

January 15, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 14953-review.doc).

Title: Expression of circulating microRNA-20a and let-7a in esophageal squamous cell carcinoma

Author: Fu-Cheng He, Wei-Wei Meng, Yun-Hui Qu, Ming-Xia Zhou, Jing He, Pin Lv, Liang Ming

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 14953

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) Reply to reviewer 1

Comment : You should further expend the samples of ESCC patients to validate the specificity and sensitivity of the diagnostic value of miR-20a and let-7a in ESCC.

Reply: We fully agree with you and thank you for the suggestion. Our experiments were repeated independently at least three times, and the data were reliable and stable. Accurate sensitivity and specificity require a large sample survey, so it might be difficult to collect enough specimens and do this experiment in two weeks. We hope our study can be shared earlier.

(2)Reply to reviewer 2

Comment 1: Novelty. MicroRNA, especially miR-20a and Let-7a have been widely studied. In addition to screening modalities of these microRNAs detection, we suggested to add mechanism studies to support the upregulation of miR-20a on cancer progression or tumor suppression properties of Let-7a in esophageal cancer. It is not clear why siRNA knockdown or ectopic expression were not used in Western blot experiments which would have provided the more convincing experiments to show the involvement of miR-20a and Let-7a in esophageal squamous cell carcinoma.

Reply: We thank you for this suggestion. This experiment is part of our study. We are conducting research on the mechanisms at the cellular level.

Comment 2:Low sensitivity In the present study, miR-20a was reported to have 55% of sensitivity. Sensitivity indicates the ability of screening to detect cancer in pre-clinical

phase. Therefore, a candidate of screening modality should have high sensitivity.

Reply: We fully agree with you and thank you for the suggestion. The cut-off value has two points: when it was set at 4.65, the sensitivity and specificity were 55.7% and 87.5% respectively, while when it was set at 4.77, the sensitivity and specificity were 64.3% and 75.0% respectively. As suggested, we use another cut-off value.

Comment 3: Patient criteria Criteria for exclusion in this study should be mentioned. For example, the author didn't mention the treatment status of the patients that might affect the results.

Reply: We thank you for pointing out this problem. Patient criteria Criteria for exclusion in this study have been mentioned in the "Study population" of "MATERIALS AND METHODS" in this manuscript. All the patients were untreated prior to surgery, so the results may not be affected by treatment.

Comment 4: Blinded study The author should mention if the samples from patients and controls were coded and studied blindly to prevent bias.

Reply: We thank you for pointing out this problem. Blinded study has been mentioned in the "Study population" of "MATERIALS AND METHODS".

Comment 5: The study will be substantially strengthened if real-time PCR analysis could be performed to demonstrate the increased miR-20a and Let-7a transcripts in esophageal squamous tumor samples. Introduction The authors have clarified the background and significance of the study very clearly. However, there is still lack of supporting evidence regarding the mechanisms of miR-20a and Let-7a in cancer progression. Results Table 1 is well presented. It supports the hypothesis very clearly with statistical significance. However, the author should propose the threshold level to define miR-20a up-regulation and Let-7a down-regulation. The comparison of medians in table 2 shows insignificant difference. Please try to compare means of fold changes to see the differences between parameters. In addition, some grammatical errors are present in the manuscript. Please consider the grammars and make sure everything is correctly written. In addition, some incomplete sentences are still found (please refer to highlighted sentences in the manuscript).

Reply:

Thank you for your insightful suggestion, and the study on the mechanisms of miR-20a and Let-7a in cancer progression is in progress.

We fully understand your concern. The threshold level had been added in the "Statistical analysis" of "MATERIALS AND METHODS", and We set two fold as threshold to measure miR-20a and let-7a as up-regulated or down-regulated. Our team is doing research on the mechanisms of miR-20a and Let-7a currently.

We had re-written the manuscript and got the help of professional translation company.

(3)Reply to reviewer 3

Comment 1: I should retitle "Expresión of circulating microRNA-20a and let-7a in

esophageal squamous cell carcinoma"; it is more comprehensible.

Reply: We are very glad to take the reviewer's advice, and have retitled the manuscript.

Comment 2: The interpretation of your own results in Discussion is scarce: you could write something about the reproductibility of the laboratory tests, about the limitation of the comparison with healthy people instead of a control group, about the fact that-let-7a is decreased when tumor but is lower in advanced stages, about some aspects (clinic practice, treatment) in which your results could be useful in future.

Reply: We thank you for raising these issues, we have added the interpretation of the results in discussion and use "healthy people" instead of " control group". We are doing the studies on the mechanisms of miR-20a and Let-7a currently, and more interpretation will be stated in the future article.

Comment 3: Be careful with spaces in the writing.

Reply: We thank you for pointing out this problem. We have now reviewed and edited our manuscript with the aid of an English-speaking editor.

3 References and typesetting were corrected

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

Sincerely yours,

A handwritten signature in black ink that reads "Fu Cheng He". The signature is written in a cursive, flowing style. The first name "Fu" is written with a large, sweeping 'F'. The last name "He" is written with a large, sweeping 'H'. The middle name "Cheng" is written in a smaller, more compact script between the first and last names.

Fu-Cheng He, MD, PhD

Department of Medical Laboratory

the First Affiliated Hospital of Zhengzhou University

Zhengzhou 450052

China

Fax: +86-371-66913528

E-mail: hefucheng@126.com