ANSWERING REVIEWERS

March 5, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 5597-review.doc).

Title: Inhibition of Girdin enhances chemosensitivity of colorectal cancer cells to oxaliplatin

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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
For reviewer 1:
1, **Question**: Materials and methods, Protein extraction and Western blot analysis for Girdin protein quantification, the description of the quantification method should be more specific.
Indication the answer: We have now added the description of protein extraction and quantification method in the materials and methods.

2, **Question**: Materials and methods, Evaluation of chemosensitivity to oxaliplatin why and how the treatment dose was chosen is not clear. Do these doses comparable to the clinical treatment dose? A corresponding reference should be given.
Indication the answer: According to the reference, clinical recommendation of oxaliplatin usage is around 85mg/m2. Body surface area and blood amount of an adult is 1.9 m2 and 5000ml, respectively. Based on these, we calculated the concentration of oxaliplatin is around 30μg/ml. We setted various concentrations of oxalipaltin below this dosage for mimic of clinical usage. We have already added the reference into the manuscript.

3, **Question:** In results section. Again, the reasons why 10μM and 3μM oxaliplatin were chosen to investigate how Girdin and TOP2B influence the chemosensitivity of DLD1 cells is not clearly defined. Please add a sentence to explain why these dosages were chosen.
Indication the answer: As knockdown of Girdin only did not show obvious inhibition rate and 3μM oxaliplatin addition to DLD1 either showed no obvious inhibitory effect according to Figure 1. So we chosed 10μM to examine the effect of Girdin knock down on oxaliplatin sensitivity. Conversely, adriamycin showed strong inhibition effect on DLD1, so we chosed less concentration of oxaliplatin for combinational treatment. We added the description of these reasons in the manuscript.

4, Question: Due to the heterogeneity of tumor cell, author should screen more drug resistant colorectal cancer cell lines to verify their conclusions
Indication the answer: We have tested the effect of combinational treatment with oxaliplatin and

adriamycin in other three CRC cell lines. They also showed inhibition effect on them. We put these data on the supplementary data.

5, Question: Fig 3B showed that DLD1 cell viability decreased to 50-60% and 10-20% when treating with 9μM and 24μM adriamycin only, respectively. One point that should be addressed in this regard is: If adriamycin could be a useful colorectal cancer drug candidate? What's the effect on normal cells treated with this molecular?
Indication the answer: Adriamycin is recommended for gastric cancer by NCCN guide. And the effect of adriamycin on advanced colorectal cancer was also investigated in clinical trial. It could be a useful drug candidate for CRC. As there were no normal CRC lines, we did not examine adriamycin effect on them.

6, Question: Another issue is the effects Girdin knockdown and inhibition of TOP2B on normal cells? This should be discussed in discussion section.
Indication the answer: As there were no normal colorectal cancer cell lines, we could not test the effect of Girdin knockdown on them.

For review 2:
No questions

For review 3:
1, Question: Figure 2D needs a statistical analysis to check the difference between scramble shRNA and Girdin shRNA.
Indication the answer: We have now performed a statistical analysis to these data and already added these on manuscript.

2, Question: Near the end of the discussion, the authors stated that Girdin suppression and oxaliplatin had synergistic effect. This needs more rigorous tests such as isobologram by Chou and Talalay. However, the authors may want to simply state that oxaliplatin effect is enhanced by Girdin inhibition if data for such tests are not available.
Indication the answer: We are grateful to the reviewer's comments on this. The Chou and Talalay test is usually used to define synergistic effect of two drugs with a variety of concentrations. However, this may be not suitable for analysis of the combinational effect of gene inhibition and certain drug. Actually, oxaliplatin in combination with Girdin suppression resulted in increased cell death compared with addictive effect of individual agents. We have realized that the synergistic effect was not a rigorous description in the text. So we state that oxaliplatin effect is enhanced by Girdin inhibition instead of synergistic effect in discussion.

3, Question: In the Results section, there needs to be an introduction of TOP2B and its function before introducing its otherwise abrupt downregulation, though it was explained somehow in the Discussion.
Indication the answer: We have already added the description of TOP2B function in the results section.

4, Question: The graphs need more professional editing to be consistent, e.g. the gird line and its absence in Figure 3. Also the y axis lacks consistency in labeling. The first two paragraphs of Discussion may be more appropriately moved to Introduction with a more condensed form. The mid part of the

2nd paragraph, "…. Subsequent PI3K/AKT activation…" has a grammar problem. There are a few places that require polishing of language. For exemple, in the last paragraph of Introduction, " we firstly reported" could be better expressed as "we reported for the first time"

Indication the answer: We have improved the graph editing for consistency. Also we employed professional language editor to improve grammar problems.

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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