Author’s response to reviews

Title: Comprehensive Analysis of Differentially Expressed Genes Associated with PLK1 in Bladder Cancer

Authors:

ZHE ZHANG (zhangzheurology@gmail.com)
GUOJUN ZHANG (zhanggj@sj-hospital.org)
ZHIPENG GAO (63099390@qq.com)
SHIGUANG LI (shiguangli@hotmail.com)
ZELIANG LI (63384797700@qq.com)
Jianbin Bi (bibibibi@126.com)
Xiankui Liu (333999289829@qq.com)
Zhenhua Li (zhenzhenhuahua@yeah.net)
Chuize Kong (kongchuize_cmu@sina.cn)

Version: 1 Date: 27 Jul 2017

Author’s response to reviews:

Editor Comments:

1. Please have the text edited by a professional language editing service or a native English speaking colleague. There are many issues with grammar, wording, spelling, and/or punctuation that need to be addressed.

   Thank you for your advice. The text has been edited by Nature Research Editing Service.

2. Please represent authors' names using their full initials, not their full name, in the Authors’ Contributions section. If there are any duplicated initials, please differentiate them to make it clear that the initials refer to separate authors.

   In “Author Contributions” section, Page 21, Line 20, the author’s names were corrected with their full initials.
3. Please remove the funding information from the Acknowledgements and include it in the Funding section instead. If you have no further acknowledgements please put “Not Applicable” in the Acknowledgements section.

Thank you for your advice. It has been corrected. (Page 22, Line 4)

4. Please upload your additional file in an editable .docx or .xlsx format. Alternatively, please amend your Availability of data and materials statement to say that data will be available from the authors upon reasonable request.

Thank you for your suggestion. The availability of data and materials statement has been added.(Page 22, Line 11)

Reviewer reports:

Claire Perks (Reviewer 1): This is an interesting and important paper for the field.

I have some minor comments:

Dear Professor Claire Perks, I am so thankful to your review about my article. It is very helpful to me.

1. Fig 1B- the blot for 5637 is not very representative- does not look higher than R or B as is indicated in text and fig 1C.

Thank you for your advice. Fig 1B and 1C were replaced.

2. Are the tissues used in Fig 5A just representative ones? Couldn't find this information or how this tissue was processed?

Thank you for your advice. We examined the protein expressions of PLK1 and the five genes in 50 bladder cancer tissues and 20 normal bladder epithelial tissues by western blotting.(Page16, Line5) The data in figure 5A were in 2 normal bladder tissues and 2 bladder cancer tissues. All the data from western blotting were analyzed and showed in figure 5B. In “Materials and Methods -Clinical samples(Page 5, Line1)” it showed that how the cancer and normal tissues were collected and classified. The protocol about western blotting was showed in Page 7, Line17.
Alcides Chaux (Reviewer 2): In this study, authors evaluate the effect of silencing PKL1 expression (a well-known oncogene) in the proliferation, invasion and migration of bladder cell lines (normal urothelium and cancer cells). The study also included the evaluation of these downstream effects in human tissue samples from patients with urothelial carcinoma, in association with clinicopathologic and outcome features. Recommendations for improving the manuscript are provided below:

Dear Professor Alcides Chaux, I am so thankful to your review about my article. It is very helpful to me. Moreover, it makes me to improve my ability how to use statistical methods. Thank you so much.

- **ABSTRACT**: Please include the results of the correlation analyses in the Abstract, so readers will have more information related to the study,

Thank you for your suggestion. The results of the correlation analyses has been added in the Abstract (Page2, Line10).

- **INTRODUCTION**: Please consider rewriting the first paragraph, as the English text is confusing. The second paragraph can be shortened, leaving the length of the information regarding PLK1 to the Discussion. In the last paragraph of the section, do not include the results. Please clarify in one line that the study was carried out with human tissue samples as well as cell lines.

