Reviewer's report

Title: Effects of small interfering RNA targeting thymidylate synthase on survival of ACC3 cells from salivary adenoid cystic carcinoma

Version: 1 Date: 21 June 2008

Reviewer: Nobuhiko Oridate

Reviewer's report:

The authors tried to elucidate the potency of thymidylate synthase as a therapeutic target in adenoid cystic carcinoma. They demonstrated that ACC3 cells had higher TS expression than non-cancer cell lines and that the induction of TS siRNA inhibited cell proliferation, which was associated with S-phase accumulation. They showed an increase in p21, active caspase-3 and spermidine/spermine N1-acetyltransferase by the induction of TS siRNA. They also found that a significant cytostatic effect by treatment with this siRNA in a xenograft model. Although I agree with their conclusion that TS may be a therapeutic target in ACC, there are several issues to be clarified as listed below before considering the publication in this journal.

Major Compulsory Revisions:
1) The authors used only one type of TS siRNA in this manuscript. It would be much better if they could use more than one siRNA to ensure the effects observed in this study was purely due to specific inhibition of TS expression. It would be also good to demonstrate the recovery of phenotype by transfection of siRNA-resistant TS cDNA. At least, the authors need to discuss this limitation.

2) The authors suggested the induction of apoptosis by the TS siRNA introduction in figure 5 C and D. However, cell cycle analyses (figure 3) did not show no obvious increase in sub-G1 population. The authors need to discuss this discrepancy.

Discretionary Revisions
3) It would be better to explain more in detail the association of S-phase accumulation and increased apoptosis induced by TS inhibition. Was induction of apoptosis resulted from up-regulation of p21 or activation of S-phase checkpoint instead of G1 checkpoint? Is there some other mechanism(s) to explain for these results?

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

Quality of written English: Acceptable

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.