Author's response to reviews

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

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Author's response to reviews:

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Dear Editorial Board members,

We appreciate the helpful comments and constructive critiques from all three reviewers. We have addressed all the critiques. However, we were unable to specifically knockdown the wild-type PTEN allele due to the technical difficulties associated with knocking down alleles with only a single nucleotide difference. Also, MDA-MB-453 has constitutively active p-Akt and silencing the wild-type allele is unlikely able to enhance the activation of the PI3-K pathway further. The following are point-to-point responses to each concern raised.

Reviewer: Michel Longy

My main concern is that the authors don’t indicate that MDA-MB-453 is a cell line representative of a very peculiar kind of breast cancer, named “molecular apocrine”, androgen receptor positive and estrogen receptor negative ( Doane, Oncogene 2006;25:3994 – Robinson, EMBO 2011;doi:101038). This characteristic should be discussed, particularly as molecular apocrine breast carcinoma is more frequent in patients with Cowden disease (Banneau, Breast cancer res. 2010;12:R63).

Response: We have included this helpful insight into the revised manuscript.

1. In the “Background” section (page 4) it is not true that the location of PTEN mutations is different in Cowden disease and in Banayan Riley Ruvalcaba (Bonneau, Hum Mutat. 2000; 16:109).

Response: We have removed this sentence.

2. In the “Discussion” section (page 15) the hypothesis of a germline origin of the E307K mutation is attractive. Are medical records concerning the patient from
whom the cell line was derived available? Clinical findings in favour of Cowden disease could support this hypothesis.

Response - Despite repeated attempts to contact the Pathology Department at MD-Anderson Cancer Center for the primary specimen, we failed to elicit a response from them and we do not have additional pathological evidence to support our hypothesis.

3. In the “Discussion” section (page 16) if the main effect of the E307K mutation is the nuclear exclusion of Pten, it is difficult to conceive a dominant mechanism either positive or negative for this mutation. A gene dosage effect with haplo-insufficiency may be also implicated.

Response: We agree that haplo-insufficiency is more likely given the recent finding of PTEN gene dosage as a major determinant of tumor progression. We have included this hypothesis in the revised manuscript.

Reviewer: Andrew Green

The authors state in their discussion that MDA-MB-453 is the only cell line with this PTEN mutation but do not provide any evidence that they have screened other breast cancer cell lines except MCF-7. Data for other cell lines needs to be included or the sentence revised.

Response: Based on two previous studies (Hollestelle et. al. Molecular Cancer Research 2007; 5:195-201; Gu et. al. Cancer Research 2011; 71:2821-2825), the E307K mutation was only discovered in MDA-MB-453 but not other breast cancer cell lines. These references are included and the sentence revised.

Whilst MDA-MB-453 is derived from a 42 year old female it is a very tenious link to suggest that the PTEN mutation may represent a germ-line mutation.

Response: We agree. In the absence of germ line tissues, we have revised this sentence.

Statistical analysis is missing from Figure 2. Please include to demonstrate differences.

Response: Statistical analysis has been included.

Minor Essential Revisions

Methods. Please state which antibody was used for Western Blotting for PTEN as it is currently unclear.

Response: The sources of anti-PTEN antibodies have been indicated in the Materials & Methods and in the legends.

Fig 1. The asterisk below the PTEN is very small and needs to be enlarged.

Response: Asterisk has been replaced with an arrow.
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.


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.

Response: Paradoxically, MDA-MB-453 has high Akt activity (Fig. 3). This may due to the presence of a potent oncogenic PI3-K H1047R mutation (Hollestelle et. al. Mol. Cancer Res. 5:195-201 2007). In addition to the PTEN E307K mutation, we believe the remaining wild-type PTEN allele may not be sufficient in blocking the activation of Akt.

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 unbiquitination of PTEN as well as its effect on AKT activity.

Response: As mentioned above, we expect the knockdown of wild-type PTEN allele will not further increase Akt activation. However, we have spent considerable efforts in designing siRNA to silence the wild-type allele. Unfortunately, this effort was successful since WT and 307 alleles only differ by a single basepair and the target region may not be optimal for gene silencing. An alternative is by somatic gene knockout. However, this is a tremendous undertaking due to the cost and time (>1 year) for constructing and selecting for allele-specific knockout. Our laboratory simply does not have the resources to carry this out. We hope this reviewer can understand out predicament.

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.

Response: Agree, we do not have evidence to suggest an oncogenic effect of the E307K mutant. We have deleted that sentence.

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.

Response: PTEN gene is one of the most highly sequence tumor suppressor apart from TP53. MDA-MB-453 is the only breast cancer cell line known to harbor E307K mutation (Hollestelle et. al. Mol. Cancer Res. 5:195-201 2007). A search at the PTEN mutation database at Sanger Institute (http://www.sanger.ac.uk/perl/genetics/CGP/cosmic?action=gene&ln=PTEN) failed to yield additional cancer cells with the E307K mutation. Also, a search for PTEN SNP polymorphism at the NCBI database (http://www.ncbi.nlm.nih.gov/snp) failed to detect the G to A substitution.

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

Response: We have recently optimized the lipofection efficiency in MCF7 cells and have repeated the experiment in Fig. 5B. Statistical analysis from two experiments performed in duplicates revealed a 19.7% reduction in nuclear localization of PTEN E307K. One-tailed t-test analysis to predict a decrease in nuclear localization revealed a significant difference between WT and E307K. However, no significant difference was observed when two-tailed analysis was performed. We have included all this information in the result and legend sections of the revised manuscript.