Reviewer’s report

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

Version: 0 Date: 18 Jul 2017

Reviewer: Alcides Chaux

Reviewer's report:

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:

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

- **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.

- **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.

- **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?)

- 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."

- TABLES: Tables 1-4 can be considered 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.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

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