

Peer Review File

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Reviewer A

The paper titled “Bufalin suppresses esophageal squamous cell carcinoma (ESCC) progression by activating the PIAS3/STAT3 signaling pathway” is interesting. Bufalin may inhibit the proliferation, migration, and invasion of the ECA109 cells through the PIAS3/STAT3 signaling pathway. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) The abstract is not sufficient and needs further modification. The research background did not indicate the clinical needs of the research focus.

Reply: Thanks for this constructive comment. We have revised the abstract of the manuscript. Changes in the text: we have modified our text as advised (see Page 1, line 29-31).

2) It is suggested to increase the detection of apoptosis indicators, which may make the whole study more complete.

Reply: Good suggestions. We already have a research plan, and the next step will be to study the effect of Bufalin on the apoptosis of esophageal tumor cells.

3) There are many drugs or factors that regulate the ESCC. Why did the author choose bufalin for research? What is the biggest advantage of bufalin? It is recommended to add relevant content.

Reply: Thanks for these constructive comments. We have added to this in the introduction section.

Changes in the text: we have modified our text as advised (see Page 3-4, line 100-118).

4) The discussion section is relatively simple, and it is recommended to supplement the research progress of bufalin in tumors.

Reply: Good suggestions. We have revised the discussion section of the manuscript.

Changes in the text: we have modified our text as advised (see Page 15-16, line 498-527).

5) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Up-regulation of SOCS4 promotes cell proliferation and migration in esophageal squamous cell carcinoma, Transl Cancer Res, PMID: 35116557”. It is recommended to quote the article.

Reply: Good suggestions. We have cited “Up-regulation of SOCS4 promotes cell proliferation and migration in esophageal squamous cell carcinoma, Transl Cancer Res, PMID: 35116557” in the introduction part of this paper.

Changes in the text: we have modified our text as advised (see Page 3, line 68).

6) In addition to PIAS3/STAT3 signaling pathway in this study, what other signaling pathways may be involved? It is recommended to add relevant content to the discussion.

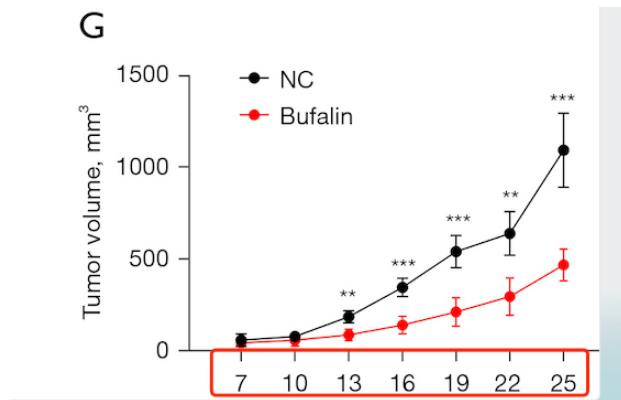
Reply: Thanks for these constructive comments. We have revised the discussion section of the manuscript.

Changes in the text: we have modified our text as advised (see Page 16, line 532-538).

Reviewer B

1. Figure 1

Please provide the description of the x-axis.

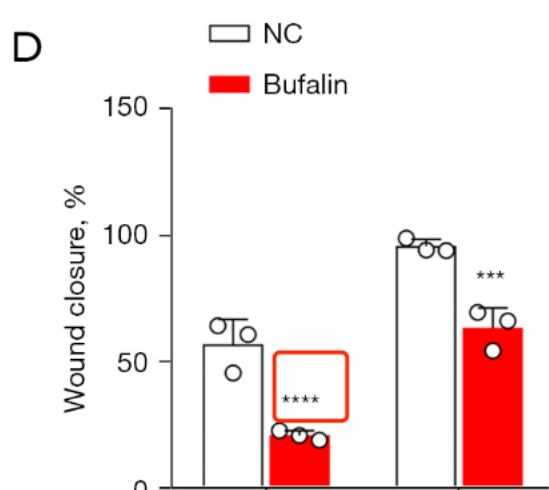


Reply: We have revised Figure 1 of the manuscript. X axis represents the number of days after subcutaneous inoculation of ECA109 cells.

Changes in the text: We have modified our text as advised (see Page 24, line 801/ 809-810).

2. Figure 2

Please explain **** in the legend.



Reply: We have revised the Figure 2D of the manuscript.

Changes in the text: We have modified our text as advised (see Page 25, line 814).

3. Figure 7

Please also provide the scale bar in the 7B.

assay (A) and EdU assay(B), Scale bar = 500 μm . (C) The statistics result of EdU assay.

Reply: We have provided the scale bar in the 7B of the manuscript.

Changes in the text: We have modified our text as advised (see Page 30, line 875).

4. Figure 8

a) Please also provide the scale bar in the 8C.

887 measured by transwell assay (A), Scale bar = 500 μm and wound healing assay (C),

888 Scale bar = 100 μm . (B) The statistics result of transwell assay assay. (D) The statistics

Reply: We have provided the scale bar in the 8C of the manuscript.

Changes in the text: We have modified our text as advised (see Page 31, line 886).

b) Please check if this part should be legend of 8F.

891 western blot. Actin was used as an internal control. Data are presented as mean \pm SD

892 (error bars). Statistical significance was tested by unpaired Student t-test. *, $p < 0.05$;

Reply: We have revised the legend in the 8F of the manuscript.

Changes in the text: We have modified our text as advised (see Page 32, line 894-896).

5. References/Citations

a) Please check if the author's name matches with the citation.

538 the zinc finger protein Gfi-1 and PIAS3⁶⁵). Dabir et al. showed that PIAS3 was also

539 involved in the dephosphorylation of activated STAT3⁶³). It has been suggested that

Reply: We have revised the citation of the manuscript.

Changes in the text: We have modified our text as advised (see Page 17, line 548).

b) Please double-check if more studies should be cited as you mentioned "studies".

103 tumors^{24, 25}). Studies have found that Bufalin can inhibit the proliferation of vascular

104 endothelial cells in a variety of tumor tissues, affect its neovascularization, and then

105 inhibit tumor growth⁶). In leukemia-related studies, it was found that Bufalin could

106 significantly inhibit the growth of leukemic cells and induce their differentiation and

107 apoptosis⁷). Studies have shown that Bufalin can activate members of Caspase family

Reply: We have revised the description of the references in this section of the manuscript.

Changes in the text: We have modified our text as advised (see Page 4, line 101-107).