Reviewer’s report

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

Version: 2 Date: 28 January 2014

Reviewer: Janice Pluth

Reviewer’s report:

I appreciate that the authors have made revisions in the paper to address some of the previous reviewers comments. However there still are multiple issues as noted below that were not fully addressed. Perhaps the authors misunderstood the prior review comments, but a primary issue is that the cell line also contains a BRCA1 mutation which affects the cellular endpoints under study, namely radiation sensitivity and PARP sensitivity, and not that the BRCA1 mutation has anything to do with the low NBS levels noted.

The study as it is currently set up is flawed in that it is unable to investigate how the R215W missense mutation functionally impacts the breast cancer cells, given the presence of the BRCA1 mutation. Just from a cursory search of the literature it is obvious that the BRCA1 mutation can influence the endpoints under study:

“cells without BRCA1 showed decreased TCR and radiosensitivity.” From Abott et al., 1999 JBC. And …

“A major breakthrough in targeted treatment of BRCA1-mutant cancers was heralded by the finding that BRCA1 and BRCA2- mutant cells are exquisitely sensitive to poly(ADP-ribose)poly- merase (PARP) inhibitors” (Farmer et al., 2005; Helleday et al., 2005).

From Popova et al., Ca Res, 2012: “Large-scale state transitions (LST) defined as chromosomal break between adjacent regions of at least 10 Mb were found to be a robust indicator of BRCA1 status in this setting.”

“…among near-tetraploid cell lines, HCC1395, HCC1937, HCC38, HCC1599 and BC227 carried the highest number of LSTs, which is again consistent with their BRCA1/2-inactivated status.”

Major Compulsory Revisions:

1) The fact that the cell line contains a BRCA1 mutation should be noted up front in the abstract, and the evidence supporting BRCA1’s influence on these endpoints as noted above should be discussed and cited. The only way to completely ensure that the cellular effects noted are linked to the NBN mutation at R215W, would be to complement the BRCA1 mutation in the HCC1395 line and compare it to the non-transfected cell line for the endpoints measured in this paper. If this is not done than the researchers are currently studying the combined effect of both mutations and thus it should be discussed as such in the very beginning and through out the paper.
2) The statement in the conclusion “We here report on the first NBN mutant breast cancer cell line which will serve as a useful tool for future molecular studies of NBN function” does not seem to be true, this cell line can not be used for studies of NBN function unless the BRCA1 mutation is complemented as it will always confound the phenotype exhibited.

3) The authors state: “Although we cannot formally exclude the possibility that a BRCA1 mutation augments the effect of NBN p.R215W, the functional BRCA1 deficiency does not cause these effects as is also evidenced by the observations of normal NBN levels in other cell lines such as HCC38 or HCC1806 which are functionally deficient in BRCA1.” But what is not mentioned is the radiation sensitivity and PARP sensitivity, these endpoints ARE affected by BRCA1 mutations, this should be CLEARLY stated here, not the fact that it does not change NBS protein levels.

4) Regarding Fig. 5, it is unclear given HCC1395’s poor growth and cloning efficiency, why it would be plated at the same 5000 cells per well in quadruplicates as the MCF10A line, as noted in methods. Information regarding the percent confluency at the time of Olaparib addition for each line should be noted. An additional experiment using a higher density of HCC1395 cells should be performed if percent confluencies at time of drug addition are not the same between the lines.

5) Others have previously published that NBN and other proteins that affect HR, besides BRCA1/2, may also show some PARP sensitivity (McCabe et.al., Ca Res 2006). This work should also be discussed and cited in the paper.

Level of interest: An article of limited interest

Quality of written English: Acceptable

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

Declaration of competing interests:

I declare I have no competing interests