Peer Review File

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Reviewer A

Comment 1: What were the DEGs or pathways commonly seen, or what kind of gene profiles or pathways might contribute to differentiating those specific disease onsets compared with others?

Reply 1: Thank you very much for your detailed comments. Considering your suggestion, we compare the pathways of macrophages among the three renal diseases (lupus nephritis, renal crystal formation and renal ischemia-reperfusion injury). Common and unique pathways that were significantly altered in different diseases was generated. Among the 47 pathways identified from all the datasets, the oxidative phosphorylation pathway, VEGF signalling pathway and JAK/STAT signalling pathway were common to all datasets. Of the two pathways unique to the GSE27045 dataset, the renin-angiotensin system pathway is a classical pathway and was thus considered worthy of further study. Of the 34 pathways specific to the GSE51466 dataset, the glycolysis and gluconeogenesis pathways are well known. Of the 13 pathways specific to the GSE75808 dataset, the calcium signalling pathway is a classical pathway. We added the content in result and discussion part.

Changes in the text: we added our text as advised (see Page 3, line 59-64; Page 13-14, line 286-294; Page 15-16, line 319-335; Page 16-17, line 348-354; Page 17-18, line 368-383).

Comment 2: The reason why the authors chose these four datasets is unclear. The species difference should be a huge limitation.

Reply 2: To reveal the role of macrophages involved in different renal diseases, we simply focus on the expression profiling by array data of renal macrophage. Thus, after searching the GEO database using the keywords ‘renal’ and ‘macrophage’, we found three GEO datasets (GSE27045, GSE51466 and GSE75808) that met our criteria. The samples were from murine models.

Changes in the text: we added our text as advised (see Page 6-7, line 131-134).

Comment 3: Some methodology needs to be elaborated. Methods for normalization and background adjustment of GEOdata, type of statistical test in GEO2R, and correction types used for GO and KEGG pathway enrichment should be explained.

Reply 3: Since the three chosen GEO dataset were founded on different array, we analyzed the differentially expressed genes respectively, instead of merging them by normalization and background adjustment. Our statistical tests for identifying differentially expressed genes data was analyzed using the GEO database's GEO2R tool.

GEO2R identifies the differentially expressed genes by variance analysis and t-test using the R project, Linear Models for Microarray Analysis (limma) R package, for statistical computing. The differentially expressed genes were then analyzed with DAVID online database(http://david.ncifcrf.gov) to identify enriched GO terms and KEGG pathways with Benjamini-Hochburn corrected P value. In our figure, we showed the most highly significant pathways or terms by ranking raw p value, which was not conflict with adjust p value.
Changes in the text: we added our text as advised (see Page 9, line 178).

Comment 4: M1/M2 classification of macrophages is outdated. The authors should make reclassification them based on the most recent relevant guideline.
Reply 4: Thank the reviewer for this important comment. We also recognized that M1/M2 classification of macrophages were not accurate. Recent studies suggest that there is a spectrum of macrophage activation states. The characteristic of macrophage activation states in vitro should be defined in another way. Thus, we focus on the signaling pathways involved to describe activation states of renal macrophage. Due to the unclear markers of M1 and M2 macrophage, we delete the heatmap in the result part.
Changes in the text: we modified our text as advised (see Page 4-5, line 82-89).

Comment 5: Please explain the details of each GEO dataset. (Affymetrix GeneChip or Agilent sureprint…)
Reply 5: The detail of each GEO dataset has been added, including GSE27045 dataset (Affymetrix GeneChip Mouse Genome 430 2.0 Array), GSE51466 (Agilent-028005 SurePrint G3 Mouse GE 8x60K Microarray) and GSE75808 (Affymetrix Mouse Genome 430 2.0 Array).
Changes in the text: we added our text as advised (see Page 7, line 139-142; Page 7, line 148-150; Page 7-8, line 154-156).

Comment 6: “GO pathway” is not a pathway. Please simply rephrase it as “GO”.
Reply 6: “GO pathway” has been modified to “GO”.
Changes in the text: we modified our text as advised in Page 10, line 202.

Comment 7: Is there any relevant human GWAS report that investigates or summarizes similar renal disease/ macrophages? Considering the audience of TAU (I assume most of them are urologists, clinicians), interpretation of this bioinformatic analysis should be simplified or compared/ connected to renal diseases, which we are well-known clinically.
Reply 7: Thank you very much for your suggestion. We failed to find the relevant

content in GWAS. To connect the result of bioinformatic analysis to renal diseases, we added specific pathway involved in each renal disease in the discussion part.
Changes in the text: we added our text as advised (see Page 15-16, line 319-335; Page 16-17, line 348-354; Page 17-18, line 368-383).

