Peer Review File

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Reviewer A

In the present study, the authors describe the cellular effects of Mebendazole (MBZ) in lung cancer models. The study is interesting, but some points need to be improved.

1 - The title could be improved from a grammatical point of view and according to the results to "Mebendazole induces apoptosis and inhibits migration via the reactive oxygen species-mediated STAT3 signaling downregulation in non-small cell lung cancer".

Answer: Thank you so much for the detailed review. We have made change according to your suggestion in the revised manuscript.

2 - The authors did not demonstrate the effects of MBZ on normal lung cells.

Answer: Thank you so much for figuring it out. It is necessary to study the toxicity of MBZ to normal cells, Tapas has demonstrated that MBZ can inhibit the proliferation of lung cancer cells, but has little toxicity to WI38 and human umbilical vein endothelial cells (PMID: 12231542).

3 - All tumor cells used are mutated for KRAS, what is the effect of MBZ on KRAS wild-type cells?

Answer: Almost all lung cancer experiments used KRAS mutant cells, except Ji-ichiro et al. used KRAS wild-type cells H1299 for a small amount of research (PMID: 12479701). We will further investigate the role of MBZ in KRAS wild-type cells.

Reviewer B

MBZ already has known induce apoptosis and anticancer effects in A549, H460 cells.

Authors provide insights into targeting STAT3 directly or inhibiting upstream regulators may be a viable treatment strategy for NSCLC.

Line 232: 0 μM

Authors should add FBS and antibiotics concentration in the medium.

The authors do not give a detailed description of transfection and luciferase assay.

I recommend explaining the detailed method.

Answer: Thank you for the detailed review. We have carefully and thoroughly proofread the manuscript accordingly. Modifications have been made.

In xenograft assay, authors expressed administered every other day for 2 weeks. Can you express this in Fig5A?

Answer: Thank you so much for figuring it out. We have modified the mistake in the method.

I recommend changing the expression level of STAT3 as "Exploring the anticancer role of MP06 peptide in NSCLCs" due to the authors just investigating the anticancer potential of the peptides.

Answer: Thank you so much for your detailed review. We focused on the anticancer role of MBZ in NSCLCs in this study. Regarding the MP06 peptide, we did not discuss its role in the manuscript.

The data is identical to the results of reference papers and it is necessary to highlight the JAK/STAT3 targeting part of the argument. Therefore, authors should add data on changes in JAK/STAT3 expression through animal samples. Discussion section needs to be improved. There are no clear conclusions about the mechanism of JAK-STAT3 for MBZ.

Answer: Thank you so much for the suggestion. It is necessary to record the expression proteins of JAK/STAT3 signaling pathway in histology and immunohistochemistry, and we will supplement relevant experiments in subsequent studies. Some changes have been made in the discussion section.

Reviewer C

The anticancer property of mebendazole, a well-known antihelminthic agent, has also been investigated as a point of interest in studies.

The study employs a range of well-established assays, including CCK-8 assay, Transwell assay, colony formation assay, wound-healing assay, and flow cytometry. This comprehensive approach allows for a thorough examination of various cellular aspects. Also, in vivo models provide a more holistic understanding of the potential therapeutic effects of MBZ. Although the anticancer properties of mebendazole have been researched for the past 20 years, the inclusion of combined and diverse experiments in your study enhances its value.

It would be valuable to discuss any limitations or potential confounding factors in the study. This adds transparency to the research and helps readers interpret the results more accurately.

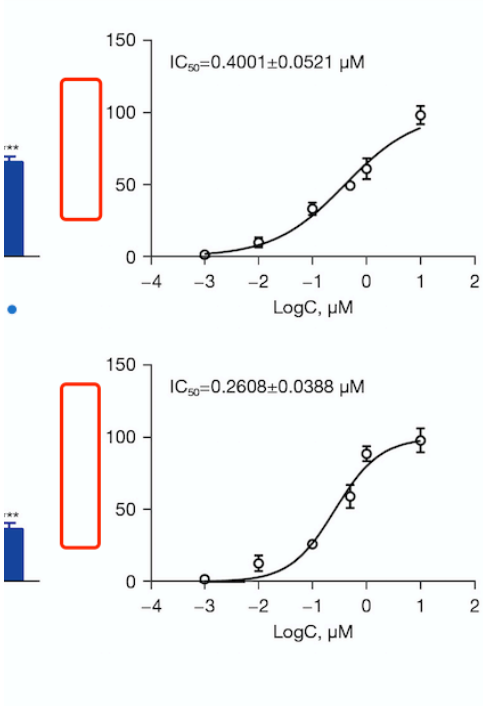
Thank you.

