Author's response to reviews

Title: Resveratrol suppresses epithelial-to-mesenchymal transition in colorectal cancer through TGF-beta1/Smads signaling pathway mediated Snail/E-cadherin expression

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Cover letter with responds

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Resveratrol suppresses epithelial-to-mesenchymal transition in colorectal cancer through TGF-beta1/Smads signaling pathway mediated Snail/E-cadherin expression
Qing Ji, Xuan Liu, Zhifen Han, Lihong Zhou, Hua Sui, Linlin Yan, HaiLi Jiang, Jianlin Ren, Jianfeng Cai and Qi Li
BMC Cancer (Section: Cell and molecular biology)

Dear Prof Ciro Isidoro,
Many thanks for your e-mail on 8th Jan 2015 regarding our submission entitled “Resveratrol suppresses epithelial-to-mesenchymal transition in colorectal cancer through TGF-beta1/Smads signaling pathway mediated Snail/E-cadherin expression”.

Editor's comments:
Dear Prof. Qi Li,
Thank you for submitting your manuscript entitled "Resveratrol suppresses epithelial-to-mesenchymal transition in colorectal cancer through TGF-beta1/Smads signaling pathway mediated Snail/E-cadherin expression". Three experts have now reviewed your manuscript, and their comments are presented below. The reviewers indicate that your paper could be of considerable interest to the field, but that it needs substantial revision before further consideration. Therefore, I urge you to carefully consider each of the comments and address them to the fullest extent possible, as a paper with only minor revisions is not likely to do well in a subsequent round of review. I wish to draw your attention to the criticisms n. 1 and 2 from Dr Chen and n. 2 from Dr Gao. Please, consider to have your manuscript copyedited for English grammar by a professional editor.
Answer:
Attached is a revised version of this manuscript (R1) incorporating all modifications and supplemental experiments as suggested by the reviewers, with the following point-by-point response to each of the reviewers’ constructive suggestions and comments. All changes have been highlighted with blue background in the text of the manuscript. In addition, we have carefully revised the whole manuscript by critical proofreading, and corrected all the spellings and grammatical errors by Elektra McDermott (Developmental Editor, Springer).

Referee 1:
Reviewer's report:
In this report, the authors demonstrated that resveratrol, an effective component often found in grape skin and some traditional Chinese medicinal herbs, can inhibit the cell growth and the migratory/invasive capability of CRC LoVo cells. More interestingly, Resveratrol repressed the lung and liver metastasis of these cells in a tail vein metastasis model as well as an orthotopic CRC tumor model. They further explored the mechanism underlying these effects of resveratrol, and revealed that, it attenuated the TGF-beta1-Smad2/3-Snail-E-cadherin signaling and blocked the EMT process in the CRC cells. This paper presented some novel evidence delineating the anti-tumor activity and associated mechanism of resveratrol, that indeed warrants its publication. However, some issues need to be addressed before being accepted for publication.
Major compulsory revisions:
1. This paper needs a professional language editing. There are a lot of grammar errors and misworded sentences, especially in the Result section.

Answer:
We have carefully revised the whole manuscript by critical proofreading, and corrected all the grammar errors and misworded sentences, under the guidance of Elektra McDermott (Developmental Editor, Springer).

2. For Figure 3A, the difference for the morphology between control and TGF-beta1
treated cells is rather minimal. So to better present the data, the authors need to show a higher-powered pictures of these cells to substantiate their conclusion about more EMT appearance in the TGF-beta1 treated cells. They may use the arrows to indicate the significantly different cells.

**Answer:**

We have presented higher-powered pictures of these cells to substantiate our conclusion about more EMT appearance in the TGF-beta1 treated cells, and the arrows were used to indicate the significantly different cells.

3. It is not clear how the authors prepared the lung metastasis samples. They need to detail the protocol in the Method and Material section, e.g. how thick the lung sections were, how many areas/lung section are observed and counted for metastasis spots, etc.

**Answer:**

We have supplemented the detailed protocol in the Method and Material section about how thick the lung sections were and how many areas/lung sections are observed and counted for metastasis spots. In detail: Both of the lung organs were excised, fixed with 10% neutral buffered formalin, and paraffin-embedded. The lung sections were fully cut, and each section was set to 6 µm. All the lung sections were stained with hematoxylin-eosin (HE), following by counting the number of lung metastases, and assessing comprehensively the extent of metastasis.

4. The content of Figure legends needs to be corrected and many description of how the experiments were done should belong to the Method section, e.g. Figure2 A. legend. The authors should consult a professional biomedical writer to revise these places of the paper.

**Answer:**

We have corrected the content of Figure legends and attributed many description of how the experiments were done to the Method section.
5. Some figures that showed pictures of the tissue sections ought to be added indicating arrow markers, to better direct readers’ attention, e.g. Figures 1C and 2A.

Answer:
We have added indicating arrow markers to show the part of metastatic tissues which would help better directing readers’ attention.

Referee 2:
Reviewer's report:
It is an interesting paper about the effect of resveratrol on CRC. The author described the promising therapeutic effect of Resveratrol in vivo and in vitro. Based on previous study, canonic Wnt pathway and EMT is the author's focus on effect of Resveratrol. Multiple molecular biological technique were used to test the hypothesis including luciferase assay and mutagenes is. However, there are some minor points required to be clearer:

1. LoVo cell line is the representative cell. The author should explain the reason why they choose the cell line instead of SW480, HCT 116, and others. More cell lines should be applied to test the paper.