Thank you for your advice. The first paragraph, the second paragraph and the last paragraph have been rewritten. And it was emphasized that the study was carried out with human tissue samples as well as cell lines (Page5, Line19).

- **METHODS**: Please adjust the classification of urothelial carcinomas following the WHO system, i.e., use "papillary urothelial carcinomas" (low or high grade) for non-invasive tumors, and "invasive urothelial carcinomas" for invasive tumors. As it is, the classification given by the authors is not entirely clear. Please also indicate how the human tissue samples were handled, as they can have an impact in the downstream process. In the statistical methods, it is not clear why the authors chose the tests they did, and why they used a mixture of parametric (Student's t test) and non-parametric (Spearman's rank test) tests. Also, the requirement of a P value for "significant differences" must indicate if one or two tails were considered. Please clarify these issues.

Thank you for your suggestion. The classification of Urothelial carcinomas has been corrected (Page5, Line7-14). In the statistical methods, we have corrected our wrong methods (Page11, Line7). Multiple samples were compared using analysis of Variance Analysis. The two-two
comparisons among multiple variables were analyzed using Turkey’s multiple comparisons test. The two-two comparisons between two independent variables were analyzed using Student’s T test. Spearman's rank correlation analysis was used to analyze the correlations between two variables or the variable and the clinicopathological parameters. In the article, we used P value representing the significant differences of two-tailed test (Page 11, Line 6).

- RESULTS: Please describe cell lines that were used in the study in the previous section, not in the Results section. Also, use this section to state the results, avoiding interpretation unless it was deemed necessary for further experimental steps. If so, indicate this in the Methods section. Please be more specific when providing the results of the study; sentences such as "It was determined that PLK1 was involved in the regulation of cell proliferation, invasion and migration by some signaling pathway" are non-informative. Please provide hard data, either in the text or in tables. Finally, in the last subsection ("Association of the protein expressions five key genes with clinicopathologic characteristics of the bladder cancer patients"), new methods are introduced that were not specified in the Methods section. Please describe all the methods in the corresponding section, leaving the Results section for results only, and clarify all the variables in play (v.g., "metastasis" refers to local lymph node metastasis, regional metastasis, or visceral metastasis? How tumor recurrence was defined and assessed?)

Thank you for your advice. In these section, some inaccurate sentences were corrected. In the last subsection, western blotting was used to examine the expression of every protein. There is not new method used. "metastasis" and “recurrence” have been explained in the Material and Methods section (Page 5, Line 17).

- DISCUSSION: Please avoid or edit sentences that do not carry out statement relevant directly to the study (v.g., "Several of these changes have been described, and yet many more are being detected."). "Moreover, there were some researches to prove the role of BUB1B in cancers."

Thank you for your suggestion. In these section, some inaccurate sentences were corrected.

- TABLES: Tables 1-4 can be considered supplementary materials.

Thank you for your advice. Tables have been supplementary materials.

- FIGURES: In Figures 1, 4, 5 and 6, do not use bar plots for comparing numeric variables. Use boxplots or density plots instead. If P values are to be used for drawing conclusions,
please consider adjusting them for family-wise error rates. In Figure 5, the scatterplots show 2 distinctive clusters, indicating that assuming linearity might not be justified. Please review this issue. Also, in the text referring to correlation tests, an R value is provided, while in the plots the same is done for an R-squared value. Please clarify these issues, as the Spearman coefficient and the R2 of the linear correlation model are two different statistics.

Thank you for your advice. Figures 1, 4, 5, 6 have been shown by boxplots. In Table 5, we showed that Turkey’s multiple comparisons test was used to analyze the two-two comparisons among multiple variables. In Figure 5, the reason why there are two distinctive culsters is the samples coming from two groups: normal tissues and bladder cancer tissues.

In figure 5c-5g, the scatterplots have been redrawn by spearman correlation analysis and the new spearman coefficient rho was given. Thank you very much.