Peer review file

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Reviewer A

Comment 1: I must congratulate authors for great effort and novel discovery. Yet requiring full validation and in-depth investigation, data provide wide field for further research and discovery. Definitely, main strength of this publication is computation analysis which is well executed by the team.

Reply 1:

Thank you very much for your comments and constructive suggestions. Your comments have helped us quite a lot to improve the quality of our study. We have made point-by-point responses to your comments and made corresponding revisions in the manuscript in the "Track Changes" mode.

Comment 2: Regarding methods and material. I believe that author utilized existed dataset. It is very convenient, but rise more questions than answers. What aortic aneurysm author talking about? Ascending aorta? Descending aorta? Arch? What clinical cause of the aneurysm? Marfan syndrome? Degenerative disease? Atherosclerosis? Most of all, is the author using aortic tissue? Plasma? Without knowing these basic clinical parameters, it is very hard to talk about application of this knowledge or further investigations.

Reply 2:

Thanks for your comments. Your questions are quite important and interesting. We need to clarify these basic clinical parameters before talking about the application of this knowledge or further investigations. In our study, 3 datasets (GSE110527, GSE26155 and GSE9106) of TAA were used.

For GSE110527 (miRNA dataset), the TAAs occurred in ascending aorta; the

etiology includes aortic dissection (n=2), Marfan syndrome (n=2), degenerative disease (n=2), bicuspid aortic valve (n=9) and unknown (n=4); tissue data were used(1).

For GSE26155 (mRNA dataset for select important target genes), the TAAs occurred in ascending aorta and tissue data were used. However, the clinical cause of TAA in GSE26155 was not given neither in the published article nor in the GEO website. Authors of this dataset only stated that "Patients with significant coronary artery disease according to angiography and patients with Marfan syndrome were excluded" in their supplemental materials(2). Besides, we did not have access to transcriptomic data of patients with bicuspid aortic valve. Therefore, we could only infer that the clinical causes of TAA in GSE26155 did not include Marfan syndrome and bicuspid aortic valve.

For GSE9106 (mRNA validation set), the TAAs occurred in ascending aorta (n=47), descending aorta (n=9) and other location (n=2). The clinical causes were also not provided neither in the published article or GEO website. However, the author states that patients with Marfan syndrome were specifically excluded (2, 3). The plasma transcriptomic data we used is the only available data of this dataset.

We must admit that discrepancies exist of samples in our study and these discrepancies mainly include:

1) Most of the TAAs in our study are located in ascending aorta, however, in the validation cohort (GSE9106), 9 TAAs were located in descending aorta.

2) In the miRNA dataset GSE110527, 2 patients with Marfan syndrome were included. In addition, the clinical cause of TAA were different among different datasets although the most of these TAAs (except 2 in GSE110527) did not have known underlying genetic disorders (such as Marfan syndrome).

3) Our results were mainly derived from aortic tissue (GSE110527 for construction of weighted miRNA co-expression network and GSE26155 for selecting important target genes), but the transcriptomic data of validation set was based on plasma tissue.

As you have mentioned, TAA is a complex disease with significant heterogeneity, it may result from genetic variations such as Marfan syndrome and Loeys-Dietz

syndrome or other factors such as syphilis infection and giant cell arteritis (4, 5). In addition, TAAs in different localizations also have differences in pathogenesis (3, 6).

We tried to find the detailed grouping information regarding localizations and clinical causes of TAA from published articles and GEO website to conduct a subgroup analysis to show the impact of heterogeneity, however, detailed grouping information was not provided although authors of datasets provided some summary information of grouping. For the validation set, we had made a compromise to choose it in our study, since there were no other available and appropriate datasets using aortic tissue for validation. We have also explained these in the limitation in the revised manuscript (Page 24, line 488 to line 495).

