

Peer Review File

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As the preferred drug for single chemotherapeutic application in pancreatic cancer, gemcitabine demonstrated low sensitivity and strong chemotherapy resistance in patients. Therefore, the search for other drugs with high efficiency and low side effects for the treatment of pancreatic cancer has become of high importance. In the manuscript “Chemotherapeutic efficacy of curcumin for pancreatic cancer as an alternative of gemcitabine: A comparative metabolomic study”, authors assessed the therapeutic effects of curcumin on pancreatic cancer as an alternative of gemcitabine and explore its underlying biochemical mechanism.

Couple questions are required to be answered before accepted.

(1) In the report (Anticancer Agents Med Chem. 2013 Jul 1;13(6):952-6), you have reported that CUS could inhibit tumor proliferation in mice models. But, in the introduction, it was showed that there are no few studies on its therapeutic effect on the individuals including human or animal model with pancreatic cancer. Why?

Response: We are sorry for the inappropriate description of this point. In the revised manuscript, we have changed the comments on this issue and the related reference was also cited.

Changes in text: We re-described this point as “Moreover, CUS demonstrated obvious inhibition to tumor proliferation in mice models, however, the specific mechanism, especially

its metabolic changes responsible for the therapeutic effect still keep unknown.” (See Page 7 Lines 99-101 in the revised version)

(2) In the introduction, please enrich the progress of the treatment for pancreatic cancer.

Response: Valid point, according to the reviewer, the information on the progress of the treatment for pancreatic cancer has been enriched in the revised manuscript.

Changes in text: “There has been very limited progress in the treatment of pancreatic cancer over the last few decades, with its 5-year survival rate increasing from 2.5% in 1975–1977 to 8% in 2010–2018. In the absence of promising results for single-agent or combination chemotherapy in the last decades, prognosis remains poor. With multi-drug systemic treatment, the median survival rate is about 8 months.” (See Page 5 Lines 59-64 in the revised version) “It is therefore projected to become the second leading cause of cancer mortality before 2030 due to improving therapies for other cancers compared with those for pancreatic cancer. At present, only 10-20% of clinical cases of pancreatic tumors are resectable, but up to 80% of patients undergoing resection develop disease recurrence even after adjuvant treatment. In patients with borderline resectable/unresectable pancreatic cancer, administration of chemotherapy may increase the chance of resection and, consequently, improve survival outcomes.” (See Page 5 Lines 65-72 in the revised version)

(3) The cell isolation method should be described briefly in the part of methods. Why the

Panc-1 cells were supplemented with gentamicin?

Response: In our original manuscript, the cell isolation method was described in the section of Methods. The cell isolation was described in the subsection of “Establishment of subcutaneous xenograft model of pancreatic cancer” as “When the Panc-1 cells in logarithmic

growth phase were  $2 \times 10^7$  cells/flask, they were digested with 0.25% trypsin and harvested into a centrifuge tube. The cells in trypsin solution were centrifuged at 1500 g for 10 min. The supernatant was discarded, and the cells ( $2 \times 10^7$  cells) were washed for 3 times with phosphate buffered saline (PBS) and resuspended in 0.2 mL culture medium.” (See Page 8 Lines 128-133 in the revised version)

The addition of antibiotics to cell culture media can effectively prevent cells from dying from bacterium, fungus, mycoplasma or microzyme. The most common antibiotics are gentamicin, streptomycin, penicillin, principen, kanamycin, fradiomycin, and so on. In our study, 80 U/mL gentamicin with broad-spectrum was chosen as the antibiotics in RPMI 1640 medium.

(4) The numbers and the volume of injected cells into the mice model are what? Please elucidate clearly in the methods.

Response: There were  $2 \times 10^7$  cells in 0.2 mL culture medium injected into the mice model, which was described in the section of Methods in the revised manuscript.

Changes in text: “The supernatant was discarded, and the cells ( $2 \times 10^7$  cells) were washed for 3 times with phosphate buffered saline (PBS) and resuspended in 0.2 mL culture medium.” (See Page 8 Lines 131-133 in the revised version) and “After skin degerming, the cell suspension ( $2 \times 10^7$  cells in 0.2 mL culture medium) was subcutaneously injected into the right shoulder back of the nude mouse with a sterile syringe, followed by normal feeding.” (See Page 9 Lines 134-136 in the revised version)

(5) How to determine the dose of CUS (1.0 mg/kg, 0.5 mg/kg)? How to determine the numbers of mouse in each group? And how to group?

Response: A number of preliminary experiments have been carried out to determine the safe

concentration of CUS in mice. The highest safety concentration was determined to be 1.0 mg/kg. Therefore, in our study, 1.0 mg/kg was chosen as the high dose, and a low dose concentration of 0.5 mg/kg was used in the comparison group.

Animal experimental design follows the "3 R principle", including reduction, replacement and refinement. Reduction means minimizing the number of experimental animals. But the statistical requirements must be met at the same time. There is no absolute requirement for the number of experimental animals. Statistical requirements in the metabolomic analysis generally require at least six available data per group to make sense, resulting about usually 10 animals per group. In our study, there were groups including Control (the healthy nude mice intraperitoneally injected with 10 mL/kg sterile saline), Pancreatic cancer group (Negative control group, the xenograft mice intraperitoneally injected with 10 mL/kg sterile saline), High dose curcumin group (High-dose experimental group, the xenograft mice intraperitoneally injected with 1.0 mg/kg curcumin), Low dose curcumin group (Low-dose experimental group, the xenograft mice intraperitoneally injected with 0.5 mg/kg curcumin) and Gemcitabine group (Positive control group, the xenograft mice intraperitoneally injection with 50 mg/kg gemcitabine). Mice were randomly assigned to these groups with 9-12 mice in each by simple randomization.

Changes in text: The dose selection was described as "The high dose (1.0 mg/kg) of curcumin was based on its safety concentration and a lower dose (0.5 mg/kg) was used as the comparison." (See Page 9 Line 153- Page 10 Line 154 in the revised version)

"... and the mice were randomly divided into 5 groups according to their intervention strategy." (See Page 9 Lines 145-146 in the revised version)

(6) The figure 3 was not clear enough. Please replace it with a new. Scale bar is needed to add in the figure S1.

Response: According to the reviewer, a new Figure 3 has been provided, and scale bar has also been added in the Figure S1 in the revised manuscript.

(7) It is better to provide the tumor growth curve each group.

Response: According to the reviewer, the tumor growth curves each group have been provided as Figure S2 in the supplementary materials, and the growth curves of body weight were also provided. And the related description was also demonstrated in the text in the revised manuscript.

Changes in text: “Their growth curves of body weight and the tumor in the each group were displayed in Supplementary Figure S2.” (See Page 14 Lines 239-241 in the revised version)