Author's response to reviews

Title: Overexpressed E2F1 in human gastric carcinoma is associated with anti-cancer drug resistance

Authors:

    Lin Hai Yan (yanlinhai000@163.com)

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

Thank you very much for editorial Team is willing to publish our manuscript in *BMC Cancer*. We appreciate very much the Reviewers’ valuable comments, and we have reviewed the comments by the reviewers, found them to be very clear and insightful, and have revised the manuscript accordingly. We detail our point-by-point responses in the following pages. We hope that our revised manuscript will be accepted by the *BMC Cancer* editorial team. If you still have any questions to our responses, don’t hesitate to inform us, and we will answer them as soon as possible. **The revised parts are highlighted in red font in the revised manuscript.** Please find enclosed the edited manuscript containing an electronic copy of the full-text manuscript in Word format (file name: *MS: 6375790271134334-Review-2.doc*).

**Title:** Overexpressed E2F1 in human gastric carcinoma is associated with anti-cancer drug resistance

**Author:** Lin-Hai Yan, Wei-Yuan Wei, Yu-Bo Xie and Qiang Xiao

**Manuscript No:** MS: 6375790271134334

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 (Johann Rotheneder)
The comments of the reviewer 1 are extremely valuable.

**Comments (1):** The surprising finding in the original manuscript that overexpression of E2F1 results in lower rather than in higher p73 (Tap73) levels has been revised now. The new Figure 3A shows the expected upregulation of p73 by E2F1. But the new data have not found their way into the discussion and conclusion sections: In the “Discussion” section (page 12, lane 395) it is stated: In our result, E2F1-overexpression lentiviral vector induced ZEB1 and ZEB2 expression up-regulation and down-regulation of TAp73 and GAX. In the “Conclusion” section (page 13 lane 404) it is stated: We conclude that up-regulation of E2F1 promotes the development of multidrug resistant in gastric carcinoma via inhibition of apoptosis related gene expression (TAp73 and GAX).

**This must be corrected.**

**Responses (1):** The reviewer is right that we were sloppy on our way of presentation which causes confusion to the readers, we revised the description in the discussion and conclusion sections according to Johann Rotheneder’s opinions. If you still think some content should be added, don’t hesitate to inform us, and we will add them as soon as possible. (See line 409-410, 420-422)

**Comments (2):** The revised finding that E2F1 overexpression does stimulate p73 contradicts the finding that E2F1 decreased the percentage of apoptotic cells. This must be discussed.

**Responses (2):** The reviewer is absolutely correct, according to Johann Rotheneder’s suggestion, we revised the discussion section, we explain E2F1 overexpression does stimulate p73 contradicts the finding that E2F1 decreased the percentage of apoptotic cells, the apoptotic of SGC7901/DDP cells may through p53/p73-independent pathways. According to the above
Methyl methanesulfonate (MMS) has been shown to induce apoptosis in various cell types through p53/p73-dependent pathways. However, pharmacological and genetic blockade of p53/p73 functions still results in similar or delayed sensitivity to MMS treatment, suggesting the presence of p53/p73-independent apoptotic mechanisms [21]. This may explain the finding that overexpression of E2F1 decreased the percentage of apoptotic cells, thus apoptosis of SGC7901/DDP cells may occur through p53/p73-independent pathways.' If you still think some content should be added, don't hesitate to inform us, and we will add them as soon as possible. (See line 393-400)

Comments (3): To better understand the function of E2F1, we performed a yeast two-hybrid screen using E2F1 as the bait and identified MRP as a MRP-interacting protein. I assume this should mean: MRP as an E2F1-interacting protein.

Responses (3): The reviewer is absolutely correct, in our study, E2F1 and MRP maybe associate with each other directly, it also mean: MRP as an E2F1-interacting protein. According to the above comments, we added the following statement in the revision: MRP as an E2F1-interacting protein. If you still think some content should be added, don't hesitate to inform us, and we will add them as soon as possible. (See line 331)

Reviewer: 2 (Doron Ginsberg)

The comments of the reviewer 2 are extremely valuable.

Comments (1): The authors did not test the effect of E2F1 overexpression in additional gastric carcinoma-derived cell lines, as I requested, so the paper is still based mainly on data from a single cell line, SGC7901/DDP. Nevertheless, some of my concerned regarding the original version were addressed in the
revised version and therefore I think the paper can now be published in BMC Cancer.

Responses (1): The reviewer is absolutely correct and we thanks for reviewer’s comments. In this study, we demonstrated that up-regulation of E2F1 significantly inhibited sensitivity of SGC7901/DDP cells to anticancer drugs, and decreased the percentage of apoptotic cells. Up-regulation of E2F1 potentiated S phase arrest of the cell cycle. Furthermore, it significantly decreased intracellular accumulation of doxorubicin. We conclude that up-regulation of E2F1 promotes the development of multidrug resistant in gastric carcinoma via inhibition of GAX gene expression, and increases the expression of MDR1, MRP, and TAp73. Finally, our observations suggest that E2F1 might serve as a molecular target for the therapy of MDR in gastric carcinoma indeed, given the role of E2F1 in MDR of gastric carcinoma. It is possible to envision that targeting this gene might treat gastric carcinoma. To better understand the E2F1-dependent mechanisms that are engaged during the development of multidrug resistant in gastric carcinoma, we will established E2F1-induced multidrug resistant models using more cell lines in our next study.

Reviewer: Editor’s Request

We recommend that you copyedit the paper to improve the style of written English. If this is not possible, you may need to use a professional language editing service. For authors who wish to have the language in their manuscript edited by a native-English speaker with scientific expertise, BioMed Central recommends Edanz (www.edanzediting.com/bmc1).

Responses: We feel so sorry about our poor English writing and thanks for reviewer’s suggestion. We have submitted our manuscript to professional English language editing company (Edanz Company) to English proof-read our
manuscript. Please see the editing certificate which are from English language editing company. It can verify that our final manuscript has been English proof-read by that company.

3. References and typesetting were corrected

Thank you again for considering our manuscript.

Sincerely yours,

Lin-Hai Yan, MD, PhD
Departments of Gastrointestinal Surgery
Tumor Hospital
Guangxi Medical University
Nanning
Guangxi Province 530021
Fax: 0086-771-5358325
E-mail: yanlinhai000@163.com