Jan 24, 2014

Dear Editor,

Thanks for the relevant comments of the reviewers and the editor. We have read these comments carefully. These comments pointed out many deficiencies of our manuscript and reminded us some new ideas. According to these comments we retrospected our experiment and manuscript again and again. Now the manuscript has been improved according to the suggestions of reviewers and editor. Please find enclosed the edited manuscript in Word format (file name: 15610–review.docx).

Title: The Effects of microRNA-1 on hepatocellular carcinoma tumor endothelial cells

Author: Chao Hu, Shi-Qiang Shen, Zhong-Hui Cui, Zu-Bing Chen, Wei Li

Name of Journal: World journal of Gastroenterology

ESPS Manuscript NO.: 15610

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

Reviewer #1:

(1) The manuscript needs a reading for correction of spelling and grammar errors, as well as to be concise on verbal use.

The manuscript has been submitted to AJE (American Journal Experts) for editing and the spelling and grammar errors has been corrected.

(2) The abstract should have abbreviations prior to its definition, e.g., miR-1 (1st line), HCC (2nd line).

The definitions of abbreviations have been added in the abstract.

(3) Authors should use the same notation along the manuscript when referring to the same thing; e.g. use of MiR-1 and miR-1; use of "l" and "L" for litter, and others. Please, choose one notation and be consistent along the manuscript.

Revision has been made according to the suggestions.

(4) **Along the manuscript, authors should use the symbol font to insert the micro symbol (μ).**

Revision has been made according to the suggestions.

(5) Page 6, Methods (Infection of TECs by lentivirus", authors should refer to the excitation and emission wavelengths used, or should indicate the filter used.

The filter used has been added in methods. (page 7, line17-18)

6) **Page 8, Methods (Flow cytometry), why the authors use the designation of D-Hanks? Isn't it Hanks Balanced salt solution (HBSS) or is another, please specify.**

Comparing with the HBSS, D-Hanks does not contain the Ca2+, Mg2+ which may influence the digestive effect of trypsin.

a. "digestion was stopped by addition of culture supernatant". Why the authors used the culture supernatant to stop the digestion?

The reason of using the culture supernatant to stop the digestion is to reduce the total volume. The centrifuge tubes we used were 5ml. If we added the complete culture medium additionally, the total volume may be more than 5ml.

b. Line two from the bottom, please consider correcting, "after washing with PBS, 1 ml 1 x binding buffer…" to ""after washing with PBS, 1 ml of 1 x binding buffer…", and the followings…

The description was reorganized and it seemed more clearly. (page 9, line 7 from the buttom)

c. Please indicate de origin (manufacturer) of Annexin V-APC; please indicate the excitation, emission wavelengths used (or the lasers and filters used) to acquire cytometry data…

The manufacturer of Annexin V-APC, the excitation and emission wavelengths of the Annexin V-APC fluorescent signals were added in the text. (page 10, line 1-5)

(7) Page 9, Please correct the pore size of Transwell chamber, it should be 8 μm and not 8 mm (cell migration and cell invasion assays)

Revision has been made according to the suggestions. (page 10, line 9)

(8) RESULTS (cell morphology) legend of figure 1 is a repetition of the text on result section. Please include the magnification used on Fig 1 legend.

Revision has been made according to the suggestions.

(9) RESULTS (determination of lentivirus….) and legend of Figure 2.

a. There is a repetition of text between the legend and the body of the manuscript.

The description was reorganized according to the suggestions. (page 11, line 11-13)

b. Rearrange text of figure 2 in a more comprehensive way. It is not so clear that photos (A1 and A2; B1 and B2; C1 and C2) were taken from the same field as they should be.

The description was reorganized according to the suggestions. (page 11, line11-13)

c. Please consider rearranging the images in figure, for instance 1st line bright field and 2nd line the corresponding fluorescence image, as I presume A1 and A2 are from the same field as well as B1 and B2. Comparison would gain if photos were close to each other.

The images in Figuer 2 have been rearranged according to the suggestions.

