Peer Review File

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Major Comment:

Comment 1: The authors described circulating miRNAs as potential promising biomarkers for the molecular detection and surveillance of lung cancer. But they do not address a fundamental problem that concern miRNAs evaluation in body fluids: the normalization. To date, there is no standard and shared normalization method to compare and reproduce miRNas expression level in blood samples. This may hamper the potential application in clinical routine. In my opinion, authors should discuss about this issue.

Reply 1: We fully agree with the reviewer. We now discussed this issue as suggested (page 12-13 highlighted lines 274-280). MiRNAs extraction and standardize quantitative methods are also the source of heterogeneity. Now there are several kinds of miRNA extraction kits, the miRNeasy kit is the recommended detection kit, but not all studies used this kit. U6 snRNA, cel-miR-39, and miR-16 are the generally standardize references, but it is not unified. In the future, the uniform preparation procedure, miRNA extraction method and standardize quantitative reference should be applied in large sample studies to identify the diagnostic accuracy of circulation miRNAs in LUAD.

Change in the text: page 12-13 highlighted lines 274-280.

Comment 2: The authors selected dataset assaying the expression levels of miRNAs in serum, plasma, exosomes, and blood, as starting material. Pooled data obtained from different starting material is could be misleading. They should prove that

expression level of miRNAs derived from different starting material are not influenced by the original biological material and the relative technology used for miRNAs isolation, or authors may perform separate analysis.

Reply 2: We appreciate the reviewer's suggestions. We now discussed this subject (page 12 highlighted lines 262-274). Exosomes, plasma and blood are currently accepted sample modalities for non-invasive detection of miRNAs, but there are different miRNA profiles from different sample types. Hemolysis can lead to the release of intracellular miRNAs into extracellular that contaminate extracellular miRNAs concentrations, as intracellular miRNA concentrations are higher than extracellular ones, (56, 57). In addition, the miRNA concentrations can be affected by cell debris which is not completely removed, if the centrifugal speed and time of preparation procedure is insufficient (58). However, strong studies have recently verified that exosomes derived from tumor cells can be specially identified by surface markers, which effectively avoid the contamination of abovementioned factors (59, 60). Therefore, more and more researches have been focusing on exosome miRNAs using for diagnosing cancers including LUAD. In the present study, subgroup analysis of sample types was not conducted due to few datasets included, we only assessed the diagnostic performance of the abovementioned miRNAs in all circulation sample types.

Change in the text: page 12 highlighted lines 261-274.

Minor comment:

Comment 1: It is not very clear why authors selected the miRNAs miR-21, miR-155, miR-210, miR-126, miR-486, miR-182, and miR-17 to further investigate. They should better explain the reason why they focussed on these miRNAs. Moreover, it was reported that miR-486 is a haemolysis-related miRNAs, then it is not suitable to use as cancer biomarker.

Reply 1: We thank the review for this comment. We now re-describe the standpoint (page 6 highlighted lines 123-127). Circulating miR-21, miR-155, miR-210, miR-126, miR-182, and miR-17 are the most frequently reported miRNAs diagnostic biomarkers for NSCLC. But the expression levels and deregulation directions are inconsistent in different studies, and whether these circulating miRNAs biomarkers can accurately identify LUAD or be applied for predictions in clinical practice is still inconclusive.

We deleted the relevant data of miR-486 as suggested (page 3 highlighted lines 53-56, page 6 highlighted lines 123-125, page 6 highlighted lines 130-132, page 7 highlighted lines 149-151, page 9 highlighted lines 190-193, page10 highlighted lines 225-227, page 13 highlighted lines 282-284, page 14 highlighted lines 303-305, and Figure 1).

Change in the text: page 6 highlighted lines 123-127, page 3 highlighted lines 53-56, page 6 highlighted lines 123-125, page 6 highlighted lines 130-132, page 7 highlighted lines 149-151, page 9 highlighted lines 190-193, page10 highlighted lines 225-227, page 13 highlighted lines 282-284, page 14 highlighted lines 303-305, and Figure 1.

Comment 2: Figures do not always correspond to the legends. Correct order should be reported.

Reply 2: Thank you for your suggestions. We modified figure legends orders (page 22 highlighted lines 481-484).

Change in the text: page 22 highlighted lines 481-484.

Comment 3: Reference n°2 is really old (Noguchi M, Morikawa A, Kawasaki M, et al. Small adenocarcinoma of the lung. Histologic characteristics and prognosis.

Cancer 1995; 75: 2844-2852). if possible, I recommend to change it with a more recent one.

Reply 3: we appreciate the review's suggestions. We replaced reference n°2 with more recent one as suggested. (page 15 highlighted reference n°2).

Change in the text: page 15 highlighted reference n°2.

Comment 4: The references cited for the sentence "The global incidence of LUAD has risen in the last few decades, especially in women, non-smokers, and young patients […]" doesn't support the issue of the increased incidence in young people. Appropriate references should be indicated.

Reply 4: We thank the reviewer of suggestions. We modified the description of this sentence (page 5 highlighted lines 97-99). The global incidence of LUAD has risen in the last few decades, especially in young patients, with the prevalence in young patients even reaching between 57.5% and 77.9%.

Change in the text: page 5 highlighted lines 97-99.