**Author’s response to reviews**

**Title:** Phoyunnanin E inhibits migration of non-small cell lung cancer cells via suppression of epithelial-to-mesenchymal transition and integrin αv and integrin β3

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**Version:** 1  **Date:** 20 Nov 2017

**Author’s response to reviews:**

Dear Editor;

I am enclosing a revised manuscript entitled “Phoyunnanin E inhibits migration of non-small cell lung cancer cells via suppression of epithelial-to-mesenchymal transition and integrin αv and integrin β3” for evaluation. I am grateful for all valuable comments and advices that editor and reviewers have given and carefully amended the manuscript accordingly. All revised contents in the manuscript were labeled in red color. I am pleased to further revise the manuscript if the need has arisen.

Sincerely yours,

Pithi Chanvorachote, Ph.D.

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Reviewers' comments:

Reviewer:

• Mohamed Abdel-Daim, Ph.D. (Reviewer 1): The manuscript should be revised for typographical linguistic errors.

Response: Thank you for your concerning comments. We have revised for typographical linguistic errors as recommend. The certificate of English editing is attached in supplement file.

• Saleemulla Khan (Reviewer 2): Dear authors It is a good work keep the good work going. Please give real data in abstract. Otherwise it is a good work Congratulations

Response: Thank you for your suggestion. We have given real data in abstract as recommend.

• Yang Fan Yang (Reviewer 3): Dear editor, thank you for the opportunity to reviewing this manuscript. The physiological process that allows cells to change their morphology, lose their polarity, and become motile is termed the epithelial-mesenchymal transition (EMT). In recent years, studies related to drugs that ablate the EMT in cancer cells metastasis have been popular. Extract from whole plant Dendrobium venustum exhibited significant antimalarial and antiherpetic activities. The present experiment firstly reveals that the phoyunnanin E, a compound isolated from Dendrobium venustum, possesses antimigration activities of non-small cell lung cancer via suppression of epithelial-mesenchymal transition and integrin αv and integrin β3. H460 cells were treated with various concentrations of phoyunnanin E to examine its effect on the viability by the MTT assay. The concentrations which showed least effect on the viability of H460 cells were used to evaluate the metastatic potentials in anchorage-independent condition, migratory activity via wound healing assay and the expression of epithelial and mesenchymal markers by western blotting. Furthermore, the phoyunnanin E treated H460 cells exhibited significant lower level of expression of integrin αv, α5 and β3, p-FAK and downstream signaling pathway (p-AKT, Rac1, Cdc42 and RhoA) from western blotting.

In general, the topic is novel and the paper is well structured. Still there are some flaws about process of argumentation.

1. To further verify the anti-metastasis effect in lung cancer of phoyunnanin E, animal models of lung cancer should be established and tumor inhibited experiment in vivo should be conducted.
Response: Thank you very much for this valuable comment. The reviewer is quite right about the additional experiment would benefit the quality of this paper.

We would like to emphasize more that this work has focused on the novel properties and the underlying mechanisms of the compound for anti-motility and EMT suppression. We have found that the compound exhibited a good effect in inhibition of migration of the cell by suppression of migratory up-stream pathway likes FAK/AKT through the decrease of specific integrins. Furthermore, we provide the supportive data that such a compound could suppress EMT process of lung cancer cells. In providing such information, we do think that the discovered novel information could be sufficient at this stage and due to our focus on molecular mechanisms. To add more, in vivo, may be beneficial, but the results may depend on so many undefined factors that could dilute the present study. As we do agree that additional assay would benefit the quality of the work, we therefore performed invasion assay and added the results to the revised manuscript.

2. The concentrations of phoyunnanin E which showed least effect on the viability of H460 cells were examined by the MTT assay, which lie between 0-20μM according to the results. However, the concentrations of phoyunnanin E for other human lung cancer H292 and A549 cells and human normal keratinocytes lack data supporting.

Response: Thank you, we added the information as recommend. We showed the results of the viability of the others human lung cancer H292 and A549 and human normal keratinocytes which showed in Figure 3.

3. To confirm the effect of phoyunnanin E on the expression of integrin αv, α5 and β3, p-FAK and downstream signaling pathway, further experiments, for example gene expression, and rescue experiment are required. Above all, I suggest a major revision before the manuscript is considered acceptable for publication. Best regards,

Yangfan Yang

Response: Thank you for your valuable comment. We do quite certain that the gene expression of named proteins is not quite required here. As the function is the direct results of protein action, however, the gene expression in certain cases can be omitted or inhibited by several means likes RNAinterferance and etc. We have linked the present of integrin proteins as well as
the direct down-stream activation level of FAK and AKT. We believe that the reviewer may know that the integrins \( \alpha v, \alpha 5 \) and \( \beta 3 \) are the surface receptor that the direct downstream target is FAK and AKT. We have reported already in figure 7. According to the well-known mechanism of integrin in cell migration refer from:


Therefore, other explanations may out of the scope. Likewise, p-FAK mediates cells motility through the activation of the downstream Akt signaling pathway. These data can confirm that phoyunnanin E has an effect on cell migration via suppression of integrin \( \alpha v \) and \( \beta 3 \).

- Raghavendra L Hallur, Ph.D (Reviewer 4): The paper may accepted with following corrections

1. One para description about D. venustum shall be included in the introduction part

Response: Thank you for your concerning comments. We descripted about D. venustum as recommend.

2. Some of the methods don't have any citation and proper citations shall be included
Response: Thank you, we added the citation as recommend.

3. Figures are not clear and author shall include minimum 300-600 dpi figures

Response: Thank you, we adjusted the dpi figures as recommend.