Author’s response to reviews

Title: 2-Ethoxystypandrone, A Novel Small-Molecule STAT3 Signaling Inhibitor from Polygonum Cuspidatum, Inhibits Cell Growth and Induces Apoptosis of HCC cells and HCC Cancer Stem Cells

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Author’s response to reviews:

Cover Letter

Dear Editor,

We are grateful for your consideration of our manuscript. We also very much appreciate Reviewers for their careful reading of the revised manuscript and for their constructive comments that help us further improve the quality of our manuscript.

We have made corrections according to reviewers’ suggestions. The changes have been marked in red in the text and our detailed responses to the specific comments are described as following. We hope that all these revisions to our manuscript will facilitate the decision to publish this study in BMC Complementary and Alternative Medicine.

We look forward to your decision.
Yours sincerely,

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Revisions

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Editor Comments:

Please attend to the remaining concerns raised by the reviewers.
Answer: We thank Editor and Reviewers for their careful reading of our revised manuscript and for their constructive suggestions that help us further improve the manuscript. We have made revisions according to their suggestions. Please see the changes marked in red in the manuscript.

Reviewers’ comments and Responses:

Reviewer 1:

1. Please show that basal phosphorylation levels of STAT3 are the same between HepG2 and HepG2/STAT3 cells.

Answer: We don’t think the requested data are relevant to our manuscript. It will not affect our conclusion.

2. Please cite appropriate references showing that STAT3 phosphorylation induces apoptosis and/or inhibition of cell proliferation. Wang et al (Cancer Res, 2009) did not show it. They only showed phenomena of STAT3 dephosphorylation and anti-apoptotic effects.

Answer: Phosphorylation of STAT3 does not induce apoptosis. The paper we cited was to show that inhibition of STAT3 phosphorylation induced cell apoptosis (Wang Y. et al., Cancer Res, 2009, 69 (18):7302-7310.), which is consistent with our submitted manuscript.

3. Please explain why the time lag occurred. STAT3 phosphorylation was inhibited within 2 h, but apoptosis was induced after 24 h. Is it usual? If so, please cite appropriate references.

Answer: The apoptosis occurred shortly after the inhibition of STAT3 phosphorylation. We could detect the PARP cleavage as early as at 4 hours. The reason for us to show cell apoptosis at 24 hours was because the PARP cleavage and cell apoptosis was much more significant at 24 hours (Please see the reference: Yu X, et al., Eriocalyxin B Inhibits STAT3 Signaling by Covalently Targeting STAT3 and Blocking Phosphorylation and Activation of STAT3. PLoS ONE 2015,10(5): e0128406.). This data again is not relevant to our manuscript.
Reviewer 2:

"Background" section:
1. please check "......HCC cells (IC50 =3.69 ± 0.51 μM–20.36 ± 2.90 μM)...

"Flow Cytometric Analysis" section:
1. please check the font at "then analyzed by flow cytometry (BDCanto ï ,USA)..

Answer: Authors thank Reviewer for his careful reading and for his helpful suggestions. We have made corrections according to reviewer’s comments, please see page 5 line 18 in Background Section and page 14 lines 4,5, 13 in Flow Cytometric Analysis Section.