(10) RESULTS (miR-1 expression….). please refer to figure 3 in the text. The values shown on the figure 3 graphic indicate the value of 1 to the control, that is not the value indicated in the text "NC group ($2^{-\Delta\Delta Ct=1}$) and the CON group ($2^{-\Delta\Delta Ct= 1.05\pm0.13}$) ($P<0.01$)." I think the values are exchanged. Also in figure 3, make a reference to the right panel in the legend or in the text...

There is a mistake in the text and we have corrected the mistake. It should be "CON group ($2^{-\Delta\Delta Ct=1}$) and the NC group ($2^{-\Delta\Delta Ct= 1.05\pm0.13}$) ($P<0.01$)".

11) RESULTS (detection of TECs….). please consider initiating the text by "As observed on figure 4…" to avoid having a "sentence" with (Figure 4)…

Revision has been made according to the suggestions.

a. Were the significant differences only observed at day 5, or at day 2 and 3 they were also significant?

The description was added in the text. It should be all significantly inhibited in the MD group in the 5 days.

12) RESULTS (flow cytometry….) and figure 5.

a. Authors should mention the excitation source of x-axis and y-axis. Should mention the excitation source for annexin, and if read at XX or YY axis. Authors should mention what is read in each quadrant, as they don't mention what is read at each axis. Thus it is difficult to interpret and to correlate the bars and the cytometry graphics….

Revision has been made according to the suggestions. (page 10, line 1-5) (in the part of "methods")

b. The reason of the above comments is that Looking at the cytometry results, the differences between B (NC) and C (MD) do not have the amplitude plotted on the graphic, as they are very similar. And the cytometry plots A (cont) and B (NC) are very different. Please confirm the data.

Because each group had three duplicate wells, so there were three images in each group. We randomly picked one image in each group before, so it appeared the phenomenon described in your suggestion. Now we reselected the images in each group and resubmitted the Figure 5.

(13) Conclusions.

a. Please italicize the designation of genes.

Revision has been made according to the suggestions.

b. I think that on the last paragraph the authors, writing "Wseten-blot" wanted to mean Northern-blot (technique used to study gene expression) and not Western-blot (technique used to study protein expression)…

Thank you for your suggestion. Revision has been made according to the suggestions.

(14) References: ref 7 is incomplete.

Revision has been made according to the suggestions.

Reviewer #2:

(1). The aim is to investigate the effect of miR-1 on biological behaviors of the hepatocellular carcinoma tumor endothelial cells， meanwhile， the hepatocellular carcinoma tumor endothelial cells is one factor of tumor microenvironment, so the author should introduce tumor microenvironment in the introduction part.

The contents about tumor microenvironment have been added in the part of introduce. (page 5, paragraph 2)

(2). Discussion: The first and second paragraphs mainly are just reviews and belong to the introduction part.

The first and second paragraphs in "discussion" have been reorganized according to the suggestion.

(3). A number of questions need to be addressed in order to elucidate whether the experimental data really support the conclusions. It is highly

recommended to carry out some experiments in vivo or co-culture TECs infected by Lentiviral MiR-1 shRNA and HCC in order to explore the biological behaviors effect of HCC.

The experiments in vivo are operating now, but because there is almost no reference about the experiments of TECs in vivo, we have many difficulties about the experiments. For example, plant TECs under the skin of Nude Mouse can't form a tumor, we need to plant the mixture of TECs and the HCC cells. But the proportion of the cells is unclear. What's more, the time of injection TECs is also unclear. All of these problems need us to explore. So in this manuscript, we only described some results about cell experiments.

(4). English has to be corrected in some instances where there are many grammar mistakes.

The manuscript has been submitted to AJE (American Journal Experts) for editing and the spelling and grammar errors has been corrected.

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EDITORIAL CERTIFICATE

This document certifies that the manuscript listed below was edited for proper English language, grammar, punctuation, spelling, and overall style by one or more of the highly qualified native English speaking editors at American Journal Experts.

Manuscript title:
The effects of microRNA-1 to the hepatocellular carcinoma tumor endothelial cells

Authors:
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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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