Although we failed to find the detailed grouping information regarding localizations and clinical caused of TAA to conduct subgroup analysis and reveal the impact of heterogeneity, our study still had implications for translational applications and further investigations. TAAs with different clinical causes and localizations still share some common pathophysiological features such as loss of vascular smooth muscle cells (VSMCs), extracellular matrix (ECM) degradation by proteases and proteoglycan deposition (5, 7, 8). Our study used multiple datasets and revealed that pathways and genes associated with apoptosis might play an important role in TAA pathogenesis. We also discussed apoptosis in VSMC related to aortic aneurysm (Page 23, line 457 to line 469) in the revised manuscript. Therefore, we can infer that therapies targeting apoptosis might also slow the progression of TAAs (Page 25, line 514 to line 525).

Changes in the text ("Track Changes" mode):

1) We have explained the heterogeneity of datasets in limitation in the revised manuscript and modified our text in Page 24, line 488 to line 495.

2) We have discussed apoptosis in VSMC related to aortic aneurysm in the revised manuscript and modified our text in Page 23, line 457 to line 469.

3) We have explained the implications of our study in conclusion and modified our text in Page 25, line 514 to line 520.

References:

1. Boileau A, Lino Cardenas CL, Courtois A, et al. MiR-574-5p: A Circulating Marker of Thoracic Aortic Aneurysm. Int J Mol Sci. 2019;20(16).
2. Folkersen L, Wågsäter D, Paloschi V, et al. Unraveling divergent gene expression profiles in bicuspid and tricuspid aortic valve patients with thoracic aortic dilatation: the ASAP study. Mol Med. 2011;17(11-12):1365-73.
3. Wang Y, Barbacioru CC, Shiffman D, et al. Gene expression signature in peripheral blood detects thoracic aortic aneurysm. PLoS One. 2007;2(10):e1050.
4. Isselbacher EM. Thoracic and abdominal aortic aneurysms. Circulation. 2005;111(6):816-28.
5. Goldfinger JZ, Halperin JL, Marin ML, et al. Thoracic aortic aneurysm and dissection. J Am Coll Cardiol. 2014;64(16):1725-39.
6. Albornoz G, Coady MA, Roberts M, et al. Familial thoracic aortic aneurysms and dissections--incidence, modes of inheritance, and phenotypic patterns. Ann Thorac Surg. 2006;82(4):1400-5.
7. Zhang L, Wang HH. The genetics and pathogenesis of thoracic aortic aneurysm disorder and dissections. Clin Genet. 2016;89(6):639-46.
8. Michel J-B. Biology of Vascular Wall Dilation and Rupture: Oxford, UK: Oxford University Press; 2017.

Comment 3: I believe if author use data derived from aortic wall, reported transcript likely related to VSMC. The VSMC as stromal cells might be involved in many pathological processes in the vessel wall. Suggest to add very nice recently published review by Shanashan et all (CM Shanahan et al Frontiers in Immunology 11, 3053. Role of Vascular Smooth Muscle Cell Plasticity and Interactions in Vessel Wall Inflammation). Interpretation of authors data would need close look in to this prospect and discuss apoptosis in VSMC related to aortic aneurysm

Reply 3:

Thanks for your comments. Our main results were derived from aortic wall. VSMSs are important in the pathophysiology of TAAs. We have discussed apoptosis in VSMC related to aortic aneurysm and added this review in our revised manuscript (Page 23, line 457 to line 465).

Changes in the text ("Track Changes" mode):

We have discussed apoptosis in VSMC related to aortic aneurysm and added this recently published review in our revised manuscript and modified our text in Page 23, line 457 to line 465.

Comment 4: The role of miRNA more investigated in murine aneurysmal model, however human VSMC need additional test and analysis. It would be great addition to manuscript if this difference clearly stated in the manuscript (CC Woo…International journal of molecular sciences 21 (1), 11) The interaction between 30b-5p miRNA and MBNL1 mRNA is involved in vascular smooth muscle cell differentiation in patients with coronary atherosclerosis.

Reply 4:

Thanks for your suggestions. We have added this point, stated this difference clearly and added Chin Cheng Woo et al.'s study in our manuscript (Page 23, line 465 to line 469).

Changes in the text ("Track Changes" mode):

We have stated that the role of miRNA more investigated in murine aneurysmal model and human VSMC need additional test and analysis and added Chin Cheng Woo et al.'s study in our revised manuscript. We have modified our manuscript in Page 23, line 465 to line 469.

