Reviewer's report

Title: The role of IFITM3 in the growth and migration of human glioma cells

Version: 1 Date: 7 August 2013

Reviewer: Jonathon Parker

Reviewer's report:

Major Compulsory Revisions

The authors should be lauded for their investigation of potentially novel involvement of the IFITM3 gene in the growth and invasion of glioblastoma, a particularly insidious and treatment resistant brain cancer. However, there are some major flaws in the scientific approach and data interpretation within the manuscript which need to be addressed.

1.) Immunohistochemical studies are not properly controlled. The authors do not provide positive (i.e. tissue with previously known IFITM3 protein expression) or negative (i.e. peptide blocking) controls for the specificity of the anti-IFITM3 antibody in this particular experimental context. It is especially concerning there is such high background staining in the WHO IV (glioblastoma) tissue. Further, the authors provide no experimental data to suggest the IHC signal detected is specific to glioma cells within the tumor tissue. Glioblastoma is a very cellularly heterogeneous tumor which includes reactive astrocytes, entrapped neurons, microglia, endothelial cells, and other cell types. Thus, it is plausible the IHC signal the authors state is specific to glioma cells, may represent expression in a stromal cell type. It would be highly recommended to perform dual antibody experiments to demonstrate co-localization of IFITM3 immunoreactivity with one or several known glioma cell expressed proteins.

2.) Insufficient data to support knockdown of IFITM3 via shRNA is resulting in gene specific effects in glioma cells. First, the authors need to demonstrate that knockdown of IFITM3 via the lentiviral shRNA construct results in decreases protein expression, to supplement the finding of decreased mRNA. Further, the authors should strongly consider the use of multiple hairpin constructs (at least one additional) to demonstrate specific knockdown of the gene in question. The specificity of knockdown would be further supported by interrogating message levels of the IFITM1 and IFITM2 genes which are known homologs with significant core sequence homology. Lastly, the authors do not clearly state whether the control shRNA is a scramble of the IFITM3 shRNA which would be the most appropriate negative control in this series of experiments.

3.) The paper must be restructured in terms of the results sections. The heading of the results sections should represent clearly the findings of a series of experiments designed to support an argument within the paper, and not merely a summary of assays performed.
Minor Essential Revisions

1.) On page 5, line 2, the authors refer to multiple studies performed in mice, yet only one primary research publication is cited. More supporting literature citations would be necessary to substantiate the claims made.

2.) The pore size (i.e. micron diameter) of the transwell inserts is not specified. This parameter is critical in the study of glioblastoma cell migration, as those transwell inserts with the small pore size (i.e. 3 microns) best assess the in vivo invasive behavior of glioma cells. This is due to the small extracellular spaces they provide for cells to move through, which are similar to those in the brain tissue of glioblastoma patients.

3.) Figures 1,2,3, and 5 which include photomicrographs need scale bars.

**Level of interest:** An article of limited interest

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests