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: 13 June 2008

Reviewer: Shu-Chun Lin

Reviewer's report:

Thymidilate synthase (TS) is essential for DNA replication and is frequently overexpressed in cancers. Conventional chemotherapeutic drug 5-FU can target TS. However, 5-FU also generates side effects by disturbing other metabolism systems. This study investigated the phenotypic effects of TS knockdown by si-RNA delivery in ACC3 salivary malignant cell, and identified the induction of S-phase arrest and apoptosis. Authors also found the decrease of tumorigenesis in a xenographic animal model. The preclinical study was particularly well done and impressive. Overall, the study was comprehensively performed and the findings may bestow insight of TS blockage for potential therapeutic extension. The quality of the manuscript is also rather high. The following weakness needs improvement.

1. All studies were carried out on only one head and neck cancer cell. Apparently, this cell can not represent all head and neck cancer cells exhibiting a great extent of diversity. The findings should be considered to be preliminary. At least one more cell is required to confirm the key findings of this study. Also, the rationale why ACC3 was used to address the TS issue and the background information of ACC3 needs to be further included.

2. Figure 2: No western blot or IF data was present to show the knockdown of TS protein expression, which is also mandatory for this paper.

3. Figure 3: The 29% S-phase fraction in control cells being identified is far too high. Although cancer cell have rapid proliferation, it is hard to believe that some 1/3 ACC3 could be in S phase. Examination should be performed to see if this is an artifact induced by transfection procedure by comparing to parental cells. BrdU incorporation assay should also be performed to clarify the query. Usually, G1 phase accumulates following S arrest, authors should present the fluctuation of all phases (including sub-G1) to better evidence their findings.

4. Figure 4 shows that both p21 and Casp-3 were activated by TS knockdown. This reviewer may assume the activation of p53 following TS knockdown underlies such changes since both events are p53-inducible. What is the status of p53 in ACC3? wild type or mutant? I would think that the cell cycle arrest triggered the repair systems in ACC3 that activated wild type p53 for all subsequent phenotypes. Thereby, authors should address more on p53 in this paper.

5. Since knockdown of TS decreased cell viability (~40%), induced cell cycle
arrest (~25%) and apoptosis (from 0.5% to 3%), senescence that secondary to cell cycle arrest and p21 up-regulation could be a more important event following TS knockdown than apoptosis.

6. Please show Mean +/- SD for all real-time PCR data.

**Level of interest:** An article of outstanding merit and interest in its field

**Quality of written English:** Acceptable

**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.