THE EMBO JOURNAL

Manuscript EMBO-2009-71812

Combinations of genetic mutations in the adult neural stem cell compartment determine brain tumour phenotypes

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Review timeline:

Submission date:	04 July 2009
Editorial Decision:	24 August 2009
Revision received:	11 October 2009
Accepted:	14 October 2009

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 24 August 2009

First of all I truly apologize for the very unusual delay in getting back to you with a decision. As outlined before, this was caused by one rather late incoming referee report that we only received upon persistent chasers. I do have to emphasize though, that this third opinion was needed to reach a justified and balanced decision.

As you will see from the reports enclosed below, all referees judge the results as timely and at least potentially interesting, but do currently differ in their support for your study. Specifically, refs #1 and #3 emphasize the technically demanding experiments on postnatal neural progenitors as informative and sufficiently supported. In contrast, ref #2 raises concerns on the conclusiveness that s/he bases on potential variable CRE efficiency. This scientist also questions the overall insight, as the tumour causing gene deletions remain correlative and not further molecularly defined (maybe additional mutations contribute and further details on PNET-inducing conditions have not been revealed). This referee does also object to the presentation/discussion of the data, an issue that might significantly contribute to the currently negative opinion on your work. On balance and in light of the rather more encouraging views of both refs#1 and #3 (as well as our own in-house assessment), we would still be willing to re-assess a thoroughly revised version of your work in the near future. Such a version should overcome the obvious presentation issue (also brought up by ref#1), clearly highlight the major advance and novelty compared to earlier studies and emphasize the multiple controls that your manuscript already contains. I also have to remind you that it is EMBO_J policy to allow a single round of revisions only, which means that the final decision on acceptance or rejection will entirely depend on the next and final version of your manuscript.

Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORTS:

Referee #1 (Remarks to the Author):

In this manuscript, Jacques et al address an important unresolved issue in the literature on malignant astrocytomas: What is the cell of origin for these cancers? How do tumours arise in an organ that is well isolated from environmental carcinogens and - to a first approximation - mitotically inert? Prior art in this area has focused on susceptibility of embryonic or neonatal neural progenitors to malignant transformation. While interesting, these earlier studies are of dubious relevance to adult astrocytomas (median age of onset in the mid 50s). Jacques et al use stereotactic injections of adeno/cre or andenoGFAP/cre to ablate tumour suppressor genes of interest in postnatal neural progenitors. Their studies show 1) that postnatal neural stem cells are competent for malignant transformation, 2) that postnatal astrocytes or committed astrocyte progenitors are not competent for transformation and 3) that combinatorially distinct genetic lesions give rise to histopathologically distinct tumour types.

In addition, these authors address the important "seed-versus-soil" issue that has not been touched in other studies. There is a growing consensus that astrocytomas arise mainly from neurogenic regions of the brain...but is susceptibility to transformation a cell-autonomous property? One could imagine that stem cells and committed glial progenitors (the major replication competent cell population of the CNS) are equally susceptible to the genetic aspects of malignant transformation but that the local environment in non-neurogenic regions does not support tumour progression. Jacques et al address the seed-versus-soil question by ablating tumour suppressor genes within stem cells and committed astrocytes in vitro and then implanting the targeted cells back into the brain. Ablated stem cells make tumours but astrocytes do not. Therefore the resistance of astrocytes to transformation is a cell autonomous characteristic.
An impressive amount of technically demanding work is displayed in this article. The subject matter is timely and will interest a broad general readership. The central claims are well supported by the data. Against this backdrop, I have only one scientific comment and one editorial concern:
Scientific comment - The authors observe that ablation of RB is associated with PNETs rather than astrocytomas in their model system. However, the RB signaling axis is attenuated in most human astrocytomas via deletion of p16, amplification/activation of CDK4 and other mechanisms. This genetic disconnect between their model and real life should be acknowledged and discussed.
Editorial concern - The manuscript has a greater than usual number of typographical/grammatical errors. The abstract alone has two errors "...it is still unknown how genetic changes their type (line 1)" and "... an important role of Rb do drive the PNET phenotype (line 11)". Most of these errors are harmless but a few are egregious eg "Activation (sic) of the Rb protein family (Rb, p107 and p130) in parenchymal astrocytes ...(p.17, line 21)". The authors of course meant to say "inactivation of the Rb protein family...". This manuscript will require careful editorial attention.

Referee #2 (Remarks to the Author):

The authors attempt to show that distinct types of brain tumours arise from different compartments of the brain and that the nature of the tumours is determined by different genetic changes . To come to this conclusion, the authors use conditional genetic ko mice with combinations of RB, p53 and PTEN and use Adeno Cre to delete/inactivate the genes .

This approach is not novel but must depend on the availability of conditional KO mice (which limits its utility) and the penetrance (Tumour development) will depend on the extent of infection and amount of CRE in the infected cell . On top of that high expression of CRE is detrimental,

giving rise to chromosomal instability . It is also difficult to know if additional mutations have occurred in the cell which leads to tumour formation . Similarly the latency of tumour may also depend on the extent of Ad Cre activity and not necessarily on the genetic KO bkgd .

While overall technical component of the ms is adequate, i am not sure as to what novel conclusions emerge from this work . For instance the authors on page 12 say that GFAP expressing B-type SVZ stem cells are the origin of intrinsic brain tumours, which will be a very important conclusion, but then on page 13 soft-peddle this by saying that "can give rise to either PNET or Gliomas " based on the combinations of genes disrupted. This conclusion has been made by many other investigators .

I had very hard time reading and understanding this paper in part back & forth discussion of non contiguous figs and extensive reliance on supplementary data . I also had hard times with some of the tables, for instance S Table 1 , under RB/PTEN/P53 Null bkgd(3rd column), how did the number 45.5% came (it should be 30%).
In summary I do not think that this manuscript, depending on the limitations of the technology is adding significant new information for publication in EMBO journal .

Referee #3 (Remarks to the Author):

This is a very well thought out and well conducted study. Although there are many studies which now suggest that brain tumours arise from SVZ precursor zones, and not differentiated zones, this paper is probably the best, and is a first to probe tumour initiation in adult mice. The data to support precursor initiation of these tumours is strong. Another interesting finding is the genotype-tumour phenotype correlation. My only query is with regard to the timings of injection of virus, the authors should state in the text and the methods at what age were the animals injected with adenovirus.

1st Revision - Authors' Response 11 October 2009

Referee 1 clearly emphasises the importance and novelty of our findings, and he recognises a significant additional insight into the origin of brain tumours in comparison to existing studies. This referee had a number of minor concerns, all of which we have addressed either by re-writing or by additional experiments.

- *The authors observe that ablation of RB is associated with PNETs rather than astrocytomas in their model system. (.....) This genetic disconnect between their model and real life should be acknowledged and discussed.*
  This is a valid point, which we have addressed by analysing representative numbers of gliomas in a TaqMan quantitative RT-PCR expression assay. We found a significant upregulation of CDK4 transcripts, in keeping with typical changes in human gliomas. We have added these data as part of a figure and have added them to the results and discussion section.
- *"Editorial concern - The manuscript has a greater than usual number of typographical/grammatical errors".*
  We have carefully revised our manuscript. We have corrected grammatical and spelling errors and have carefully revised all numbers and references in the text, tables and figures.

Referee 2 raises a number of minor concerns, which mainly relate to experimental details and the usefulness of generally established technologies. We have addressed all the comments in the revised version. We have also revised the order of the figures, by moving supplemental material into the figures in the section and by revising the tables.

The referee had a number of specific comments:

- *"This approach is not novel but must depend on the availability of conditional KO mice (which limits its utility)"*.
  The use of conditional gene inactivation is indeed not novel but is a widely used and extremely well characterised system used to elucidate disease mechanisms. Importantly, this approach has been used in many seminal studies of disease mechanisms, including brain tumour pathogenesis. We feel that using a well-established system is actually an advantage, exactly because it is thoroughly characterised. Using an entirely novel system would probably introduce a number of unknown variables and would raise additional concerns / questions about the methodology. However, we feel that the combination of *in vivo* and *in vitro* techniques to address the origin of brain tumours is indeed novel.
- *" ... Cre infection and cre toxicity…"*:
  This is a very important point which we had considered when performing our experiments but we did not specifically address this issue in the previous version of the manuscript. Indeed we had carried out several series of control experiments using heterozygous LoxP lines of all genotypes. We did not observe tumour formation in any of the lines, hence excluding cre–induced mutagenesis as the cause of these experimental brain tumours. The second argument, that *"cre can induce genomic instability"* is valid. Therefore, our conclusions are based on large cohorts before stating that there is a consistent genotype–phenotype correlation. The referee also comments on the possibility "*that tumour latency may depend on the cre levels rather on the combination of genotypes*". This is an extremely unlikely scenario, as we controlled for adenovirus titres and injection volumes throughout the experiments, and also given the sound statistical significance. However, it is possible that variability of tumour latencies within one genotype may be a result of variable cre efficiency. This possibility is discussed in the manuscript. The referee further comments *"It is also difficult to know if additional mutations have occurred in the cell which leads to tumour formation"*. We had discussed this possibility as a very likely and biologically explicable scenario. We specifically made the point that the initial mutations will provide a growth advantage to the stem cells which the leads to a further selection of cells which acquire additional mutations, resulting in tumour formation and progression from microneoplasia into tumours. This mechanism also is the biological basis for brain tumour progression in humans.
- *"The authors on page 12 say that GFAP expressing B-type SVZ stem cells are the origin of intrinsic brain tumours, which will be a very important conclusion, but then on page 13 soft-peddle this by saying that "can give rise to either PNET or Gliomas " based on the combinations of genes disrupted. This conclusion has been made by many other investigators*.
  The referee confirms that our statement "*GFAP expressing B-type SVZ stem cells are the origin of intrinsic brain tumours "* is a very important conclusion. This statement is still correct, as both PNET and gliomas are both intrinsic brain tumours and in our view becomes even more important, as we can demonstrate that the same type of stem cell is the cell of origin for PNET or gliomas. We are not aware of any previous work that *"... this conclusion has been made by many other investigators"*. In fact we show here for the first time that the same stem/progenitor population can give rise to phenotypically different tumours. We have explained these finding more clearly in the revised discussion.

Referee 3 is very supportive of our study and finds our data strong, and the conclusions important and novel. The referee wants more information …

- *... about a "correlation between age at injection and the tumour latency"*.
  We had these data included in the results section of our previous version. We are now presenting the data graphically in Fig. 3 together with survival and mitotic count. Our data

indicate that the age at injection does not influence tumour latency. Material and methods now contain more information about the age at injection.

Summary of major changes in the revised version.

- We have addressed all referees comments.
- We moved data from the supplementary material section into the main section and have also added new data. We have also changed the order of the figures, to address the concern of one referee.
    - One supplementary figure is now integrated into Fig 1 (G-J).
    - One supplementary figure is now integrated into Fig. 2 (P, Q, R, S).
    - Figure 3 contains a new graph (3C), illustrating the relationship between age at injection and tumour latency.
    - Figure 6J contains new data, showing the involvement of the Rb pathway in experimental gliomas.
- We have revised the Tables in the supplementary section and corrected all errors from the previous version.
- We have added the qRT-PCR data to the result and the discussion section
- We have added control experiments addressing the Cre toxicity into the results section.
- The abstract is rewritten
- The Discussion is entirely re-written and restructured, and subdivided by subheadings. The flow is more logical and clear.

