Author's response to reviews

Title: Loss of heterozygosity of TRIM3 in malignant gliomas

Authors:

Jean-Louis Boulay (Jean-Louis.Boulay@unibas.ch)
Urs Stiefel (Urs.Stiefel@unibas.ch)
Elisabeth Taylor (Elisabeth.Taylor@unibas.ch)
Beatrice Dolder (Beatrice.dolder-schlienger@unibas.ch)
Adrian Merlo (Adrian.Merlo@unibas.ch)
Frank Hirth (Frank.Hirth@iop.kcl.ac.uk)

Version: 2 Date: 17 December 2008

Author's response to reviews: see over
Dear Dr. Le Good,

Thank you very much for the referees comments on our manuscript and your comments and instructions on how to address a revised version. We have addressed all points raised by the referees and state them in a point-by-point reply for each referee as outlined below.

We have also dealt with your concerns raised about ethical issues. We have added a paragraph in the Methods section that erroneously was lacking in our initial submission. Please accept my sincerest apologies for that. Let me emphasize that ethical approval has been and still is in place and that tumor resection by Prof. Merlo and subsequent analysis of the tumor samples was only possible with informed consent of the patients.

This is now clearly documented in the methods section where we state: “The collection of tumor samples has been approved by the Ethics Committee of Basel-Land and Basel-Stadt (EKBB). Informed consent has been obtained together with the patient's permission to conduct open brain surgery, consenting to the use of biopsies for anonymous scientific research. This procedure follows the present recommendations of the Swiss Academy of Medical Sciences as proposed in 2008 and is in line with the Helsinki declaration.”

We hope that our revisions made do convince the referees and the editorial board and that our revised manuscript is now suitable for publication in BMC Cancer.

We are looking forward to your reply.

With best wishes
Frank Hirth

POINT BY POINT REPLY TO REFEREES

Response to referee 1 (Abdullah)
1. The paper is well written but needs a little bit more statistical analysis of the data on Table One and thus the discussion that will follow what the statistical results will show and also the comparison with other previous publications.
   Reply: We have provided more details on the tumors investigated and have amended statistics in Table 1, which are discussed in more details in the discussion section.

Response to referee 2 (Demuth)
1. Interpretation of the results would benefit from simplification. Figures 1 and 2 are very busy and a better explanation of the different shaded grey areas would be helpful.
   Reply: We have tried to simplify interpretation of our results in the discussion section. We also provide a detailed key in each Figure in order to explain the different shaded areas shown.
2. Validation of the LOH findings by in situ hybridization would help to address the discrepancy between STS and SNP analysis in Figure 2A.

   **Reply:** We have not been able to provide in situ hybridization for the tumors named in Figure 2A due to the lack of sufficient material. The seemingly discrepancy between STS and SNP analysis has been the initial clue for the existence of homozygous deletions and has therefore been used as a rationale to further search for homozygous deletions. This is already explained in §2 of the results: “Allelic retention within a chromosomal interval displaying LOH has been interpreted as a potential site of homozygous deletion, where retention seems to result from the amplification of wildtype DNA deriving from non-neoplastic cells present in the tumor biopsy [37].”

3. The biological function of TRIM3 as a tumor suppressor gene is by no means addressed in this manuscript and experiments to do so are warranted (TRIM3 knockout experiments etc.). Alternatively, the conclusion needs to be modified.

   **Reply:** We have added a paragraph in the discussion in order to emphasize the relevance of LOH analyses for the identification of potential tumor suppressor genes. We also amended the last paragraph of the discussion and modified the conclusion, where we now state “Together, these data suggest TRIM3 as a 11p15.5 candidate brain tumor suppressor gene. Further investigation will be needed to elucidate the biological function of TRIM3 and its precise role in brain tumor suppression.” (see page 14).

**Response to referee 3 (Urbschat)**

1. The sample size is quite limited and the selection is not to reconstruct. They authors need to add the important information about the WHO-grade of the investigated Oligodendrogliomas and Astrocytomas! If different tumor entities were used, as in this work, the differences have to be discussed (with an evidence of the small sample size).

   **Reply:** Glioblastoma has low incidence in the population (3.5/100 000/year), and OG even lower (0.3/100 000/year). Practically, this means that the Department of Neurosurgery at the University Hospital of Basel operates an average of 2 gliomas per month. Thus, collecting 54 glioblastoma takes more than 2 year, and 10 oligodendrogliomas, about 5 years. This explains why the material is so limited and used with parsimony. However, our data from 70 tumors are sufficient to provide a deletion map.

   We have also added information about the WHO-grade of the investigated Oligodendrogliomas and Astrocytomas (see Methods page 4 and Figures 1-3) and some of the differences are discussed in the discussion section (see page 11-13).

2. The main conclusion “Loss of heterozygosity and genomic dosage alteration in malignant gliomas signify TRIM3 as a brain tumor suppressor gene” is named redundant, in exactly the same sentences. For example: Abstract: last sentence, Background: last sentence, Results: page 9, last sentence of the first paragraph and of course in the Conclusions, where it belongs to, also in the Abstract, but not in the Background-chapter and in my view also not in Results. However, this main conclusion should be revised linguistically and the statement should be worded more carefully, due to the available data.

   **Reply:** We have omitted redundancy of our conclusion in the abstract and background, taken out the sentence on former page 9 and modified the Conclusion section as well. We have linguistically revised our main conclusion and worded our statement more carefully. In order to be consistent, we also changed the title of our manuscript accordingly.

3. The Background about “malignant gliomas” is poor and about survival times and the current therapy regimen not up-to-date. In the last paragraph the authors document the data already. This is neither common nor meaningful.

   **Reply:** We have amended the Background section and updated it with more recent data regarding the treatment of glioblastoma (see page 3). We have also shortened the last paragraph of the background and no longer document the data already there.
4. Methods, first paragraph: in which form “micro-dissection” would be used? This chapter is very comprehensive and could be shortened, apart from “biopsies and DNA extraction”, particularly a more specific tumor sample description is missing (WHO-grade, clinical data). The WHO-grade of selected tumors were only mentioned in the Figures.

   Reply: We no longer use the word micro-dissection in order to avoid misunderstanding. What we meant is that DNA was extracted from resected tumor biopsies (see page 4). We have tried to shorten the Methods section without affecting clarity. We also added a more specific tumor sample description with WHO-grades and a full description of data is amended as supplementary material.

5. Results, first paragraph showed that the acquired data are very heterogenous, as expected in gliomas, but this should be receive attention.

   Reply: We have paid more attention to this point and have amended the results section accordingly (see page 8-10).

6. Discussion, page 12: Number of the investigated GBMs is unclear (54?), comparing for example page 5! The own data should be more discussed, like tumor entities, limitations of the small sample size..., as already mentioned above.

   Reply: The number of GBMs investigated is now named consistent throughout the manuscript. Moreover, we have discussed in more detail tumor entities and limitations of the sample size (see page 12/13).

7. In Conclusions the novel findings should be pointed out, but further investigations to underline the data should be suggested.

   Reply: We have pointed out the novel findings (page 12/13) and suggested further investigations (page 14).

8. Page 8: “This area also contains.....by this region (Fig. 1).” This is unclear and needs refinement.

   Reply: We have refined this paragraph (see page 8).

9. Page 11: last paragraph, “....a recent LOH study....”.This study is from 2001!

   Reply: we no longer state “ a recent LOH study” and now state “a previous LOH study”.