Reviewer Report

Title: A methodological approach to correlate tumor heterogeneity with drug distribution profile in mass spectrometry imaging (MSI) data

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Reviewer Comments to Author:

The authors present a computational method for identifying spatial regions with molecularly distinct regions between control and drug therapy using previously published data. The method is well described and paper is somewhat easy to follow. The code and attached data was reviewed as well and appears clear and would be easily translatable to other projects. A more formal implementation as an R package would be desirable as the workflow is quite complex it would benefit to make a more accessible API so less experienced users wouldn't get lost.

For step 1 in the processing: How are 'drug' related peaks guaranteed to be removed from the 'microenvironment' segmentation? In processing, it is mentioned that ion peaks with correlation > 0.5 with the drug compound were removed. This seems like it would bias the segmentation if the drug had a very discrete distribution in a very particular histological region. One can imagine a scenario where a drug is distributed in area "A" exclusively along with other endogenous compounds. These endogenous compounds would be then be removed from the segmentation pipeline simply because the drug was highly partitioned into this region. Could the peaks be derived solely from undosed control tissue? Otherwise the authors statement may be misleading.

The authors note that mass spectral validation of model-identified differential ions is not possible and that is reasonable. In general, the spatial models presented in the findings are compelling. However, as this paper deals with spatial characterization of tissue, there appears to be no spatial validation. Indeed the obvious choice of the gold standard in pathology, H&E microscopy, is present in Figure 5 but the size of the images is so small it is negligible for spatial validation. Secondly, there are numerous published MSI examples(DOI: 10.1021/jasms.8b04879, doi:10.1074/mcp.O115.053918, https://doi.org/10.1038/srep36814) where there are clean and distinct, immediate visual association of segmented MSI images to histological regions in H&E, but here the segmentation doesn't seem to replicate much of the structure visible in Figure 5, at least AS PRESENTED. This comment isn't to push for models integrating H&E as an input but to have some qualitative result describing the types of cells present in the tumor regions associated with the major clusters. While molecular histology is valid, it is unusual for it to not mimic classical histology.

Minor comments

All figures containing should have a scale bar indicating the physical dimension of the images.

Small errors:

Introduction

* Techniques of election <- is not proper English. Perhaps 'A valuable technique' would be less

awkward.

Methods

Are the methods appropriate to the aims of the study, are they well described, and are necessary controls included? Choose an item.

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