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: 3 Date: 30 August 2007

Reviewer: Jose Segovia

Reviewer's report:

General

Gliomas are very aggressive tumors with a very poor clinical prognosis. For these reasons several different therapeutic approaches, including gene therapy, have been developed in recent years. This paper focuses on one of these approaches, namely, the use of RNAi to knockdown a protein involved in cell-cycle regulation. The authors analyze the expression of CDC2 in a series of human gliomas, and conclude that the expression of this protein increases in the more malignant tumors. Based on these results, the investigators propose that reducing or eliminating the expression of the protein will be an effective clinical treatment. To accomplish this objective, the authors use retroviral vectors to express shRNAs against CDC2. Although the concept of the treatment proposed is interesting, and some of the results are very positive, the paper needs a careful rewriting, and important control experiments must be included.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

I believe there is a confusion regarding “recombinant retroviral vectors”, which I understand from this manuscript to be the plasmids that contain the minimal retroviral sequences, as well as the cassette to express the shRNA, and the actual retroviral vectors, or viral particles or, simply, virus. This comment is based on the following: On page 6 it is stated that “cells were transfected using a modified Lipofectamine TM 2000 protocol”. It is not stated which cells, but if these are the glioma cells, then it is not a viral vector but a plasmid that is used to transfect them. Then on page 11, it is indicated that effective recombinant vectors were packaged on Phoenix cells, which is the method to obtain retroviral vectors. In general I find the description of the construction of the different plasmids and viral vectors insufficient, for instance: what promoter is used? Also there is no indication of viral titer obtained in vitro, only at the end of the discussion a very high titer for a retrovirus of 2.15x10^7 RCFU injected into the gliomas, is mentioned. I was not able to understand how the viruses were injected subcutaneously in vivo. Were the injections of 100 µl performed in 10 different sites (on a 0.5 mm diameter tumor) every day for three days, and that procedure repeated 3 times (would that make a total of 900 µl, that is almost 20 million viruses?) I cannot understand this procedure. Also there are no controls of
the efficacy of the viral particles in vitro.

I also believe the controls for RNAi experiments are not appropriate. In the in vitro transfection assays three different shRNAs were used, as well as “blank retroviral vector”, and the transfecting reagent. I think that a plasmid expressing a non-related shRNA (or scrambled) should be used to insure the specificity of the RNAi process. On page it is indicated that C1, C2 and C4 oligonucleotides recognized sequences within the CDC2 ORF, and in page 10 it is stated that C4 is “off-targeted” and, thus it is used as a negative control. Regarding the in vivo experiments with implanted gliomas, there is injection of the retrovirus, but the only control is injecting the same volume of PBS. Controls regarding the effect of the retrovirus should be included. There is also ample discussion regarding the generation of new stem cells from gliomas and fetal tissue, but except for mentioning that they do not express CDC2 no data are obtained from them, and no characterization of the cultures is provided (i.e. expression of CD133, nestin or glial or neuronal markers.)

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Figure 7 is used to demonstrate the efficacy of the shRNAs to reduce the expression of CDC2. Figure 7C shows the result from a western blot, but it does not indicate the treatments.

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Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

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

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