Reviewer B
Comment 1: the manuscript would benefit if it would focus on a specific research question, e.g. comparing the activity of a specific set of pathways in different datasets of the same kidney condition (instead of summing up results of very different datasets).
Reply 1: Thank you very much for your detailed suggestion. We compare the pathways of macrophages among the three renal disease (lupus nephritis, renal crystal formation and renal ischemia-reperfusion injury). Common and unique pathways that were significantly altered in different diseases was generated. Among the 47 pathways identified from all the datasets, the oxidative phosphorylation pathway, VEGF signalling pathway and JAK/STAT signalling pathway were common to all datasets. Of the two pathways unique to the GSE27045 dataset, the renin-angiotensin system pathway is a classical pathway and was thus considered worthy of further study. Of the 34 pathways specific to the GSE51466 dataset, the glycolysis and gluconeogenesis pathways are well known. Of the 13 pathways specific to the GSE75808 dataset, the calcium signalling pathway is a classical pathway. We added the content in result and discussion part.
Changes in the text: we added our text as advised (see Page 3, line 59-64; Page 13-14, line 286-294; Page 15-16, line 319-335; Page 16-17, line 348-354; Page 17-18, line 368-383).

Comment 2: Please shorten your introduction by 30%, same for your abstract
Reply 2: Considering your suggestion, introduction and abstract has been shortend.
Changes in the text: we have modified our text as advised (see Page 3, line 59-64; Page 4-6, line 73-126).

Comment 3: The use of the "M1" and "M2" classification is slowly becoming obsolete, because the distinction between macrophage phenotypes may not be as clear. You might consider to change these definitions.
Reply 3: Thank you for this important comment. We also recognized that M1/M2 classification of macrophages were obsolete. Recent studies suggest that there is a spectrum of macrophage activation states. The characteristic of macrophage activation states in vitro should be defined in another way. Thus, we focus on the signaling pathways involved to describe activation states of renal macrophage.
Changes in the text: we modified our text as advised (see Page 4-5, line 82-89).

Comment 4: Please specify the systematic approach by which the datasets were selected (why these?)

Reply 4: To reveal the role of macrophages involved in different renal diseases, we simply focus on the expression profiling by array data of renal macrophage. Thus, after searching the GEO database using the keywords 'renal' and 'macrophage', we found three GEO datasets (GSE27045, GSE51466 and GSE75808) that met our criteria.

Changes in the text: we added our text as advised (see Page 6-7, line 131-134).

Comment 5: Don't only show the p-value for every pathway, please include an abundancy score, as it is more relevant.

Reply 5: The normalized enrichment score (NES) was added in the result of Gene Set Enrichment Analysis (GSEA).

Changes in the text: we added our text as advised (see Page 10-11, line 215-222; Page 12, line 245-255; Page 13, line 278-285).

Comment 6: Did you correct for multiple testing.

Reply 6: The differentially expressed genes were analyzed with DAVID online database(http://david.ncifcrf.gov) to identify enriched GO terms and KEGG pathways with Benjamini-Hochburn corrected P value. In our figure we showed the most highly significant pathways or terms by ranking raw p value, which was not conflict with adjust.p value.

Changes in the text: we added our text as advised (see Page 9, line 178).

Comment 7: In GSE65326, bulk RNA from biopsies was sequenced. This seems to be a random choice of dataset if you want to study macrophage phenotypes.

Reply 7: Thank the reviewer for this important comments. We've recognized that GSE65326 should not be involved in our study that focused on macrophage. Therefore, we deleted it.

Changes in the text: we deleted the text as advised in abstract, introduction, result and discussion part.

Comment 8: Please elaborate on the methods by which RNA was sampled in all datasets (see STROBE q8).

Reply 8: In the GSE27045 dataset, F4/80^{hi} macrophages cells were isolated from NZB/W mice at early stage of lupus (young), during lupus nephritis (sick) and after induction of remission (rem) using flow cytometry. RNA was extracted and processed for hybridization on Affymetrix GeneChip Mouse Genome 430 2.0 Array. In the GSE51466 dataset, renal CD11b^{+}/CD11c^{+} macrophages in stone model mice and CSF-

1-deficient(op/op) stone model mice were sorted and investigated on Agilent-028005 SurePrint G3 Mouse GE 8x60K Microarray. In the GSE75808 dataset, $CD11b^{+}/Ly6C^{+}$ monocyte/macrophage populations were sorted by flow Cytometry at 4 hour, 1 day and 9 day after AKI. Samplea were processed based on Affymetrix Mouse Genome 430 2.0 Array.

Changes in the text: we added our text as advised (see Page 7, line 139-142; Page 7, line 148-150; Page 7-8, line 154-156).