

January 15, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 15334-review.doc).

**Title:** MicroRNA-124 (miR-124) inhibits Cell Proliferation, Metastasis and Invasion in Colorectal Cancer by Downregulating ROCK1

**Author:** Zuowu Xi, Shiyong Xin, Liqing Zhou, Haixin Yuan, Qian Wang, Kaixuan Chen

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 15334

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:** In their manuscript , MicroRNA-124 (miR-124) inhibits Cell Proliferation, Metastasis and invasion in Colorectal Cancer by Downregulating ROCK1”, Xi et al. aim to analyze the role of miR-124 in the pathogenesis of CRC.

1.The language is overall very poor with additionally a massive amount of small mistakes in typing, grammar and style. This manuscript has to be seen by an editing service at best and by a native speaker of English at the very least.

**Reply:** This manuscript has been edited by a native speaker of English according to the reviewer’s comments.

2.Please provide the number of the ethics committee approval.

**Reply:**Our study was approved by the Ethics Committee of Henan University of Traditional Chinese Medicine on Human Research (Approval number: H20130816).

3.what means “UVP software“?

**Reply:** UVP software is a software of Gel electrophoresis and analysis system for Ultrasensitive protein detection and imaging produced by American UVP company.

4.In the results part, the sentence “Similarly, there was a no difference in the level of ROCK1mRNA between the CRC tissues and the metastatic CRC cases compared to the normal tissues and non-metastatic CRC tissues( $P>0.05$ )(Fig. 1F, G and H).” is not understandable.

**Reply:**We have revised the sentence according to the reviewer’s comments.

5.Overall, the data of the authors would substantially be improved, when data of miR-124 and ROCK1 expression in CRC metastases would be provided. Similarly, basic molecular-characterization data for the tumors analyzed must be provided (i.e. which molecular subtype of CRC). Of course, this must also be correlated with the expression data of miRNA-124 and ROCK1).

**Reply:**We have added the data of miR-124 and ROCK1 expression in CRC metastases according to the reviewer’s comments.(Figure 1 and 2).

As the reviewer said, basic molecular-characterization data for the tumors analyzed and the correlation with the miRNA-124 and ROCK1 should be provided. However, due to the project design and budget restrictions in our present study, we mainly investigated the interactions between miR-124 and ROCK1 and the correlation between the miR-124 and the pathology in human Colorectal Cancer (CRC). And we will investigate the basic molecular-characterization data for the tumors analyzed and the correlation with the miRNA-124 and ROCK1 in our next study.

6. Fig. 1A shows a few examples of CRC cases – was this analysis performed for all 68 pairs? This would be a strong argument – please provide these data.

Reply: The Fig. 1A shows the representative picture of western-blot for the 68 pairs sample. The Figure 1B was the results of statistical analysis performed for all the 68 pairs samples.

7. Please provide real numbers for “age” in Table 1.

Reply: We are so sorry for our clerical error about the numbers for “age” in Table 1, and we have revised the table 1.

8. All functional data have been provided for HCT116 only – please add data for at least two cell lines with a different molecular type; i.e. chromosomally-instable and CpG-island methylator-phenotype (since HCT116 is a classical microsatellite-instable cell line).

Reply: We have added data for another cell line with a different molecular type according to the reviewer’s comments. (The result section of manuscript)

9. Reduce redundancies between the introduction part and the discussion.

Reply: We have reduced the redundancies in the introduction and discussion according to the reviewer’s comments.

10. What studies are the authors referring to in the discussion “...together with our correlative results in previous clinical studies on miR-124 and ROCK1...”?

Reply: In our past clinical work, we found that the CRC patients with high expression of ROCK1 and low expression of miR-124 always have a poor prognosis. These patients need a strict systemic therapeutic strategy after operation such as immunotherapy, angiogenesis inhibitor drugs, chemotherapy, and radiotherapy with a regular investigation to improve prognosis.

**Reviewer 2:** This manuscript is an interesting work about a potential linkage between mir124 and ROCK. Although the overall quality is good there are a number of comments.

1. In the title the authors state an inhibition of metastasis, but this is only investigated by clinical correlation which seems to be weak evidence.

Reply: We have revised the title according to the reviewer’s comments.

2. Experimental design: Which controls have been used for normalization?

Reply: In our study, U6 and GAPDH was used as endogenous control. We have revised the manuscript.

3. Were mimics and inhibitors commercially available; if yes indicate the provider, if not indicate the details of construction.

Reply: The mimics and inhibitors produced by Invitrogen was commercially available in our study, and we have added the name of provider in the manuscript.

4. In all “statistical histograms” the standard deviation appears to be extremely low. Just by comparing the variability of the shown western blots it is difficult to believe such very high reproducibility of the results. Are the technical replicates of the same preparations or independent replicates? How many replicated were done in all experiments?

Reply:As the reviewer said , the standard deviation in our statistical histograms were low.The possible reasons for the results : Firstly: some Outliers were canceled, when we performed the statistical work for the 68 pairs tissue samples; Secondly, the statistical results in our study were based on a limited number of cases , and require confirmation from the analysis of larger cohorts .

In our study, three technical replicates of the same preparations were done under the same experimental conditions in all experiments.

5. “GC cells” should be explained at first usage?

Reply:We have revised the “GC cells” to “Gastric Cancer (GC) cells”.

6.The stars in all figures for significance are very difficult to see and should be optimized.Figure 4 a is not readable and can be omitted;

Reply:We have revised the Figures.

7.A number of spelling mistakes needs to be corrected.

Reply:We have corrected the manuscript. and this manuscript has been edited by a native speaker of English according to the reviewer’s comments.

### **Reviewer 3:**

1.This interesting paper is very long, mainly due to repetitions in the Introduction (lines 15-20) and Discussion sections that could be avoided.

Reply: We have reduced the redundancies in the introduction and discussion according to the reviewer’s comments.

2.Introduction: explain GC and LN the first time they are mentioned Mat. & Met.: the characteristics of the patients from which the specimens were taken should be presented (number, sex, median age, n of colon and rectal cancers....)

Reply:We have revised the manuscript according to the reviewer’s comments and added the characteristics of the patients in the manuscript.

3.Results: Lymph nodes metastasis are not reported in the table, as is Duke’s stage. Moreover employing both TNM and Duke’s staging system could be confusing, it would be better to choose one and I would suggest the TNM. The Authors state that they investigated miRN-124 in 68 specimens but they not specify if the same specimens were used to evaluate ROCK1. Several part of the Results should be moved to Mat. & Met. in particular of the paragraph: Knockdown of miR-124 gene induce cell proliferation, and miR-124 inhibited metastasis , invasion and clonogenic survival of CRC cells.

Reply:We have revised the manuscript according to the reviewer’s comments. In our study, we investigated miRN-124 and ROCK1 in the same 68 specimens.

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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China.

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