Reviewer’s report

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

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Reviewer: Maurizio Genuardi

Reviewer’s report:

In this manuscript, the authors undertook investigations aimed at defining the consequences of PTEN dysregulation on the WNT pathway and on cytokine expression in non tumor cells. To this purpose, they examined peripheral blood leukocytes from three patients with clinical manifestations of PTEN-associated syndromes in whom molecular analyses were suggestive of a constitutional PTEN defect.

The results obtained provide indirect evidence for the existence a link between partial PTEN inactivation, #-catenin levels and transcriptional activity, and expression of IL10, TNF# and TNF receptors. In principle, this would be a nice demonstration of the effects of PTEN alterations on other signalling molecular pathways performed ex vivo on non transformed human cells from PHTS patients.

MAJOR COMPULSORY REVISIONS

-While a clinical diagnosis of PHTS is reasonable for case 1, the manifestations reported for cases 2 and 3 are less convincing, especially for patient 3. Hence the causal relationship with a PTEN defect, particularly given that this is an RNA alteration without a demonstrated DNA defect, is only hypothetical. The main clinical manifestation in case 3 is hamartomatous polyposis: were other conditions associated with hamartomatous polyposis excluded? The association with a congenital malformation (horseshoe kidney) is more suggestive of juvenile polyposis, especially in the absence of additional typical PHTS manifestations. Was genetic testing for SMAD4/BMPR1 performed?

-The authors should provide more compelling evidence for considering the reduced PTEN expression levels in cases 2 and 3 as indicative of an undetected constitutional defect/mutation. For instance, genomic deletion analysis was performed using a single probe located in intron 9. More thorough analysis, by MLPA or by more extensive Real Time PCR with multiple probes, should be performed to rule out the possibility of large genomic rearrangements that can reduce mRNA stability or involve the region containing exon 5-6 junction, that was specifically tested with the RT-PCR probe. Mutation/methylation analysis of the 5’ non coding region is also warranted to identify alterations causing reduced expression.
Mixing RNAs from different controls does not allow accurate evaluation of interindividual variability in expression. The 20 control samples should be analysed individually, to provide a more realistic estimate of standard deviations. Theoretically, it is possible that some normal samples had mRNA levels in the range of PHTS cases, but this could be masked by the use of control mixtures.

MINOR ESSENTIAL REVISIONS

- Background, page 3, last sentence of 2nd paragraph: delete “therefore”
- Methods. Page 5, 1st paragraph: delete 1st sentence “When .. familial cases”, as the concept is obvious.
- Methods: specify how many cells from each subjects were used for western blotting.
- Results, page 9, paragraph beginning with “As shown ..”. The order of figures 1c and 1d should be inverted (alternatively, the text description can be inverted). In the same paragraph, “... was a carrier of .... whole gene deletion”: neither promoter mutations/methylation nor partial deletions can be excluded.
- Results, page 10: it is interesting that the reduced APC levels are found in the two cases that do not have a PTEN mutation identified on DNA. This could be due to alternative mechanisms (possibly mutations in other genes), that cause reduction in both APC and PTEN mRNA levels.
- Results, pages 10-11. The first few sentences of the section entitled “Cytokine dysregulation is observed ....” (where “cytochine” should be changed to “cytokine”), from “Finally ...” to “Moreover, many literature data correlate with ...” contain general concepts that are more appropriate for the introduction or the discussion.
- Discussion, page 13, 1st paragraph, 2nd sentence: “The authors showed .... PGE2 production”. Please clarify.
- Discussion, page 13, 2nd paragraph: the issue of the need for quantitative mRNA screening is certainly important. However, as previously specified, the two patients on which only mRNA level reduction was observed are also those that have a less significant phenotype.
- Figure 1a should be deleted, as it does not show relevant data.
- Legend to figure 1c and d (please note the inverted order of 1c and 1d mentioned above): “Control 1 and 2 are two healthy subjects supposedly not deleted for the target genes” # replace “supposedly” with “apparently”.

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