Peer Review File

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Reviewer Comments-Reviewer A

Chen et al. propose an impact on proliferation, apoptosis, migration and invasion in an NSCLC cell line, A549, by miR-183-5p and the proposed target LOXL4.

The manuscript contains a lot of data which merits publication. I have a couple of comments that need to be addressed.

- It is unlikely that LOXL4 would promote proliferation if also promoting migration and invasion. In fact, the data on that LOXL4 would promote proliferation is not convincing. In Figure 3E, overexpression of LOXL4 does not lead to a higher percentage of cells in S-phase compared to the control condition. The CCK-8 assay is not a perfect assay for measuring proliferation and the manual assessment of EdU-positive cells is prone for errors. Authors should comment on this in the discussion.

Reply 1: Thank you for your comment. In Figure 3E, overexpression of LOXL4 does not lead to a higher percentage of cells in S-phase compared to the control condition. However, the percentage in G0/G1-phase and S-phase (the summation) was significantly reduced by overexpression of LOXL4. It suggested that LOXL4 promotes the transition of cells from G0/G1-phase to S-phase, or from S-phase to G2/M phase. Conversely, when we knocked the LOXL4 down, the percentage in G0/G1-phase was significantly increased and that in S-phase was significantly decreased. Therefore, it's almost certain that the expression of LOXL4 could inluenced the transition of cells from G0/G1-phase to S-phase. We have added the corresponding content in the discussion.
Changes in the text: Line 472~479.

- The data displayed in Figure 6 is very confusing. Is miR-183-5p tumor suppressive or tumor promotive? From the data presented earlier in the manuscript, I would assume that the impact of miR-183-5p mimics would be tumor suppressive? Still, Figure 6B shows increased tumor volume with miR-183-5p mimics. Moreover, miR-183-5p mimics leads to a decrease in both Ki67 and TUNEL staining (Figure 6C), i.e., reducing both proliferation and apoptosis. Authors need to clarify whether the data is mislabeled and revise the manuscript accordingly.

Reply 2: Sorry about the error in the figure 6B and 6C. miR-183-5p functions as a tumor suppressive role in NSCLC. We have double checked the tumor volume, Ki67 and TUNEL staining. We confirmed that the data is mislabeled and we have revised the the manuscript accordingly.
Changes in the text: Revised figure 6B and 6C.

Reviewer Comments-Reviewer B

Non-small cell lung cancer (NSCLC) progression is mediated by changes in gene expression induced by microRNAs. However, the underlying mechanisms remain to be elucidated. In the manuscript "miR-183-5p regulates ECM and EMT to promote non-small cell lung cancer progression by targeting LOXL4", authors investigated the roles of miR-183-5p and its target gene in lung cancer development.

Couple questions are required to be answered before it will be accepted.

(1) In the abstract, what was the meaning of "immunofluorescence or Western blotting as appropriate"? Please state clearly.

Reply 1: Thank you for your comment. This is polished by an English-speaking scientific researcher. "Relative levels of miR-183-5p and lysyl oxidase-like 4 (LOXL4) expression in lung cancer cells or tissues were measured by quantitative RT-PCR, immunofluorescence or Western blotting as appropriate". Relative level of miR-183-5p expression was measured by quantitative RT-PCR only, while that of LOXL4 expression could be measured by quantitative RT-PCR, immunofluorescence and Western blotting. We are not good at English writing, but we think that there should be no problem with this description.

Changes in the text: No changes.

(2) In the introduction, it was proposed to add related reference (DOI: 10.21037/atm-22-345) about the LOXL4.

Reply 2: Thank you for your suggestion. We have added it.

Changes in the text: "More importantly, the expression of LOXL4 during NSCLC development was found to be targeted by multiple miRNAs such as miR-135a-5p, miR-210 (19) and miR-328-5p (20)." (Page 5, line 7-9)

(3) In the introduction, "NLCLC" was showed. I thought it should be "NSCLC".

Reply 3: Thank you for your mention. "NLCLC" has been changed to "NSCLC".

Changes in the text: Page 4, line 15-18.

(4) What were the roles of ECM in the NSCLC? Please state in the introduction.

Reply 4: Thank you for your suggestion. We have added it.

Changes in the text: "The remodeling of ECM plays an essential role in allowing epithelial cell detachment from the basement membrane, which further promotes EMT progression and cancer metastasis (16)". (Page 4, line 31-33)

(5) Why to choose female mice? And state clearly the body-weight of used mice.

Reply 5: Because we found that male rats are too aggressive. Tumor planted in male mice are often damaged due to fighting. The body-weight was added in the text.

Changes in the text: Page 9, line 14.

(6) Please perform statistical analysis about the Western blot, scratch test and EdU staining.

Reply 6: Thank you for your suggestion. We added statistical analysis in figure 1, 3 and 5.

Changes in the text: No changes.

(7) In the figure 2 legends, please state clearly the *P versus which group.

Reply 7: It was compared with HBE group or miR-183-5p NC group.

Changes in the text: "*P<0.05; ***P<0.001; vs. HBE group or miR-183-5p NC group." (Page 21, line 16)

(8) What were the correlations between ECM accumulation and EMT? Please state in the discussion.

Reply 8: Thank you for your suggestion. We have added it.

Changes in the text: "As an important component of tumor microenvironment, ECM alterations affected EMT progression. Conversely, cancer cells entering the EMT process can promote ECM alterations through multiple signal pathways." (Page 15, line 18-20)