Author's response to reviews

Title: Inhibition of STAT3 signaling and induction of SHP1 mediate antiangiogenic and antitumor activities of ergosterol peroxide in U266 multiple myeloma cells

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Author's response to reviews: see over
Dear Dr. Jigisha Patel MRCP, PhD, Editor-in-Chief:

We thank for the valuable comments about our manuscript entitled, “Inhibition of JAK2/STAT3 signaling and induction of SHP1 mediate antiangiogenic and antitumor activities of ergosterol peroxide in U266 multiple myeloma cells (Manuscript ID 1158690452571707)”. According to the comments, our manuscript was revised point by point and the answers to the comments were provided as follows:

Reviewer: Alessandra Russo

- It well known that some natural compounds could be interfere with MTT salts. Therefore, the medium would be removed before to add the MTT salts.
  
  (Response) We removed the culture medium before adding the MTT solution. This has been mentioned in methods section in this revised MS.

Reviewer: Bharat B Aggarwal

1. There are no significant changes in SHP-1 transcript (Fig. 3D); however protein levels are elevated at 4 hr treatment with EP. It needs explanation.
  
  (Response) We have carried out the quantification of SHP-1 RT-PCR in Fig. 3D by using Image J software. Results revealed that mRNA level of SHP-1 was increased ~ 9 fold at 4 hr treatment. Quantification results have been added in this revised MS.

2. “Cytotoxic assay” is addressed in “Method” section: however the cytotoxicity data is missing.
  
  (Response) Sorry for making you confused. The cytotoxicity data have been added in Fig. 4A.

3. Does VEGF induce the phosphorylation of STAT3 in HUVEC cells? If so, does EP modulate STAT3 in HUVEC?
  
  (Response) Ebrahem et al. in 2006 showed VEGF can phosphorylate STA3 in HUVECs. We also observed that VEGF treatment (20 ng/ml) clearly increased phosphorylation of STAT3 in HUVECs. As shown in Fig. 4F in this revised MS, EP treatment inhibited the VEGF-induced phosphorylation of STAT3, indicating that EP can modulate STAT3 in HUVECs.

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4. How do the authors know that anti-angiogenic effect of EP is STAT3 driven?

(Response) As mentioned in response for No. 3, we have shown that EP clearly inhibited VEGF-mediated STAT3 phosphorylation in HUVECs in this revised MS (Fig. 4E). Our data also showed that EP treatment suppressed VEGF-induced tube formation in HUVECs (Fig. 4E). These data support that anti-angiogenic effect of EP was STAT3 driven.

5. How do authors decide the dose of EP for animal study?

(Response) Thanks! We decided the optimal doses for animal study after toxicology screening in mice.

6. Fig. 5C, how many mice are enrolled in vivo study?

(Response) We used 5 mice per group (n=5). This has been mentioned in method section and figure legend.

Minor comments are as follows

1. This manuscript is carelessly edited before its submission.

(Response) This revised MS has been thoroughly edited by Dr. Chan-Yan Chen at Harvard Medical School.

2. “Method” section: The detail information on antibodies is required (e.g., source, amino acid residue for phosphorylation).

(Response) The information has been added.

3. Typo error in 8th line, page 5.

(Response) Corrected (phosphor → phospho).

4. Typo error in 3rd line from bottom, page 9 (phosphorylation).

(Response) Corrected (phosphorylation → phosphorylation).

5. “Velcade” should be replaced to “bortezomib”.

(Response) Replaced.

6. Page 9, “Fig. 2C” should be replaced to “Fig. 1C”.

(Response) Replaced.
7. Fig. 2A, 2B and 3E lack statistical analyses—with such deviations in data.
   (Response) The SD has been added.

8. What is mean of “***” and “**” in Fig. 5C?
   (Response) We have mentioned the mean in the figure legend.

9. The magnification of IHC should be addressed (Fig. 5C).
   (Response) The magnification has been addressed in the figure legend.

We sincerely hope that we addressed our reasonable responses to the concerns raised by three reviewers and look forward to receiving a good news from your journal.

Sincerely yours,

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