nature portfolio

Peer Review File

Single-cell immune profiling and validation of PBMCs in the onset of and recovery from herpes zoster

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This is an important initial study to understand the heterogeneity in gene expression responses to herpes zoster, a common infection that occurs in many immunocompromised individuals.

However, there are several parts of this study that need to be improved, including a potential mechanism and better in depth analysis (UMAP plots only provide information on clustering as they are designed to find associations).

Overall, we recommend either adding ATAC-seq analysis or increasing the number of patients in this study as this seems more like a preliminary study with surface level analysis and dependence on KEGG ontology analysis.

Additional recommendations for the manuscript:

We recommend rewriting the abstract: highlight major findings, don't point out numbers of samples, and provide enough background about the purpose of the study.

Your audience is more general than a traditional medical journal. Therefore, the introduction needs to be rewritten to provide better context for readers who may understand immunity but not the specifics of HZ. For example, introduce the infection – define its nucleic acid content, lifecycle, relation to chicken pox, etc. and put in the context of other herpes viruses that have been well studied. Then discuss postherpetic neuralgia, symptoms, complications, etc.; how much of the population (worldwide?) does this affect? What about patients that are inoculated against chicken pox? What does the literature say about latency of infection? Discuss what is known about infection with HZ (activation of immune cells seems to fairly standard compared to other herpes viruses) – you already have this content in the intro, it just needs to be moved. Then discuss susceptibility to getting the infection in immunocompromised patients – what does the current literature say? Is this a common infection the co-occurs with other morbidity? Then the purpose of the study is to identify... etc.

Make sure to provide information on acronyms

Don't summarize all of your findings in the introduction – 1-2 broad statements about what you found is appropriate, while setting up the reader for the results – some of the statements in the last paragraph of the intro can go in the abstract

Line 19 add "herpes zoster infection"

Given the audience, explain "postherpetic neuralgia"

Results:

The study design could be better presented in the first section

Use the same acronyms throughout (example – HZ and HP are used interchangeably for herpes zoster)

What was the timing of blood draw? What stage of infection for HZ? Have the three healthy donors never had HZ or just

currently did not have HZ when the blood draw occurred? Provide more details

Unclear what “mirroring changes” are..

Are the three patients in the first paragraph different from the 6 patients in the onset or recovery from HZ infection? Why are there a different number of cells 66K versus 42K? Are there only 3 patients? No need to provide means when you can provide a table of their ages, etc.

Too broad of conclusions drawn from UMAP analysis -- we recommend going back to bulk expression once you have identified the subcluster to delineate changes among groups.

Further, what new information does the scRNA-seq analysis tell us over flow data or bulk RNA seq? We already knew that there were different populations of cell types. This information is lost in the text of the results.

There are several typos and grammatical errors throughout the text; we recommend having an editor read through to fix and rewrite portions of the manuscript.

Reviewer #2

(Remarks to the Author)
In this work, the authors investigate the landscape of immune cells in the pathogenesis of herpes zoster virus infection through single-cell RNA-Seq. By analyzing peripheral blood mononuclear cells (PBMCs) at different stages of the disease, researchers identified changes in the immune cell profiles during the onset and recovery phases of herpes zoster. These alterations included shifts in the proportions of various cell subpopulations such as monocytes, B cells, CD8+ effector T cells, and neutrophils. They also validated their discoveries with routine blood data from herpes zoster patients and flow cytometry analyses. Overall, the results here present a useful resource for the research community if the analyses are conducted properly. The paper needs the following improvement:

Major comments:
1. Since the major dataset of this paper is the single-cell RNA-Seq dataset. However, I did not find statements of the data availability. Without the available dataset, the reviewer will not be able to judge the data quality. The authors should add the statement of data availability.
2. The paper's conclusion heavily relies on the data analyses of the scRNA-Seq. The authors did not provide the scripts to reproduce the analyses, making it hard to judge whether the analyses were conducted properly. The authors should add the statement of code availability.
3. Many of the comparisons required statistical analyses (e.g., Fig 2h-k; Fig 3d-f; Fig 4d)to make sure the changes observed are statistically significant (e.g., whether it is consistent among the three individual patients).
4. When the authors t-tests or u-tests to perform DEG analyses, they should state whether the p-values used are FDR-corrected or not.

Minor comments:
The figure quality can be improved by making all figure fonts consistent and font sizes not too small.

Version 1:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
I want to thank the authors' efforts addressing my previous comments. However, they still do not provide the scripts or codes in the current version. Since this paper's conclusions are heavily relied on data analyses, the results will not be easily reproducible without the data analyses scripts. I strongly recommend the authors providing their data analyses codes even they used routine pipelines before this paper get accepted.

Dear Reviewers

We would like to thank you for careful and thorough reading of this manuscript and for your thoughtful comments and constructive suggestions, which have helped to improve the quality of this manuscript. Our responses are as follows:

Reviewer #1

1. This is an important initial study to understand the heterogeneity in gene expression responses to herpes zoster, a common infection that occurs in many immunocompromised individuals.

However, there are several parts of this study that need to be improved, including a potential mechanism and better in-depth analysis (UMAP plots only provide information on clustering as they are designed to find associations).

Overall, we recommend either adding ATAC-seq analysis or increasing the number of patients in this study as this seems more like a preliminary study with surface level analysis and dependence on KEGG ontology analysis.

Answer: We appreciate your valuable comments. Your input has helped shape the article and added to the depth of research and understanding within.

We collected blood samples from three patients with herpes zoster and three healthy controls for ATAC-seq analysis, through which we revealed that the top 10 motifs enriched in the HP group were mainly from ZF transcription factor family and ETS transcription factor family, both were previously reported to have a role in herpesvirus infection. We also integrated scRNA seq and ATAC-seq at bulk level and revealed that among all motifs with increased chromatin accessibility and corresponding genes, the expression of some key factors including IGTAM, IFNG, and TLR were upregulated, and chromatin accessibility was also increased. Furthermore, we found that the activation of T cells and neutrophils were highlighted in HP by the motif enrichment analysis. This also reinforces the critical role of T cells and neutrophils in herpes zoster infection.

To further deepen the mechanistic understanding of herpes zoster infection, we also performed Pseudotime analysis using single-cell data to further identify t-cell subpopulations that change during development. Furthermore, a significant difference was found between HA, HP and RP (Fig. 5m). The cells from HA group were mainly concentrated in state1, while the HP and RP group were primarily enriched in state2 and state3. By cell communication analysis, we found that rps19_C5AR1 was enriched in t-cell and neutrophil interactions. Neutrophils exhibited a tendency to

communicate with MPs and platelets (Fig. 6g). Among the identified interactions, a significant number were associated with CXCL8 and its corresponding receptor CXCR1.

2. We recommend rewriting the abstract: highlight major findings, don't point out numbers of samples, and provide enough background about the purpose of the study.

Answer: We appreciate the reviewer's suggestion. We have rewritten the abstract.

3. Your audience is more general than a traditional medical journal. Therefore, the introduction needs to be rewritten to provide better context for readers who may understand immunity but not the specifics of HZ. For example, introduce the infection – define its nucleic acid content, lifecycle, relation to chicken pox, etc. and put in the context of other herpes viruses that have been well studied. Then discuss postherpetic neuralgia, symptoms, complications, etc.; how much of the population (worldwide?) does this affect? What about patients that are inoculated against chicken pox? What does the literature say about latency of infection? Discuss what is known about infection with HZ (activation of immune cells seems to fairly standard compared to other herpes viruses) – you already have this content in the intro, it just needs to be moved. Then discuss susceptibility to getting the infection in immunocompromised patients – what does the current literature say? Is this a common infection the co-occurs with other morbidity? Then the purpose of the study is to identify... etc.

Answer: We appreciate the reviewer's suggestion. We've rewritten the introduction.

4. Make sure to provide information on acronyms

Answer: Thank you for your careful work. We have made the correction accordingly and added acronyms on page 19 line 23.

5. Don't summarize all of your findings in the introduction – 1-2 broad statements about what you found is appropriate, while setting up the reader for the results – some of the statements in the last paragraph of the intro can go in the abstract

Answer: Thank you for your careful work. We have made the correction accordingly in the introduction.

6. Line 19 add "herpes zoster infection"

Answer: Thank you for your careful work. We have made the correction accordingly.

7. Given the audience, explain "postherpetic neuralgia"

Answer: Thank you for your careful work. We have made corresponding corrections on page 2, line 4.

8. The study design could be better presented in the first section

Answer: We appreciate the reviewer's suggestion. We've rewritten the study design on page 3, line 22- page 4, line 2.

9. Use the same acronyms throughout (example – HZ and HP are used interchangeably for herpes zoster).

Answer: Thank you for your careful work. We have made the correction accordingly. We used herpes zoster as an abbreviation for herpes zoster and hp as an abbreviation for herpes zoster patient. As per your suggestion, we will use the entire time rather than the acronym to refer to herpes zoster to avoid confusion.

10. What was the timing of blood draw? What stage of infection for HZ? Have the three healthy donors never had HZ or just currently did not have HZ when the blood draw occurred? Provide more details. Unclear what "mirroring changes" are.

Answer: Thank you for your careful work. The time point of blood collection in the patient with herpes zoster (HP) group was at the patients' initial visit to the outpatient clinic, which was in the onset stage of confirming the diagnosis of herpes zoster. Healthy controls did not have herpes zoster infection at or before the time of blood collection. To serve as the patient recovered from herpes zoster (RP) group, the blood sample after recovery were collected 90 days after first negative diagnose date.

We are sorry for unclear description and thank you for careful proofreading. It turns out that we had misspelled minor as mirror, and the clerical error has been addressed.

