Author's response to reviews

Title: Resveratrol protects leukemic cells against cytotoxicity induced by proteasome inhibitors via induction of FOXO1 and p27Kip1

Authors:

Xiao-Fang Niu (niuxiaofang521@126.com)
Bao-Qin Liu (baoqinliu@163.com)
Zhen-Xian Du (dzx_doctor@hotmail.com)
Hai-Yan Zhang (zhy_doctor@hotmail.com)
Yan-Yan Gao (angela_042214@163.com)
Ning Li (lining@hotmail.com)
Chao Li (lichao@hotmail.com)
Hua-Qin Wang (wanghq_doctor@hotmail.com)

Version: 2 Date: 5 December 2010

Author's response to reviews: see over
Dear Editor

Thank you for your comments concerning our manuscript. I am returning herewith the revised manuscript.

We have studied the comments carefully and have incorporated the majority of the suggestions made by the reviewers in our revised manuscript, with the necessary corrections requested by them. We feel that we have dealt adequately with the comments raised by the reviewers.

We greatly appreciate all of your help concerning improvements of our paper. We believe the manuscript revised according to the good suggestions of the reviewers has been improved satisfactorily. We hope you would be satisfactory with the revised manuscript and hope it will be acceptable for publication in BMC Cancer.

We seek your kind consideration of our revised manuscript. Thank you very much.

Hua-Qin Wang
China Medical University
Department of Biochemistry & Molecular Biology
Key Laboratory of Cell Biology, Ministry of Public Health
Key Laboratory of Medical Cell Biology, Ministry of Education,

Responses to the editor

Editorial comments:
Please include a 'Competing interests' section between the Conclusions and Authors' contributions. If there are none to declare, please write 'The authors declare that they have no competing interests'.
As pointed out, we added it in the revised version (underlined).

Please include an Authors' contributions section before the Acknowledgements and Reference list.
As indicated, we included it in the revised version (underlined).

Responses to the reviewers point by point
Reviewer: Professor Alessandra Mocali
Major Compulsory Revisions:
1. In the first paragraph of Results authors say that “resveratrol alone caused no obvious apoptosis on K562 cells within 24h when applied even at concentrations of 100 µM (Figure 1D), indicating that although it was sufficient to inhibit proliferation, resveratrol per se was not sufficient to induce apoptosis in K562 cells within 24h.” Other authors (Chakraborty et al., Cancer Sci, June 2008, vol. 99, no. 6, 1109–1116) reported that resveratrol induces apoptosis in K562 cells. They used 40 µM resv for 24, 48 and 72h. I think that it would be better to repeat experiments at least after 48h, otherwise authors should explain why they performed experiments only for 24h. This citation would be also added.

As suggested, we have cited the indicated reference in the revised version (underlined). We don’t think our data are contradictory with Chakraborty’s results. In their study, they used 40 µM resveratrol for 24, 48 and 72h, and found that only about 16% apoptotic cells after treated for 48 and 72h (note: they did not present the data for 24h, about 4% apoptotic cells were observed in 0h treatment. Furthermore, there was no difference between 48h and 72h), the net apoptotic cells were 12% after 48h and 72h treatment. In our study, about 11% apoptotic cells were observed after 24h exposure of resveratrol (Figure 1D), while 5% apoptotic cells were observed after treatment with vehicle, the net apoptotic cells were about 5-6% after 24h treatment. We think that although longer exposure of resveratrol may cause some more apoptotic cells, the difference might not be very marked. As the referee suggested, addition of results after 48h may be better, while investigation only after 24h in cytotoxicity model is widely acceptable. In the current study, we think the data after 24h is permissible and enough to support our conclusion, therefore we would like your permission.

2. Data show that resveratrol acts as antagonist against MG132 through the blockage of cell cycle progression at the G1/S transition and thus preventing the cell from proliferation. However, this anti-proliferative effect might be positive for cancer cell treatment with proteasome inhibitors, should it correlate with cell commitment to differentiation. This hypothesis would request some discussion and/or experimental evidence by the authors (e.g. the expression of differentiation markers). Lacking in vivo experimental evidences and also for above reported reasons, expressions as “attenuation of antitumoral action” by resveratrol should be replaced with “attenuation of apoptotic effect of proteasome inhibitors”.

