Author’s response to reviews

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

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Author’s response to reviews: see over
Reviewer's report

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

Version: 3  Date: 12 May 2014

Reviewer: janice Pluth

Reviewer's report:
It is appreciated that the authors have made a number of revisions to the latest draft that have improved the paper. However, there are still two critical revisions regarding the conclusion, and a number of more minor edits, which should make the paper read better and are detailed below.

Authors’ reply: We thank the reviewer for her time and appreciate her helpful suggestions to improve the text of this manuscript.

Major Compulsory Revisions:
The conclusion still does not accurately relay what has been determined in the work and its impact. The conclusion should accurately reflect the findings and their impact. The authors have identified the mutation in NBN to have a biologically functional impact, but it should be stated that it is in the context of a defective BRCA1 background. Any studies looking at NBN function will always need to keep that in mind, as it is impossible to determine without complementation studies that the effect being observed is not a result of the combined defect. In addition, the conclusion as written does not really say anything, what is a “natural model” or “genuine NBN functions”? These are not scientific statements.

(1) Conclusion statement pg 3 in abstract:
“Our results indicate functional relevance for the p.R215W mutation and provide a natural model for its molecular analysis in the context of genuine NBN functions.”
A suggestion for a more accurate conclusion would be: Our results indicate that the R215W mutation in the HCC1395 breast cancer cell line impacts NBN function, making this cell line a potentially useful cellular model for studying defective NBN protein within a mutant BRCA1 background.

Authors’ reply: We have changed the wording as suggested.

(2) Also at end of paper similar issue would need to be edited:
Conclusion pg “We here report on the first NBN mutant breast cancer cell line which may serve as a useful tool for future molecular studies of genuine NBN functions.”
More accurate to state:
We report for the first time the identification and characterization of a breast cancer cell line containing a NBN mutation that affects its function, providing a potentially
useful cellular model for studying defective NBN protein within a mutant BRCA1 background.

Authors’ reply: We have changed the wording as suggested.

Minor Essential Revisions:

(1) In Background
Mutations in NBN, the gene for Nijmegen Breakage Syndrome (NBS), are considered as breast cancer susceptibility alleles but a breast cancer cell line with NBN mutations has not been described so far.
Suggest:
Mutations in NBN, the gene for Nijmegen Breakage Syndrome (NBS), are thought to predispose women to developing breast cancer, but a breast cancer cell line containing mutations in NBN has not yet been described.

Authors’ reply: We have changed the wording as suggested.

(2) Under Key words: What is a “DNA double strand break disorder”? do you mean DNA DSB defect? Or DNA DSB deficient repair?? Typically you do not see DNA DSB “disorder”.

Authors’ reply: We have corrected this term and write “DNA double strand break repair disorder”

(3) On Page 4:
“However, further biological models for this missense mutation in breast cancer are warranted to fully clarify its role and impact.”
The sentence is worded it is confusing, “a biological model for the missense mutation in breast cancer…??”
It would be clearer to state “a cellular model of this missense mutation in an epithelial breast cancer line would be useful in clarifying its role in disease.”

Authors’ reply: We have changed the wording and write: “…further biological cellular models for this missense mutation would be useful to fully clarify its role in breast cancer.”

(4) On Page 5
“Human breast cancer epithelial cell lines HCC1395 and HCC1937 were cultured in…”
Would be good to indicate here in Methods section what mutations are present in each of these lines.

Authors’ reply: We haven’t included the mutations at this point. The cell lines had been bought from ATCC without regard of their genotype, and as we haven’t introduced the identification of the NBN mutation yet, its mention would anticipate the Results section.
In Results section Page 9:
“At the reduced level of about 30-40% of wildtype, the NBN protein could still be phosphorylated as judged by an electrophoretic mobility shift after irradiation with 6 Gy (Figure 1A).”
The sentence is not clear as written, would be clearer to state:
“The pR215W mutation reduced NBN protein expression to 30-40% of wildtype levels, but the protein appeared to be phosphorylated correctly following damage as noted by electrophoretic mobility shift after irradiation with 6 Gy (Figure 1A).”

Authors´ reply: We have largely followed this suggestion and write: “NBN was observed only at 30-40% of wildtype levels in HCC1395 cells, but the protein appeared to be phosphorylated correctly following damage as noted by an electrophoretic mobility shift after irradiation with 6 Gy (Figure 1A).”
Again, we didn’t name the mutant since its identification appears only later in the text.

On Page 11:
“showed a significant reduction of γH2AX foci area in HCC1395 (Figures 3B-E).
More clear to state “a significant reduction in the size of γH2AX foci were noted as determined by measuring total fluorescence?? Or how was area calculated?”
Note: additional detail is also needed in methods to define how area was actually determined; the information currently in methods does not detail this:

Authors´ reply: We have followed the suggestion and write "A closer inspection using confocal laser microscopy revealed a smaller and a more fuzzy appearance of foci in HCC1395 cells compared with MCF10A. A significant reduction in the size of γH2AX foci was noted as determined by measuring the γH2AX foci area in HCC1395 (Figures 3B-E)." (p11)
We also clarify: “For a more detailed inspection of the size and area of foci, images were taken as z-stacks by using a Leica TCS SP2 confocal microscope (40x or 63x magnification) and image acquisition was carried out using CorelPhotoPaintX4 Software and evaluated using ImageJ software. The number of pixels was determined as a proxy for the foci area.” (p7).

On Page 14
Our results support, in a patient-derived breast cancer model, previous evidence from targeted mutagenesis experiments in retrovirally transduced fibroblasts where p.R215W NBN was unable to bind γH2AX shortly after IR and was diffused into the nucleus, strongly suggesting that p.R215W is functionally relevant in protein-protein interactions [21].
The sentence is a bit long and difficult to follow, this may be clearer:
Our results support a previous study, using a targeted mutagenesis approach [21], which showed a mutation at R215W prevented the binding of NBN to γH2AX following radiation, and suggests a functional relevance for this mutation in protein-protein interactions.

Authors´ reply: We have changed the wording as suggested.
At present, the role of NBN p.R215W as a breast cancer susceptibility allele is still controversial and the magnitudes of the relative risk for cancer are insufficiently defined, but the accumulating evidence that p.R215W represents a severe mutation might stimulate larger genetic case control studies in additional patient series. It is not clear what a “severe mutation” is, maybe better to state: At present, the functional impact of the p.R215W NBN defect in breast cancer susceptibility is still controversial, but evidence is accumulating that this mutation might predispose individuals to disease.

Authors’ reply: We have changed the wording as suggested.