Reviewer's report

**Title:** Overexpression of CDC2/CyclinB1 in gliomas, and CDC2 depletion inhibits proliferation of human glioma cells in vitro and in vivo

**Version:** 4  **Date:** 21 December 2007

**Reviewer:** Michael Berens

**Reviewer's report:**

**Summary**
Serially-collected specimens from a brain tumor showing progression from low â## to â## high grade revealed increased levels of CDC2. An IHC survey of glial tumor in a tissue microarray suggested the increase in CDC2 associated with glial tumor progression. Knock-down of CDC2 using shRNA depletes protein and mRNA for CDC2, accompanied by arrested proliferation of p53mutant glioma cell lines. Viral delivery of shRNA to knock-out CDC2 in human glioma xenografts leads to retardation (temporarily) of subcutaneous tumor growth and prolongation of animals harboring intracranial gliomas.

**Critique**

**Major Compulsory Revisions**
none

**# Minor Essential Revisions**

1. That perturbations in levels of CDC2 occur in gliomas is not particularly surprising in light of the well-documented elevated cell proliferation associated with increasing grade of malignancy. Additionally, proliferative impairment coincident to the loss of CDC2 from shRNA knock-down confirms the well-established, critical role of CDC2 in a cell navigating cell cycle. The suggestion that aberrant CDC2 expression justifies this gene being labeled as an oncogene in gliomas is poorly founded.

2. The manuscript still warrants considerable editing to ensure proper use of English.

3. The description of the orthotopic injections (page 9) indicates â##1mm3 of SHG44 xenogeneic graft tumor tissue was injected into the frontal lobe ofâ##; mice.â## This is a very large injection, and the authors should confirm this is an accurate report of the technique.

4. The use of the phrase â##off targetâ## effects of shRNA (page 10) is unconventional. In light of the failure of C4 to knock-down CDC2, a better term would be â##inactiveâ##. Off-target effects of shRNA interventions are conventionally considered the impaired expression of genes unrelated to the intended target.

5. The report of the â##Therapeutic potential of CDC2 deletion on human
gliomas: in vivo studies## (page 11) uses the language, ##weight and volume of other xenogeneic graft tumor treated with recombinant C3 retrovirus were significantly reduced##, but the term ##significantly## is a statistical reference, and appropriate analysis of the differences in tumor weights are not provided. Either do the analysis of the measurements, or rephrase to indicate that tumor weights and volumes seemed different.

6. In the Discussion (page 13), the brief prolongation of survival of animals with intracranial gliomas consequent to C3 treatments but the subsequent abrupt fall-off in survival suggests that the infection rates of the virus are poor. This point is made, but the therapeutic challenges of viral elimination of key cell cycle genes in tumors is an enormously challenging problem, especially if the target gene is necessary for normal cell function or renewal.

7. Table 1 is very confusing as to the fractional entries in the body of the table. What do the numerators and denominators represent?

# Discretionary Revisions
none

What next?: Accept after minor essential revisions

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

Quality of written English: Needs some language corrections before being published

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