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SPECIFIC COMMENTS TO AUTHORS

Cerebral small vessel disease (CVSD) accounts for 20–30% of ischemic stroke cases that are the deadliest disease in China and the second most disabling disease worldwide. In this study, the authors aimed at exploring the protective role of human exosomal miR-320e in treating cerebral small vessel disease. The authors used exosomes derived from patients with CVSD, cell models, western-blot analysis, real-time PCR, and RNA-seq to demonstrate their hypothesis. The results showed that exosomal miR-320e was downregulated in patients with CVSD, and exosomal miR-320e suppresses the Wnt/β-catenin pathway and may play a protective role in CVSD progression. So, in my opinion, this paper is well-written. The experimental design is reasonable, and the results reflects the conclusion as well. I recommend its acceptance after the minor revision. The detailed comments are: 1. In this study, the authors extracted exosomes from blood by filtering. As far as know, ultracentrifugation is also a good and common way for exosome extraction. So, is there any particular reason to use filtering? What are the key advantages of it compared to other way, like ultracentrifugation? 2. Page 15-17, there are some redundant tables. 3. The authors should carefully check the format and typo issues. For example, “Vascular integrity is gradually lost with aging and other risk factors.-catenin pathway can regulate vascular neogenesis” (Page 5).

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SPECIFIC COMMENTS TO AUTHORS

The authors extracted exosomes from cerebral small vessel disease (CVSD) patients and use them to investigate the protective role of exosomal miR-320e in the progress of CVSD and the subsequent stroke. After reasonable designing and performing the experiments, the authors demonstrated that exosomal miR-320e can effectively alleviates cognitive impairment and depression in CVSD through Wnt2 targeted inhibition of the Wnt/β-catenin pathway. This result also draws a conclusion that exosomal miR-320e is a potential drug for stroke treatment. In short, the topic of this manuscript is timely and interesting. The authors have organized the manuscript rationally, with good methodology and well-written English. However, some important editing needs to be done before publication: 1) Since the authors aim at investigating the protective role of exosomal miR-320e in the progress of CVSD. I wonder whether the utilization of HUVECs can represent the cerebral blood vessels? 2) Undoubtedly, the authors have provided us a nice manuscript, several formatting errors should be addressed before publication. A significant example is, on page 15-17, the table templates of the journal are still there.

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Thank you for your time and effort in reviewing our manuscript. We appreciate your constructive feedback and have carefully considered each of your suggestions.

Reviewer #1:

The use of HUVECs was primarily due to their ease of access and widespread use in cerebral vasculature studies. However, we agree with your concern and have now clarified in the revised manuscript that while HUVECs can be used as a preliminary model, further investigation with more relevant cell types would certainly strengthen our findings.

We apologize for the oversight regarding the formatting errors. We have now carefully revised the manuscript, corrected all formatting errors including the removal of redundant table templates on pages 15-17, and ensured it adheres to the journal's guidelines.

Reviewer #2:

We appreciate your insight on the method of exosome extraction. We used filtration due to its simplicity and high yield. However, we understand that ultracentrifugation is a more commonly used method and have now included a discussion in the manuscript comparing these two methods.

The redundant tables on pages 15-17 have been removed.

We apologize for the typographical errors. We have thoroughly reviewed and corrected all formatting and typo issues throughout the manuscript. The specific sentence you highlighted from page 5 has been rewritten for clarity.

We believe that these revisions have strengthened our manuscript and we hope that it is now suitable for publication.