Author's response to reviews

Title: Frankincense oil derived from Boswellia carteri induces tumor cell specific cytotoxicity

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Author's response to reviews: see over
Re: MS 9378358372323491
Frankincense oil derived from Boswellia carteri induces tumor cell specific cytotoxicity Mark B Frank et al.

Dear Dr. Puebla:

Please find attached an electronic revised version of the above referenced manuscript for your consideration. We were pleased to receive the initial review which was quite positive. We have now responded to these concerns in the revision. We respond to these concerns in the order they appear.

Reviewer 1: Katarina Hostanska

1. We fully agree with the reviewer that an effect of only one bladder cancer cell line is not enough to postulate specificity of frankincense oil. We plan to expand the study by evaluating multiple types of human bladder cancer cell lines of varied aggressiveness and stage to investigate the impact of frankincense oil on tumor cell cytotoxicity. We had the following sentence included in the Discussion "Future studies are required to determine whether frankincense oil has similar effects on other bladder cancer cell lines of varying severity such as RT4, T24, and 5637, followed by in vivo studies using bladder cancer animal models."

2. We re-evaluated the data obtained from microplate reader, but could not explain why SEM seen in UROtsa cells is larger than J82 cells. However, we noticed that in some experiments, frankincense oil treated UROtsa cells had elevated absorbance using XTT assay. We will pay more attention on this issue in the future.

3. Statistical analysis has been performed to compare differential responses between J82 and UROtsa cells using ANOVA followed by post hoc Dunnett's test as suggested by the reviewer.

4. IC$_{50}$ values (the 50% inhibitory concentrations of frankincense oil) for J82 and UROtsa cells were 1:600 and 1:1,250, respectively. This information has been included in the revised manuscript.

5. We agree with the reviewer that there was suppression in viable cell following 1:600 frankincense oil treatment. The lack of statistical significance may due to high SEM values. Trypan blue exclusion demonstrated that 1:600 dilution of frankincense oil has significant cytotoxicity effects (Figure 2B). We have change the statement to make them consistent.

6. Fetal bovine serum (FBS) was not a component in UROtsa cell culture medium, whereas 10% FBS was supplemented in the medium for J82 cells. In the discussion, we suggested that medium components may not be a factor to provide UROtsa cell resistant to frankincense oil treatment.
7. Second method for the detection of apoptosis using TUNEL analysis (as suggested by reviewer 2) has been included in the revised manuscript, instead of annexinV/PI stain suggested by the reviewer.

8. The title and abstract, as well as the results and conclusion were maintained, since inconsistent messages were provided by reviewers 1 and 3.

Reviewer 2: Yongkui Jing

9. We totally agree with the reviewer that it is important to identify active component in frankincense oil. Contents of frankincense oil have been determined for this batch by GC/MS performed by an independent laboratory. These components will be compared between different batches of frankincense oil in the future; and tumor-cell specific cytotoxicity from different batches of frankincense oil will be compared. Relative significance of each component, based on its variation present in different batches, will be estimated after several batches of frankincense oil are evaluated for their cancer cell cytotoxicity.

10. The viability of non-adherent cells in the medium together with adherence cells have been determined determined by trypan blue exclusion as suggested by the reviewer. The results has been included present in Figure 2B.

11. TUNEL assay has been used to determine frankincense oil-induced cell death in J82 cells. The result has been included in the revised manuscript (Figure 4A).

Reviewer 3: Monica Liebert

12. We agree the reviewer that the timing of evaluation is critical in DNA fragmentation analysis. Although we still think that apoptosis occurs following frankincense oil treatment in J82 cells based on TUNEL analysis (Figure 4A), the language for DNA fragmentation is softened in the revised manuscript. Roles of ATG2 and ATG5 in frankincense oil-induced cell death in bladder cancer cells will be further investigated in the future to understand the potential interaction and/or classification of frankincense oil with other currently used agents.

13. We have planned to test frankincense oil-induced cytotoxicity in multiple bladder cancer cell lines as addressed in 1. Additionally, Dr. Xue-Ru Wu at New York University School of Medicine agreed to provide transgenic mouse bladder tumor models to confirm the tumor-specific cytotoxicity in animal models.

14. A brief list of the Gene Ontogeny (GO) classification has been included in the text (Table 3). The complete list of gene table from the GO analysis was retained in Supplemental Table 3.

Microarray data have been deposited in Gene Expression Omnibus (GEO) with accession number GSE14002. The accession number has also been provided in the revised manuscript.
For tracking changes made in the revised manuscript, we submitted a separate file with all changes marked in RED including additions and deletions (double strikethrough). Texts in the original submission are in BLACK.

We truly hope that our revisions are acceptable for publication in *BMC Complementary and Alternative Medicine*.

Sincerely yours,

HK Lin, Ph.D.