Author’s response to reviews

Title: Linc01234 promotes cell proliferation and metastasis in oral squamous cell carcinoma via miR-433/PAK4 axis

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Author’s response to reviews:

Response to reviewer 1:

There is no control group. It is important to find if such changes are seen in normal cases as well. Otherwise study is good.

Response: We really appreciate this constructive suggestion. We have added the control group in these figures.

Response to reviewer 2:

This manuscript is informative and may help in understanding the role of lncRNAs in OSCC. However, there are some concerns, which need to be clarified and further discussed in the manuscript.

1. Authors have not shown any supporting data, which can clarify the effect of Linc01234 knockdown/over expression on the level of expression of PAK4.

   Response: We really appreciate this constructive suggestion. We have examined PAK4 expression in SCC25 and CAL27 cells with Linc01234 knockdown. RT-qPCR and WB results were showed in supplementary figure 1.

2. Authors should have compared the endogenous expression of miR-433-3p and PAK4 in different cell lines.
Response: Thanks for your kind advices! We have compared the endogenous expression of miR-433-3p and PAK4 in different cell lines. The RT-qPCR and WB results were showed in supplementary figure 2.

3. Effect of PAK4 knockdown on the proliferation/migration/invasion of different OSCC cells can be studied.

Response: We are extremely grateful for your comments. We have detected the effect of PAK4 knockdown on the migration and invasion of SCC25 and CAL27. It’s has been reported that PAK4 overexpression promotes the proliferation and/or survival of OSCC cells. See https://www.ncbi.nlm.nih.gov/pubmed/19594544. In addition, some articles proposed that PAK4 overexpression enhanced migration and invasion of different cancers. See https://www.ncbi.nlm.nih.gov/pubmed/30808546 https://www.ncbi.nlm.nih.gov/pubmed/28205613 https://www.ncbi.nlm.nih.gov/pubmed/23893240

Furthermore, we also perform the transwell assays in OSCC cells with PAK4 siRNA and the results were showed in supplementary figure 3.

4. Why authors have shown HNSCC patient data, when manuscript is concerned about OSCC?

Response: We really appreciate your question. Oral cancer, predominantly oral squamous-cell carcinoma(OSCC), is the most common head and neck neoplasm (head and neck SCC, HNSCC), affecting 270 000 people worldwide each year.

5. In the results section, while describing Figure 2C, authors have written Linc01234 siRNA transfected cells showed a glowing green fluorescence. Please check the sentence.

Response: We are extremely grateful for your comments. We have modified this sentence in our article.

6. Provide Statistical analysis details i.e no. of repeats for each experiment in the respective legends.

Response: We really appreciate your suggestion. We have provided the details of statistical analysis in our article.

7. Please go through the following writing/labeling errors in the manuscript:

   a. Provide catalog no. of all the cell lines used in the manuscript.

Response: Thanks for your kind suggestion. We have added the catalog no. of cell lines. Such as: SCC9 (ATCC® CRL-1629), SCC25 (ATCC® CRL-1628), CAL27 (ATCC® CRL-2095). The catalog no. of HSC-3 and NOK cells bought from Otwo Biotech is HTX2055 and HTX2992. The catalog no. of CAL33 cells bought from Cobioer is CBP60579.
b. In the results section, Figure 1B is written for tissues and Figure 1C is written for cell lines. But in the images, Figure 1B is for cell lines and 1C for tissues.

Response: We really appreciate your suggestion. We have corrected these errors in result section.

c. In the Figure 3 legends A and B should be written for migration and invasion assays while C and D should be written for wound healing assays.

Response: We really appreciate your advices. We have corrected these writing errors in Figure 3 legends.

d. In the legends of Figure 1B and Figure 5E, the data is from which experiments should be mentioned (RT-PCR / Western Blot).

Response: Thank you very much for your suggestion. We have added RT-qPCR experiment in Figure 1B and Figure 5E legends.

e. Label Figure 4D diagram with Linc01234 (wild/mutant).

Response: We really appreciate your suggestion. We have changed the Gigure 4D label with Linc01234 wild/mutant.

f. Write Linc01234 in same pattern through out the manuscript.

Response: We really appreciate your suggestion. We have corrected the pattern of Linc01234 in our manuscript.

g. Authors should be more careful towards writing of the manuscript (Grammar/Spelling). In the last sentence of the Discussion section, write findings instead of founding.

Response: We really appreciate your suggestion. We have corrected these writing errors.