Reviewer's report

Title: Characterization of a novel PTEN mutation in MDA-MB-453 breast carcinoma cell line

Version: 3 Date: 15 July 2011

Reviewer: Jing Wang

Reviewer's report:

This manuscript by Sign et al. entitled "Characterization of a novel PTEN mutation in MDA-MB-453 breast carcinoma cell line" reports the identification and characterization of a novel mutation in the PTEN gene at codon 307 in MDA-MB-453 cell line resulting in the substitution of glutamate (E) to lysine (K), which caused the mutant protein to migrate with a faster mobility on SDS-PAGE. Further analysis revealed that while the PTEN E307K mutant displayed similar lipid phosphatase and growth suppressing activity when compared to the wild-type PTEN (WT), the PTEN E307K mutant was present at a higher level in the membrane fraction and suppressed Akt activation to a greater extent than the WT protein. The authors also report that the PTEN E307K mutant was polyubiquitinated to a higher level by NEDD4-1 and displayed reduced nuclear localization. This is an interesting finding.

(1) However, it is unclear why a mutation opposite to the one reported for residue 289 (K289E) in the C2 loop results in similar behavior of the PTEN protein, i.e., reduced nuclear localization and increased ubiquitination (Trotman et al., 2007). According to that report, one would expect that E307K mutation, if not neutral, would increase PTEN nuclear localization. The authors need to address and discuss this discrepancy.

(2) Since this mutation was present in a heterozygous state in MDA-MB-453 cells, it seems that the presence of a wild-type copy of PTEN would be sufficient to confer normal PTEN activity.

(3) One key experiment to perform would be to specifically knock-down the endogenous wild-type PTEN in MDA-MB-453 cells and assess the effect of the remaining mutated copy on nuclear localization and ubiquitination of PTEN as well as its effect on AKT activity.

(4) The conclusion that "This mutation may predispose breast epithelial cells to transformation events" is unfounded since the authors did not conduct any in vitro or in vivo assays to assess the effect of this mutation on transformation or tumorigenecity of cancer cells.

Specific comments:

(1) In Figure 1, there is a need to confirm that this mutation is not found in the control population and to check whether it can be found in other cancer cell lines.
and tumors. This will increase the significance and relevance of this mutation.

(2) In Figure 5, the authors should perform statistical analyses for the cellular distribution of the E3037K mutant versus the wild-type.

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

**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 that I have no competing interests