Peer Review File

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Reviewer A

The authors investigated whether lobaplatin has an antitumoral effect on bladder cancer cells by performing several different in vitro and in vivo experiments. The findings look promising for bladder cancer. However, the results might be better evaluated if the experiments were conducted along with cisplatin which is the most commonly used chemotherapeutic agent in bladder cancer treatment and has a similar mechanism of action to lobaplatin as a DNA crosslinking agent. Besides, migration experiments are somewhat out of place in the context of a manuscript focusing on the drug's cytotoxic and apoptotic effects. In general, the work was performed and written precisely.
In addition to these comments, there are some points that need to be clarified. Please find my specific comments below:
1. What is the reason that the authors chose 8ug/ml of LPB for subsequent experiments while both cell lines showed specific IC50 concentrations different from 8ug/ml?
Reply 1: Concentrations well below the IC50 concentration were chosen for conducting experiments in this study, this is because concentrations higher than the IC50 concentration may cause cytotoxicity, thus interfering with subsequent studies on the mechanism of apoptosis. Therefore, concentrations below the IC50 concentration were chosen for the experiments.

2. It would be nice to see the effect on migration in comparison to a positive control, such as another chemotherapy drug or tubulin inhibitor.
Reply 2: Yes, that's right.

3. The authors stated that the inhibitory effect of LPB on the migration of cells is dose-dependent. However, it looks like the effect is independent of the increasing doses of the drug in the panels, particularly for T24.
Reply 3: The width of the scratch experiment was analysed using image j. The experiment was repeated three times to ensure the reliability of the results obtained.

4. Is there any explanation for the decrease in body weight of the control group in T24 inoculated mice at the first week? Because it is the opposite for the 5637 cells injected group.
Reply 4: As can be seen from the figure, the difference in body weight of the mice was not significant. Initially, some of the mice were in conflict, which may have affected

their body weights, and the situation disappeared when the mice were divided into cages of 3 mice each.

5. How many days did it take to reach the 200mm3 volume of the tumor? It is necessary to know this for evaluating the results. Also, on which day was the drug injected?
Reply 5: The number of days required to bring the tumour volume up to 200mm^3 varies as different bladder cancer cells are used to establish the tumour model, typically taking 10-15 days.

6. In the abstract, it makes no sense to mention that the level of the proteins was suppressed in a dose-dependent manner because the cells were only treated with a single dose (8ug/ml) of LPB.
Reply 6: We have modified our text as advised
Changes in the text: Page 2, line 61 and 64.

7. In Figure 4, it would be nice to name the plots in 4b and 4e as 'relative densitometric analyses'. When 'relative expression fold change' was mentioned, it gave the impression of mRNA expression levels analysed by qPCR.
Reply 7: Thank you for your suggestion, we have made changes to Figure 4.
Changes in the text: Page 16.

8. On line 310, the sentence says there are no significant changes in Akt levels and the p value shows smaller than 0.05.
Reply 8: Here it means that the above results are statistically significant at $P < 0.05$. To avoid ambiguity, We have modified the position of the P values in the text .
Changes in the text: Page 10, line 334 and 335.

9. Has the IHC been conducted in the metastatic tumour tissue? Figure 6 states that t24 and 5637 metastatic tumours were evaluated.
Reply 9: Yes, it is.

10. There are several typos in the Celsius degree symbols.
Reply 10: We tweaked it using the symbols that come with the Word document.

11. In Figure 3, quadrants of the flow cytometry data have not been explained regarding the annexin V and/or PI positivity (late apoptosis, necrotic cells), and have not been discussed in the discussion either.
Reply 11: Corresponding descriptions have been added.
Changes in the text: page 10.

Reviewer B

In the present study, the authors investigated the antineoplastic effects of lobaplatin using T24 and 5637 bladder cancer cells. There are so many points raised by the reviewer and the authors are recommended to entirely rewrite a manuscript. My specific comments are as follows.

1. L. 79, “Some patients with metastatic BC have unsatisfactory recovery”: Please correct. It is rare that patients with metastatic disease achieve complete remission. Also, please cite literature.

Reply 1: We have modified our text as advised.

Changes in the text: Page 3, line 83.

2. L. 93-95, “multiple pathways in tumor cells related to the occurrence of BC, such as…”: Please cite literature.

Reply 2: We have modified our text as advised.

Changes in the text: Page 4, line 102.

3. LL. 96-109: Redundant. Please don’t repeat what is written in the Materials and Methods section or Results section.

Reply: We deleted this part.

4. L. 154, “the experiment was performed according to the reagent instructions”: Unclear what the authors performed.

Reply 4: We have modified our text as advised.

