Author's response to reviews

Title: Comparative microRNA profiling of sporadic and BRCA1 associated basal-like breast cancers

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Author's response to reviews: see over
Dear editor,

We would like to thank the reviewers for their helpful comments. We have revised manuscript as suggested by the reviewers.

Reviewer 1
1. The authors have identified three proteins for which a specific negative correlation with BRCA1 mutation status has been established, which collectively might be useful as a diagnostic biomarker for this special category of breast cancer. However, specific biological implications have not been discussed. The authors might want to take this opportunity to expound on possible connections between these three proteins and BRCA1 itself, the phenotype associated with BRCA1 mutation, and/or the clinical behavior of these tumors. For instance, cyclin D1 has been found to serve as a direct target of Notch1 in triple negative breast cancer, an antibody targeting neuropilin-1 has entered clinical trial, and the authors’ own previous publication describes an association between FOXP1 and familial breast cancer.

A paragraph regarding the biological implications of our findings has now been included in the discussion (p.17, line 16)

2. The use of the scoring system (0 – 7) adds considerably to the depth of information gained from the immunohistochemical analyses of the three proteins of interest, beyond categorical positivity or negativity. Although the degree of statistical significance of the IHC score is given (Table 3), it might be of considerable additional benefit if the distribution of scores was provided, either in graphical form or described in the text. For instance, are the scores bimodal? (suggesting more than one distinct population), or does a closer examination reveal that either one of the contributing parameters (% of cells positive or intensity of immunoreactivity) correlates particularly well with BRCA1 status?

The scores have now been submitted in graphical form (p.15, line 3, supplementary table 4).
3. The authors might consider mentioning the degree of overlap between the gene lists included in Supplemental tables 4 and 5 (or perhaps highlighting those genes in common, e.g. C9orf86 and XIAP which are included in both).

Of the 1218 genes predicted to be regulated by miRNAs, there was an overlap of 71 genes (5.8%) between the 2 lists. This has been stated in the results (p.14, line 7).

4. The principle component analysis graph (Supplemental figure 4) provides a valuable visual representation of the authors’ findings regarding the relationship between BRCA1 mutant status and the basal breast cancer phenotype overall. The authors might consider including this graph within the main manuscript.

This graph is now included in the main manuscript as Figure 3 (p.14, line 14).

1. Please clarify the status of CD99 expression in the BRCA1 versus other basal breast tumors. In the Results section, the text states that CD99 is “predicted to be upregulated” in BRCA1 vs. sporadic basal cancers. Yet the next sentence includes CD99 in the list of proteins which exhibit reduced positivity in BRCA1 mutant cancers, although the statistic provided (85% vs. 37%) and the table itself (Table 3) agree with the first sentence that CD99 is actually increased in BRCA1 mutant tumors. The next paragraph describing the data obtained with the second validation cohort states “an opposite association for CD99 … with sporadic basal cancers showing increased expression compared to BRCA1 cancers”, though the data for CD99 is not included in Table 4. The Discussion states that CD99 was reduced in BRCA1 mutant tumors in the initial cohort. -- It is clear that the results obtained for CD99 differed between the two experimental groups examined, however the text is not absolutely clear and it seems at least one of the statements may be incorrect. Minor issues – not for publication:

The expression for CD99 in the validation cohort is now included in Table 4. The difference in expression for CD99 is now explicitly stated in the discussion (p.17, line 10).

1. Conclusion section: “… highly relevant to clinical trials investigated …” should probably be “investigating…”

This has been amended in the manuscript (p.19, line 12).
Reviewer 2

1. Introduction and methods of Abstract are incomplete, conclusion drawn in abstract and its implication is somehow incomplete.

The introduction, methods and conclusion in the abstract have been redrafted (p.2, lines 3, 6 and 24).

2. Rational behind opting FOX1, cyclinD1 and NRPI to perform immunohistochemistry is not mentioned appropriately. In addition, how BRAC1 is associated with these proteins in not mentioned/explored.

Selection of antibodies was based on their previously described associations with BRCA1 status, wherever possible. This is now stated in the manuscript (p.10, line 6).

3. Finally authors suggested that targeted therapies such as PARP inhibitors would be highly relevant, how this conclusion was drawn is not evident in manuscript.

There is evidence to suggest BRCA1 deficient cancers may be sensitive to PARP inhibitors (Rottenberg et al., PNAS 2008). Hence stratification of basal-like cancers, based on the “BRCA1-ness” of their miRNA signature may be relevant to clinical trials investigating targeted therapies, such as PARP inhibitors. This is now stated in the conclusion (p.19, line 9).

4. Careful edition of the manuscript is required.

The manuscript has now been re-edited. Changes include:

- Re-drafting of the abstract (p.2)
- Amendment of typographic error “investigated” to “investigating” (p.19, line 12).

We hope that the manuscript is now suitable for publication.

Regards,

Max Yan

Stephen Fox