Reviewer's report

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

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Reviewer: Maria Zajac-Kaye

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The paper by Shirasaki et al investigated the effect of siRNA directed against thymidylate synthase on the survival of salivary adenoid cystic carcinoma cells. The authors transfected the ACC3 cell line with commercially purchased siRNA targeted for TS and showed inhibition of TS expression that was linked to the inhibition of cell proliferation. In addition, the authors injected the siRNA into ACC3 tumor xenografts and showed reduction of tumor volume following siRNA injection. The experiments are well performed, however, the authors should address several major issues:

1) The authors did not show that TS protein levels were reduced following siRNA transfection. An immunoblot analysis showing reduction of TS levels in transfected cell line as well as in the tumor xenografts would strengthen the results.

2) The data would be greatly strengthened by testing additional cell lines. For example, why was only ACC3 tested? There are many other related tumor cell lines that could be tested as well as other salivary gland adenocarcinoma cell lines.

3) It would be more convincing if the authors used two different siRNA target sequences especially since the effect on cell viability is modest.

4) lack of error bars on figures 2b and 4b suggest that this data comes from only one experiment. It would be preferable to obtain additional data including another target sequence as mentioned above.

5) There are several sentences that should be edited for the clarification of the text. For example “TS gene amplification was 11-fold higher in ACC3 cells than keratinocytes” do the authors mean gene amplification or gene expression.

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