Comment 5: Although miRNA is important, other epigenetic mechanism should not be downplaying and should be discussed/mention in this context. (R Gurung.et al. International Journal of Molecular Sciences 21 (17), 6334. Genetic and Epigenetic Mechanisms Underlying Vascular Smooth Muscle Cell Phenotypic Modulation in Abdominal Aortic Aneurysm)

Reply 5:

Thanks for your comments. Other epigenetic mechanism is also quite important in aneurysm pathogenesis and is worth exploration in future studies. We have

discussed/mention this and cited R Gurung.et al.'s review in our revised manuscript (Page 25, line 499 to line 503).

Changes in the text ("Track Changes" mode):

We have discussed other epigenetic mechanism and cited R Gurung.et al.'s review in our revised manuscript and modified our text in Page 25, line 499 to line 503.

Reviewer B

Comment 1: This article presents a rather interesting analysis of miRNA and mRNA expression patterns in thoracic aortic aneurysms. Unfortunately, the results are not fully explained in their current form and it is difficult to fully appreciate the scientific contribution of the research. Below are some of the main points that need to be improved before considering this work for publication.

Reply 1:

Thank you very much for your comments and constructive suggestions. We are very sorry for the unclarities and redundancies of our manuscript and the drawbacks in the figures and tables. Your comments have helped us quite a lot to improve the quality of our manuscript, figures and tables. We have made point-by-point responses to your comments and made corresponding revisions in the manuscript in "Track Changes" mode.

Comment 2:

Major concerns:

1. Line 71-72 - The sentence is not true. Please update your knowledge and read at least three articles on this topic, such as:

A. Bi S, Liu R, He L, Li J, Gu J. Bioinformatics analysis of common key genes and pathways of intracranial, abdominal, and thoracic aneurysms. BMC Cardiovasc Disord [Internet]. 2021 Dec 1 [cited 2021 Jan 27];21(1):14. Available from: https://bmccardiovascdisord.biomedcentral.com/articles/10.1186/s12872-020-01838-x

B. Chen S, Yang D, Lei C, Li Y, Sun X, Chen M, et al. Identification of crucial genes in abdominal aortic aneurysm by WGCNA. PeerJ [Internet]. 2019 Oct 8;7:e7873. Available from: https://peerj.com/articles/7873

C. Gasiulė, Stankevičius, Patamsytė, Ražanskas, Žukovas, Kapustina, et al. Tissue-Specific miRNAs Regulate the Development of Thoracic Aortic Aneurysm: the Emerging Role of KLF4 Network. J Clin Med [Internet]. 2019 Oct 3 [cited 2021 Jan 27];8(10):1609. Available from: https://www.mdpi.com/2077-0383/8/10/1609

Reply 2:

Thanks for your suggestions. We have corrected this statement in our revised manuscript by referring these 3 articles (Page 8 and Page 9, line 138 to line 149).

Changes in the text ("Track Changes" mode):

We have updated our knowledge and corrected this statement and modified our manuscript in Page 8 and Page 9, line 138 to line 149.

Comment 3: The first paragraph of the discussion should probably be included in the results as it is a summary of the research.

Reply 3:

Thanks for your comments. We have included the first paragraph of the discussion in the last paragraph of results section as a summary of the main results of our research (Page 17, line 328 to line 339).

Changes in the text ("Track Changes" mode):

We have included the first paragraph of the discussion in the last paragraph of the results section and modified our manuscript in Page 17, line 328 to line 339.

Comment 4: The discussion does not answer the questions posed in the article, it lacks an explanation of the mechanism of changes in expression detected as a result of the research. It's too long and chaotic. It does not add any meaningful information to the reader.