Answer: Thank you so much for your recognition of this study.

Reviewer D

1. Figure 1

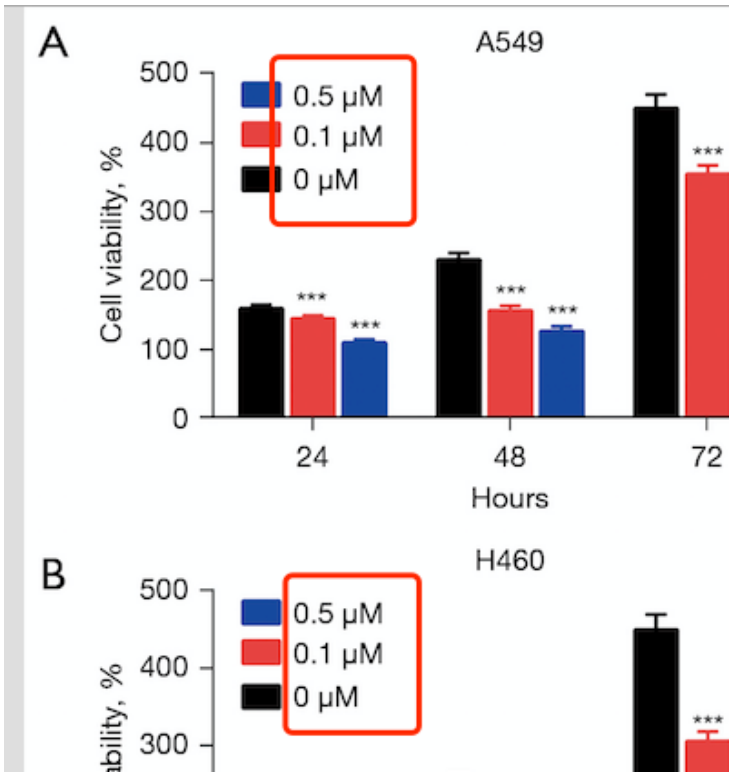
a. Please add the description of Y-axis (with unit).



Reply: We have been modified.

b. Please confirm if the figure legend is correct.

491 **Figure 1** MBZ inhibited A549 and H460 proliferation. A549 (A) and H460 (B) cells
492 were administered MBZ at titers of 0, 0.001, 0.01, 0.1, 0.5, 1, and 10 μM. At 24 h, 48



Reply: We have been modified.

c. Please indicate the observation method of figure 1C in the figure legend.
Reply: We have been modified in the method and the legend.

2. Figure 2B

Please indicate the magnification in the figure legend.
Reply: We have been modified.

3. Please spell out the full term of "PI" in the figure 3 legend.

Reply: We have been modified.

4. Reference/citation

There are total 33 citations in the main text, but only 31 references in the reference list. Please check and revise. Please note that references should be cited consecutively and consistently according to the order in which they first appear in the text.

343 docetaxel, often break the stability of oxidant and antioxidant systems (30,31). As a
344 result, ROS production is able to silence some signaling cascades; for instance, the
345 AKT–mTOR signaling axis (32) and the JAK–STAT3 signaling cascade (33),
346 resulting in tumor cell apoptosis. We therefore hypothesized that MBZ-induced

481 31. Lin Z, Pan J, Chen L, et al. MiR-140 Resensitizes Cisplatin-Resistant NSCLC
482 Cells to Cisplatin Treatment Through the SIRT1/ROS/JNK Pathway. Onco
483 Targets Ther 2020;13:8149-60.
484
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486
487
488 **Figure legends:**

Reply: We have been modified.