Author's response to reviews

Title: Antitumor effect of Kanglaite(R) injection in human pancreatic cancer xenografts

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Author's response to reviews: see over
Dear Dr Tom,

We found the referee’s comments most helpful and have revised the manuscript according to the comments of the referees. Revised portion are highlighted in yellow.

With best wishes,
Shi Liu

To Dr Qing-Yi Lu,

1. In Introduction, the statement “In the recent years, traditional Chinese medicine has been widely used as a complementary and alternative medicine in cancer treatment in the United States and Europe [2]” is not an accurate description of the use of TCM in cancer treatment in USA and Europe.

We modified this description into “In recent years, traditional Chinese medicine has been widely used in China as adjuvant treatment during chemo- and radiotherapy for cancer [2].” in introduction (Introduction, paragraph 1, line 17-21).
2. The active ingredient of KLT was reported being a compound of triglyceride containing four of fatty acids (C16, C18, C18-1, C18-2). KTL has been shown to improve the life span and quality of life of patients when combined with chemotherapy or radiotherapy with some evidence. It is not convinced that KLT alone would cure lethal human malignant such as the pancreatic cancer.

We added a section in introduction: While KLT alone cannot cure lethal human malignancies such as pancreatic cancer, there are no previous findings on the effect of KLT on the PI3K/Akt/mTOR pathway in treating pancreatic cancer. Therefore, we studied the anti-cancer effect of KLT on the PI3K/Akt/mTOR pathway in treating pancreatic cancer (Introduction, paragraph 4, line 13-19).

3. The injection doses used in present study are similar to other studies. What is the equivalent dose of 25 ml/kg to human being? How do it compared with clinical dosage used in China? These can be added as part of the discussion.

We added a part of discussion: The injection doses used in this study are similar to those in other studies. The equivalent dose of 25 mL/kg to a human being is 2.77 mL/kg. The clinical dosage of KLT used in China is
200 mL/person (70 kg), which is approximately 2.86 mL/kg (Discussion, paragraph 2, line 19-25).

4. Authors presented tumor volumes with significant decrease for mice injected with KLT in comparison to the untreated controls (Fig 1B). The number of mice in each group used to calculate the SD needs to be specified. The fact that all the standard deviations in Fig 1B and (Fig 2 and 3 as well) are so small makes the data very questionable.

The number of mice in each group used to calculate the SD is ten. We feel very sorry to say that the author who is responsible for the data collection and statistic used the wrong formulae of tumor volume and apoptotic index, so we checked the data carefully and recounted the data in Fig1B and Fig 2B (Results, paragraph 1, line 3-25; Results, paragraph 2, line 33-39), as a result, we recounted the tumor inhibition ratios as well (Discussion, paragraph 2, line 1-5). Furthermore, there is no mistake in Fig 3 after a careful investigation. We also exhibited the data of tumor weights in Fig1D (Results, paragraph 1, line 15-23).

5. For Fig 1C, all 10 mice from each group (not one) should be aligned to show the decreasing in tumor volumes after treatment.
We feel very sorry to say that we do not have an image of all 40 mice from each group together after treatment, therefore, in Fig 1C, all 40 tumors from each group had been aligned to show the decreasing in tumor volumes after treatment.

6. How the samples are pooled or how many samples are used to obtain PCR data and Western Blot data are not clear.

All 40 tumor specimens from each group (100-150 mg each) were used to obtain PCR data and Western Blot data and we added this into the methods section (Methods, paragraph 4, line 33-35; Methods, paragraph 5, line 27-29).

7. Authors presented results showing that KTL downregulates the expression of pAkt and p-mTOR to modulate the PI3K/Akt/mTOR signaling pathway. It would be more convincing to include in vitro studies to demonstrate the modulation of the signaling pathway.

We had studied the anti-cancer effect of KLT on the PI3K/Akt/mTOR pathway in treating pancreatic cancer cell, nevertheless, we feel very sorry to say that this part of data had been submitted to a Chinese journal and we have no droit to use the data in this manuscript.
8. The number of reference cited written in Chinese which is not accessible to the reviewers should be limited.

We have deleted most of references cited written in Chinese and there is only one citation in Chinese remained.

9. Methods need to be better described, font consistent and grammar checked.

Methods have been better described and grammar has been modified by a professional native English editor.

To Dr Zhijun Wang,

1. In figure 1B, the data were expressed as mean +/- SD as per the text. Is that possible to get such small variability in the tumor size of each group? Based on the figure, the greatest RSD is less than 10% which is almost not possible for a xenograft model. I urge the authors to check whether SD should be SE.

We feel very sorry to say that the author who is responsible for the data collection and statistic used the wrong formulae of tumor volume and
apoptotic index, so we checked the data carefully and recounted the data in Fig1B and Fig 2B (Results, paragraph 1, line 3-25; Results, paragraph 2, line 33-39), as a result, we recounted the tumor inhibition ratios as well (Discussion, paragraph 2, line 1-5). Furthermore, there is no mistake in Fig 3 after a careful investigation. We also exhibited the data of tumor weights in Fig1D (Results, paragraph 1, line 15-23).

2. In figure 3D, the values of the control are not the 100%. Are all the data compared with the control samples in day 0? Please clarify in the figure legend.

This description was added into the figure legend: After treated with KLT for 21 days, pAkt and p-mTOR expression levels were significantly decreased compared with the controls (*P<0.05); furthermore, the expression levels of PI3K, Akt and mTOR were unchanged compared with the controls (P>0.05) (Figures, paragraph 3, line 5).

3. The English should be further edited. Some examples are as follows and the authors should check the entire manuscript. “amounts of protein were” should be “amount of protein was” (page 7) “Differences in both parameters were analyzed for significance by Student’s t-tests” should be “Difference of parameter between two samples was compared using
Student’s t-test”. (Under section of Statistical analysis) The word of “reduction” was used to describe the tumor growth. I believe that “retardation” would be more appropriate, since all the tumors were growing.

The English has been modified by a professional native English editor.