

Dear Professor Ma:

Manuscript NO: 72525

Title: Circulating miR-627-5p and miR-199a-5p are Promising Diagnostic Biomarkers of Colorectal Neoplasia

Thank you for your letter and the reviewer's comments concerning our manuscript. These comments are all valuable and very helpful for revising and improving our paper. We have studied the reviewers' comments very carefully and have tried our best to improve the manuscript. The followings are our point-by-point responses to the original reviewers' remarks underneath each comment. Revised portions are marked in yellow in the revised version of the manuscript. I hope that the adjustments made to the manuscript are satisfactory, and I am looking forward to your correspondence!

All the best.

Yours Sincerely,

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Point-by-point responses to review critiques

Reviewer 03730829:

The article is novel and interesting; However; =Sample size calculation and power of the study is important so that the results of the study can be relied on. =Discussion; you need to compare the results of the study with other studies; - I suggest important and relevant studies to be discussed in Discussion: -Mol Biol Rep. 2020 Apr;47(4):2509-2519. doi: 10.1007/s11033-020-05334-5. Epub 2020 Feb 22. Role of serum Metadherin mRNA expression in the diagnosis and prediction of survival in patients with colorectal cancer

Response:

Thank you very much for your kind response to this manuscript. We should like to express our appreciation to you. According to your suggestion, we have discussed some relevant studies in the manuscript. Corresponding statements have been modified in the discussion section of the revised manuscript on page 18, highlighted in yellow. We also carefully proof-read the language, data, and references in our manuscript. I hope that the revised manuscript is now suitable for publication.

Reviewer 05934777:

1. Major Authors conclude that both mir-627-5p and mir-199a-5p are derived from tumors based on the findings of Fig.4. However, Fig. 4 shows that expression levels of both mir-627-5p and mir-199a-5p are lower than FHC, suggesting that the origins of these micro RNAs are normal colorectal tissue and tumor microenvironment upregulates both microRNAs expression/secretion from normal tissue. Authors also show that these microRNAs have tumor suppressor activity in Fig.6. This finding also suggests that these two microRNA are derived from normal tissue, not tumor. Although I also agree with your conclusion that mir-627-5p and mir-199a-5p are useful for colorectal carcinoma early detection, your data cannot

completely exclude the possibility that these microRNAs are secreted from normal tissue. Therefore, you have to show more direct data in order to determine the origin of these microRNAs. Or you have to delete your conclusion that these microRNAs are tumor origin, since your data is immature.

Response:

Thank you very much for your valuable questions and comments. We studied carefully on your comments and we agree with you that we indeed made an immature claim that elevated serum miRNAs were tumor-derived. The origin of extracellular circulating miRNAs was indeed complex. Studies have demonstrated that circulating miRNAs can simply derive from the passive release of cytosolic components during cell death or other cellular activities[1]. They can also be actively secreted into the biological fluids by different cell types inside the tumor. Besides, circulating tumor cells also contain intracellular miRNAs that can be also found in the blood plasma or serum[2]. In our study, we used three methods to explore the origin of circulating miRNAs: (1) an independent cohort of 34 CRC patients, 33 advanced adenoma patients and 24 healthy controls was enrolled to explore the tissue expression of miR-627-5p and miR-199a-5p, and the results revealed that the levels of both miRNAs were significantly decreased in CRC patients; (2) the serum levels of both miRNAs were measured in another independent cohort of 15 CRC patients before and 1 month after surgery removal. Their expression level was significantly decreased in postoperative serum samples compared with that in the preoperative samples; (3) the expression of both miRNAs were detected in the culture media of three CRC cell lines (HCT116, RKO, and SW480) and our results showed that the expression of extracellular miRNAs increased with culture time and cell numbers. The last two results showed that both extracellular miRNAs might be released from cancer cells. However, the tissue expression of both miRNAs did not show the same trend. The incongruence between intracellular and extracellular profiles of

miR-199a-5p and miR-627-5p questioned the existence of a specific secretion mechanism for extracellular miRNAs and a series of in vivo and in vitro experiments should be performed to answer the question. According to your suggestion, we delete our conclusion that both miRNAs were tumor origin and add this as one of the limitations of our study. Thanks again for your valuable comments.

2. Minor Authors used CCK-8 for determination of cell proliferation. I think that CCK-8 kit can measure cell viability, not cell proliferation. CCK-8 contains WST-8 dye and dehydrogenase in mitochondria converts WST-8 dye to WST-8 formazan. This WST-8 formazan can be detected by microplate reader. Therefore, CCK-8 can only detect viable cells. So, please use cell viability instead of cell proliferation. If you would like to use cell proliferation, please refer appropriate reference.

Response:

We are very sorry for the incorrect statement and thank you for giving us the opportunity to explain it. Following your suggestion, we have replaced the statement "cell proliferation" with "cell viability". More details have been modified in the revised manuscript, highlighted in yellow.

Reference

- [1] A. Turchinovich, L. Weiz, B. Burwinkel "Extracellular miRNAs: the mystery of their origin and function," Trends in biochemical sciences, vol. 37, no. 11, pp. 460-465, 2012.
- [2] M. Marcuello, V. Vymetalkova, R. P. L. Neves et al., "Circulating biomarkers for early detection and clinical management of colorectal cancer," Molecular aspects of medicine, vol. 69, no., pp. 107-122, 2019.