Answer:
LoVo cell line from ATCC is a representative cell with high metastatic ability. In our previous research, two cell lines including LoVo and HCT116 were both applied, which could be supported by our published articles, e.g. Ji et al., PlosOne, 2013 and Ji et al., Br J Cancer, 2013. In the course of our experiment, we found resveratrol of low concentration could suppress EMT in colorectal cancer LoVo cells. However, in HCT116 cells the phenomenon of TGFβ induced EMT was not as clear as in LoVo cells. Therefore, in the following EMT study, we focused on the LoVo cells, and we have just got the preliminary conclusion that resveratrol suppresses EMT through TGF-beta1/Smads signaling pathway mediated Snail/E-cadherin expression. In our future research, we would choose more than one cell line to verify our finding.

[1] Qing Ji, Xuan Liu, Xiaolin Fu, Long Zhang, Hua Sui, Lihong Zhou, Yan Wang, Jian Sun, Jianfeng Cai, Huirong Zhu, Jianmin Qin, Jianlin Ren, and Qi Li. Resveratrol Inhibits invasion and


2. As we know, lacz plasmid is more useful in observing the specific metastasis of body than GFP. In vivo test, the author should explain why GFP plasmid is applied instead of lacz plasmid.

**Answer:**

In our experiment, GFP plasmid was applied for in vivo imaging to dynamically observe the change of the metastases in the lung section of the mice.

3. The dosage and intragastric way of Resveratrol. The author should explain why they choose once administration, after one or two weeks after the construction of model instead of constant administration.

**Answer:**

Our dosage and intragastric way of resveratrol were performed according to Majumdar et al (2009), in which resveratrol of 150 mg/kg body weight was administered by gavage every day for 3 weeks. We have left out the detailed intragastric way of resveratrol, and have supplemented in the Materials and Methods section.


4. From Fig 3a, it is difficult to distinguish the morphological shape of cells after Resveratrol treatment. Please provide more representative figures.

**Answer:**

We have presented more representative figures.
5. For Fig 7, Snail should repress the expression of E-cadherin, there may be a mistake. Please correct it.

**Answer:**

We have corrected the mistake in Fig 7.

**Referee 3:**

Reviewer's report:

The authors present a detailed report on the proposed working of resveratrol on inhibiting EMT in colorectal cancer LoVo cells both in vitro and in vivo. However the manuscript needs to be revised before it can be published in the journal.

**Major Concerns:**

1. Resveratrol has been previously demonstrated to inhibit the PI3K/Akt signaling pathway. Having said that the Akt, Wnt and TGF beta pathways all contribute towards the development of EMT in cancer cells. The authors present a work focused only on the TGF beta pathway without exploring the fact that resveratrol has been established to inhibit the PI3K/Akt signaling pathway. It is good to rule out the possibility that the effects seen here are not due to the primary inhibition of resveratrol of the PI3K/Akt pathway. Or at least discuss this in the discussion.

**Answer:**

According to the reports, resveratrol could affect many signaling pathway including Akt, Wnt and TGF beta pathways in the process of anti-tumor or inhibiting the EMT. Here, we presented a work focusing only on the classical TGFβ/Smad pathway. In this paper, TGFβ1 was used to induce EMT *in vitro*, which could directly act on the receptor of TGFβ, thereby leading to downstream activation of Smad signaling pathway that correlated closely with EMT. Although we could not rule out the possibility that resveratrol has inhibiting effect on PI3K/Akt signaling, our provided results showed clearly the inhibiting effect of resveratrol on TGFβ/Smad mediated EMT. More research in future would be performed to understand completely the
effect mechanism of resveratrol on EMT and anti-tumor.


2. Smad 2 has been reported to protect the cells from undergoing EMT (Hoot et al., 2008), however the authors provide results demonstrating that Smad2 promotes EMT. Therefore, it is important to provide reasoning towards these results obtained in the discussion.

**Answer:**

Although Smad 2 has been reported to protect the cells from undergoing EMT (Hoot et al., 2008), in which *keratinocyte-specific* Smad2 ablation results in increased epithelial-mesenchymal transition during skin cancer formation and progression. However, from most of the other reports, it demonstrated that Smad 2 could promote the EMT process, especially from the reports by Geng et al, Pang et al, Sengupta et al, Islam et al, Jingushi, and so on.


Minor Points:
1. Page 6, Line 143, the authors should clearly described the tumor size instead of using “an enough size”.

Answer:
We have clearly described the tumor size with “100 mm$^3$” instead of using “an enough size”.

2. Page 7, Line 171, the authors should avoid to start a sentence using digital number “5”.

Answer:
To avoid starting a sentence using digital number “5”, we have adjust the sequence of the sentence with “LoVo cells ($5 \times 10^5$, in F12K medium with 0.5% FBS)”.

3. Page 11, Line 274, it is not suitable to use “dose-dependent manner” for the in vitro study. It should be better to use “concentration-dependent manner”.

Answer:
We have changed “dose-dependent manner” to “concentration-dependent manner” in
vitro study.

Please do not hesitate to contact me, should you have any further questions.

With the best regards,

Sincerely,

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