Reviewer’s report

Title: Wnt Signaling in Triple Negative Breast Cancer is Associated with Metastasis

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Reviewer: Brian D Lehmann

Reviewer’s report:

The investigators analyzed gene expression data from two breast cancer cohorts and show overrepresentation of WNT signaling components in basal-like breast cancer. While a link between wnt signaling and TNBC is interesting, the authors do not sufficiently demonstrate this with the data included in this manuscript. They provide minimal evidence for their findings using a mixture of two cell lines (MDA-MB-231 and HCC38) for functional studies evaluating migration and invasion in WntC59 treated cells and B-catenin knockdown. There are formatting and organizational errors that detract from the science reported. For instance, Figure 6 is labeled as S10, tables S10 and 11 are actually S2 and S3 and inclusion of response to previous reviewers comments in the methods section. This study at best is incremental and does not firmly establish a link between wnt signaling and metastasis in TNBC.

Specific comments
(1) The use of wnt (+) is not clearly or uniformly used throughout the manuscript and is essential to understanding any conclusions. Sometimes wnt(+) refers to the LWS-81, sometimes it is the shrunken centroid of the WNT/b-catenin classifier. This use becomes increasingly important in understanding the kaplan-meier graphs, as figure 1 shows there are more than just two phenotypes of wnt signaling and these differ between datasets (figure 1A and B).

(2) Discretionary Revisions-Throughout the manuscript TNBC and basal-like are used interchangeable, however these are not synonymous and describe two separate patient cohorts. It is true that 80% of TNBC display basal-like gene expression, so this grouping is at best 80% correct.

(3) Methods RNA, preparation, quality control and DASL assay.
Minor Essential Revisions-“we have added a supplementary table with the gene-list... to the revised-MS S10 and S11 for the convenience of the reader” should be removed and included in the response to reviewer’s comments in the previous submission. I think that supplemental S3 and S4 are these figures, as there are no S10 and S11 tables.

Figure 1.

(4) Minor Essential Revisions The names of the genes should be included so reviewers/readers could make their own minds up whether they are wnt inducible
genes. This gene list came from lung cells and there may be tissue-specific gene expression.

(5) Major Compulsory Revisions - Overall it is very unclear as two which population has activated wnt. Figure 1 demonstrates that there two populations of WNT (−) in the QC-BCP group and two populations of WNT (+) in the GA BCP group. These clusters appear to display a mix of wnt activated and repressed genes.

Figure 2.

(6) Major Compulsory Revisions - It is unclear on how the Wnt expression is quantified. Is this just the aggregate expression of all 81 genes in the LWS dataset? This could be biased based on the number of genes activated vs. repressed.

(7) The finding that the Wnt pathway is overrepresented in the DASL cohorts may be a reflection of the biased selection of cancer genes, as the panels are quite small, representing approximately 500 genes.

Figure 3.

(8) Minor Essential Revisions - Figures need to be compressed into one page.

Major Compulsory Revisions

(9) Appears that majority of experiments were performed in MDA-MD-231 cells, however the authors switched to HCC38 cells for the final panel (Ci and Cii) to evaluate cytoskeletal changes in F-actin.

(10) The comparison between Ci and Cii appears to be at different magnifications and have few representative cells (2 to 4) to describe large phenotypic changes.

(11) The use of additional cell lines treated to all of the conditions (#-catenin knockdown, WntC59 and sulindac sulfide treatment) would provide evidence for the conclusions drawn.

(12) The authors should consider showing that the wnt pathway was successfully inhibited under all conditions by using a wnt luciferase reporter (TOPflash) or RNA/protein analysis of downstream targets.

Figure 4.

(13) Major Compulsory Revisions

What are wnt (+) and wnt (−) in the analysis? This analysis is entirely dependent of that classification and it is clear from figure 1 that there are multiple clusters with mixed wnt activated and repressed genes.

Figure 5.

(14) Minor Essential Revisions - This figure does not add significantly to any of the findings and should not be included.

Figure 6.
(15) Minor Essential Revisions- This figure appears to be labeled S10. Figure should also be formatted as to not include graphs with NA, as they add no information and are distracting.

(16) Major Compulsory Revisions- While this analysis is meant as an extension to figure 4 highlighting metastasis to various organs. This analysis is highly dependent of the definition of Wnt(+) and Wnt (-), in which the authors have do not providing convincing evidence in figure 1 for the separation of patients into these groups

Figure S1.

(17) Major Compulsory Revisions- It is unclear what figures C and D are showing from the figure legends and text. Are these two independent replicates? If so why are they so different? In panel F what are the 34 and 12 genes that are differentially regulated in TNBC?

Figure S2.

(18) Major Compulsory Revisions-What is the overlap in WNT canonical pathway genes in the HCP (n=77) vs. BCP (n=42). It is alarming that just a decrease in 35 genes could make such a profound statistical influence on wnt pathway (p=0.6246 vs. 0.0048). Clearly the composition of the DASL platforms (HCP with 1488 genes and BCP with 1536 genes) introduces substantial bias and the results carefully interpreted.

(19) Also while these pathways may appear to be enriched, Figure S1F only identifies 110 genes statistically enriched in TNBC between both platforms.

Figure S3.

(20) Discretionary Revisions- Why is it that the wnt pathway is not significantly enriched in the Georgia cohort? Is this analysis highly variable with breast cancer subtype composition?

Figure S7.

(21) Major Compulsory Revisions-This figure does not appear to add anything and actually demonstrates that the wnt (+) signature extends across all subtypes of breast cancer contrasts with your overall conclusion that this TNBC specific.

**Level of interest**: An article of limited interest

**Quality of written English**: Needs some language corrections before being published

**Statistical review**: Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I have no competing interests.