2
As suggested, we added some discussion in the Discussion Section in the revised version (underlined).

As corrected, we altered the expression of “attenuation of antitumoral action” to “attenuation of apoptotic effect of proteasome inhibitors” in the revised version (underlined).

Minor Essential Revisions:
1. Some more recent citations on the usage of proteasome inhibitors in cancer therapy would be added and discussed.

As suggested, we added some recent citations and made some discussion in the revised version (underlined).

2. There are some citations without the year of publication.

As corrected, we added the year of publication in the revised version.

3. All the text requires some editing, in particular:

Abstract:
-Line 2, “the anticancer actions of several other cancer drugs” should be: ….some conventional chemotherapy drugs
- Conclusions section of the Abstract is too emphasized, while not sufficiently supported by experimental data.

Methods: Last sentence “all experiments were repeated three times, and data were expressed as the mean ± SD from a representative experiment” contains a mistake.

As corrected, we altered the expression in the revised version (underlined).

Reviewer: Professor Andreas Gescher

Major Compulsory Revisions:
1. Intro - The authors do not give a robust hypothesis which was tested here and which would rationalize why the work was carried out in the first place. Why was resveratrol combined with MG at all? Was it a serendipitous observation?

Since accumulating data suggested that resveratrol could sensitize various cancer cells to some other conventional chemotherapy drugs and radiotherapy, we tried to investigate whether resveratrol could sensitize leukemia cells to proteasome inhibitors and performed the current study. In the revised version, we added some introduction in the Introduction Section (underlined).
2. Agent concentrations - MG132: the conc of 5 microM used needs robust justification. Can this value be achieved in the human (or animal) plasma? Resveratrol: The effect of resveratrol commences at 2microM and is optimal at 10-20microM. The authors need to place this observation in the appropriate context of the concentration of resveratrol achievable in the human biophase. In a recent clinical trial of resveratrol heroic doses of 2.5 to 5g gave peak plasma levels of just 3-4 microM. This finding suggests that more reasonable doses (up to 1g) of resveratrol furnish levels which are clearly insufficient to adversely affect proteasome inhibitor action. This issue needs to be discussed because it ameliorates the potential clinical impact of the findings.

In the current study, we focused on in vitro study, in cell model, as high as 20µM MG132 and 100µM resveratrol is generally permissible. As MG132, we found that 5µM MG132 resulted in about 50% of cell death, so we used this concentration for the further investigation. As the referee correctly pointed out, the concentration of MG132 or resveratrol achieved in the human plasma should be considered when the conclusion from cell model extrapolates to in vivo phenomena. The microenvironment in vivo is quite different from that in vitro, possibly the same achievable concentration in vivo might have different effect as those in vitro. Referring to plasma level of resveratrol, it was reported that in human, about 70% of orally administrated resveratrol (25mg) is absorbed with a peak plasma level of ~2µM and a half-life of ~10h (Walle T et al. Drug Metab Dispos. 2004; 32:1377-1382). As the referee suggested, we added some discussion in the revised version (underlined).

Minor Essential Revisions
1. Fig legends: How many independent experiments and repeats within experiments were performed?
All experiments were repeated three times and three independent samples within experiments were performed. We added relative description in the Figure legends in the revised version.

2. The sentence in the intro that " resvera...is highly enriched in a variety of food sources, such as grapes, peanuts and red wine...." is incorrect. What does "highly enriched" mean? State correct content and translate into human intake whihc of course is minute.

In the skin of grapes, the concentration of resveratrol is 50-100µg per gram (Goswami SK and Das DK. Cancer Lett. 2009; 48:713-723). As a bioactive molecule, resveratrol is generally reported to be abundant in grapes. As the referee
pointed out, we deleted “highly” in the revised version.
3. The discussion needs focussing on the issues in hand.
As suggested, we changed some discussion in the revised version (underlined).