Peer Review File

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Reviewer A:

Cardiovascular disease are one of the most important causes of mortality worldwide. MicroRNAs (miRNAs) modulate gene expression at post-transcriptional level and increasing evidence has shown that miRNAs are involved in cardiovascular physiology and in the pathogenesis of cardiovascular disease.

Here authors investigated the protective efficacy of miR-155 in mouse model of cardiovascular disease. Authors found that miR-155 played an important role in NoxA1-related signaling pathway. miR-155 transfection into VSMC may have anti-inflammatory regulatory effect on NoxA1 expression in vivo and resulting in amelioration of atherosclerotic lesion in AS mouse model.

Overall, this is an interesting manuscript. Here is my comments:

Major Comments:

Comment 1: Are there studies in human tissue that support miR-155 in cardiovascular disease?

Reply 1: Most of the miR-155 related studies were conducted in vitro by using animal VSMC or hematopoietic stem cells. However, a small number of publications used CAD patients' PBMC to test the expression of miR-155, IL-6, etc. vs health control. (e.g., DOI：10.7754/Clin.Lab.2018.171222).

Changes in the text: The corresponding explanation of clinical application between miR-155 and CAD was clarified in discussion part on page 18, line 514-527.

Comment 2.The author should describe in detail how to choose NoxA1 as the target of miR-155 (based on the references or bioinformatics analysis).

Reply 2: Yes, the detail of how we choose NoxA1 as the target gene of miR-155 was described.

Changes in the text: The mechanism of selecting NoxA1 was added on page 7, line 190 to line 203.

Comment 3.Which cells or tissues are miR-155 expressed? Does miR-155 expression co-localize with NoxA1?

Reply 3: The miR-155 was abundantly expressed in the human spleen and thymus and detectable in the liver, lung, and kidney. It is also detectable in most of the hematopoietic cells and that's the reason why miR-155 has been widely investigated in hematopoiesis. Based on existed references, no direct study was conducted to verify the colocalization of miR-155 and NoxA1, however, is tightly correlated with ROS system regulation and serves as the critical regulator of the phox complex.

Changes in the text: The related explanation was shown in discussion part page 14-15.

Minor Comments:

Comment 1. Figure1c needs to add protein marker.

Reply 1: Yes, the protein marker was added.

Changes in the text: The protein marker was input on Figure 1c.

Comment 2. It is recommended to merge the legends of Figure1 and Figure3.

Reply 2: Yes, the merge was conducted accordingly.

Changes in the text: The legends of Fig 1 and Figure 3 were merged, respectively.

Comment 3. The resolution of pictures in figure3 is low.

Reply 3: Yes, part of the figures was changed with relatively high resolution.

Changes in the text: The new figure was replaced. The other figures resolution cannot be highly improved due to equipment limitation.

Reviewer B:

The manuscript "miR-155 Acts as an Inhibitory Factor in Atherosclerosis-associated Arterial Pathogenesis by Down-regulating NoxA1 related signaling pathway in ApoE-/- Mouse" aims to investigate the protective efficacy of miR-155 on down regulating NoxA1 gene expression, its inhibitory effect on VSMC migration and thus ameliorating the progression of arterial atherosclerosis in AS mouse model. Therefore, to further explore the regulatory effect of miR-155 on neointima formation in AS and locate potential anti-atherosclerosis target.

Overall, authors provide a meaningful approach for clinical application of atherosclerotic diseases. However, some minor changes in both text and figures are recommended:

Comment 1. In "key words" part, "neointima" should be substituted for "neointima formation" based on MeSH search. (https://meshb.nlm.nih.gov/search)

Reply 1: Yes, the key word was changed.

Changes in the text: The key word was change from "neointima formation" to "neointima" on page 4, line 90.

Comment 2. In discussion part, a bit more discussion focused on miR-155 and ROS system could be made to further clarify their relationship and other parts of discussion will be simplified accordingly.

Reply 2: Yes, the regulatory mechanism between miR-155 and ROS system was added, and other parts of the discussion was revised accordingly.

Changes in the text: We have modified the discussion part on page 14, line 413, line 420-421, line 412-413, page 15, line 429-430, line 443-444 and page 16, line 461-462.

Comment 3. In Fig.2, the staining method of the arteries should be further clarified in the "methods" part.

Reply 3: Yes, the method was added in the METHOD part as mentioned.

Changes in the text: The detailed method of oil red staining was added on page 11, line 303-304.

Comment 4. Some figures like Fig. 1-D, Fig. 2, 4, and 5, brief statement of the statistical significance between or among each group should be added.

Reply 4: Yes, the footnote of the figures above mentioned was added accordingly.

Changes in the text: The footnote of each figure was input below the figures, respectively.

Comment 5. A bit deeper explanation may be required on the clinical significance for guiding future treatment of AS via miR-155 at early screening stage or gene therapy.

Reply 5: Yes, the further relationship between miR-155 and AS diagnosis and treatment was added.

Changes in the text: We added the related part in the discussion part on page 18, line 522-527. In addition, one more reference was also added on page 21, line 628-629.

