Cell Systems

Transparent Peer Review Record

SYLARAS: A Platform for the Acquisition, Statistical Analysis, and Visual Display of Systemic Immunoprofiling Data and its Application to Glioblastoma

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Summary

Initial Submission: Received Dec. 16, 2019
Preprint: https://doi.org/10.1101/555854
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Scientific editor: Quincey Justman, Ph.D.

First round of review: Number of reviewers: Three
Three confidential, zero signed
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Data freely available: Yes
Code freely available: Yes

Editorial decision letter with reviewers' comments, first round of review

Dear Peter,

I hope you and yours are doing ok during this difficult time. I'm very pleased to let you know that the reviews of your revised manuscript are back, the peer-review process is complete, and only a few minor, editorially-guided changes are needed to move forward towards publication.

In addition to the final comments from the reviewers, I've made some suggestions about your manuscript within the "Editorial Notes" section, below. Please consider my editorial suggestions carefully, ask any questions of me that you need, make all warranted changes, and then upload your final files into Editorial Manager. We hope to receive your files within 5 business days, but we recognize that the COVID-19 pandemic may challenge and limit what you can do. Please email me directly if this timing is a problem or you're facing extenuating circumstances.

As you look forward to acceptance, please do consider submitting one of the protocols you've developed in this paper to STAR Protocols, or extending this offer to one of your trainees. STAR Protocols is geared towards trainees and its key purpose is to provide complete and consistent instructions for how to conduct reproducible experiments. If you have any questions, please email starprotocols@cell.com.

I'm looking forward to going through these last steps with you. More technical information can be found below my signature, and please let me know if you have any questions.

All the best,

Quincey

Quincey Justman, Ph.D.
Editor-in-Chief, Cell Systems

Editorial Notes

General Overview: The flow cytometry in this paper is an absolute tour de force, as I'm sure I don't have to tell you! We also see the "platform" quality of this paper as its key strength. Figure 7, though, is problematic. There are two key problems, which I'll list, along with a suggestion for how to solve them.

> Problem 1: Absent functional validation, the RNAseq data are really weak. We're also simply not inclined to publish gene lists generated by comparatively cursory analysis.
>
> Solution 1: Remove the RNAseq data from the manuscript, reserving it for future work. Also, I'm inclined to think that the microscopy goes further towards suggesting function, and it's also a nice complement to the flow data. I suggest moving parts of Fig. S8 to Fig. 7.
>
> Problem 2: We agree with Reviewer 3 that the human data are simply too weak to include at this stage.
>
> Solution 2: Remove the human data from the paper.

These changes need to be reflected in the title, abstract, and text of the paper as well. I'll send you a .docx file indicating the needed changes; thankfully, they are very clear cut.

Finally, to address Reviewer 3's concerns about study design, please include a Limitations section of your Discussion that speaks to choices made and not made.

Thank you!

Reviewer comments:

Reviewer #1: This review is in reference to the manuscript entitled, 'Systemic immune response profiling with SYLARAS implicates a role for CD45R/B220+ CD8+ T cells in glioblastoma immunology'.

Overall, the manuscript is written very well. It also presents interesting new data highlighting the intratumoral accumulation of CD45R/B220+ CD8+ T cells. The one major feature the authors do not show data for is what effect, if any, the depletion of these cells have on overall GBM immunology. Showing this will be critical, as the current observations are only descriptive and associative.

Overall, however, this is very nice and comprehensive work.

Reviewer #2: An extremely thorough immune profiling with traditional fluorescence-based cytometry. An very complete "pipeline" for others to use in similar mouse models or human cases-- so no need to create ones own software system. In addition, for GBM, this is a beautiful resource that provides a baseline for study or other inquiries.

My only comments are minor and should not affect acceptance of the manuscript.

1. Some mention of how difficult or easy it would be to scale up the single cell measurements (in terms of the software) for CyTOF and coming split pool multiplexing of antibodies to replace CyTOF. Does one have a similar ease of use of the software?
2. They should mention somewhere, frankly, that bar-coding of the wells could have enabled more consistent staining of their samples. It doesn't take away from their general software pipeline, nor the final results, but barcoding of the samples is a proven winner for minimizing sample to sample variation.

Reviewer #3: The immune phenotype associated with glioblastoma remains a very active area of investigation. This manuscript employs the SYLARAS software tool (SYstemic Lymphoid Architecture Response ASsessment) with a data set collected with SYLARAS. This effort quantified immune cells in primary and secondary lymphoid organs and in the tumor microenvironment of mice engrafted with a single syngeneic glioblastoma cell line. This is an excellent start to the effort, but there are substantial issues that should be addressed prior to further consideration.

Major concerns:

1. The entire manuscript effort really focuses on a single cell line (GL261). The Glioma 261 (GL261) orthotopic model for murine glioma was established in 1970 via chemical induction with methylcholanthrene. This model and others were reviewed in: Oh T, Fakurnejad S, Sayegh ET, Clark AJ, Ivan ME, Sun MZ, Safaee M, Bloch O, James CD, Parsa AT. Immunocompetent murine models for the study of glioblastoma immunotherapy. J Transl Med. 2014 Apr 29;12:107. GL261 has been widely used in many studies, but, like all models, has significant limitations. It harbors a Kras mutation, which is uncommon in adult human glioblastoma. It also appears to have greater response to immunotherapies than human tumors. Although there was very limited validation in human glioblastomas of the findings, it is really essential to validate in additional murine models. The choices would include other syngeneic models (CT-2A, GL26, GSC005, etc.) or genetically engineered models (GEM).
2. The GL261 growth is extremely fast (as has been previously reported). Even the "late" stage is not very long and very different than the growth patterns in human tumors. This brevity of growth likely alters the immune responses.
3. Syngeneic tumor grafts require traumatic inoculation. The vehicle control is reasonable, but another cell type (e.g. fibroblasts, etc.) would have been a better control.
4. Cell lines are inherently different than human tumors. A human tumor develops with progressive accumulation of mutations and continuous adaptation to the immune response. Cell lines are already fully formed cancers and do not share this evolution. GEMs would be useful to consider.
5. The multidimensional flow cytometry is a powerful technique, but it would be helpful to consider orthogonal analysis with single cell RNA sequencing, either by their own efforts or the multiple published reports.
6. The title, abstract and text imply function of the B220+ cells. The absence of functional data suggests that this set of claims is premature. The writing is overly definitive.
7. The transcriptional comparisons are not very sophisticated or deep. Finding some differences is not very useful, as any comparison of two groups will find differences. The questions would be how difference are they and whether there are functional differences.
8. The human data are not rigorous. The tissue microarray is useful, but small areas of tumor are simply not complete pictures. Glioblastomas are very heterogeneous. Also, immunostaining will show differences in cell populations from flow cytometry.