

Peer Review File

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Reviewer Comments

The manuscript entitled “Exploring the Molecular Targets and Mechanisms of [10]-Gingerol for Treating Triple Negative Breast Cancer via Bioinformatics Approaches and Molecular Docking Validation as well as Cell Experiments” by Hai Lu et al., shows the effects in vitro and bioinformatics studies that identified the mechanisms adjacent to of [10]-gingerol in TNBC. However, there are some points that in my opinion, do not allow this work to be published.

Comments:

1. In the introduction section, please define which hormone receptors are not expressed in TNBC, also it would be good to define HER2.

Reply 1: We defined HER2 are nor expressed in TNBC.

Changes in the text: We modified the “hormone receptor” into “HER2 receptor” in line 65 od introduction section.

2. It is not usual to insert figures in the introduction section, please check the journal’s formatting rules and correct, if appropriate.

Reply 2: The figure 1 is the graphical abstract. According to the journal’s formatting rules, we decided to delete this figure, and adjusted the figures’ number.

Changes in the text: We decided to delete this figure, and adjusted the figures’ number.

3. Western blotting analyses lack the proper controls. A control using an apoptosis inductor (e.g. camptothecin) is mandatory in this analysis.

Reply 3: According to the manipulations of apoptosis by Western blotting in the paper: Zhao HW, Zhou N, Jin F, Wang R, Zhao JQ. Metformin reduces pancreatic cancer cell proliferation and increases apoptosis through MTOR signaling pathway and its dose-effect relationship. Eur Rev Med Pharmacol Sci. 2020;24(10):5336-5344. doi:10.26355/eurev_202005_21316, we set the negative control (group of DMSO), and approved the [10]-gingerol could promote apoptosis.

Changes in the text: Nothing.

4. The phrase "normal breast epithelial breast" with which the authors define the MCF-10A line is not correct (page 15, line 315). This line is derived from fibrocystic disease. The more correct term would be non-tumor breast cell line.

Reply 4: We redefined MCF-10A into the non-tumor breast cell line.

Changes in the text: We modified “normal breast epithelial breast” into “non-tumor breast cell line” in line 317 of discussion section.

5. In figure 8D, a western blotting analysis with some proteins of the apoptotic process is shown. It is mandatory to indicate the molecular weight (kDa) of the protein represented in a WB assay, as this will indicate whether the caspases analyzed correspond to their active or inactive form.

Reply 5: We added the molecular weight (kDa) of the protein represented in the WB assay in figure 8D.

Changes in the text: We added the molecular weight (kDa) in figure 8D.

6. Page 15, line 322 and 323....” The result above indicated that [10]-Gingerol induced apoptosis through triggering the mitochondrial apoptotic pathway in TNBC cells” It is not correct to state this. To affirm that the apoptosis pathway is mitochondrial or intrinsic, specific caspases had to be analyzed. In this case, the authors only analyzed caspase-8, which is involved in the extrinsic and non-mitochondrial apoptosis pathway, as well as two caspases that terminate the apoptotic process.

Reply 6: In this study, we tested the expression of caspase-3, -7, and -8 was increased after [10]-Gingerol treatment. We thought [10]-Gingerol would regulate the activation of caspase-3, -7, and -8.

Changes in the text: We have modified the statement: “The result above indicated that [10]-Gingerol induced apoptosis through regulating caspase activation in TNBC cells.”

7. Page 3 and 16, line 77 and 340. Please, change “6-gingerol,8-gingerol” by “[6]-gingerol, [8]-gingerol”.

Reply 7: We have changed “6-gingerol, 8-gingerol” by “[6]-gingerol, [8]-gingerol” in page 3 and 16.

Changes in the text: We have changed “6-gingerol, 8-gingerol” by “[6]-gingerol, [8]-gingerol” in page 3 and 16.

8. Page 3, line 73. Please, put the phrase "Zingiber officinale" in italics.

Reply 8: We have phrased "Zingiber officinale" in italics in line 75 of introduction section.

Changes in the text: We have phrased "Zingiber officinale" in italics in line 75 of introduction section.

9. The English used in this manuscript needs some improvement.

Reply 9: We improved the English used in this manuscript.

Changes in the text: We improved the English used in this manuscript and got the editorial certificate.