Author's response to reviews

Title: An evaluation of the anti-angiogenic effect of the Korean medicinal formula "Sa-mi-yeon-geon-tang" in vitro and in ovo

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Author's response to reviews: see over
Jan 22th, 2015

Dear Editor of *BMC Complementary & Alternative Medicine*.

We are now submitting the 1st round revised version of original manuscript (Ms. No. 8189004911504545) entitled, “An evaluation of the anti-angiogenic effect of the Korean medicinal formula “Sa-mi-yeon-geon-tang” in vitro and in ovo” authored by Yi, Bang, and myself. We are also providing point-by-point replies to the reviewers’ comments. We tried to answer all issues and expect our revised manuscript to be accepted for publication in the “*BMC Complementary & Alternative Medicine*” as a “Research Article”. The manuscript was English-edited by American Journal Experts, Editorial Certificate Verification Key 4E1F-F04B-028D-6A5C-9FDD.

We really appreciate your time for reviewing our revised manuscript.

Best regards,

No Soo Kim, Ph.D.
Replies to Reviewer #1’s comments:

**Comment 1.** What is the substantiation i.e. reference/s for the statement on page 3, line 22 that natural products are effective in cancer prevention?

**Reply to Comment 1.** The authors added the following three references in the revised manuscript to support the statement (Page 3, Line 23).

Liu SH, Cheng YC: Old formula, new Rx: the journey of PHY906 as cancer adjuvant therapy, *J Ethnopharmacol* 2012, **140**:614-623 (Ref#6 in the revised manuscript).


Wang XL, Ma F, Wu XZ: Anticancer effects of 5-fluorouracil combined with warming and relieving cold phlegm formula on human breast cancer, *Chin J Integr Med* 2012, **18**:599-604 (Ref#8 in the revised manuscript).

**Comment 2.** Where does the reader find the previous work quoted on page 4, line 14 i.e. reference please.

**Reply to Comment 2.** “Our previous work” means “our preliminary in vitro screening study” and the results were not published in the scientific journal. To make it clear, the authors rephrased “In our previous in vitro activity screening” to “In our preliminary in vitro activity screening” in the revised manuscript (Page 4, Line 14).

**Comment 3.** What does the term clumps on page 4, line 6 mean … clumps of what?

**Reply to Comment 3.** The term “clumps” on page 4, line 6 means hard mass or lumps. The authors changed the term “clumps” to “the abnormal hard mass” in the revised manuscript (Page 4, Line 6).
Comment 4. Along with the assays for cell viability described on page 5, line 14 and cell mobility on page 6, line 17 onwards, please describe your method of reading cell proliferation.

Reply to Comment 4. The authors inserted the method for determining cell proliferation and viability in the “Methods” section (Page 5, Line 15-Page 6, Line 7).

Comment 5. What is/are the vehicle/s on page 5, line 22; page 7, line 5; page 7, line 16

Reply to Comment 5. SMYGT was dissolved in PBS for in vitro assays and therefore, the authors used PBS as a vehicle for a negative control. The authors defined the vehicle in the revised manuscript (Page 6, Line 13; Page 7, Line 19; Page 8, Line 8).

Comment 6. Please attribute the MetaMorph image analysis software the first time it is mentioned on page 6, line 3 and delete the attribution from page 6, line 23

Reply to Comment 6. As suggested by the reviewer #1, the authors revised the manuscript (Page 6, Line 17; Page 7, Line 14).

Comment 7. Randomly selected fields on page 7, lines 8 and 21 need more description i.e. magnification, area included in observation, were orientation markers like grid lines used, how many fields per well, etc.

Reply to Comment 7. As suggested by the reviewer #1, the authors added the information of randomly selected fields in the revised manuscript (Page 7, Lines 23-Page 8, Line 1; Page 8, Line 14).

Comment 8. The dose titration studies ought to be described in Methods and the reader ought to not first learn first about this body of work in the Results, page 9, line 18

Reply to Comment 8. As suggested by reviewer #1, the tested dose range of SMYGT was described in the “Method” section of the revised manuscript (Page 6, Line 12).
Comment 9. The use of a positive control inhibiting neovascularisation, sulforaphane, ought to be described in Methods and not introduced in the Results section page 9, line 18 or the legend of Figure 1, page 19, line 15

Reply to Comment 9. As suggested by Referee #1, the authors described sulforaphane as a positive control in the “Methods” of the revised manuscript (Page 6, Line 14; Page 7, Line 12 and Line 19-20; Page 8, Line 10).

Comment 10. Were there negative controls in the CAM assay as well as positive – page 6, line 12?

Reply to Comment 10. As commented by reviewer #1, the authors described the information of negative control in the “Methods” section of the revised manuscript (Page 7, Line 1-5).

Comment 11. What evidence is there for the statement that abnormal embryo development was not observed on page 10, line 4? How many embryos were observed? What species?

Reply to Comment 11. We used “abnormal embryo development” for “death of embryos” after drug treatment. To make it clear the authors changed to “dead embryo” in the revised manuscript (Page 10, Line 18).

Comment 12. Wording on page 11, lines 13 to 14 imprecise enough to confuse. Although SMYGT did inhibit MMP2 activity at each time point, it did so by comparison with vehicle control at each time point. Comparison of SMGT inhibition at different time points does not appear to be time dependent; or rather inhibition seems to be less at later time points. I cannot tell if protein product was generated in identical experimental conditions for each time point.

Reply to Comment 12. As commented by reviewer #1, the MMP2 activity was increased as time goes by because the MMP2 were secreted from the cells and were accumulated in the culture medium. However, the accumulated MMP2 were significantly decreased by SMYGT treatment when compared to vehicle treatment at each time point. The slight difference of
MMP2 activities between vehicle and SMYGT treatments were due to the fact that the MMP2 activity at 24 h was saturated in the zymogram activity gel assay.

**Replies to Reviewer #2’s comments:**

**Comment 1.** Minor Essential Revisions: In page 11, line 3, "As shown in Figure 2C, SMYGT potently inhibits ....", word "inhibits" should be revised "inhibited".

**Reply to Comment 1.** As suggested by Referee #2, the authors have changed “inhibits” to “inhibited” in the revised manuscript (Page 11, Line 18).

**Replies to Reviewer #3’s comments:**

**Comment 1.** The authors present convincing evidence on the anti-angiogenic effect of the Koran medicinal formula "Sa-mi-yeon-geon-tang". However, the inhibitory effect on pFAK does not seem to have a clear dose-response.

**Reply to Comment 1.** The authors replicated the western blot analysis of FAK phosphorylation and replaced the image of pFAK with new version in Figure 3(A) right panel of the revised manuscript to show a clear dose-response of HUVEC to SMYGT.