

Peer Review File

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Reviewer A

Mao S. et al. conducted this study to investigate the role of exosomal miRNA in SCLC metastasis. They identified that SCLC cell-secreted miR-375-3p of which level in blood was elevated in SCLC patients could increase the permeability of vascular endothelial cells and facilitate the transendothelial migration of SCLC cells. It could break the tight junction of vascular endothelial cells by down-regulation of claudin-1. They suggested that miRNA-375-3p has a great potential to be a novel biomarker monitoring metastasis and guiding clinical therapeutics of SCLC patients.

Comment 1: In cohort 2, there are only 12 stage IV SCLC. Hence, only a few deviated values could greatly affect the result as shown Fig 1h. More than 70% of SCLC are extensive stage (stage IV) at the time of diagnosis. Why did the cohorts of this study have more SCLC of limited stage (stage I~III)? Did you select intentionally?

Reply 1: We didn't select patients intentionally. There are two reasons for the small sample size of stage IV patients in cohort 2. Firstly, this study was mainly conducted in the Department of Thoracic Surgery which lacking of stage IV patients, the plasma samples of stage IV SCLC patients were obtained with the aid of the Department of Medical Oncology, which made it more difficult to collect the plasma samples of stage IV patients. Secondly, we collected blood samples from SCLC patients who did not receive any antitumor therapeutics before blood sample collection, many stage IV SCLC patients in our hospital (National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Beijing, China) have received antitumor therapies before in other hospitals and were excluded from our study. So, there are only 12 stage IV SCLC in cohort 2. In order to make up for this problem, we used serum samples in cohort 3 to enlarge our samples to validate our results. We appreciate for reviewer's valuable suggestion very much and we will try out best to enlarge our plasma samples to further validate our conclusion in the future.

Changes in the text: There are no changes in the text.

Reviewer B

In the manuscript by Mao S, et al., Exosomal miR-375-3p breaks vascular barrier and promotes small-cell lung cancer metastasis by targeting claudin-1. The authors suggested that exosomal miR-375-3p has a great potential to be a novel biomarker monitoring metastasis and guiding clinical therapeutics of SCLC patients. Although the results are of great interest and the manuscript is well written, the lack of validation are a few limits for generalizing these results. Specific points are listed below.

Comment 1: Authors focused on exosomal miR-375-3p in metastasis of SCLC. However, authors described the circulating miR-375-3p as well as exosomal miR-375-3p in text (e.g.

abstract and result). I think that authors should use the exosomal miR-375 instead of circulating miR-375 to avoid the confusion.

Reply 1: Following the reviewer's valuable suggestion, we have changed "circulating" to "exosomal" to avoid the confusion (see Page 3/13/14/22/33, line 63/284/313/493/733).

Changes in the text: Page 3/13/14/22/33, line 63/284/313/493/733.

Comment 2: In the figure 1c, authors should use negative control (e.g. calnexin) to validate the purification of exosome.

Reply 2: Following the reviewer's valuable suggestion, we detected the expression of calnexin using western blotting to validate the purification of exosomes, the results were added in figure 1c and supplementary figure 1e (see Page 14/33, line 293/736).

Changes in the text: Page 14/33, line 293/736.

Comment 3: The rationale to choice of miR-375-3p is weak because many miRNAs were enhanced in SCLC compared with normal and non-metastasis. Authors should need the deep discussion about choice of miR-375-3p in this paper.

Reply 3: We chose miR-375-3p because of the following three reasons. Firstly, the expression of exosomal miR-375-3p was upregulated in the plasma of SCLC patients with tumor metastasis compared with normal and non-metastasis (shown in figure 1d-1e). Secondly, miR-375-3p was the most abundant miRNA in the plasma of SCLC patients (shown in figure 1f). Thirdly, exosomal miR-375-3p has been reported to play important roles in other tumors (described in the Introduction, see Page 6, line 120-132). We have added this discussion about choice of miR-375-3p in the Results (see Page 14, line 299-302).

Changes in the text: Page 14, line 299-302.

Comment 4: In the figure 2e, authors should add the scale bar.

Reply 4: Following the reviewer's valuable suggestion, we have added the scale bar in figure 2e.

Changes in the text: There are no changes in the text.

Comment 5: In the figure 4 and 6, authors showed that human miR-375-3p can play the induction of blood vessel permeability by targeting claudin-1 in mouse endothelial cells. Although the sequences of many of the miRNAs are homologous among organisms, any miRNA can have the difference sequences according to organisms. Thus, authors should describe the conserved sequences of miR-375-3p.

Reply 5: Following the reviewer's valuable suggestion, we compared the sequences of miR-375-3p between human and mouse and found that they are homologous, the highly conserved sequences of miR-375-3p in human and mouse were shown in figure 5d. We have added this data in the Results (see Page19-20, line 427-428).

Changes in the text: Page19-20, line 427-428.

Comment 6: Recently, many studies demonstrate the role of circulating miR-375 as well as exosomal miR-375. In addition, Yoda S, et al. suggested that claudin-1 is a novel target of miR-375 in non-small cell lung cancer. Thus, author should add these references in introduction and

discussion.

Reply 6: Following the reviewer's valuable suggestion, we have added the corresponding references including Yoda's study in the Introduction, Results and Discussion (see Page 6/22/24, line 120-132/488-490/524-527).

Changes in the text: Page 6/22/24, line 120-132/488-490/524-527.