Author's response to reviews

Title: Cisplatin sensitivity is enhanced in non-small cell lung cancer cells by regulating epithelial-mesenchymal transition through inhibition of eukaryotic translation initiation factor 5A2

Authors:

Guodong Xu (guodongxu413@163.com)
Xinbao Shi (xinhaoshi212@126.com)
Lebo Sun (lebosun1216@163.com)
Qingyun Zhou (qingyunzhou123@sohu.com)
Dawei Zheng (dawei.zheng1018@yeah.net)
Huoshun Shi (huoshunshi@qq.com)
Ni Li (sara08188@sina.com)
guofeng shao (guofengshaolihulili@163.com)
xianning zhang (zhangxianning@zju.edu.cn)
hui yu (rosetty0319@hotmail.com)

Version: 2
Date: 1 July 2014

Author's response to reviews: see over
Dear Editor,

Thank you for your review of our manuscript (MS: 1307269684103314). We appreciate the concerns and suggestions provided by the reviewers, and have revised our manuscript accordingly. Our point-by-point responses are provided below, and text that has been added or modified from the original text is shown in the revised manuscript with the changes bold and underlined. At this time, we have re-submitted the revised manuscript through the Author Center. Upon review of our revised manuscript, we hope that you will find it acceptable for publication in *Pulmonary Medicine* and we look forward to your response.

Sincerely yours,

Guofeng Shao
Reviewers' Comments to Author:

Reviewer: 1

Title: Cisplatin sensitivity is enhanced in non-small cell lung cancer cells by regulating epithelial-mesenchymal transition through inhibition of eukaryotic translation initiation factor 5A2

Thanks a lot for reviewer’s carefully and patiently reading of our manuscript. And here are the answers for your suggestions on this manuscript in details. We will use bold and underline text to highlight all the changes to the manuscript.

1. The authors are providing data that GC7 is able to enhance the sensitivity of NSCLC cells to cisplatin. Furthermore, they have shown that GC7 treatment affects EMT in NSCLC cells. Based on additional experiments using siRNA against eIF5A-2 the authors claim that GC7 effects are due to a specific inhibition of eIF5A2. Since eIF5A-1 is expressed in these cells and probably inactivated by GC7 as well, authors have to show that the observed effects are really specifically induced by inactivation of eIF5A2 and are not, at least in part, based on parallel inactivation of eIF5A1. These can be performed by an isoform-specific siRNA mediated knockdown of eIF5A1. Without these additional experiments it is not possible to clearly separate between GC7 mediated eIF51- and eIF5A2-effects. Furthermore, data showing evidences for an inhibition of hypusine modification (e.g. 2-dimensional Western blot analysis) of eIF5A2 compared to eIF5A1 are missing. Those data could strengthen the conclusion that eIF5A2 is the key mediator for the GC7 mediated effects. So far, the statement: “Cisplatin sensitivity is enhanced in non-small cell lung cancer cells by regulating epithelial-mesenchymal transition through inhibition of eukaryotic translation initiation factor 5A2” is not entirely supported by the data presented in the current form of the manuscript.

Answer: We appreciate for your valuable comments. We added Western Blot analysis
and CCK-8 analysis, we also examined the protein expression of eIF5A-1 and eIF5A-2, and added the sensitivity of NSCLC cells to cisplatin by knockdown of eIF5A-1 siRNA (Figure S1). From the results, CCK-8 showed that when cisplatin treatment was combined with GC7 after eIF5A-1 siRNA transfection, there was little change in the cisplatin sensitivity of both cell lines. Western Blot indicated that eIF5A-1 was expressed in the control cells of both cell lines; however the expression of eIF5A-1 was higher in NCI-H1299 cells compared to A549 cells. Thank you for your comments again.

2. The authors have already nicely published a manuscript about “Down-regulation of eIF5A-2 prevents epithelial-mesenchymal transition in non-small-cell lung cancer cells” in 2013 (J Zhejiang UnivSci B. 2013 Jun;14(6):460-7. doi: 10.1631/jzus.B1200200). In that work, which is online accessible, the authors focused on just one non-small cell lung cancer cell line (A549 cells) and have already shown that a siRNA mediated knockdown of eIF5A2 affects different aspects of EMT in this particular cell line. Even if the new manuscript provides many new experiments (including a second cell line, GC7 and cisplatin treatment) it is mandatory that the authors refer to the former publication. Furthermore, the author should clearly discuss what is different between both publications and whether there might be overlaps between both manuscripts.

Answer: Thank you for your comments. In this study, we found that GC7 almost have the same effect of eIF5A-2 siRNA, but eIF5A-2 siRNA is more expensive than GC7. GC7 not only can be used for mass production, but also can be used to treat for disease.

