Author's response to reviews

Title: Butein inhibit metastatic behavior in mouse melanoma cells through VEGF expression and translation-dependent signaling pathway regulation

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Author's response to reviews: see over
Dear Editor:

We sincerely appreciate your giving us many constructive comments on our manuscript. We carefully revised our manuscript according to the raised issues and also performed some complementary experiments. The following explanation may meet the concerns raised. Our Ms has been edited for English language, grammar, punctuation, and spelling by Enago, the editing brand of Crimson Interactive Pvt. Ltd.

Reviewer 1:

Q: Given that B16F10 cell do not resemble the mutation pattern found in human melanomas and poorly resemble the pathway activation found in clinical melanomas, could the authors comment on that?

Respond: Thanks for your reminding. The following sentence was added in page 18 line 2-13 [According to the Flaherty et al. study, approximately 50% of melanomas harbor activating BRAF mutations. Human melanomas display abnormal activation of the MAPK cascade due to these mutations and may exhibit sensitivity to MEK inhibition. Although the mouse melanoma cell, B16F10, were without mutations in the BRAF and NRAS genes, the MAPK cascade and PI3K/Akt signaling pathway are still the major mediators for melanoma metastasis. So far, the best characterized of metastasis models is the B16 melanoma model. B16F10 cells had an effective metastasis rate of 5 x10^{-5} per cell per generation. Beside human melanoma cell transplanted null mice, this metastasis model in conjunction with in vitro modeling and manipulation of tumor cells have enable investigators to development of agents that can be used to prevent or treat overt metastatic disease.]

Q: The western blot images in figure 4 are not very clear and their profiles, except for FAK, do not clearly depict the data presented in the graph. Moreover, it is important to demonstrate that the level of these proteins (independent of phosphorylation) is the same.

Respond: According to your suggestion, we examined the level of total protein expression and remake the western blotting. As shown in Fig. 4, the expression of these proteins was not altered by butein treatment.

Q: Can the authors elaborate on what is the target molecule or mode of action of butein? How can it affect both MAPK and AKT pathways? Are other pathway also affected by butein.
Respond: There is no available information for how butein interact with other protein kinase and produce its inhibitory effect. Since both of ERK and PI3K/Akt are serine/threonine kinases family, butein may inhibit their kinase domain to suppress their activity. However, this hypothesis still needs further investigation. We are not the only group who found butein inhibit both MAPK cascade and PI3K/Akt signaling pathway. Zhang et al. also reported that Butein sensitizes HeLa cells to cisplatin through the AKT and ERK/p38 MAPK pathways by targeting FoxO3a. We summary of the main signaling pathway and finding of this article in one graphical abstract and show in Fig. 7.

Q: What is the effect of butein in normal cells?

Respond: We have test the effect of butein in human wash platelet and human endothelial progenitor cells (EPCs). Butein did not affect the platelet aggregation and only slightly inhibit EPCs viability in 10µM. Our results reveal that butein specific inhibit the tumor cell proliferation and migration without affect normal cell function.

Q: In the conclusion the authors state: “... butein exhibited anti-tumor activity in vivo, including pulmonary metastasis”. I would say that this was shown only, not “including” pulmonary metastasis.

Respond: According to your advice, we change the sentence to [More importantly, butein exhibited anti-tumor activity by in vivo pulmonary metastasis model.]

Reviewer 2

Q: In this study, it seems to be extreme important result from Figure 4, indicating butein suppressed on PI3K/Akt/mTOR, ERK and FAK pathway. Unfortunately, figure (missing the expression of concentration) of western blot are indistinctness, provably, many readers of this journal cannot believe that of bare figure. Thus, authors should indicate more clear result with dose-dependent concentration.

Respond: According to your suggestion, we examined the level of total protein expression and remake the western blotting. As shown in Fig. 4, the expression of these proteins was not altered by butein treatment.

Q: Figure 2 and 3: Should add scale bar.
Respond: Thanks for your reminding, the scale bar was added to Fig.2 and Fig.3.

Q: Explanation of figure 6B is lacking through the text.

Respond: According to your suggestion, we rewrite the sentence and discuss this part to make more clearly explanation (Page14 Line15-17)

Q: In Figure 3: Is individual scratched area same? Please confirm the area in the group (A-F) again. Also, how did count the wounded closure count in (G)?

Respond: The cells move into the scratched area was the indicator for cell migration. We did not use the distance of scratched area as the indicator for cell migration. Since we use the migratory cell number to evaluate the cell migration inhibition activity, the difference of scratched area distances were not affect our results.

Q: Should check below and also carefully spell check through the text including English grammar.
#Page 8, lines 18: 5x10^4 cell/ well#5x10^4 cells/ well
#Page 11, lines 6: Butein(1#3#10 mg/kg)#Butein (1, 3, 10 mg/kg)
#Page 17, lines 2: Previously study shown #Previous studies showed

Respond: Our Ms has been edited for English language, grammar, punctuation, and spelling by Enago, the editing brand of Crimson Interactive Pvt. Ltd.

We hope that the revised manuscript will meet the requirement for publication in this journal. Your kind arrangement for the evaluation of our revised manuscript will be greatly appreciated.

Best regards,

Ching-Hu Chung
CERTIFICATE OF EDITING

This is to certify that the paper titled *Butein inhibit metastatic behavior in mouse melanoma cells through VEGF expression and translation-dependent signaling pathway regulation* commissioned to us by Ching-Hu Chung (馬偕醫學院) has been edited for English language, grammar, punctuation, and spelling by Enago, the editing brand of Crimson Interactive Pvt. Ltd.

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Disclaimer: The author is free to accept or reject our changes in the document after our editing. However, we do not bear responsibility for revisions made to the document after our edit on **12 Nov 2015**.

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