Peer Review File
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Reviewer comments

This study by Ma et al. aims to assess a programmed cell death gene panel in the context of LUAD to predict patient prognosis. Using expression data from TCGA and gene sets from a prior publication, the authors utilize various computational approaches to define a PCD gene set associated with LUAD patient prognosis.

The concept of this study is excellent. The approach to utilize PCD gene sets in this context is fairly novel and can obviously be expanded to many other contexts in a similar manner. Because PCD is a critical component of the cancer program, the utility of this study is very high. The computational approach, methods, and results are all adequate for publication. However, as described below, the authors have not really proved that they are actually using a PCD gene set in their study. In fact, their results demonstrate that they are really using a gene set populated by genes NOT described as PCD genes. By carefully reading the numbered comments below, the authors must a) define what they mean by PCD genes and then b) defend that their definition is biologically accurate. Currently, it looks like the gene set that they used included numerous genes that may only be tangentially related to PCD... as in many of these genes act far upstream of any PCD rather than being traditionally associated with PCD. Simply citing one single paper that also defined many of these genes as PCD genes is insufficient. This paper that they cite does the same thing: simply state that they have curated a PCD gene set without proving it. In this present study, these authors' own resource that they use to choose genes (Gene cards) does NOT define the genes in their final gene set as PCD genes. This is a major problem. If it is true that the gene set(s) that the authors have used really are recognized in the PCD community as PCD genes, then the authors simply need to prove that. At the very least, the authors could tie their final gene set back to the Tang et al 2019 Cell Research review which carefully defines PCD machinery and the authors of this present study could point out that many of their genes are components (or family members of components) shown in this well-recognized review. The main issue is for the authors to convince the field that they have a genuine PCD gene set. Then, this will be an important and useful resource for the field that others will cite and also use. Without doing that, this will be just another publication with a not-very-trustworthy gene set that will likely remain obscure. Because this overall study concept is so good, the authors should invest the time into proving that they have a gene set that will be broadly useful for the field.

1) In the introduction, the authors should introduce the gene sets that they used which are related to the 12 forms of programmed cell death. While all of the genes do not need to be listed or described here, the authors definitely need to establish that they have identified appropriately comprehensive and curated gene sets for the purpose of this study.
Reply 1: “We added a comprehensive introduction to the gene sets that we used which are related to the 12 forms of programmed cell death (see Page 6, line 155-160).”
Changes in the text: Page 6, line 155-160

2) The authors need to more robustly cite the field of PCD in the context of cancer and LUAD. This paper only has 27 citations. As mentioned previously, this is study is an excellent concept, yet the authors need to demonstrate that they have adequately assessed the state of the PCD field as it relates to cancer/LUAD so that their study can be properly contextualized.
Reply 2: “We added more robust introduction to the PCD in the context of cancer as advised (see Page 4-5, line 95-133).”
Changes in the text: Page 4-5, line 95-133

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3) In the results related to figure 2, the authors should comment on why no GO terms related to any PCD pathways were found. This again highlights the requirement for the authors to more robustly characterize their gene sets. Did the gene sets they used from the cited publication really capture PCD pathways, or were many genes included that are only tangentially related to PCD? A brief Gene Card analysis of the list of 18 genes related to figure 3 demonstrates that many (most?) of these genes are not obviously associated with PCD. Many of these genes are much better characterized with the GO terms shown in figure 2 or as checkpoint genes rather than PCD genes. This is a major flaw that needs to be addressed. Does this study actually assess PCD genes or does it assess genes far upstream of PCD that are only tangentially associated with PCD? In order for this paper to be accepted, the authors need to make a strong argument for PCD... otherwise this is just a paper that has chosen a pre-biased sample of important cancer-related genes and ranked them.
Reply 3: “We added some data to show GO terms that are related to PCD pathways. They were just not shown in Figure.2 as the top 30 pathways (see Page 10, line 300-301).”
Changes in the text: Page 10, line 300-301
