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Manuscript ID: molecules-2048632

Manuscript Title: A natural CHI3L1-targeting compound ebractenoid F inhibits lung cancer cell growth and migration and induce apoptosis by blocking of CHI3L1/AKT signals

Dear Editor,

Thank you for your letter dated on Nov, 28, 2022. We thank you for allowing us to submit revised manuscript, and we also thank the referees for the constructive and positive comments on our manuscript entitled, **"A natural CHI3L1-targeting compound ebractenoid F inhibits lung cancer cell growth and migration and induce apoptosis by blocking of CHI3L1/AKT signals".** We have performed several additional experiments and made several other modifications for the manuscript to address the editor and reviewer's comments/suggestions.

To assist in evaluating changes made to our manuscript, portions that are revised have been and marked up using the red fonts. Below are our point-by-point responses to the referee's comments (**Red**).

It would be grateful for us that this revised manuscript would be reviewed and accepted for consideration of publication in the "**Molecules**".

Looking forward to hearing from you.

Sincerely Yours,

Jin Tae Hong, Ph.D.

College of Pharmacy,

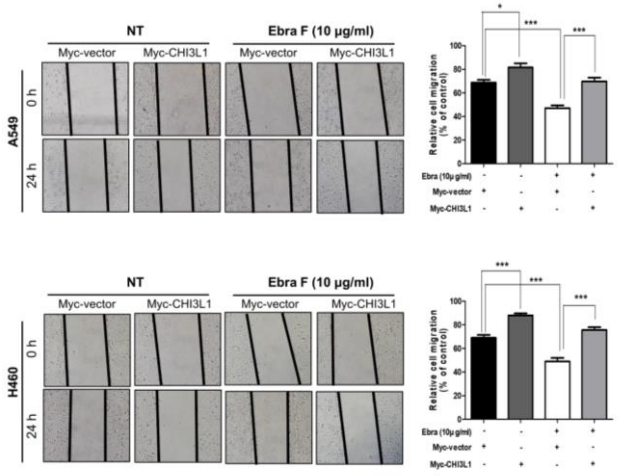
Chungbuk National University

South Korea

Response to Reviewer 3 Comments

Point 1: All the data on the combination effects, such as those presented in Figure 6, 7, 8, does not provide any evidence on the underlying mechanism as interpreted by the authors. For example, in Figure 6, the fact that "The combined treatment of CHI3L1 siRNA and ebractenoid F more significantly reduced the expression of migration proteins compared to the treatment of ebractenoid F or CHI3L1 siRNA alone", can neither confirm nor reject the hypothesis that the effect of ebractenoid F on cell migration is mediated by CHI3L1. Moreover, the fact that adding CHI3L1 siRNA can further inhibit cell migration, as shown in the manuscript, strongly suggests that CHI3L1 is not the only mediator of ebractenoid F's effect on cell migration. To gain mechanistic insight, I suggest the authors perform rescue assay, instead of combination assay, to pinpoint the regulatory cascade.

Response 1: Thank you for your suggestion and this is very important comment. There was not enough time, so cell migration assay was performed. Please understand. In rescue experiments, A549 and H460 cells were treated with ebractenoid F and then transfected with the myc-CHI3L1 plasmid or control plasmid (myc-vector). As a result, cell migration inhibited by ebractenoid F treatment could be recovered by myc-CHI3L1 transfection. The results suggest that ebractenoid F-induced migration inhibition may be rescued by CHI3L1 overexpression. This result was reflected in the manuscript (supplementary figure)



Point 2: The images for wound healing assays presented in Figure 2A, 2B and 6C are not very convincing. The proportion of migrated cells does not seem to differ much based on the current image. Please consider replacing with more representative images. Also, for Figure 2A and 2B, why not show images from 20 ug/ml treatment?

Response 2: Thank you for your comment. The results of Figures 2A and 2B were replaced with experimental results of 20 μg/ml. We also changed the results of the wound healing assay to make them look clearer.

Point 3: Section 2.5 has the wrong title, this section has nothing to do with CHI3L1, it is all about apoptosis.

Response 3: Thank you for pointing out. We made a mistake while editing a manuscript. The 2.5 title has been modified to Ebractenoid F induced apoptotic cell death of lung cancer cells.

Point 4: Section 2.3 and 2.6 have the same title.

Response 4: Thank you for pointing out. We made a mistake while editing a manuscript. The 2.6 title has been modified to Ebractenoid F interacts with CHI3L1.