Reviewer's report

Title: Pathway activity profiling of growth factor receptor network and stemness pathways differentiates metaplastic breast cancer histological subtypes

Version: 0 Date: 19 Jun 2019

Reviewer: Dung-fang Lee

Reviewer's report:

In this manuscript entitled "Pathway activity profiling of growth factor receptor network and stemness pathways differentiates metaplastic breast cancer histological subtypes", McQuerry et al., applied NanoString nCounter platform to analyze gene expression in 19 MpBC and 8 invasive ductal TNBC FFPE samples. They first used HMECs overexpressing genes of interest or GFP and applied ASSIGN to identify signature genes related to AKT1, BAD, HER2, IGF1R, KRAS G12V, RAF1, BCL2L11, and SNAI1. Using these signature genes, they compared the differentiated gene expression among MpBC, TNBC, and different MpBC subtype, and concluded (1) Mesenchymal MpBC subtype has an increase of SNAI1 and BCL2L11 pathway; (2) In comparison with TNBC, EMT and collagen genes were upregulated but late cornified envelope and keratinization genes were downregulated in MpBC; (3) MpBC patient with high mesenchymal and ECM genes are correlated to poor clinical outcome. Though authors' results are convincing and findings are important, there are several scientific points needed to be addressed before publication.

Major points:

1. Can authors use their data to create a potential MpBC gene signature which can help readers to distinguish MpBC and TNBC?

2. Authors compared the gene expression between MpBC and invasive ductal TNBC, and identified numerous gene signatures correlated to MpBC. However, it is not clear if they also express in normal breast duct cells. The patient adjacent normal tissue should be included in this study as a control.

3. Piscuoglio et al and Krings and Chen had investigated MpBC genome and transcriptome recently. It is great if authors can compare their findings with these two previous publications, and discuss the potential genes may be involved in MpBC etiology.

4. The Figures 1-4 are not clear. Authors should provide higher resolution figures.

5. Instead of SPARC gene expression, authors should examine if other genes (at least 1-2) identified in their studies are also correlated MpBC disease recurrence and survival.
Minor points:


7. Authors included HER2 in the initial HMEC overexpression study. Since MpBC is often triple-negative, is there a specific reason authors choose HER2?

8. In line 298, authors mention "CD24, whose lack of expression is associated with stemness phenotype, was also down-regulated in MpBC samples". Please provides references for (1) lack of CD24 is associated with stemness phenotype (2) CD24 is downregulated in MpBC.

9. In line 298, authors mentioned In line 299-301, authors mentioned "Genes up-regulated in MpBC included immune genes IL6 and IL8, EMT-related genes 300 FN1 and CTGF, and genes involved in 301 extracellular matrix synthesis and adhesion: COL1A2, COL5A1, COL5A2, ICAM1, and HAS2." Can authors provide known or published data supporting these genes are related to MpBC or breast cancer?

**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?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

**Quality of written English**
Please indicate the quality of language in the manuscript:

Acceptable
**Declaration of competing interests**

Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests.

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors’ responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal.