

August 25, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 12621-review.doc).

Title: miR-133a functions as a tumor suppressor in gastric cancer by repressing IGF1R

Author: Yu Gong, Jun Ren, Kun Liu and Li-Ming Tang

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 12621

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated.

2 Revision has been made according to the suggestions of the reviewer.

Comments from Reviewer No.61678

Dear Editor, Authors Thanks for sending the manuscript "miR-133a functions as a tumor suppressor in gastric cancer by repressing IGF1R." for revision - Title represent the aim and content - Good idea and relatively new field of research - Well organized - Significant grammatical errors need revision. Thanks.

Response: Thanks for the positive comments on our study. We have made our paper revised by professional English language editing company named American Journal Experts.

Comments from Reviewer No.71702

Emerging evidence has shown that microRNAs are involved development and dissemination of gastric cancer. miR-133a could serve as a potential tumour suppressor in several distinct cancer types that includes esophageal squamous cell carcinoma, bladder cancer, prostate cancer, colorectal cancer, osteosarcoma and breast cancer. However, the specific role of miR-133a in gastric cancer is largely unknown. The work documents a very interesting aspect and data looks convincing. But I have few concerns that need to be addressed before the work is accepted for publication.

Response: Thanks for the positive comments on our study. The specific concerns were addressed below.

(1) How were the samples selected for the study? Describe.

Response: This experiment is a retrospective study. All patients engaged in curative resection of the primary tumor at Nanjing Medical University Affiliated Changzhou No.2 People's Hospital from January, 2012 to December, 2013. All clinical specimens were

histopathologically confirmed and the corresponding normal tissues are over 5 cm away from tumors. We have made some revisions in section of Clinical specimens (Highlights of Page 5 Line 3: “This experiment is a retrospective study in which” was added; Highlights of Page 5 Line 6: “from January 2012 to December 2013.” was added; Highlights of Page 5 Line 9-11: “and the corresponding normal tissue samples were collected more than 5 cm away from the tumors.” was added)

(2) What were the exclusion and inclusion criteria for the selection of samples? Elaborate.

Response: Inclusion criteria: (1) Age from 18 to 69 years old (2) All patients were diagnosed as gastric cancer by histopathology. (3) Patients did not receive any other adjuvant therapy before surgical resection. (4) All the patients' performance status scores ≤ 2 points according to Eastern Cooperative Oncology Group scale.

Exclusion criteria: (1) coexistence of other serious diseases which are not under control, (2) coexistence of other malignant tumors.

(3) HEK293T cells - gastric epithelial cell line? Confirm.

Response: HEK-293T cell line is one of the human embryonic kidney cell lines. We used it for dual-luciferase report assays according to methods of several papers (Wada R, Akiyama Y, Hashimoto Y, Fukamachi H, Yuasa Y. miR-212 is downregulated and suppresses methyl-CpG-binding protein MeCP2 in human gastric cancer. *Int J Cancer*, 2010,127(5):1106-14; Zheng YB, Luo HP, Shi Q, et al. miR-132 inhibits colorectal cancer invasion and metastasis via directly targeting ZEB2. *World J Gastroenterol*, 2014,20(21):6515-22; Shen J, Niu W, Zhou M, et al. MicroRNA-410 Suppresses Migration and Invasion by Targeting MDM2 in Gastric Cancer. *PLoS One*, 2014,9(8):e104510).

(4) What prompted the authors to investigate the possible role of IGF1R? Did the authors adopted a non-bias approach for selection of predicted-targets? Specify.

Response: To investigate the downstream target gene of miR-133a in GC, we consulted the original articles and found that miR-133a suppressed ovarian cancer cell proliferation by directly targeting IGF1R. Thus, we thought perhaps IGF1R was a direct target gene of miR-133a in GC because of its positive role in carcinogenesis. The potential miR-133a binding site in the IGF1R 3'-UTR was predicted using TargetScan (www.targetscan.org) and Miranda databases (www.microRNA.org). Our study identified IGF1R was a direct target gene of miR-133a in GC. Furthermore, Qiu T et al. found another target gene named Sp1 of miR-133a in GC. These findings indicate that miR-133a may have multiple

targets in GC. Thus, further studies are essential to fully clarify the role of miR-133a in GC.

- (5) High-throughput technologies, such as miRNA oligonucleotide arrays and quantitative RT-PCR have demonstrated significant associations between miRNA expression levels and tumor type, grade, response to treatment and prognosis. Does this indicate the potential for miRNAs to serve as useful markers of disease state? The possibility of using miR-133a as a biomarker for gastric cancer must be discussed.

Response: We agree with the reviewer and we have made some revisions about the possibility of using miR-133a as a biomarker for GC in the discussion. (Highlights of Page 13, Line 16-18: “These results indicate that miR-133a downregulation may be a potential diagnostic biomarker and that it may predict cancer progression in GC patients.” was added)

- (6) What is the translational significance of this work? Understanding of the molecular networks controlled by miRNAs should be exploited for future cancer treatment. Discuss the feasibility of this strategy in light of complexities involved in a disease such as gastric cancer.

Response: In our study, the expression level of miR-133a was significantly down-regulated in gastric cancer tissues compared with corresponding normal tissues. And the significant reduction of miR-133a was associated with degree of differentiation, local invasion and TNM stage in GC. These results indicated that miR-133a downregulation might be a potential biomarker of diagnosis and predicting cancer progression in GC patients. In the future, miR-133a may be used as a novel therapeutic target for GC in miRNA-based gene therapy.

Comments from Reviewer No. 2460289

NO.

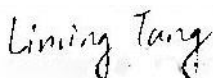
Response: Thanks for the positive comments on our paper.

3 References and typesetting were corrected.

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

Sincerely yours,

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