We thank the reviewers for these important questions and appreciate the reviewer’s helpful suggestions to our research.

Comments to the Author

By analyzing the circulating EMVs from subjects with IS, high stroke risk and controls, the manuscript confirmed EMV-miR-125a-5p was correlated with stroke. The authors have revised a lot according to previous reviewers’ suggestions. I have the following concerns.

1. The major concern for this study is the authors used CD105 and CD144 to isolate circulating EMVs. However, CD105 is not a specific marker for endothelial cells, it is also a marker for mesenchymal stromal cells, which makes the conclusions not convincing. For the original paper regarding to isolate circulating endothelial microparticles (doi:10.1002/ana.21681), the authors used CD31, CD42b, annexin V (AV), and CD62E to isolate circulating endothelial microparticles.

   Answer: Thanks for this question. EMVs (diameter: 100 nm-1μm) are derived from plasma membrane of endothelial cells (ECs), which contain endothelial proteins such as CD62E, CD31, CD54, CD105, CD146, annexin V and CD144. Isolation and identification of EMVs relies on the use of these specific markers. However, except CD62E and CD144, most of them lack exclusive endothelial expression. CD31 is present on activated platelets and their derived MV; CD146 is present on activated T-cells; CD105 has been found on mesenchymal stromal cells and activated monocytes/macrophages. Of note, CD144 was proposed as one of the most specific markers for EMVs detection. Since one surface antigen CD105 expression cannot define EMVs specifically, we applied the surface antigen CD144 conjugated to Q-dots to simultaneously label the microbeads captured MVs in this study. We added these information in discussion section (page 16) of the revision.

2. The authors have deleted a lot of information on the Methods part to make the methods oversimplified. Some of which I think are very important. For example, how TEM and NTA are performed? For stroke animals, did the author check the blood flow and what is the criteria for the success of the animals? Are any animals excluded from the experiment? And what is the criteria for the exclusion?

   Answer: Thanks for pointing out these questions. We added information for TEM and NTA assay in the Methods section (page 7). In the present study, Transient middle cerebral artery occlusion (tMCAO) surgery was used to establish ischemic stroke (IS) mouse model. At 48 h after tMCAO, the mice were used for various measurements, including cerebral blood flow (CBF), infarct volume, and neurological deficit score (NDS). The CBF was measured by PeriCam PSI system as we previously described. In brief, mice were anesthetized and a crossing incision was made on the head to expose the whole skull. CBF on the intact skull was observed under a PeriCam PSI system. The relative CBF was calculated using the formula: CBF of ipsilateral side / CBF of contralateral side × 100%, and the mice with relative CBF ≤ 80% were included in this study. Additionally, we marked the low cerebral perfusion zone of IS...
mice in representative images of CBF. In the present study, mice with no infarct zone or with severe complications were excluded. These information were added in methods section (page 9) and revised Fig.8.

3. Figure 1 showed the characterization of the result. From the NTA data we can see multiple types of EMVs, with different diameters. Why? Do these different EMVs contribute differently to stroke?

**Answer:** Thanks for these questions. This study, our NTA analysis results showed that circulating EMVs have a diameter of 100 nm-800 nm, which were in accordance with previous reports showing that MVs were ranging from 100 nm to 1000 nm. It is well known that MVs can regulate target cell functions through transferring their cargos (RNAs, proteins, lipids). Evidence from us and others has shown that the roles of EMVs were highly depended on their levels and cargos. However, whether EMVs with different diameters exert different roles in stroke are still unknown, which will be investigated in our future research work.

4. In figure 1c, only one marker Annexin V was used to characterize EMVs, which is not sufficient, other markers such as TSG101, CD63 should be used, and negative marker such as GM130 should be added.

**Answer:** Thanks for this question. Extracellular vesicles, including exosomes (EXs) and microvesicles (MVs), have been recognized as newly paracrine communicators through the component cargo. EXs and MVs were different in size, biogenesis, and specific markers. EXs (diameter: 30 nm-100 nm) are generated as intraluminal vesicles (ILVs) in the lumen of endosomes and secreted by the fusion of multivesicular bodies (MVBs) with plasma membrane. Numerous specific markers of EXs have been discovered, such as CD63, TSG101, HSP70, CD9, etc, and GM130 (Golgi marker) has been also used as a negative marker for EXs. MVs (diameter:100 nm-1000 nm) are formed by outward budding of the plasma membrane, and express specific marker Annexin V and membrane protein of the source cells. Thus, Annexin V was used to characterize EMVs in this study.

5. There are many typos in this manuscript, In Line 46, was variety should be was varied. In figure 8b, Shan should be Sham.

**Answer:** Thanks for pointing out these mistakes in our manuscript. We changed “was variety” to “was varied” in page 14 and “Shan” to “Sham” in Fig.8b. Additionally, we checked and corrected the wrong description in our manuscript, such as changed “thecarotid” to “the carotid” in page 6, and added “Effects of” before the sentence “EMVs and their carried miR-125a-5p on infarct volume, NDS and CBF” in page 23.

References:

1. Dignat-George F, Boulanger CM. The many faces of endothelial microparticles. *Arterioscler*


---


---

Supplemental material

BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s)
doi: 10.1155/2016/8357567


