Author's response to reviews

Title: Bufalin, a component in Chansu, effects anti-proliferation and anti-invasion for hepatocellular carcinoma

Authors:

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Author's response to reviews: see over
Dear Tom Rowles,

It's my great honor to hear about our manuscript has now been peer reviewed. After reading the comments accessible in PDF format and your e-mail, I find great illumination in them. After all modification proposals in PDF format have been corrected according to the reviewers’ comments for one-to-one correspondence, and we also use the professional language editing service Edanz (www.edanzediting.com/bmc1) which BioMed Central Editorial recommends. And then I send this e-mail. I hope that my modified file is suitable for publication in BMC Complementary and Alternative Medicine. I’m looking forward to hearing you soon. If there are any problems or suggestions about my manuscript, you can contact me at any moment.

With best wishes,

Yun-Ke Yang

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The paper has shown interesting observation and have demonstrated the utility of bufalin in hepatocellular carcinoma. This work is good and it may have potential to acceptance contingent upon few suggested modifications.

Comments:
1. Author should mention about the available treatment and other phytochemicals, chemopreventive agents worked out invitro and invivo.
   Response: Thanks for your suggestion. We add the sentence in page 4 line 9-14: 5-Fluorouracil and mitomycin-C, widely used chemotherapeutic drugs, have limited overall effects in the treatment of HCC owing to resistance. Nowadays, patients with advanced HCC are treated with a comprehensive series of vascular interventional therapy, but their median life spans are not obviously prolonged [4]. Chinese traditional medicine may change the status quo, and the herbal compound Songyou Yin may improve the efficacy of chemotherapy in HCC [5].

2. It looks from the statement from introduction that “patients with advanced HCC have median survival… no effective treatment for these patients “ the present study doesn’t ensure any effective treatment? Authors are advise to modify the sentence/restrict with certain statement i.e to identify any bioactive components that can be effectively suppress HCC cell growth in-vitro.
   Response: Thanks for your suggestion. We modify the sentence in page 2 line 2-3: Hepatocellular carcinoma (HCC) is a common and aggressive cancer, and the treatment options are limited for patients with advanced HCC. And in page 4 line 7-8: Patients with advanced HCC have a median survival of about 6-8 months, and there are limited effects in the treatment for these patients [3]. And then draw out the subject in page 4 line 9-14: 5-Fluorouracil and mitomycin-C, widely used
chemotherapeutic drugs, have limited overall effects in the treatment of HCC owing to resistance. Nowadays, patients with advanced HCC are treated with a comprehensive series of vascular interventional therapy, but their median life spans are not obviously prolonged [4]. Chinese traditional medicine may change the status quo, and the herbal compound Songyou Yin may improve the efficacy of chemotherapy in HCC [5].

3. Our results revealed that bufalin significantly decreased the “invasion” potentials of HCCLM3-write “invasive” potential.

Response: Thanks for your suggestion. We modify the sentence in page 10 line 16-18: Our results revealed that bufalin significantly decreased the invasive potential of HCCLM3 and HepG2 in a dose-dependent manner (Figure 5).

4. This study was conducted in to two different phases;
1) Effect of bufalin- decrease cell proliferation, cell invasiveness, cell adhesion

The phenotypic observation ie cell proliferation, cell invasiveness, cell adhesion with respect to bufalin treatment was not corroborated with expression of certain mentioned molecules which were expected to play role.

Response: Thanks for your suggestion. We increase the sentence of discussion in page 13 line 17-20: A current literature review shows the importance of cross-talk between the PI3K/AKT and β-catenin pathways as a therapeutic target in treatment of malignant tumors [16].

5. The cells in which the bufalin was treated, the reduction of proliferation and invasiveness was observed, in the same treated cells they have to conduct the molecular players such as AKT’s role. Rather author have represented separately.

Response: Thanks for your suggestion. We change the description of our results in page 15 line 16-page 16 line3: In brief, our results suggest that AKT is the main regulatory protein of the AKT/GSK3β-β-catenin/E-cadherin signaling pathway, which regulates the expression and phosphorylation of GSK3 β and the nuclear translocation of β-catenin. β-Catenin functions as a negative regulation of the downstream molecule E-cadherin, which modifies the expression of downstream target proteins MMP2, MMP9. This signaling pathway activation and protein regulation are related to hepatoma cell proliferation, migration, invasion and adhesion. Thus, bufalin exhibits multiple anti-tumor effects on hepatoma cells because
the mechanisms underlying bufalin action appears to mediate AKT/GSK3β/β-catenin/E-cadherin signaling and affect regulation of protein expression.

6. Author has to clearly state akt inhibition by bufalin actually decrease proliferation, invasiveness through E cadherin over expression etc. This needs a simple reformatting the paper with observed phenotype and representing the molecular reasoning.
Response: Thanks for your suggestion. We modify our discussion in page 15 line 7-12: These results confirm the previous observations that overexpression of E-cadherin in human prostate cancer and mouse skin cancer reduced tumor cell invasiveness by decreasing MMP-2 and MMP-9 expression levels [17] [18]. Therefore, upregulation of E-cadherin and a concomitant reduction in MMP-2/MMP-9 might negatively regulate cell proliferation, cell invasiveness, cell adhesion of HCCLM3 and HepG2.

7. Under the head –Effect of Buffalin in GS3Kβ
There is no enough assay to say this statement “These results confirm that bufalin inhibited the nuclear translocation of β-catenin via the PI3K/Akt/GSK3β signaling pathway”
Response: Thanks for your suggestion. The expression of “Effects of bufalin on GSK3β and β-catenin expression and β-catenin nuclear translocation in hepatoma cells” is not very exact, so we modify the sentence in page 12 line 5-6 : These results confirm that bufalin inhibited Wnt signaling by decreasing the nuclear translocation of β-catenin.

8. Fig 15/slide 15
GSK3β expression blots are not convincing, please redo it and send a convincing result. In case of pGDK3β the blots are not having any changes at different time points, at 12 hours (HCCLM3) it has shown reduction and at 24 hours it looks normal. This particular slide, whole experiments may be advised to redo and send convincing results.
Response: Thanks for your suggestion. We redo GSK3β and pGSK3β blots, our new results can be found in Figure 8.
9. Fig 21 slide MMP expressions may be repeated.
Fig 22 slide The MMP2, MMP9 expressions were analysed upon treatment with Bufalin and LY. The expression pattern or blotting of MMPs are not convincing, it is suggested to re-do it and send a convincing results of the same.

Response: Thanks for your suggestion. We redo our Western blot analysis to MMP9 and MMP2. Our new results can be found in Figure 9.
10. Author may say few evidential supports of differences in treatment response between the two cell lines and in discussion more emphasis may be given to argue their own results.

**Response:** Thanks for your suggestion. We add the sentence in discussion in page 15 line 13-15: **Similar trends were observed in both of these hepatoma cell lines in various experimental processes, which further verified the importance of AKT/GSK3β/β-catenin/E-cadherin signaling in HCC.**

11. Needs some language corrections before being published.

**Response:** Thanks for your suggestion. Our manuscript has been re-edited by a professional language editing service Edanz (www.edanzediting.com/bmc1).
Reviewer’s report
Title: Bufalin, an effective anti-proliferation and anti-invasion component in Chansu for hepatocellular carcinoma
Version: 1 Date: 24 March 2013
Reviewer: Bin Lu

Reviewer’s report:
In this paper, Qiu and coworkers present data to elucidate the inhibitory effect of bufalin, a major component of the traditional Chinese medicine Chansu, on the cell proliferation, migration and invasion of HCC cells. Furthermore, the authors found that bufalin inhibited the nuclear translocation of β-catenin via the PI3K/Akt/GSK3β signaling pathway, and regulated the expression of metastasis-related gene E-cadherin, MMP-2 and MMP-9.

Overall, this work is interesting and well-performed, which will be useful for readers to understand the effect of bufalin on tumors including HCC. I think that the manuscript may be suitable for publication in BMC.

Discretionary Revisions:
The ordinate title should be indicated in the figures (3C, 4D, 4C, 4D, 5C, 5D, 6C,6D).
Response: Thanks for reviewer suggestion. We have increased the ordinate title of each Figure.