Changes in the text: Page 5, line 169- 171.

5. L. 187, “protein extraction kit”: Unclear which kit the authors used.

Reply 5: We have modified our text as advised.

Changes in the text: Page 6, line 206.

6. LL. 194-195, “the membrane was evaluated using an Odyssey dual-color infrared laser imaging system”: Please describe in more detail. This system does not detect HRP-conjugated antibodies, so what did the authors next before detecting the bands using this device?

Reply 6: Using the Odyssey Dual-Colour Infrared Laser Imaging System, which uses an infrared fluorescent dye to label the secondary antibody, the secondary antibody is scanned and imaged directly after incubation, eliminating the need for enzyme (ERP or

AP)-labelled secondary antibodies.

7. LL. 216, “The tumor tissues obtained in 2.8”: Incomprehensible.
Reply 7: We deleted this part.

8. LL. 227-231, “The intensity of staining is based on the staining characteristics of the majority of cells (shades of staining in contrast to background staining): 0 points for no staining…”: Please show the theoretical basis of this evaluation method.
Reply 8: References have been cited for the method.

9. Fig. 1: Please also show the photomicrographs of the cells.
Reply 9: We have reviewed the published papers and found that the image meets the requirements of the paper and the experimental results[1].

10. Fig. 1: Please show the graphs of the control group.
Reply 10: Dear reviewer, The first dot in both line graphs points to 100% cell viability, which is the control group! The dose of 0 μg/mL is the control group.

11. Fig. 2: Please repeat the experiment using various concentrations of lobaplatin.
Reply 11: All experiments were repeated three times to obtain. The figure shows that we have used 0,1,2,4,8 μg/mL for experimental studies. These four groups are the doses with quantitative relationship.

12. The apoptosis assay results seem to be incompatible with cell viability assay results.
Reply 12: If the mismatch is that the apoptosis rate is not high enough, the Flow of experiments is 24h for 8 doses of apoptosis, please see the curve of 24h in Figure 1.

13. Fig. 4: The experiment should be repeated using various concentrations of lobaplatin.
Reply 13: As stated in the text, the concentration that we chose based on the quantitative-effect relationship curve.

14. Fig. 4: Unclear why two different loading controls (GAPDH and actin) were used.
Reply 14: Written wrong here, thanks to the reviewer for reminding me!
Changes in the text: Page 16.

15. Fig. 4: The authors did not show the method of densitometry. How many times were the experiments repeated?
Reply 15: Well, Grey scale analysis was performed using imagej and all experimental

replicates were repeated 3 times.

16. Figs. 5A and 5D: Please include a scale in the photographs.
Reply 16: Marked as required.
Changes in the text: Page 16.

17. Fig. 6: Please confirm the results using western blotting.
Reply 17: Only paraffin sections are now suitable for experiments; other specimens have failed.

Minor:
1. A company's name should be shown along with the city, state, and country where the company is located. This should be consistent throughout the text.
Reply 1: We have collated.
Changes in the text: Page 4, from line 128 to 153.

2. Please spell out the following abbreviations at their first use both in the abstract and in the text: FITC; PI; SPF; RPMI; GAPDH; HRP; PBS; PVDF; TBS-T; ANOVA.
Reply 2: Additions have been made as required.

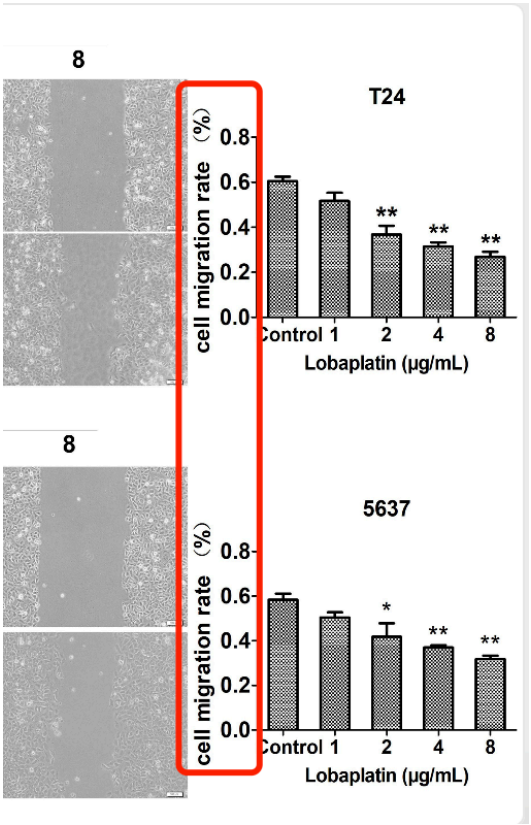
3. LL. 194-195, "Odyssey dual-color infrared laser imaging system": Please show the manufacturer.
Reply 3: Lincoln, Nebraska, United States.
Changes in the text: Page 5, line 153.