11. Are the three patients in the first paragraph different from the 6 patients in the onset or recovery from HZ infection? Why are there a different number of cells 66K versus 42K? Are there only 3 patients? No need to provide means when you can provide a table of their ages, etc.

Answer: We are sorry for unclear description and thank you for picking it up. In addition to the 3 control samples, there are 6 samples collected in total, which included three samples whose blood sample were collected at their initial visit to the outpatient clinic to serve as HP group, and three samples whose blood sample were collected 90 days after first negative diagnose date to serve as RP group.

42K cells is the total number of cells of 6 samples from HP group and RP group. And 66k cell include 9 samples from HP group, RP group and HA group.

The 6 samples mentioned above were from 4 patients. The three HP samples collected were from three individual patients at their initial visit to the outpatient clinic, which was in the primary stage of confirming the diagnosis of herpes zoster infection. Two

patients in the HP group had blood taken after recovery to serve as samples for the RP group, however, the third patient from the HP group dropped out of group, and we had an additional case added to the RP group.

12. Too broad of conclusions drawn from UMAP analysis -- we recommend going back to bulk expression once you have identified the subcluster to delineate changes among groups.

Answer: Thank you for the productive proposal, we fully agree, and we have now directly followed your advice.

13. Further, what new information does the scRNA-seq analysis tell us over flow data or bulk RNA seq? We already knew that there were different populations of cell types. This information is lost in the text of the results.

Answer: Thanks for your insightful and professional advice. We'd like to improve our work by following your professional advice. Single-cell sequencing differs from traditional high-throughput sequencing in that it is performed on a single cell within a population of cells. It has the advantage of accurately analyzing the gene expression of each cell, being able to accurately differentiate between cell populations and perform inter-cell classification comparisons, as well as being able to find the expression of rare cells. Based on the existing enrichment analysis, we have added Pseudotime analysis and cell communication analysis, which can help us better understand the mechanism of herpes zoster infection and increase the depth of research. As limited by the length and scope of this paper, we decided to concentrate our work in the subpopulations with more significant differences, there are some results we hope to include in the next paper.

14. There are several typos and grammatical errors throughout the text; we recommend having an editor read through to fix and rewrite portions of the manuscript.

Answer: We have asked a native English speaker to polish the manuscript thoroughly.

Reviewer #2

1. Since the major dataset of this paper is the single-cell RNA-Seq dataset. However, I did not find statements of the data availability. Without the available dataset, the reviewer will not be able to judge the data quality. The authors should add the statement of data availability.

Answer: The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA008316) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa-human. We added this information to the revised manuscript on page 19 line 4.

2. The paper's conclusion heavily relies on the data analyses of the scRNA-Seq. The authors did not provide the scripts to reproduce the analyses, making it hard to judge whether the analyses were conducted properly. The authors should add the statement of code availability.

Answer: We appreciate your suggestion. We have added the statement of code availability on page19, line 11- line 13.

3. Many of the comparisons required statistical analyses (e.g., Fig 2h-k; Fig 3d-f; Fig 4d) to make sure the changes observed are statistically significant (e.g., whether it is consistent among the three individual patients).

Answer: We appreciate the valuable advice provided and completely agree with your comments. Comparative analysis between groups was added and performed using Student's t-tests, with a threshold for statistical significance set at $p < 0.05$.

4. When the authors t-tests or u-tests to perform DEG analyses, they should state whether the p-values used are FDR-corrected or not.

Answer: Thank you very much for your professional advice. We have taken your advice and specified corrected p-values were label as p.adj in the paper.

5. Minor comments: The figure quality can be improved by making all figure fonts consistent and font sizes not too small.

Answer: Concerning figure quality, we fully agree, and we have now directly followed your advice.

Dear Reviewer;

Thank you very much for reviewing our manuscript entitled “Single-cell immune profiling and validation of PBMCs in the onset of and recovery from herpes zoster” (manuscript ID: COMMSBIO-24-1993A). The comment was valuable and very helpful for revising our paper and improving our research. We have carefully made point-by-point responses to all the comments as listed below and have revised the manuscript accordingly. The changes in the revised manuscript are highlighted in red. We hope that our responses address all the concerns from the reviewer and that the manuscript is now acceptable for publication.

I want to thank the authors' efforts addressing my previous comments. However, they still do not provide the scripts or codes in the current version. Since this paper's conclusions are heavily relied on data analyses, the results will not be easily reproducible without the data analyses scripts. I strongly recommend the authors providing their data analyses codes even they used routine pipelines before this paper get accepted.

Answer: We appreciate this valuable advice. These points would greatly improve the reliability of our results. This article contains no original code. The codes used in this article are shown in https://github.com/zhengshang1300/Single-cell-immune-profiling-and-validation-of-PBMCs-in-the-onset-of-and-recovery-from-herpes-zoster. It has been added on Page 19 Line 17 in revised manuscript. And we sincerely hope that the revisions can meet your requirements and make the revised manuscript more suitable to be published in Communications Biology.