Author’s response to reviews

Title: Abalone visceral extracts inhibit tumor growth and metastasis by modulating Cox-2 levels and CD8+ T cell activity

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Author’s response to reviews: see over
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The BioMed Central Editorial Team

Re: MS-9978807323816845

Dear Editor,

Thank you for your help in reviewing the above manuscript, “Abalone visceral extract inhibit tumor growth and metastasis by modulating Cox-2 levels and CD8+ T cell activity” by Choong-Gu Lee et al., for the BMC Complementary and Alternative Medicine. We are very grateful for the inputs from the reviewers and happy that their comments are positive overall and quite helpful to further investigate our work.

We have revised our manuscript to address the comments raised by the reviewers. This revised manuscript includes modified texts and a point-by-point response to reviewers’ comments is attached. We hope that revised manuscript is now suitable for publication in the BMC Complementary and Alternative Medicine.

Sincerely yours,
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A point-by-point response to reviewers’ comments

Reviewer: Bernd L Fiebich

1) Major Compulsory Revisions

One of the key conclusion by the authors is, that due to the decrease of COX-2 by abalone, tumor growth and metastasis is reduced as well as down-stream parameters from COX-2 such as VEGF etc. However, the authors did not show any data to prove this conclusion.

a) A COX-2 inhibitors needs to be included in the tumor growth experiments to demonstrated, that COX-2 and prostaglandins are involved in tumor growth and metastasis.

Response: We appreciate the comment. In this study, we used 4T1 mammary adenocarcinoma cells for tumor implantation. A study performed by Connolly EM et al, has already demonstrated that treatment of either non-selective Cox inhibitor or selective Cox-2 inhibitor significantly reduced primary tumor growth as well as inhibition of metastasis by 4T1 breast cancer cells (Br J Cancer 2002, 87(2):231-237). In addition, knockdown of Cox-2 levels directly reduced level of PGE\(_2\) synthesis and inhibited cell growth in 4T1 cells (Carcinogenesis 2008, 29(11):2227-2235). These studies suggest that reduction of Cox-2 expression could decrease PGE\(_2\) expression especially in 4T1 breast cancer cells that were used in our study as well. Based on these previous findings, we inferred that either functional inhibition or reduction of Cox-2 expression by abalone extract could suppress growth and metastasis of 4T1 mammary carcinoma cells. Thus, in Result and Discussion part, we addressed those issues in the revised manuscript accordingly.

b) The read out parameter of COX-2 are prostaglandins. Thus, if COX-2 plays any crucial role in tumor growth, this will be mediated by prostaglandins and their respective receptors EP1-4. The authors show some reduction of COX-2 protein, which does not necessarily has to correspond with reduced prostaglandin release, since there is COX-1 present in most cells and there is also still enough COX-2 protein to produce prostaglandins. The important control experiment is missing showing the effect of abalone extract on prostaglandin levels. Moreover, COX-1 should be involved in the Western blot.
Response: We appreciate the comment. We showed that treatment of abalone extract reduced Cox-2 expression level both in primary tumor and metastasized lung. A study reported by Neil JR et al, has demonstrated that stable expression of Cox-2 shRNA in 4T1 cells consequently diminished synthesis of PGE$_2$ (Carcinogenesis 2008, 29(11):2227-2235). Even though we did not measure the prostaglandin level upon abalone extract treatment, our data are in accordance with the aforementioned report. Thus, we modified the manuscript to address this issue in Discussion part accordingly.

Regarding the issue of Cox1 and Cox-2 in tumor growth, expression of Cox-1 is constitutive in most tissues (malignant and nonmalignant tissue as well), while Cox-2 expression is upregulated mainly in 4T1 tumors (Int J Cancer 2006, 118(9):2220-2231). Mitsuhashi M et al also reported that selective Cox-2 inhibition significantly reduced PGE2 synthesis in 4T1 tumors (J Leukoc Biol 2004, 76(2):322-332). Based on the previously reports, we mainly examined the effect of abalone extract on Cox-2 levels as a target of controlling tumor growth and metastasis in vivo in 4T1 breast carcinoma cells.

c) The authors claim that VEGF, FGF and MMP-13 are COX-2 associated molecules and down-stream factors from COX-2. This is questionable and should be removed. These molecules might be regulated by prostaglandins but not by COX-2 itself!!

Response: We appreciate the valuable comment. By following the suggestion we deleted sentences and modified the manuscript accordingly in Discussion and Conclusion parts.

d) Related to point c), the authors do not show any evidence, that VEGF, FGF and MMP-13 are dependent on prostaglandin synthesis and release and therefore indirectly on levels of COX-2. The authors have to include an experiment showing the effects of COX-2 inhibitors on those factors before drawing such a conclusion. It is more likely, that abalone interferes with signal transduction cascades common in the expression of COX-2 and VEGF, FGF and MMP-13 and are most likely not linked to each other.

Response: We appreciate the insights and comments. Previous report already showed that treatment of Cox-2 inhibitor decreased the levels of VEGF, FGF and MMP families (Pharmacol Rep 2010, 62(2):233-244). Based on this report we did not perform COX-2 inhibitors experiments. However, as pointed out by the reviewer, it is more acceptable
that abalone visceral extract may affect the upstream-signaling events of Cox-2 and VEGF, FGF and MMP-13, which mediated inhibition of tumor growth and metastasis. By following the reviewer’s suggestion, we modified the manuscript accordingly in Result and Discussion part.

2) Minor Essential Revisions
The title and in most part of the manuscript, the authors state that they have tested the effects of abalone extracts. Looking on the data, it seems like that there was only one type extract used in all experiments and not biochemical different ones.

Response: We appreciate the comment. Indeed, we used one kind of the extract. By following reviewer’s comment, we replaced “abalone extracts” with “abalone extract”.

Reviewer: valsala haridas

Major Compulsory Revisions: 
1. The authors have shown a decrease in the levels of Cox-2, but an increase in TNF-alpha in Abalone extract treated mice. Since TNF-alpha is a known inducer of Cox-2, and thereby inflammation, the authors should provide an explanation for this in the discussion.

Response: TNF-alpha and IFN-gamma are the two major effector molecules of cytotoxic CD8 T cells (Nat Rev Cancer 2004, 4(1):11-22). We showed that administration of abalone visceral extract increased proliferation of CD8 T cells and expression levels of effector molecules such as TNF-alpha and IFN-gamma and granzyme molecules (Fig. 5). However, increased effector function of CD8+ T cells may be not related with the cox-2 expression in cancer. By following reviewer’s comment we include the additional explanation in the Discussion part.

2. The authors should describe the treatment of animals with the Abalone extracts in greater detail. How were the animals given the extracts (in water or with the chow), and how often were they fed? Was body weight of the animals taken into account while giving the 5mg/dose

Response: We appreciate reviewer’s comment. We included detailed feeding protocol in the Methods part of revised manuscript.
Minor essential Revisions:
1. In the methods, Proliferation assay, line 3, please change "cultured under none or stimulation" to "cultured in the presence or absence of stimulation".

Response: We made the above corrections according to the reviewer’s comments.

Discretionary revisions:
1. In describing the JAM test, the authors need to specify if the radioactivity was measured in the supernatant or the effector cell pellet.

Response: We made corrections by following reviewer’s comment.

References


