Reviewer's report

Title: Bioinformatics analyses on the immune status of renal transplant patients, a systemic research of renal transplantation

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Reviewer: Zhuoyi Huang

Reviewer's report:

The author investigated immune status changes in renal transplant recipients and reported a number of differentially expressed genes (DEGs) but comparing chronic, acute rejection group with stable recipients and healthy samples. Their analysis was based on fifteen datasets from Gene Expression Omnibus (GEO).

The authors need to address following questions.

The major questions are:

- The study reports several DEGs in the renal biopsy of transplant recipients based on microarray datasets with limited sample size in GEO database. The authors should use clinical samples to validate the discovery of DEGs.

- The authors mentioned there are several confounding factors such as innate immunity, inflammation and microcirculation remodeling which complicates the understanding of renal allograft rejection. Is the contribution of these factors properly evaluated in the 15 GEO studies after applying the simplified filtering criteria?

- The description of the datasets is limited in the methods. Since there is no validation, a natural question about the design of current study is, how much will the result of DEGs change with the size of available dataset? Note that the authors arbitrarily removed GSE9493 from seven datasets just because of the low number of DEGs found. This raised the concern about ascertainment bias in the choice of the datasets in GEO which can affect the results. Also, how to evaluate the candidate DEGs in the 5 out of 6 datasets when comparing acute rejection vs stable recipients?
- The authors did not compare their DEGs with existing finding in the literature, which is very important to highlight the novelty of this study, and may also serve as a control set to evaluate the quality of analysis in present study.

- Based on the DEGs discovery in current study, what is new in our understanding of the mechanism or regulators in the process of allograft rejections?

- Most of the DEGs analysis suffer from small sample size in the comparison when stratified down to each case and data set (Table 1 and 2). The statistical significance of each candidate DEG is a big concern and needs to be quantified in text (together with the sample size explicitly mentioned). Specifically,

Page 6, Line 17 (IGHM, IGHV4-31 and IGHG1 were upregulated in chronic rejection patients but not in stable recipients)

- Figure 5B, IGHM and IGHV4-31 in stable are also upregulated on average (there are only 2 samples in the stable group vs 3 in the chronic rejection group, what is the p-value of the difference?).

Page 6, Line 36-39 (Expressions of MAP4K1, LILRB2 and IGHG1 were increased in acute rejection patients but not in stable transplant recipients)

- Figure 5C, from the plot, again there are only two samples of the stable patients in comparison with acute group for gene MAP4k1 and LILRB2, how significant is the fact that the expression in MAP4K1, LILRB2 in stable transplant recipients are not increased?

Page 6 Lin 47 (There were sixteen upregulated genes found in both acute and chronic rejection comparison)

- Figure 5D, most of the expression fold change is within 3-fold, given the small sample size, what is the contribution from the statistical noise?
Page 7, Line 12 (Ofnote, expressions of ... were significantly higher in the acute rejection group than those in the chronic one)

- Can the authors qualify the significance of this comparison (it is not straightforward to estimate from Table 1)?

Page 7, Line 26: There were no DEGs overlapped in combined comparisons.

- This is unexpected. While the authors provided three interpretations in the discussion, it may be helpful to evaluate the ascertainment bias in the GEO datasets and the ethnicity difference in the samples.

Methods:

Page 15, Line 3: both upregulated and downregulated were defined when log FC was higher than 0.5 after correction with a false discovery rate

- What is the false discovery rate and why such a low logFC cutoff was chosen? Since there is no validation in this study, how do the authors access the false positives in the analysis?

Minor questions about the format:

- Figure 2C, 3C, 4C are not legible


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

No
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?
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