Reply 4:

Thanks for your suggestions. We are very sorry for the unclarity in the discussion. It's very important to answer the questions posed in the article and provide a clear explanation of the mechanisms of changes in expression detected in our study in discussion section. In the revised manuscript, we have made major changes in the discussion section and mainly focused on the relationship between our findings and apoptosis and its implications in TAA pathogenesis:

1) We have deleted some of the unnecessary details about pathways in functional enrichment analysis for DEMs and DEGs and added more details about TRAIL signaling pathway and apoptotic signaling pathway (Page 17 and Page 18, line 342 to line 367; Page 19, line 368 to line 388).

2) We have reorganized the discussion about crucial genes BCL2L1, RARα and NRIP2. BCL2L1 has two isoforms, i.e. Bcl-xl and Bcl-xs, due to alternative splicing. The two isoforms have opposite effect in regulating apoptosis(1). RARα is a retinoic acid receptor, the activation of receptor this could induce apoptosis and it has cross-talk with TRAIL signaling pathway which we found in DEM functional enrichment analysis(2). We have discussed BCL2L1 (Page 20 and Page 21, line 389 to line 417) and RARα (Page 21, line 418 to line 428) in detail.

3) For NRIP2, it can up-regulate Wnt signaling pathway. Although it's not clear whether Wnt pathway participate in TAA pathogenesis through apoptosis, studies have shown that Wnt signaling pathway might be involved in aneurysm pathogenesis by inducing inflammation, up-regulating MMPs, promoting endothelial cell senescence(3). We have discussed NRIP2 in Page 21 and Page 22, line 429 to line 454.

4) We also added a short paragraph about the implications of our findings on vascular smooth muscle cells (VSMCs) since the apoptosis of VSMCs were shared among different types of aneurysms (Page 23, line 455 to line 467).

Changes in the text ("Track Changes" mode):

1) We have deleted some the unnecessary details about pathways in functional

enrichment analysis for DEMs and DEGs and added more details about TRAIL signaling pathway and apoptotic signaling pathway. We have modified our text in Page 17 and Page 18, line 342 to line 367; Page 19, line 368 to line 388.

2) We have reorganized the discussion about crucial genes BCL2L1 and modified our text in Page 20 and Page 21, line 389 to line 417.

3) We have reorganized the discussion about crucial genes RARα and modified our text in Page 21, line 418 to line 428.

4) We have reorganized the discussion about crucial genes NRIP2 and modified our text in Page 21 and Page 22, line 429 to line 454.

5) We have added a short paragraph about the implications of our findings on vascular smooth muscle cells (VSMCs) and modified our text in Page 23, line 455 to line 467.

References:

1. Grignani F, Ferrucci PF, Testa U, et al. The acute promyelocytic leukemia-specific PML-RAR alpha fusion protein inhibits differentiation and promotes survival of myeloid precursor cells. Cell. 1993;74(3):423-31.

2. Altucci L, Rossin A, Raffelsberger W, et al. Retinoic acid-induced apoptosis in leukemia cells is mediated by paracrine action of tumor-selective death ligand TRAIL. Nat Med. 2001;7(6):680-6.

3. Krishna SM, Seto SW, Jose RJ, et al. Wnt Signaling Pathway Inhibitor Sclerostin Inhibits Angiotensin II-Induced Aortic Aneurysm and Atherosclerosis. Arterioscler Thromb Vasc Biol. 2017;37(3):553-66.

Comment 5: The conclusions are not precise and basically duplicate the results.

Reply 5:

Thanks for your suggestions. We have rewritten the conclusion part. We made a more concise summary of our study and showed the implications of our findings in conclusions.

Comment 6: Please consider whether all inserted figures are necessary in the main text of the article. Figures 4 and 8 in particular would be more suitable for

supplementary materials.

Reply 6:

Thanks for your suggestions. We have reconsidered all inserted figures and thought it's appropriate to put Figure 4 and 8 into supplementary materials. The numbering of figures in the manuscript were also corrected in the revised manuscript (Figure 4→Figure S2, Figure 5→ Figure 4, Figure 6→Figure5, Figure 7→Figure 6, Figure 8→Figure S5, Figure S2→Figure S3, Figure S3→Figure S4).

Comment 7: Figures 2, 3, 5, 7, S3 are not very legible, additional descriptions should be placed next to the heatmaps, in figures showing the clusters letters should be larger.

