Reviewer's report

Title: Functional deficiency of NBN, the Nijmegen Breakage Syndrome protein, in a p.R215W mutant breast cancer cell line

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Reviewer: Janice Pluth

Reviewer's report:

In this work the authors have identified a breast mammary epithelial cancer line that has hemizygous mutations in both NBN and BRCA1 (p.R215W and p.R1751X in NBN and BRCA1 respectively). This line was used to study how this particular NBN mutation may affect its radiation sensitivity and ability to respond to DNA damage. In addition, the effect of a PARP inhibitor on cell density and growth as measured using cellular impedance was also evaluated. The authors conclude that their studies provide functional proof of the underlying importance of the R215W mutation on breast cancer risk. However, although the cellular phenotypes described are interesting, it seems difficult to conclude that this particular mutation is responsible for the phenotypes observed given the additional BRCA1 mutation present in the cell line.

Major Compulsory Revisions:

1) There is a lack of discussion and no mention of the fact that the BRCA1 mutation also discovered in the cell line may be more key in terms of breast cancer risk (and the phenotypes noted in this study), than the NBN mutation that was centered on. The authors appear to down-play the potential additional effect of the BRCA1 mutation and state because the BRCA1 mutant line HCC1937 does not have reduced NBN protein levels that the mutation in BRCA1 can not account for the low NBN levels. This may or may not be true, the mutation in NBN may be what is affecting the NBN protein levels but it could also be the combination of mutations that affects the protein levels. In addition, it is still possible and likely, that the cellular phenotype observed is a result of the combined mutations and not just due to the mutation in NBN. As the two BRCA1 mutations in the two lines (HCC1395 and HCC1937) are different it is uncertain what affect the BRCA1 mutation at R1751X may have on NBS. The effects observed in HCC1395 may be more heavily influenced by the BRCA1 mutation than with the slightly reduced levels of NBN, and the mutation noted in NBN. This seems especially likely, as a number of breast cancer tumors have been shown to have this particular BRCA1 mutation. In addition the region of this BRCA1 mutation 1646 to 1863 has been shown to be critical for the binding a number of important proteins including CtIP, BACH1, Aurora A and BRCA2, to mention a few. To completely ensure that the effects noted are linked to the NBN mutation at R215W, the HCC1395 line should be transfected with wild-type BRCA1 and compared to the non-transfected cell line for the endpoints measured in this paper. If the BRCA1 corrected line with the R215W mutation still shows these...
effects then the results would support the conclusions that were reached.

2) In Fig. 5 it is concerning that the normalized cell index for the HCC1395 line is so much lower than that for MCF10A. The difference between the DMSO control and the Olaparib treated cells would appear minor or non-existent if placed on the same scale as the MCF10A line. It would be important to give more details regarding the normal growth of the HCC1395 cells, what is their doubling time as compared to the MCF10A line? Is the sensitivity to Olaparib just a reflection of the poor growth in general for the HCC1395 line?

3) The statement on page 21 under Fig. 3A does not appear to be supported by the graph. It is stated that “Both lines, HCC1395 and HCC1937 exhibited increased residual levels of foci at 24 and 48 hours after irradiation”. However it only seems that 48 h following a 6 Gy dose are levels higher for the HCC1395 line as compared to the control.

Minor Essential Revisions:

1) In Fig.1B the numbering for the sequence is not aligned with the other two lines in the HCC1395 BL cell line. In addition, no arrow is provided to guide you to the site in this line, however it looks to be between 110 and 120, whereas for the other two lines the mutation is between 120 and 130.

2) For Fig. 2A the cloning efficiency should be provided for both the MCF10A and HCC1395 lines in the methods or results section.

3) In Fig. 2C as well as based on Fig.2B it appears that the HCC1395 line may be suffering considerable damage even without additional endogenous exposure (higher levels of pSMC1, and Chk2 phosphorylation and PARP cleavage in 0 Gy irradiated samples). This finding, perhaps related to its poor growth overall, is not discussed in the paper.

4) On page 10 in discussing the closer inspection using confocal laser microscopy, it is mentioned that a smaller area and more fuzzy appearance of foci is noted in the HCC1395 cells as compared to MCF10A, and stated that “this feature" was statistically significant for #H2AX foci. It should be more clearly stated that foci area was statistically significantly different, as the sentence as written seems to imply that the fuzzy nature was also quantified.

5) In Fig. 3D it should be clarified that these are enlarged portions of single cells (if this is the case). Would be better to highlight a box from the cell portion that is enlarged.

6) On page 4 it is stated that the HCC1395 line harbors (side note harbors is spelled incorrectly as harbours) the p.R215W mutation in a hemizygous state (meaning only one allele present) and on page 8 it is stated that this missense mutation was uncovered in the apparently homozygous state (meaning both alleles are the same). This is a bit confusing and should be clarified.

Discretionary Revisions:

1) A bit more discussion regarding the impedance measurements would be helpful and could be included in methods. What is measured by this assay and how does it relate to cell viability and proliferation? Also details regarding the
percent confluence for both cell lines at 24 h, when these studies were performed.

2) Page 9, last sentence in paragraph 2, the word “but” is not additionally needed in this sentence.

3) Although what is likely trying to be said can be figured out, the last paragraph, first sentence, on page 10 is very clumsy and not clear: “...we suspected that a synthetically lethal approach using PARP inhibition may be effective in HCC1395”.

4) Last sentence in paragraph before conclusion, should include the word “cancer patients” with the p.R215W mutation could benefit…” as obviously they would not be treated unless they had been identified as having a malignancy.

**Level of interest:** An article whose findings are important to those with closely related research interests

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

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests