Title: Jingfukang induces anti-cancer activity through oxidative stress-mediated DNA damage in circulating human lung cancer cells

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Author’s response to reviews:

Dear Editor and Reviewers:

Thank you very much for your kindness and for the reviewers’ comments concerning our manuscript entitled "Jingfukang induces anti-cancer activity through oxidative stress-mediated DNA damage in circulating human lung cancer cells" (BCAM-D-18-01101R3). Those comments are of great value and help for revising and improving the quality of the paper. We have studied the comments carefully and made corrections. The main changes in the paper and the responses to the editor’s/reviewers’ comments are as follows:
Responses to the reviewers’ comments

(Reviewer 3):

I have gone through the Ms critically and found it to be interesting and the content to be scientifically important. The results are drawn from only one cancer cell line, the lung cancer cell line. Similar results, if obtained also from a different cancer cell line, would have made an additional impact on its anti-cancer potential. The source of the cell line (CTC-TJH-01) used may be clarified more and whether the cancer patient agreed by signing "Informed Consent" should be mentioned. Anyways, I would like to see one important control to be at least performed and added in order to be able to recommend the use of Jingfukang in cancer- if a cytotoxicity test is done to examine if Jingfukang does not have a toxic effect, or even if it has, it is much less on any normal cell line (not originating from a cancer cell line) compared to the cancer cell line tested. This would serve as one important arm of the control. Therefore, this control should be included before its final acceptance for publication. There is also "language issue" at some places, just for one example: "Traditional Chinese Medicine (TCM) has been used to treat various Diseases". So a thorough language check up to remove such errors is recommended in the next version.

Reply: Thank you for your suggestion. Firstly, the article on establishment of CTC cell line (CTC-TJH-01) has been published in Cancer cell international journal, and informed consent has been mentioned in that article. “Que Z , Luo B , Zhou Z , et al. Establishment and characterization of a patient-derived circulating lung tumor cell line in vitro and in vivo. Cancer Cell Int, 2019, 19:21.”


Thirdly, we have already found a company to polish the article.
(Reviewer 4):

-What are the correlation between Cyclin A, B, D1, E1 and CDK1, 2, 4, 6 at G1 phase. Describe it details regarding check points in discussion part.

Reply: Thank you for your suggestion. We have added the corresponding content in the discussion part.

-The cell cycle diagram of Figure 1C, it is not clear whether Jingfukang arrest the cell at G1 phase. Replace these figures with new one.

Reply: Thank you for your advice. We have replaced it.

Abstract-

Methods: In this study, we investigated the action and underlying mechanisms of Jinfukang against circulating lung tumor cells.

-Place this sentence in background section.

Reply: Thank you for your suggestion. We have revised it.

-Cell viability assay

-What is the process of dose preparation?

- What is the percentage of DMSO for stock preparation?

-DMSO itself can cause cell death at more than 0.1%. Have you made -ve control?

-At which wavelength, have you read the absorbance? Mention it.

Reply: Thank you for your suggestion. The freeze-dried powder of Jinfukang was weighed and dissolved in DMSO to prepare an initial concentration of 1 g/mL. Then the concentration of the original drug was diluted to 70 mg/mL with F12K medium. In the experimental group, 10 μL of drug was added into each hole. Therefore, the concentration of DMSO is less than one thousandth. The control group was added with the same amount of medium. We have added relevant detection wavelength to the manuscript.
Colony forming assay

What is the criteria of doses selection (350 and 700ug/mL) for the rest of the experiments, although the IC50 of Jingfukang at 728.8ug/mL.

Reply: Thank you for your suggestion. We chose the doses (350 and 700 μg/mL) because it facilitates dosing and reduces errors. It is also effective on cells at a dose of 700 μg/mL.

Comet Assay

Describe the methods in brief.

Reply: Thank you for your suggestion. We have revised it.

Results

Jinfukang inhibits proliferation of CTC-TJH-01 cells

The cell cycle diagram of Figure 1C, it is not clear whether Jingfukang arrest the cell at G1 phase. Replace these figures with new one. Bold the axis title and show the legend on cell cycle diagram.

Reply: Thank you for your advice. We have replaced and revised it.

Jinfukang induces apoptosis in CTC-TJH-01 cells

Figure 2C, 2E are blurred, make it clear and bold.

Reply: Thank you for your suggestion. We have revised it.

Also insert the page no. in the manuscript file.

Reply: Thank you for your suggestion. We have revised it.

Our found that the CTC-TJH-01 cells showed, what is meaning of this sentence?

Jinfukang induces apoptosis of CTC-TJH-01 cells by promote ROS generation

Reply: Thank you for your suggestion. We have revised it.
Jinfukang induces the apoptosis of CTC-TJH-01 cells through DNA damage

Reply: Thank you for your suggestion. We have revised it.

Previous studies have confirmed that increased ROS levels can cause DNA damage and induce cell apoptosis [18];

-Describe only the results here, discussion and citation should be in Discussion part.

Similarly;

Jinfukang induces the apoptosis of CTC-TJH-01 cells through the ROS-mediated ATM/ATR-p53 pathway

It is well known that ROS-mediated oxidative damage can induce tumor cell apoptosis through signaling pathways such as the caspase, MAPK, and PI3K/AKT pathways [19, 20].

-Describe only the results here, discussion and citation should be in Discussion part.

Reply: Thank you for your suggestion. We have revised it.

Discussion

The present study demonstrates that Jinfukang can induce the apoptosis of lung cancer cells by activating the expression of AIFM2.

-Which study has demonstrated that? It seems that you have reported the previous study, so give the citation just after this sentence and also revise the language.

Reply: Thank you for your suggestion. We have revised it.

We acknowledge the editor’s/reviewers’ comments and suggestions, which are valuable in improving the quality of our manuscript.

We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper.
We really appreciate Editor’s/Reviewers’ opinions, and hope that the correction will meet the approval criteria of the journal.

Once again, we would like to express our great appreciation to you and reviewers for comments on our paper.

Sincerely yours,

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