4. L. 241, "SPSS 24.0 software": Please show the manufacturer.
Reply 4: Added.
Changes in the text: line 261.

5. L. 241, "ImageJ software": Please show how the authors obtained this software.
Reply 5: The software is open source freeware.

Reviewer C

1. Figure 2
a) Please check if the y-axis is correct. For example, cell migration rate=0.8%?

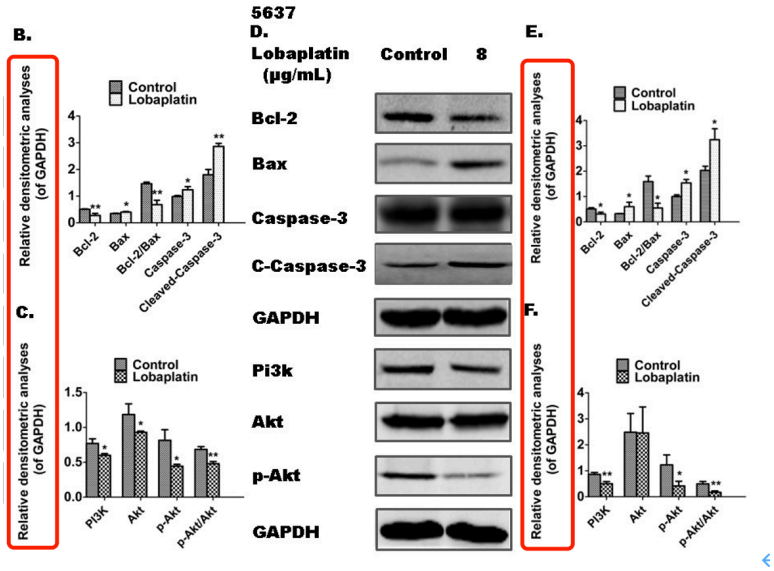


Reply a): This part is the "Cell Migration Rate", we use decimals to express it, so the "%" in the vertical coordinate is redundant, we have changed it in the graph.

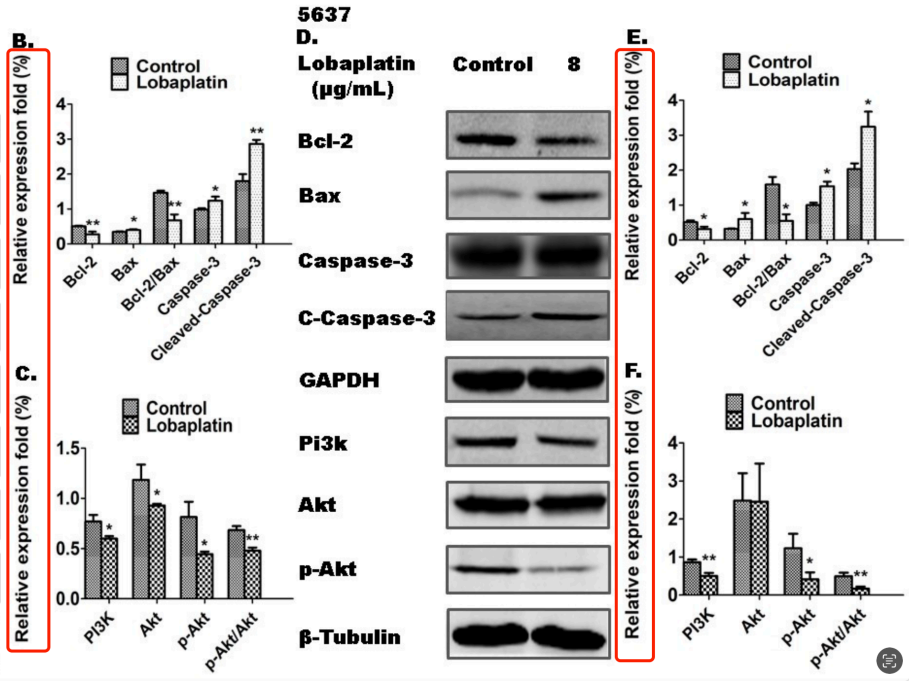
b) Please provide the observational method in the legend.
Reply b): Modified.
Changes in the text: Page 15, line 606

