ANSWERING REVIEWERS

August 25, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 12513-Edited.doc).

Title: miR-21/RASA1 axis affects malignancy of colon cancer cells by RAS pathways

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 12513

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
(1) Please simplifying the text taking into consideration that MTT, pre-miR-21-LV cells, anti-miR-21-LV cells, siRASA1, pcDNA3.1 are not terms understandable to all readers .

MTT is an abbreviation of the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dye. Pre-miR-21-LV means up-regulation of miR-21; anti-miR-21-LV means down-regulation of miR-21; siRASA1 means down-regulation of RASA1; pcDNA3.1-RASA1 means up-regulation of RASA1. The terms have been explained at first appearance in the revised manuscript.

(2) Moreover the choice of RKO cells for transfection experiments should already be explained in the methods ?

Colon cancer RKO cells were chosen for transfection because they are KRAS wild type colon cancer cells that RASA1 expression is significantly decreased. The explanation has been added to the Methods.

(3) Page 4, line 12, please change ‘diagnostic’ with ‘prognostic/predictive’ ?

It has already been modified.

(4) Please add, whenever the anti-EGFR antibody cetuximab is cited, also the other anti-EGFR panitumumab ?

It has already been modified. But Reference 12 (in line 6, paragraph 3 of Introduction section) in the manuscrip just reported cetuximab without panitumumab. Thus in this part, panitumumab cannot be added.

(5) Page 4, last line: ‘…in relation to abnormal or missing expression of some molecuxles…’ instead of ‘…in relation to abnormal expression or missing …’ ?

It has already been modified.

(6) In METHODS, 'Transfection of RKO cells with plasmid vector for RASA1 up/down-regulation' section: Please specify how GV102 vector down-regulates RASA1 and what is the molecular difference between siRASA1 and siRASA1-NC ?

GV102 is a plasmid vector. By importing RASA1 antisense sequence, we constructed the RASA1 antisense strand expression vector. Then the vector infected cells, and fused with the cell DNA to downregulate the RASA1 expression. siRASA1 was an antisense sequence. siRASA1-NC was a nonsense sequence.

(7) Please spell out RT-PCR at first appearance ?

It has already been modified.

(8) In METHODS the pGL3-promoter vector is labeled as 'negative control': Is this a real 'negative' control? Or rather a 'positive control' as light emission is not inhibited by miR21? See figure 3 ?

Yes, the pGL3-promoter vector is a positive control as light emission is not inhibited by miR-21.Thank you for reminding us.

(9) In METHODS, In cell proliferation assay section, first line: Please, specify what 'negative control and control' are (are they native cells?) ?

The negative control is cells transfected with non-coding sequence. Control is their native cells.

(10) RESULTS section, in 'Validation of lentivirus and plasmid vector transfection efficiency' : Please specify what controls are (non-coding LV? Native RKO cells?) ?

They are native RKO cells.

(11) 'Role of miR-21 and RASA1 in the RAS signaling pathway' section, third line: please change 'close' with 'switch off' ?

It has already been modified.

(12) In RESULTS, '…We analyzed the changes in Raf-1, KRAS, AKT and p-AKT, ERK1/2 and p-ERK1/2…': KRAS is not a downstream signal of RAS, but it is the RAS protein itself, therefore authors should explain the mechanism by which total RAS amount changes, rather than just its activation, through miR21 and RASA1 modulation, so they should for changes in total amount of downstream proteins (why does not it change just their phosphorilyzed status?) ?

RASA1 can regulate KRAS. In this part of experiment, the KRAS represented the activated RAS ,not total RAS. In addition, the downstream proteins change just when they have been phosphorilyzed.

(13) In figures: where the line representing standard error appears in the bar graphs, number of tests performed for each experiment should be specified

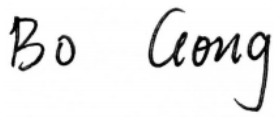
Numbers of tests have been added to the legends of figures.

3 References and typesetting were corrected

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

Sincerely yours,

Bo Gong



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