Reviewer's report

Title: STAT6 expression in glioblastoma promotes invasive growth

Version: 1 Date: 29 November 2010

Reviewer: Julie Carrier

Reviewer's report:

Reviewer's report: Major Compulsory Revisions.

Comments to Author,

In this manuscript, BC Merk and colleagues explore 1) the association between STAT6 protein expression and grade of glioblastoma cell lines and glioma patient tissues, 2) the consequence of STAT6 knockdown by shRNA on cell proliferation and invasion and 3) the publically available relationship between STAT6 mRNA expression and survival of patient with glioma.

This manuscript is of pertinence to glioblastoma and the work is well performed. There are, however, a number of issues which need to be addressed.

Major comments:

1. In its present form, results from this manuscript are somewhat superficial and some sort of mechanistic link regarding STAT6 knockdown would significantly improve the manuscript. In particular, the phenotype of STAT6 silenced cells should be characterized in more details, such as cell cycle analysis and characterization of cell cycle protein expression, pathways involved in the observed lower invasive ability of STAT6 silenced cells, etc.... Another suggestion would be to search for genes co-induced with STAT6 in microarrays from human glioma and investigate the relationship between STAT6 expression and the expression of these genes, using clones of U-1242 MG and U-87MG.

2. The relationship between STAT6 and STAT3 should be discussed: is there known crosstalk between these 2 STAT? Is the observed reduction in STAT3 associated with STAT6 knockdown due to conserved sequence targeted by one shRNA versus is STAT3 a downstream target of STAT6? In fact, it is hard to understand why possible off target effect of STAT6 shRNA have been tested by STAT3 expression for U-1242 clones and by STAT5b expression for U-87 clones.

Minor comments:

1. Figure 1, the immunoblot for STAT4 is absent.

2. Page 12, it is state that STAT6 mRNA expression have been normalized to HPRT while in the legend, it is written that it was normalized by the geometric mean of the housekeeping genes beta actin and HPRT. In fact, the method section does not describe how real-time (quantitative) PCR have been
performed.

3. Results from the tissue-microarrays are convincing but it would have been interesting to use phospho-specific STAT6 antibody since it is the active form. In addition, it would have been interesting to correlate STAT6 immunostaining with patient survival, if that information is available.

4. There are relatively few human glioma overexpressing STAT6 (10 on 343 patients) compared with 72/343 STAT6 down-regulated glioma and the authors should discuss the possibility of false positive results in microarray analysis. Furthermore, because astrocytomas, oligodendrogliomas and GBM tumors do not imply the same prognostic, the authors should clearly compare the survival of patient with up or down-regulated STAT6 expression within the same subtype of tumors.

5. Lentivirus mediated shRNA cellular infection could be associated with an interferon response and this should be assessed in STAT6 shRNA clones.

**Level of interest:** An article whose findings are important to those with closely related research interests

**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