissection to
operation and harvesting of the specimens? What was the interval and storage method from the time of harvesting until analysis? Were the intervals the same in dissectors and controls?

Thanks for your comments. Briefly, we placed aortic tissue samples as sterilely as possible into transport media and stored at 4°C. Then, samples were transferred to the lab and carefully cleared of adventitia as quick as possible (normally no more than 60 min). As mentioned above, control specimens were obtained from the patients undergoing aortic valves replacement without aortic disease. The method was the same between dissectors and controls.

Your findings relate to tissue samples. Of course, we cannot obtain tissue samples of the aorta at intervals for clinical evaluation of patients. So, how should we use your data clinically?

Thanks for the comments. Considering that the samples used in our study were obtained from developed TAD patients and difference may be existed between the in vitro and in vivo studies, further experiments using appropriate animal models are warranted to establish the role of the BRG1 in the development of TAD. Then, targeting BRG1 to regulate SMCs to a reparative phenotype might provide a possible therapy in the treatment of TAD. We have discussed this point in the revised manuscript (page 15, paragraph 1, lines 7-8)

Level of interest: An article whose findings are important to those with closely related research interests.

Quality of written English: Needs some language corrections before being published.

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests: I declare that I have no competing interests.
Reviewer: Annalisa Angelini
Reviewer's report
Reviewer's report:
Major Compulsory Revision
This is a well written paper on an hot topics, and the results are of interest in the field of aortopathies and our need to understand their pathophysiology.

We thank the reviewer for the positive comments.

I have some points that I would like the authors clarify before publication.

1) the authors should state more clearly the tissue sampling made during the surgery since ascending aortic dissection can start even 1 cm above the commissure valves, or can have the inlet break even at aortic arc level. This could imply inclusion of the dissection or a segment not involved. This could have implication considering apoptosis of SMCs.
Thanks for your suggestion. We have added the information of the tissue sample collection in methods section (page 5, paragraph 1, line 9 - line 11).

2) there are no reported data on age, gender, associated diseases, imaging procedures previously performed.
Thanks for your suggestion. Our data showed that the subjects with TADs and control showed no significantly difference in clinical features including age, gender, smoking status, hypertension or diabetes. We have added the information in methods section (page 5, paragraph1, line 5- line 8).

3) it would be of interest to know if the aortas were dilated or aneurysmatics.
Thanks for your comments. All patients were acute type A TAD without arteriectasis medical record. The ascending aortic diameters at the time of the dissection were from 3.3 cm to 6.5 cm. Statistical analysis results showed that MMPs and BRG1 expression did not correlate with the aortic diameter. These data suggest that dilative level of ascending aorta at the time of the dissection may be not an important manifestation reflecting the structural weakness of the aortic wall.

4) the authors could add a short description or a table with the major pathological findings at classical histology (HE, FvG, Alcian) to make clear for the readers the type of aorta they were evaluating. It is not easy to assess the figures which are high power view and not panoramic.
Thanks for your suggestion. We have added the description of the pathological
findings in page 9, paragraph 1, lines 3 and 4.

5) As for the figures I would like to have the same orientation of SMCs for controls and affected samples.
   We agree that it is more convincing to have the same orientation of SMCs for normal and TAD samples.

6) please modify legend of figure 4, since the authors mentioned for controls a more intense expression for SMCs staining, since in immunohistochemistry we know that this can be misleading since intensity can be influenced by many technical variables as well as different time of collection and storage. In the methods you have correctly indicated the cells involvement instead of the cell intensity.
   Thanks for your suggestion. We have modified the legend of Fig.4 (page 21, paragraph 1, line 2 - line 4)

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I declare that I have no competing interests