Reviewer's report

**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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**Reviewer:** Bharat B Aggarwal

**Reviewer's report:**

The manuscript entitled "Inhibition of STAT3 signaling and induction of SHP1 mediate antiangiogenic and antitumor activities of ergosterol peroxide in U266 multiple myeloma cells" by Rhee et al. describes the following:

1. Ergosterol peroxide (EP) derived from edible mushroom suppresses STAT3 activation through inhibition of its upstream kinases Src and JAK2 in human multiple myeloma (MM) cells.
2. The suppression of STAT3 is mediated by EP-induced SHP-1 upregulation.
3. EP reduces the secretion of VEGF leading to inhibition of angiogenesis. Andhydrocannabinol LYR-8 inhibits angiogenesis in vivo.
4. EP also inhibits the growth of human MM in nude mice.

This manuscript provides evidence on anti-angiogenic effects of EP in MM cells. The authors clearly showed that in vitro, EP inhibits STAT3 signaling pathway and mediates significant suppression of angiogenesis both in vitro and in vivo. The results are convincing and adequately discussed. However, there are some specific comments as follows:

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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.
2. "Cytotoxic assay" is addressed in "Method" section: however the cytotoxicity data is missing.
3. Does VEGF induce the phosphorylation of STAT3 in HUVEC cells? If so, does EP modulate STAT3 in HUVEC?
4. How do the authors know that anti-angiogenic effect of EP is STAT3 driven?
5. How do authors decide the dose of EP for animal study?
6. Fig. 5C, how many mice are enrolled in vivo study?

Minor comments are as follows

1. This manuscript is carelessly edited before its submission.
2. "Method" section: The detail information on antibodies is required (e.g., source, amino acid residue for phosphorylation).
3. Typo error in 8th line, page 5.
4. Typo error in 3rd line from bottom, page 9 (phosphorylatioon).
5. “Velcade” should be replaced to “bortezomib”.
6. Page 9, “Fig. 2C” should be replaced to “Fig. 1C”.
7. Fig. 2A, 2B and 3E lack statistical analyses—with such deviations in data.
8. What is mean of “***” and “**” in Fig. 5C?
9. The magnification of IHC should be addressed (Fig. 5C).

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests