Reviewer's report

Title: STAT6 expression in glioblastoma promotes invasive growth

Version: 1 Date: 13 December 2010

Reviewer: Janusz Rak

Reviewer's report:

The article by Merk et al explores the role of STAT6 in aggressiveness of human glioblastoma. While other STAT proteins have been previously examined and found to promote or inhibit tumour growth, STAT6 has not been extensively studied. The authors show that this particular signal transducer is expressed in some, but not all, glioblastoma (GBM) cell lines, and if it is, the levels are higher than in normal fetal astrocytes. The authors also document the across-the-board nuclear staining for STAT6 in a cohort of astrocytomas grade I to IV. Interestingly, Merk et al also show that EGFR stimulation causes STAT6 phosphorylation, whether directly or indirectly. They also document that shRNA silencing of STAT6 in U87 cells is associated with a decrease of invasiveness and proliferation. Finally, the study attempts to use the Rembrandt data base to suggest that STAT6 upregulation is associated with poor prognosis.

Overall this is an interesting, original and well documented paper. However there are a few possible shortcomings that the authors would be asked to address.

Major Compulsory Revisions:

1. The authors make somewhat inconsistent claims from various pieces of presented evidence. First, the way Table 1 is presented in unconvincing as in some sections of that table the authors present individual cases, which still amount to 16.6% (?). Clearly there is not enough material to stratify cases into “high” and “low”, and there is no point to do so either. The useful message from that table is that all grades of GBM are positive for STAT6, at least to some extent whereas normal brains are not. Therefore, the table should be redesigned to reflect this reality (i.e. positive and negative outcomes only). Second, there is a rift between data in Table 1 and the Rembrandt based analysis. This is because it is difficult to imagine that STAT6 would be expressed in PA (a manageable disease) and still be a sign of poor prognosis. There is not enough cases in the Rembrandt analysis to make any prognostic conclusions and either the authors should discuss this properly or delete this non-informative analysis altogether.

2. The paper is lacking much of the technical and methodological detail. There is little specific information as to under what conditions the growth and migration assays were done, and the absence of sequence, vector and procedure information in the case of shRNA silencing is startling. This should be corrected.

3. The authors should explore the properties of STAT6-silenced cells in vivo, e.g. in a xenograft model. In vitro assays in high serum media are not conclusive as
to the real impact of this molecule on tumour growth, invasion angiogenesis and other important aspects.

Minor Essential Revisions:

4. The authors should at least try and explain why STAT6 is not expressed in U251 cells and whether this has any impact on proliferation and invasion of these cells. If it doesn’t then a comment as to why not would be appropriate.

5. Making references to STATs as oncogenes or tumour suppressors should be qualified (type II) unless the authors possess evidence that these molecules have a primary transforming role (undergo mutations). On the other hand, it may be interesting to consider what are the causes of STAT6 upregulation in proliferating NHAs, and more so, in GBM cells.

Discretionary Revisions:

6. It would be appropriate to pay more attention to the emerging molecular classification of high grade gliomas and how this may impact the study on STAT6. The authors could also assess whether STAT6 positive cases are the ones expressing EGFR or else they represent secondary GBM and its hallmarks. None of this is presently considered.

7. If the authors feel compelled to comment on the present status of the GBM therapy, this should be done in a more comprehensive and balanced fashion. This field is clearly awaiting a breakthrough, but progress has been made

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests