Peer Review File

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Reviewer 1:

Comment 1: The background of the abstract was lack of background, but started with aims and a bit of method.

Response: Thanks for your comment. We felt sorry for not introducing background in the beginning of the abstract. We have added a brief background in the section in **page 3, line 40-45, colored red**.

Changes in the text: In the field of transplantation, inducing immune tolerance in recipients is of great importance. Blocking co-stimulatory molecule using anti-CD28 antibody could induce tolerance in a rat kidney transplantation model. Myeloid-derived suppressor cells (MDSCs) reveals strong immune suppressive abilities in kidney transplantation. Here we analyzed key genes of MDSCs leading to transplant tolerance in this model.

Comment 2: Step by step bioinformatics analyses were very clearly demonstrated, however there was lack of supportive analytic evidence biologically.

Response: We are thankful for your advice and recognized there was a lack of biological analytic evidence. However, in this manuscript, we are aimed to put forward key genes, corresponding proteins, and their functions of MDSC in induced immune tolerance rat models. Our results may provide the valuable background for both basic and clinical research and could be the direction of further studies.

Although we did not test the hub genes in animal models in this manuscript, we have reviewed the work by other experts. We found that these genes have strong relationship with cell function and immune tolerance, and we added these findings in our manuscript. We added this part in **page 11-12, line 252-267, colored blue.** We hope these work could meet the requirement of biological analytic evidence.

Changes in the text: By binding of tSH2 domain and the doubly-phosphorylated ITAM motifs of CD3 chains, ZAP 70 is recruited in the TCR complex (26), and contributed to T cell-mediated immunological diseases (27). The frequency of ZAP-70 cells were significantly correlated with M-MDSCs level (28). GzmB expression was found in both mice and human MDSC, B16F10 melanoma cells decreased in invasive potential co-cultured with perforin/GzmB$^{-/-}$ MDSCs (29). STAT4 are major hubs regulating MDSC-derived macrophages in anti-tumor process (30). Another study focusing on in head and neck squamous cell carcinoma showed STAT1 inhibits MDSC accumulation in T cell-mediated antitumor immune responses (31). For example, CCL5 was shown to contribute to MDSC immunosuppression by establishing a graft-to-periphery CCL5 gradient in tolerant kidney allograft recipients which controlled recruitment of Tregs to the graft, where they likely participated in maintaining tolerance (15). Another study suggested that administration of resveratrol into IL-10$^{-/-}$ mice induced immunosuppressive CD11b^{+}/Gr-1^{+} MDSCs in the colon, which correlated with reversal of established chronic colitis and downregulation of mucosal and systemic CXCR3^{+}-expressing effector T cells as well as inflammatory cytokines in the colon (32).

Comment 3: There is no any type of validation for suggested key genes, so it is difficult to obtain any convincing conclusion.

Response: As the former comment, lack of biological evidence led to shortage of convincing conclusion. Although we did not have validation test of the hub genes by ourselves, we added the findings about the key genes found in our work that related with the other immune cells and the results from the others' work. We suppose this might be the validation for the key genes, and we added it in the discussion part, in **page 11-12, line 252-267, colored blue.**

Comment 4: There should be a space between "word and (", such as a correct format "responses (8)", but many others such as "myeloid cells(7)", "tyrosines(9)" "tolerance(10, 11) " should be changed.

Response: We have made corresponding modifications in the text. Thank you.

Comment 5: "…the induction of immune tolerance by using anti-CD28" should be "…the induction of immune tolerance by anti-CD28". The title may also need to be changed accordingly.

Response: The sentence and the title of the manuscript were corrected.

Changes in the text: **In Page 11, Line 250-251, colored red:** In addition, evidence indicates that some DEGs may contribute to the induction of immune tolerance by anti-CD28.

The title was also corrected: MDSC key genes analysis in rat anti-CD28-induced immune tolerance kidney transplantation.

Reviewer 2:

Thanks for your positive comments.

Comment 1: Why do the authors select GSE28545, not other datasets? Please provide the selection process.

