Reviewer’s report

Title: Estimation of immune cell content in tumor using single-cell RNA-seq reference data

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Reviewer: Guy Adami

Reviewer's report:

Estimation of immune cell content in tumor using single-cell RNA-seq reference data by Yu et al is an extremely well written paper that covers a method to use reference GEP matrices from SC RNAseq data of individual cell types, to deconvolute bulk tumor gene expression data. By combining t-SNE and ssGSEA the authors enhanced methods for differentiating T cell types in HNSCC samples.

Most of the analyses here are with simulated tumors not bulk tumor tissue. With scRNAseq and RNAseq of sorted cells there is a similar lag in time between enzymatic separation and isolation for single cells, cell sorting and placement in an RNA preservative. This at least in theory would make bulk tumor RNA levels for some genes different than that in sorted cells. Of course the gene lists used in the study to separate cell types, may already accommodate this problem.

Figure S 10 is very important to show the method works with bulk TCGA tumors. If there is some point for the survival curve, S11 it should be made as it is already well know that oral pharynx tumors with HPV are associated with longer survival.

Page 6 Results Line 1 "However, results from scRNA-seq data suggests that the overall Treg expression signature may be underrepresented in genomic projects that are biased towards tumors with higher purity, such as TCGA."

This point is likely to be true, but the alternative of studying samples with variable amounts of stroma is probably not ideal either unless similar methods to those described here can be used estimate tumor and stromal fractions in each sample. May want to address in the discussion. Histopath of tumors reveals lots of HNSCC with large scale T cell invasion in the tumor not in stroma.

Figure S1 - Should make it a regular figure as it adds clarity to understanding of the cluster diagram of Figure 1A when patient origin of sample is included.

As a non-mathematician I am not totally clear on how much true external validation was done, though the work depicted in S8, S9 and S10 is most clearly an example of external validation.

Figures 2D and 3B are hard to understand. May need better description.
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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