27 February, 2017

Dear Dr. Jing Yu

Scientific Editor

World Journal of Gastroenterology

Please find enclosed the edited manuscript in Word format (file name: 32318-manuscript).

Title: miR-382 functions as a tumor suppressor against esophageal squamous cell carcinoma
Author: Jie Feng, Bo Qi, Ling Guo, Ling-Yun Chen, Xiu-Feng Wei, Yu-Zhen Liu and Bao-Sheng Zhao
Name of Journal: *World Journal of Gastroenterology*
ESPS Manuscript NO: 32318

I am hereby resubmitting our revised manuscript entitled "**miR-382 functions as a tumor suppressor against esophageal squamous cell carcinoma**" to be considered for publication in the World Journal of Gastroenterology. We have taken into account the suggestions of the Scientific Editor and the modifications to the manuscript. Our detailed responses to the reviewers' comments are to be found below. Our reply to the reviewers is in italic.

Reviewer 00503563:
The authors already reported the clinical significance of miR-382 in patients with esophageal squamous cell carcinoma (ESCC). In the present study, the functional role of miR-382 in ESCC was investigated. Then, the authors demonstrated miR-382 functions as a tumor suppressor in ESCC. Although this manuscript is important for the development of a new targeted therapy in patients with ESCC, there are some queries and comments.

1. In this study, Eca109 alone was used as a ESCC cell line. Why did the author use only one cell line in the present study? How about miR-382 expression in other ESCC cell lines?

Reply: *We previously examined the miR-382 expression in the specimens from 46 ESCC patients with different outcomes and found that miR-382 was downregulated in the patients with poor outcome compared to those with good outcome. Accordingly, it is better to choose the ESCC cell line in which miR-382 is downregulated comparing with the*

human normal esophageal epithelium cell line, as the study object to investigate the effect of miR-382 on ESCC cells in vitro and its possible molecular mechanism. For this purpose, we tested miR-382 expression in Eca109，KYSE-450 and TE-1 cells, which are all ESCC cell lines. The result showed that endogenous miR-382 was clearly downregulated in all tested cells comparing with Het-1A cell line derived from human normal esophageal epithelium. In our lab, Eca109 cells were more easily cultured and maintained so as to be used for all experiments in the current study. We speculate the similar phenotypes induced by overexpression of miR-382 in KYSE-450 and TE-1 cells as in Eca109 cells and will use not only Eca109 cells but also other ESCC cell lines in our further experiments.

2. Misspelling: β-Ccatenin (Results section, Page 10, 2nd paragraph)

Reply: *We deeply apologize for our spelling mistake and the error was extinguished in the revised manuscript.*

3. The authors investigated the functional role of miR-382 in mammalian target of rapamycin (mTOR) signaling pathway. Why did the authors focus on this?

Reply: *The regulation of translation is critical for controlling many major cellular processes, including cell proliferation, apoptosis, and metastasis. mTOR signaling pathway plays a key role in the regulation of translation process and has been reported being abnormally regulated in tumors. We found that overexpression of miR-382 in Eca109 cells inhibited cell proliferation, migration, invasion, EMT, as well as inducing cell cycle arrest and apoptosis. In terms of our results and the previous reports of mTOR signaling pathway's functions, we studied whether or not miR-382 functions as a tumor suppressor through the regulation of mTOR signaling pathway. As the results showed in the current study, the inhibitory influence on protein translation mediated by mTOR/4E-BP1 signaling might be involved in the antitumor activity of miR-382 against ESCC. Other signaling pathways might be also involved in the antitumor activity of miR-382 against ESCC and the further study is needed to be performed for this.*

Reviewer 03505541:

This is one of series of topic aiming to reveal more advanced and detailed functions of miR-382 on ESCC. The authors previously found that miRNA-382 (miR-382) was reduced and associated with poor survival in ESCC patients, implying that miR-382 may contribute to the development and metastasis of ESCC. They tried to establishe the possible roles and mechanisms of miR-382 in human ESCC by demonstrated that overexpression of miR-382 inhibited cell proliferation and invasion, induced cell apoptosis, and cell autophagy. They showed that the anti-tumor activity of miR-382

might be initiated by inhibition of mTOR/4E-BP1 mediated protein translation process, which is a potential therapeutic strategy for ESCC.

Reply: *We thank the reviewer's comment.*

Thank you again for consideration of publishing our manuscript in the World Journal of Gastroenterology.

Sincerely yours,

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