Response: Thank you for the remind and we felt sorry that we missed the description of the reasons for choosing GSE28545. GSE28545 is the only dataset in GEO database focusing on the difference between MDSC in syngenic and tolerant transplantation models, also having great data integrity. Following your requirement, we added the reason for choosing this dataset in **page 6, line 111-115, colored blue.**

Changes in the text: GSE28545 (species: Rattus norvegicus; platform: GPL2996; samples: GSM706861~GSM706865) (11), which compared the levels of blood MDSCs from three tolerant allogeneic kidney transplant recipients with two syngenic kidney transplant recipients at 100 days post-transplantation, which is the only dataset in GEO database focusing on the following issue with great data integrity.

Comment 2: The authors show Zap70, Stat1, Stat4, Gzmb, Pcna, Ccl5 and Cxcr3 with more than 10 degrees of connectivity. What's the value of this result? Please elaborate the meaning of this result in Discussion.

Response: Calculated by cytoscape, we found the genes with the most linked with other genes, some of which were never reported in the research of MDSC (like Pcna), and some were found connectivity with MDSC. The others link with MDSC were further discussed in Discussion in **page 11-12, line 252-267, colored blue.**

Changes in the text: By binding of tSH2 domain and the doubly-phosphorylated ITAM motifs of CD3 chains, ZAP 70 is recruited in the TCR complex (26), and contributed to T cell-mediated immunological diseases (27). The frequency of ZAP-70 cells were significantly correlated with M-MDSCs level (28). GzmB expression was found in both mice and human MDSC, B16F10 melanoma cells decreased in invasive potential co-cultured with perforin/GzmB$^{-/-}$ MDSCs (29). STAT4 are major hubs regulating MDSC-derived macrophages in anti-tumor process (30). Another study focusing on in head and neck squamous cell carcinoma showed STAT1 inhibits MDSC accumulation in T cell-mediated antitumor immune responses (31). For example, CCL5 was shown to contribute to MDSC immunosuppression by establishing a graft-to-periphery CCL5 gradient in tolerant kidney allograft recipients which controlled recruitment of Tregs to the graft, where they likely participated in maintaining tolerance (15). Another study suggested that administration of resveratrol into IL-10$^{-/-}$ mice induced immunosuppressive CD11b^{+}/Gr-1^{+} MDSCs in the colon, which correlated with reversal of established chronic colitis and downregulation of mucosal and systemic CXCR3^{+}-expressing effector T cells as well as inflammatory cytokines in the colon (32).

Comment 3: What's the difference between Fig.4 and Fig.5? Is Fig.5 a branch of Fig.4? In my opinion, Fig.5 only shows the top 15 hub genes again compared with Fig.4.

Response: Fig. 5 is a branch of Fig. 4, showing the hub genes in the whole cluster. In Fig. 4, there are 192 nodes and 469 edges in total, we showed a whole picture of the cytoscape and the readers can find any interested DEG and their correlation with other nodes as they want. However, as we were concerned, there must be a key cluster in the panorama, which showed the most important genes, probably mainly contributing to the anti-CD28-induced immune tolerance. Precisely calculated by cytoscape, the hub genes corelating mostly with other genes were presented in Fig. 5, the text mining was

based on these genes and were presented in Discussion. Thus, we consider Fig. 5 necessary in this manuscript. The reason we made Fig. 5 was added in the manuscript in **page 9, line 195-198, colored blue.**

Changes in the text: Precisely calculated by cytoscape, these genes have the strongest interactions with each other (Figure 5) and the other DEGs, and formed the key cluster of the whole PPI interaction, so we predict they will play key roles in immune tolerance in the case.

Comment 4: The authors may show the KEGG pathway graph of the most important hug genes using clusterProfiler package in R.

Response: We have already put the hub genes of KEGG pathway in Table 1, the last list "Genes" showed the related genes of different pathways, and these genes were selected by DAVID, an online bioinformatic database, which could make the analysis more efficiently.