Reply 7:

Thanks for your comments. We have put additional descriptions next to the heatmaps in Figure 2 and Figure 3. We also enlarged the font size in the heatmap. In figures showing the cluster letters, we enlarged the font size of these letters in Figure 5, 7, S1. In addition, the resolution of all original figures was raised from 600 ppi to 900 ppi or more.

Comment 8: Figure S2 after improving the quality of the subtitles more closely matches the main body of the article.

Reply 8:

Thanks for your suggestions. We have improved the quality of the subtitles in Figure S2 by enlarge the font size to more closely match the main body of the article.

Comment 9. Figure S4 is indescribable, illegible and adds nothing to the reader.

Reply 9:

Thanks for your comments. Figure S4 showed the interactions between crucial miRNAs and target genes. We have removed Figure S4 in the revised version since Table S4 also provided the same information as Figure S4.

Comment 10: Table 1 - Please add a column with information on which genes and pathways affect the detected miRNAs.

Reply 10:

Thanks for your comments. We have added a column with information on which genes and pathways affect the detected miRNAs. The genes were found based on miRTarbase and the pathways were based on KEGG pathways enrichment analysis. Since there are lots of genes and pathways that might interact with the DEMs in Table 1, we have taken an overlap between target genes and DEGs of the mRNA dataset of TAA (GSE26155) and listed the overlapping gene with the most significant p value. For pathways, we listed the most significant pathway. We also added a description about this in the revised manuscript (Page 15, line 279 to line 280).

Changes in the text ("Track Changes" mode):

We have added a description about the changes in Table 1 and modified our text in Page 15, line 279 to line 280.

Comment 11:

Minor concerns:

1. Too long Abstract, please shorten to 350 words.

Reply:

Thanks for your comments. We have shortened our abstract under 350 words (to 325 words) by deleting unnecessary details and reorganizing the conclusions.

Changes in the text ("Track Changes" mode):

We have modified our abstract in Page 3 and Page 4, line 48 to line 87.

2. All citations in the main text, incorrectly placed. Please remember that the citation

should be at the end of the sentence, BEFORE the period.

Reply:

Thanks for your comments. We are very sorry for this mistake. We have placed the citation at the end of the sentence and before the period.

3. Line 61-64; 148-149; 163-164; 175-176; 303-305 - Please correct the spelling and grammar in the sentences.

Reply:

Thanks for your comments. We have changed these spelling and grammar in the sentences. In addition, our manuscript was polished by AME Editing Service (http://editing.amegroups.cn/#editing, Verification code: MfnZOVDO).

Changes in the text ("Track Changes" mode):

1) For line 61-64 in the original manuscript, we have modified our text in Page 7 and Page 8, line 124 to line 128 in the revised manuscript.

2) For line 148-149 in the original manuscript, we have modified our text in Page 12 and Page 13, line 233 to line 235 in the revised manuscript.

3) For line 163-164 in the original manuscript, we have modified our text in Page 13, line 252 to line 254 in the revised manuscript.

4) For line 175-176 in the original manuscript, we have modified our text in Page 14, line 268 to line 269 in the revised manuscript.

5) For line 303-305 in the original manuscript, we have deleted this sentence after reorganizing the paragraphs about discussion of crucial genes.

4. Line 239-241 – Please rewrite the sentence as it is incomprehensible.

Reply:

Thanks for your comments. We reorganized the whole paragraph containing this sentence as response to "Major concerns 3". This sentence was deleted in the revised manuscript.

5. Check the references as they are not complete – 8, 12, 18, 32

Reply:

Thanks for your comments. In the revised manuscript, the reference 8, 12, 18, 32 correspond to reference 9 12, 22, 30 respectively. We have checked these references, downloaded the latest ".nbib" citation files of these references from PubMed, imported them into EndNote X9 and updated all of these citations in our revised manuscript.

6. Figure 6, S1 - better quality of description is needed

Reply:

Thanks for your comments. We have made a more detailed description of Figure 6 (Figure 5 in the revised manuscript) and Figure S1 in corresponding figure legends.