Reviewer's report

Title: Claudin 1 expression in basal-like breast cancer is related to patient age

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Reviewer: Gabriel DiMattia

Reviewer's report:

This manuscript describes a great deal of histopathological work looking at the relative levels of claudin1 and 4 in basal-like and non basal-like breast tumour samples using TMAs. They performed an exhaustive and appropriate analysis of the data to determine whether high levels of claudin1 might be associated with specific disease parameters. However found that high levels were only associated with greater than 55 years of age at presentation. They also went on to provide functional data regarding the potential significance of high CLDN1 by generating stable KD of CLDN1 in BT-20 breast cancer cells and found that some EMT markers were reduced in expression and that migration of these cells seemed to be negatively affected. They also observed an unusual subcellular localization in the TMA with claudin1 protein in the cytoplasm instead of being concentrated at the cell membrane. Overall the manuscript is well-written and the data sound. Ideally one would like to see the functional studies consistent across multiple cell lines and western blot data to backup the qPCR data.

Specific Comments:

Given that BLBC is characterized by a mesenchymal phenotype and claudins are considered essential for the overall maintenance of the differentiated state of epithelial cells it seems odd that BLBC cells would show high levels of claudin 1. I would have expected the authors to comment on this in the Introduction or Discussion, unless I missed it.

The authors indicate in the Introduction that only recently has claudin 1 been shown play an active role in tumorigenesis and I wonder if this included xenotransplantation assays with specific cell lines containing shRNAs?

"Although a large cohort of tumors (350 samples) was examined in our earlier study, they belong to mixed pathological lesions. As such, clinical relevance of claudin 1 expression to the BLBCs could not be properly addressed."

The above statement in the manuscript seems to be a major portion of the rationale for the work so I would ask the authors to provide a comparison of their data to the CLDN1 data in the provisional breast cancer TCGA dataset (i.e., cBio website). Does the TCGA dataset agree with their data or are they not comparable? I would also ask the authors to be more definitive regarding the above statement and define mixed pathological lesions.

Perhaps the authors could be more specific regarding the production of the
TMAs used in their studies? Who made them and where? Are BLBC enriched TMAs common or easily generated? If not, perhaps that should be indicated.

Referring to the shRNA expressing lines as clones may not be correct. Are these clones or a mixture of BT-20 cells with independent transgene integration events? Clones implies derivation from a single cell with a unique integration event similar to a limiting dilution cloning assay.

H-scores are essential for quantifying subjective assessment of claudin-1 levels in TMAs but what does this really mean in terms of levels of expression? IHC Examples of these data would be useful.

The down-regulation of proteins of genes that encode factors regulating EMT is an interesting result; but does this imply that the BT-20 cells have a more mesenchymal phenotype relative to other cell lines? Is there any evidence in the literature that this line exhibits a mesenchymal phenotype with loss of EpCAM and E-Cad and specific cytokeratins? Are these cells more "epithelial" upon loss of claudin 1 which doesn't make sense since epithelial cells or adenocarcinoma cells should show tight junctions. I don’t think the authors have provide sufficient context for the observations regarding epithelial or mesenchymal phenotype in the BT-20 cells in order for their data to make sense, at least to me. Moreover, the authors could provide a few specifics regarding the PCR array i.e., how genes included in the array given that they saw significant alterations in just seven genes.

MW markers in Fig. 3 western? Should we expect to see a smear? Post-translational modifications?

The authors measure cell migration using a scratch assay but what about cell viability over time or sensitivity to stress ie chemoresistance upon down-regulation of CLDN1? Can the authors comment on this?

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

No competing interests