Reviewer’s report

Title: Beta catenin and cytokine pathway dysregulation in "PTEN hamartomatous tumor syndrome" patients

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Reviewer: Anxo Vidal

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Major Compulsory Revisions

1. Methods section should be clarified. The present form that the methods are presented for mRNA and gene copy quantitation, as well as sequence analysis from both cDNA and genomic is confusing. Primers used and the precise regions analyzed by each approach should be clearly stated for best understanding. Also the method for protein extraction is missing and just “following manufacturer’s instructions” is stated, without any other reference. Since functionality of beta-catenin depends on subcellular localization, this may not be a minor issue.

2. The authors show a missense mutation in one of the patients and lower levels of mRNA in the other two. The molecular explanation for this way of inactivation is unknown but at least it should be discussed. It is clear that sequencing of cDNA can rule out mutations in the coding region as well as in the UTRs, but it is unclear which portion of the promoter (page 9, second paragraph of results) was sequenced. From Methods it looks like “a short fragment inside intron 9 was amplified”, is it enough evidence to disregard the possibility of intragenic deletions? On light of the experimental evidences provided, possible mechanisms for PTEN mRNA should be considered.

3. The authors favor a model where deregulation of the PI3K-Akt signaling is responsible for alterations found in beta-catenin and cytokine pathways. However, PTEN can act through other pathways and no evidence is provided for PI3K-Akt activation. Mechanistic proof would require pharmacologic or genetic manipulation of patients’ PBCs, which could be challenging, but the authors could show, in protein extracts from PBCs, the phosphorylation status of Akt or some of the downstream targets of the pathway. Also, since the proposed functional link between PTEN-PI3K-Akt and beta-catenin is its phosphorylation, phospho-beta-catenin analysis should be performed by western blot. Phospho-specific antibodies are commercially available.

4. The authors say that in TNFRI western the main signal is a 25kDa band that they claim to be a beta isoform of TNFR1A. However, in figure 3b only shows this band and not any others. Does it mean that other forms of TNFR1A are not detected at all? If other bands are detected they should be shown. Also, besides the molecular weight which is the evidence to support identity of this detected form as isoform beta? It seems that showing the data about mRNA expression
and sequencing of these isoforms may help.

Minor Essential Revisions

1. There are some typos that should be corrected, such as “Figure legends” or “cycline D1”. Also I believe the term “Cytochine” used in some section titles is not correct and should be substituted by “cytokine”, the one used in the main title of the article.

2. Figure 1 is mislabeled: panel c) shows genomic and panel d) mRNA quantification.

3. The authors show increased levels of messenger for c-myc or cyclin D1 in non-neoplastic blood cells, when in other instances c-myc overexpression is sufficient to drive oncogenesis. The authors could discuss the functional consequences of such alteration regarding tumor predisposition in PHTS patients.

Discretionary Revisions

1. The authors may want to discuss their results with those obtained by PTEN inactivation in mouse hematopoietic cells, such those in Guo et al Nature 2008 or Yilmaz et al Nature 2006.

2. In the present form, it is unclear which functional relationship if any exists between the beta-catenin and the TNF pathway causing phenotypic manifestations upon PTEN deregulation. The authors may want to hypothesize about this link.

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.