Reviewer's report

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

Version: 2 Date: 6 January 2015

Reviewer: Helen Healy

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Yi et al describe how a traditional Oriental compound, SMYGT, of four medicinal herbs – Sargassum, Laminariae Thallus, Prunella Spica and Ostreae Concha – changes the angiogenic biology of endothelial cells in vitro, down regulating new vessel formation. They use sophisticated scientific methods and cell and molecular readouts to discover mechanisms that may explain the efficacy of SMYGT.

They report new vessel formation is stunted in length with fewer branching without discernable affect on endothelial cell viability, proliferation and mobility. Endothelial cells treated with SMYGT do not adhere to neighbouring cells nor invade or migrate across cell culture inserts as well as untreated cells. The former may be because of inhibition of focal adhesion kinase, pivotal in the cell adhesion signalling pathway. The latter phenomenon may be attributed to the lower activity of matrix metalloproteinase 2, a pro-angiogenic protein, due at least in part to lower MMP2 mRNA detected in the endothelial cells.

The Authors ought to attend to the following prior to publication:

Minor essential revisions in Background:
1. What is the substantiation ie reference/s for the statement on page 3, line 22 that natural products are effective in cancer prevention?
2. Where does the reader find the previous work quoted on page 4, line 14 ie reference please

Discretionary revisions in Background
1. What does the term clumps on page 4, line 6 mean … clumps of what?

Minor essential revisions in Methods:
1. 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
2. What is/are the vehicle/s on page 5, line 22; page 7, line 5; page 7, line 16
3. 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
4. Randomly selected fields on page 7, lines 8 and 21 need more description ie magnification, area included in observation, were orientation markers like grid
lines used, how many fields per well, etc

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

6. 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

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

Minor essential revisions in Results:
8. What evidence is there for the statement that abnormal embryo development was not observed on page 10, line 4? How many embryo were observed? What species?

Discretionary revisions in Results:
9. 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.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests