Reviewer's report

Title: STAT6 expression in glioblastoma promotes invasive growth

Version: 2 Date: 12 March 2011

Reviewer: Janusz Rak

Reviewer's report:

With inclusion of several clarifications the revised paper is now much more convincing. It is an interesting study, overall, and while I have no major compulsory revision requests, I would like to point out a couple of interpretational issues (minor essential). If left in their present form these aspects of the manuscript could potentially detract from the reader’s appreciation of this important work.

1. The paper is written in such a way that one is not entirely sure what mechanism(s) of STAT6 contribution to gliomagenesis is actually being proposed. Is it proliferation, invasion (both tested in vitro only), immunosuppression (not tested at all, but discussed), or some other cooperative mechanism (inferred from the expression data sets)? As mentioned in the earlier review, one major leap in the authors’ line of analysis is between the in vitro assays and patient gene expression and clinical data sets. Animal experiments could bridge this gap somewhat. However, it is easy to understand that in vivo models, including orthotopic xenografts, are difficult, expensive, plagued with regulatory hurdles, time consuming, and possibly beyond the time frame allowed for the revision. With this said, the limitations of SCID mice in capturing the immune component of STAT6 activity cannot be greater then those of monolayer cell culture models, on which the entirety of the mechanistic analysis is based in the present study. Incidentally, there have been numerous informative studies on GBM invasion in immunodeficient mice. The suggestion, then, for the authors would be to consider rephrasing sections of the discussion to account for the aspects of STAT6 biology that have been studied in their papers (proliferation, invasion), and those that are relevant but have not been analysed (in vivo invasion, immunity).

2. The argument about STAT6-/- mice and tumour growth characteristics is interesting. However, the authors comment on the fact that in these mice not only spontaneous tumours, but also xenografts exhibit an impaired growth. This would suggest that a host cell mechanism is involved, the nature of which is potentially unrelated to changes induced by the authors in GBM cell lines. This should be made more clear and, if possible, supplemented with some information as to STAT6 status in stromal cells present in tumour sections.

3. The section describing Rembrandt analysis is still slightly unclear. What baseline levels of STAT6 do the terms “upregulation” and “downregulation” refer to? It cannot be the normal brain, because the authors clearly document that
STAT6 is not expressed in this setting, and most tumours (Table 1) are histologically positive for this protein, no matter what grade.

**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