Author’s response to reviews

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

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Author’s response to reviews:

Dr. Andrea H. Bild
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July 19, 2019

Dr. Linda Gummlich
Editor
BMC Cancer
Dear Dr. Gummlich,

We would like to thank your editorial staff and the reviewers for contributing helpful feedback to our manuscript, “Pathway activity profiling of growth factor receptor network and stemness pathways differentiates metaplastic breast cancer histological subtypes”/BCAN-D-19-00164. We have revised the manuscript as described in response to each point below. Thank you again for the opportunity to revise this manuscript for publication in BMC Cancer.

Sincerely,

Jasmine A. McQuerry

&

Dr. Andrea H. Bild
Professor, Department of Medical Oncology and Therapeutics Research
City of Hope
Editor’s Comments

1. Please confirm whether informed consent, written or verbal, was obtained from all participants and clearly state this in your Methods and Ethics approval and consent to participate sections. If verbal, please state the reason and whether the ethics committee approved this procedure. If the need for consent was waived by an IRB or is deemed unnecessary according to national regulations, please clearly state this, including the name of the IRB or a reference to the relevant legislation.

The Methods and Ethics Approval sections have been updated to include the statement “Written informed consent was obtained from all patients who participated in the study,” at lines 120 and 488.

2. BMC Cancer uses Vancouver style referencing, please reformat your references, numbering them in the order they appear in the text, and citing them in the text using the appropriate number in square brackets.

Thank you for the help. We have made this change in the manuscript.

3. Please check the link in the Availability of data and materials section, it currently does not lead to the data.

The link to the repository was previously set to private access and has now been set to public.

4. Please note, the role of the funding body in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript should be declared in the Funding section.

The funding bodies had no role in study design; data collection, analysis, and interpretation; or manuscript writing. A statement declaring such has been added to the “Funding” section at line 505.
5. Please include a statement in the Authors' contributions section to the effect that all authors have read and approved the manuscript, and ensure that this is the case.

The Authors’ contributions section has been updated to include the statement “All authors have read and approved the manuscript,” at line 516.

6. Please move the Figure Legends and Additional File list to under the References.

The Figure Legends and Additional File List section has been moved to the appropriate location.

7. Please state in the figure legends whether the clip art (petri dish, Eppendorf tubes, etc.) in figure 1 is your own or taken from another source.

The figure artwork was drawn by the authors. The statement “Figure artwork was created by the authors” has been added to the end of the Figure 1 legend.

Reviewer 2 Comments

REQUESTED REVISIONS:

In the description of the gene selection for the Nanostring analysis, authors state ".. as described previously" (line 130). This is somewhat confusing. If the selection was done in a previous publication, this should be stated explicitly. If the selection was done following the same pipeline, the data (including validation of gene expression, and the expression profiling data) should be described appropriately.

Thank you for pointing this out to us. The statement at line 130 referred to the method of adenoviral overexpression in HMECs, not to gene selection. To make the gene selection process more clear, we have added additional information to the methods section and have broken the methods into separate sections for the patient samples, the HMEC controls, and the gene selection. The gene expression data for the selection process can be found in Figure 2, as well as the datasets and code can be accessed at https://github.com/dfjenkins3/MpBC_genomics_paper.
Further, the whole idea of deriving pathway signature by over-expressing genes with adenoviral vectors is somewhat questionable, unless authors achieve expression levels that are comparable to those that could be seen in tumor cells. Also, while the idea seems to make sense for expression of transcriptional factors (like SNAIL), with expected ripple effect in terms of up or down regulation of different genes, the case is much less obvious for most of other genes used in the study. I think these caveats are at least worth mentioning in the discussion.

Adenoviral overexpression to generate gene expression signatures for both transcription factor and non-transcription factor genes has been well-established and referenced. Please see Bild et al. (2006), Nature 439(7074):353-7, which has currently been cited by 764 PubMed Central articles. The pathway signatures described in that article have also been incorporated into the Molecular Signatures Database curated by the Broad Institute for wide use by the public.

It will be helpful to show H&E slides with different histologies that authors describe in the manuscript.

The authors thank the reviewer for this suggestion. Histological slide images of each of the subtypes have been added as Additional File 4, Supplementary Figure 2.

Why do authors refer to IL6 and IL8 as "immune genes" (line 300). Both of these cytokines as well as their receptors can be expressed by carcinoma cells, and stromal fibroblasts.

The authors are thankful for the reviewer’s suggestion and have updated the term to read “cytokine genes” instead of “immune genes” at the current line 305.

It is not clear why authors refer to "signaling" in the Conclusions sections. This is a sloppy use of terminology, as authors examined gene expression levels, not signaling.

The reviewer raises a valid concern; thus, the term “signaling” has been changed to “gene expression profiling.”

ADDITIONAL REQUESTS/SUGGESTIONS:

In the abstract, the authors have highlighted that RNA samples from FFPE samples represent a challenge due to poor quality, it might be useful to show info on RNA quality, as analyzed by Bioanalyzer.

We have added RNA Integrity Number (RIN) scores, calculated by Agilent 2100 BioAnalyzer for each of the samples, to a new column in Additional File 1, Supplementary Table 1.

Lines 255, 260. Not sure it is fair to refer to the Nano String Ncounter panels as "novel platform". It is quite mainstream.
The authors apologize for the confusion—we agree with the reviewer that the NanoString platform is mainstream. In the manuscript, “novel platform” refers to the application of the gene expression signatures to the NanoString platform. It does not refer to the NanoString platform itself. To help eliminate confusion, this term has been removed and replaced with “the NanoString platform.”

Reviewer 3 Comments:

Major points:

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

We thank the reviewer for thinking deeply about the project and acknowledge that a signature distinguishing MpBC from TNBC would be extremely valuable. We do believe that generation of a MpBC signature is possible; however, for this analysis we would need to take a separate approach that would include higher numbers of TNBC tumors (and possibly MpBC) and regenerate an additional custom NanoString panel specific to the differences in the two breast cancer subtypes versus its current specificity for assessing pathway activity.

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.

This study focused on identification of the gene expression and pathway activity differences between metaplastic and triple negative breast cancer as well as differences between the histological subtypes of metaplastic breast cancer. The study did not focus on differences between metaplastic breast cancer and normal breast tissue. We have edited the text to highlight this point, and apologize for any confusion. In addition, our IRB does not cover use of normal tissues, only tumor, so we are unable to include additional normal tissue analysis.
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.

The reviewer’s suggestion to integrate findings from previous papers studying MpBC is well received. Krings and Chen assayed a panel of genetic mutations in MpBC and TNBC; however, our main objective was to profile gene expression and pathway differences. The Piscuoglio et al. paper profiled gene expression using RNA sequencing, and so we have added a few sentences beginning at line 417 to draw parallels between the findings from the two studies.

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

Thank you for the feedback. It appears the figures were quite compressed by the Journal’s software in the compilation of the manuscript submission pdf. The full resolution individual figure files can be downloaded by clicking the “Click here to download” link on each figure within the compressed pdf. Additionally, small changes have been made to figure 1 to improve image clarity.

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.

The authors thank the reviewer for the suggestion. Several other genes were examined, but none were significantly correlated with recurrence or overall survival. An example of a negative result, vimentin expression, is included in Additional File 6, Supplementary Figure 4, Panel A.
Minor Points:

1. The link https://github.com/dfjenkins3/MpBC_genomics_paper does not exist. The link was previously set to private access only, and the repository has now been made public.

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

Several studies have demonstrated HER2 positivity by IHC in MpBC patient tumors, e.g., Xiao et al. (2017) Oncology Letters 14(2):1971-8. Therefore, we anticipated seeing potential activity of this pathway in some patients. Unfortunately, no patient accrued to our study was categorized as HER2+ by IHC.

Additionally, because the HER2 pathway signature consists of other downstream genes whose activity may be influenced by other upstream receptors, the pathway was still included in the manuscript. Interestingly, as noted by Additional File 5, Supplementary Figure 3, ERBB2 gene expression was measurable in these patient samples categorized as HER2-negative by IHC.

3. 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.

The authors thank the reviewer for this suggestion. The reviewer’s suggestion (1) brought to light that this sentence better fits in the discussion, to where it has been moved and further expounded upon, with references, at line 357. In response to the reviewer’s suggestion (2), we have added an additional reference to results Table 2 at the end of this paragraph (Line 307), to again point readers to where our differential gene expression results across the subtypes can be found.

4. 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 FN1 and CTGF, and genes involved in 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?
We thank the reviewer for the suggestion to incorporate supporting results from other studies. Many of these genes have not yet been linked to MpBC in the literature. However, based on a literature search, we have added references at lines 360-371 regarding genes COL1A1 and HAS2 in both MpBC and breast cancer.