

Dear editor,

Thank you for your kind letter. We have revised the manuscript in accordance with the editor and reviewers' comments. All changes are colored in highlight in the revised manuscript. Here below are our point-by-point responses to reviewers' comments.

Thank you and all the reviewers for the kind advice.

Sincerely yours,

Wenqiao Zang

Reviewer #1:

1. What are the limitations of the study and its findings ? and your future directions ?

Answer: Thank you for your question. The pathogenesis of esophageal squamous cell carcinoma and regulatory network of lncRNAR are very complicated, and there must be other target genes taking part in the process, which needs further study. The limitations of the study and its findings are that we only studied one lncRNA and its target genes. Our future directions are to discover and verify more lncRNAs and target genes.

2. How might this publication impact science and /or clinical practice ?

Answer: Thank you for your question. In the study we investigated the biological roles of lncRNA XLOC\_001659 in ESCC and showed lncRNA XLOC\_001659 to be overexpressed in ESCC. Silencing lncRNA XLOC\_001659 expression inhibited the proliferation and invasion of ESCC cells. The lncRNAXLOC\_001659 -miR-490-5p-PIK3CA regulatory axis affected the growth and invasion of ESCC, suggesting that it may play a role in ESCC tumorigenesis and progression. For this reason, we speculate that lncRNA XLOC\_001659 may be a cancer-promoting gene and may be a novel diagnostic biomarker and therapeutic target for ESCC.

Because it is the first time that lncRNAXLOC\_001659 is found to play an important

role in ESCC, whether this molecule also plays an important role in other cancers or diseases remains to be further studied.

Reviewer #2: The manuscript by Li and Zang entitled “Knockdown of lncRNA XLOC\_001659 inhibited proliferation and invasion of esophageal squamous cell carcinoma via regulation of miR-490-5p/PIK3CA” reports that a upregulated long non-coding RNA XLOC\_001659 promotes proliferation and invasion via miR-490-5p/PIK3CA in EC cells.

Major concerns:

1. The authors stated that in their preliminary observation, lncRNA XLOC\_001659 increase in human esophageal cancer tissues. While no data has been shown in the manuscript, neither if lncRNA XLOC\_001659 shows any clinical relevance. These concerns should be addressed.

Answer: Thank you for your advice. To analyze the expression and clinicopathological significance of lncRNA XLOC\_001659, we examined 30 primary ESCC tissues, as well as 30 normal esophagus tissues, collected from sites adjacent to tumors. The results of RT-qPCR showed that the expression level of lncRNA XLOC\_001659 was generally upregulated in ESCC tissues compared with normal esophagus tissues adjacent to tumors (Fig.1A). The further clinical relevance results showed that lncRNA XLOC\_001659 expression level were associated with the TNM stage, not associated with age, gender and location. We found that lncRNA XLOC\_001659 expression was lower in stage I - II, but higher in stages III (Fig. 1B); This reflects that lncRNA XLOC\_001659 has a significant correlation with clinical stages. But these results are in another paper, which is about some abnormal lncRNAs expression and biomarkers screening in esophageal squamous cell carcinoma. The paper is currently under review. So these results in human esophageal cancer tissues are not presented in the manuscript again.

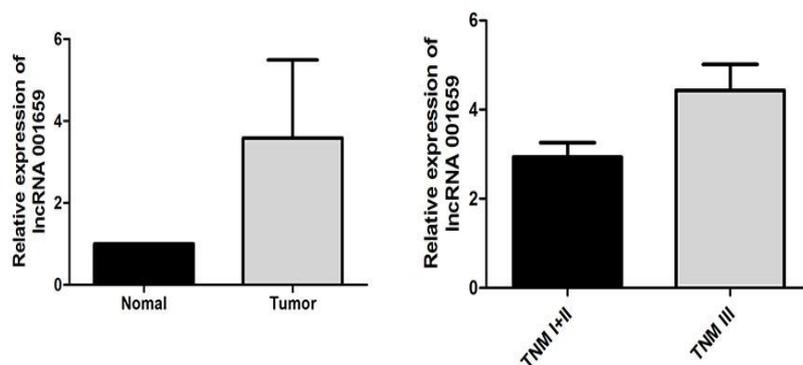


Fig.1 lncRNA XLOC\_001659 expression in ESCC tissues.

2. All the experiments are carried on in ESCC cell lines. Sometimes, the significance of the discovery may be limited without further confirmation in other systems. Is the result from cellular experiments applicable in animal models?

Answer: Thank you for your advice. Due to the limitations of experimental conditions and funding, we did not conduct animal models experiments. I am sorry about the question. But if we apply for more funding in the future, we will have further confirmation in animal models and other systems.

Minor concerns:

1. The groups in statistics and comparisons in the Figures should be clearly stated.

Answer: Thank you for your advice. We have clearly stated the groups in statistics and comparisons in the Figures in the revision.

2. There are numerous typographic and grammatic error through the text. For example, an “and” should be needed between the listed two authors.

Answer: Thank you for your advice. We have checked the typographic and grammatic error in the revision.