Reviewer: 2

Title: Cisplatin sensitivity is enhanced in non-small cell lung cancer cells by regulating epithelial-mesenchymal transition through inhibition of eukaryotic
translation initiation factor 5A2

Thanks a lot for reviewer’s carefully and patiently reading of our manuscript. And here are the answers for your suggestions on this manuscript in details. We will use bold and underline text to highlight all the changes to the manuscript.

1) The authors should appropriately discuss the different sensitivity of A549 to the potentiation induced by both GC7 and eIF5A-2 knock down to the anti proliferative activity of cisplatin. It should be useful to assess the ratio between NCI-H1299 cells compared to A549 cells. The cell lines that they have used in their experiments.

Answer: Thank you for your comments. We assessed the expression of eIF5A-1 and eIF5A-2 in the cell lines. The results indicated that eIF5A-1 was expressed in the control cells of both cell lines; however the relative expression of eIF5A-1 and eIF5A-2 was higher in NCI-H1299 cell compared to A549 cell. The ratio of eIF5A-1/eIF5A-2 is 1.38 in NCI-H1299 cell and A549 cell is 3.44. (Figure S1 C)

2) The authors should evaluate the possible synergism between GC7 and cisplatin on the growth inhibition of NSCLC using an opportune software for the calculation of the synergism expressed as combination index. For methods please see and cite the following manuscript: Bruzzese F et al. Clin Cancer Res.2006 Jan 15;12(2):617-25.

Answer: Thanks for your suggestion. We used the software to calculate the synergism expressed as combination index (Figure S2). From the result, we know that combinations are synergistic when CIs were <1 in NCI-H1299 and A549 cells.

3) The authors should assess the effects of GC7 and cisplatin on the expression of the two isoforms of eIF5A in the NSCLC cell lines.
Answer: Thank you. We assessed the effects of GC7 and cisplatin on the expression of the two isoforms of eIF5A in the NSCLC cell lines. (Figure S1E). The result showed that GC7 could inhibit the expression of eIF5A-2.

4) The expression of eIF5A1 in the siRNA transfected cells should be also evaluated in order to be sure of the specific effect of down modulation.

Answer: Thank you for your comments. We evaluated the expression of eIF5A-1 in the siRNA transfected cell. The results showed that when cisplatin treatment was combined with GC7 after eIF5A-1 siRNA transfection, there was little change in the cisplatin sensitivity of both cell lines. Western Blot indicated that eIF5A-1 was expressed in the control cells of both cell lines; however the expression of eIF5A-1 was higher in NCI-H1299 cells compared to A549 cells. (Figure S1)

5) Figure 5D and E and Figure 6 C and D. The authors should show a quantization of the results as columns in an additional figure.

Answer: We appreciate your suggestion and we made a quantization of the results as columns in Figure 5D and E and Figure 6 C and D. In Figure 5D and E, wound healing results indicated that the migration capability is more weaker after induction by TGF-β1 and GC7, compared with TGF-β1 exposure (10 ng/ml) for 24 h \( (P<0.05) \), this suggesting that GC7 can decrease the migration capability of A549 cells when it exposure to TGF-β1 (Fig 5 D). The transwell-matrigel invasion results showed that more cells invaded through the matrigel after induction by TGF-β1, compared with cells without TGF-β1 stimulation \( (P<0.05) \) in 24h; and less cells invaded through the matrigel after induction by TGF-β1 and GC7, compared with cells without TGF-β1 and GC7 stimulation \( (P<0.05) \) in 6h and 24h (Fig 5 E). In Figure 6 C and D, using the wound healing assay, we found that, when transfected with eIF5A-2 siRNA or eIF5A-2 siRNA and GC7, migration capability in the NCI-H12999 cells was clearly more weaker than that observed in the negative siRNA group \( (P<0.05) \) (Fig 6 C). The
transwell-matrigel invasion assay showed that less cells invaded through the matrigel after induction by transfected with eIF5A-2 siRNA and GC7, compared with cells without the negative siRNA group ($P<0.05$) in 24h(Fig 6 D). Thank you for your comments again


Answer: Thanks for your suggestions. We added new contents in introduction. We used bold and underline text to highlight related contents to the manuscript.


Answer: Thanks for your comments. It is a good idea, we should consider the combined use of synergies and chemotherapy commonly used strategy is a combination of multiple drugs, but this study focused on GC7, we did not refer to the synergistic combination in our experiments. In future research, we will focus on the combination therapy use of drugs. Thank you for your suggestions again.

8) Finally, a revision of the English language style is required.

Answer: Thanks for your comments. We have revised the English language to make the article more accurate. And we indicated specific changes in the text. We used bold text to highlight modify contents to the manuscript.