2. Figure 3
Please explain PI and FITC in the legend.
Reply: Added
Changes in the text: Page 16, Line from 612 to 617

3. Figure 4
a) The individual file is not the same as the main text, please send us the correct one.
Main text:

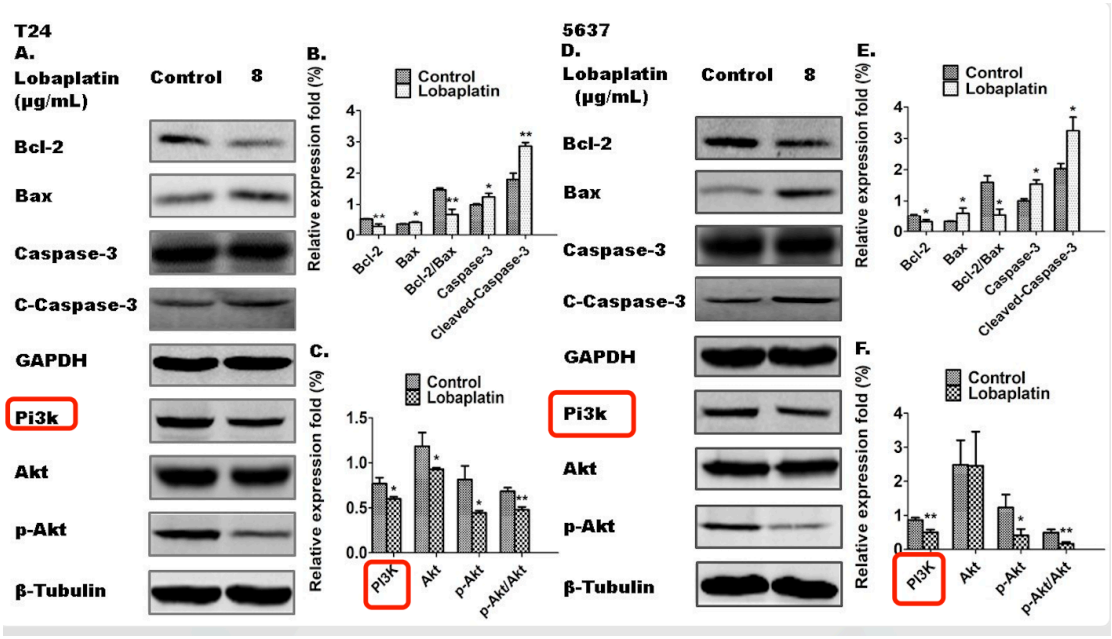


File:



Reply a): Submitted with this revision

b) Please unify the format, Pi3k or PI3K?



Reply b): Submitted with this revision

4. Figure 5

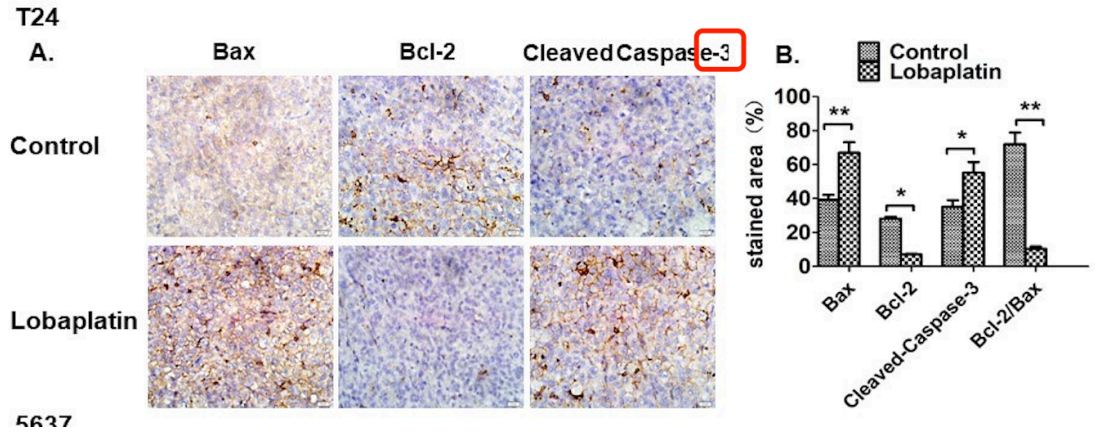
Figures, tables and videos should be cited consecutively in the text and numbered in the order in which they are discussed. Please revise the citation in the main text.

Replied: Modified.

Changes in the text: Page 10, Line 349-350.

5. Figure 6

The figure 6A was not complete, please revise.



Reply: Submitted with this revision