Author's response to reviews

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

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Author's response to reviews: see over
Letter to the Editor

Dear Prof. Melissa Norton
Editor-in-Chief
BMC Cancer

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Effects of small interfering RNA targeting thymidylate synthase on survival of ACC3 cells from salivary adenoid cystic carcinoma
Shin-ichiro Maruya, Takashi Shirasaki, Hiroki Mizukami, Seiji Kakehata, Hidekachi Kurotaki, Soroku Yagihashi and Hideichi Shinkawa

I am returning herewith the above manuscript revised according to your notice. We found the referees’ comments most helpful and have revised the manuscript accordingly. I also included a letter that responded to reviewers’ comments. We greatly appreciate if you take the publication of our manuscript into consideration.

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The comments of the three reviewers have been helpful in allowing us to revise our manuscript. We should like to thank the referees for their helpful comments and hope that we have now produced a more balanced and better account of our work. We trust that the revised manuscript is acceptable for publication in BMC Cancer. We have attempted to address the questions raised by the referees as follows:

Referee #1 (Dr. Shu-Chun Lin)

The reviewer kindly recommended using more cell lines in order to address the effect of TS silence by siRNA method in various malignancies. In this study, we focused on the efficacy of TS silence in adenoid cystic carcinoma (ACC), because this type of uncommon salivary gland carcinoma is characterized by frequent occurrence of recurrence and distant metastasis and the effective therapy has not been established. We understand that the study in several different ACC cell line is better. However, available cell lines originated from ACC is very rare. Most in-vitro investigations of ACC have been performed by using ACC3 cell line [Ref.35/ Page 16, line 7 to 9]. We selected ACC3 cell line which is well-known as a representative line of adenoid cystic carcinoma.

Referee #2 (Dr. Nobuhiko Oridate)

1. As the reviewer’s indication, we found that TS silence induced activation of p53-pathway and cell cycle at S-phase without significant increase in sub-G1 population in ACC3 cells. However, the up-regulation of p21 and S-phase accumulation seems to be a common event in cells treated with TS inhibitors [Ref. 22, 23, 28/ Page 13, line 14 to17/ Page 15, line 5 to 8]. In this context, we speculate that S-phase accumulation precedes increase in sub-G1 population. Previous investigations showed that ZD9331, a chemical TS inhibitor, induced S-phase accumulation at 24 hours and subsequently increase in sub-G1 apoptotic fraction at 48 hours in p21-null colorectal carcinoma cells in vitro and that 5-FU led to significant increase in S-phase population in samples treated for 5 days and apoptosis with TUNEL-positive cells in tissues treated for 7 days in an investigation
of clinical colorectal cancer samples [Ref. 8, 32]. These observations suggested that apoptosis with sub-G1 accumulation occurred after S-phase accumulation in cancer cells treated with TS inhibitors. Our induction of TS siRNA was transient without constitutive silence because of limitation of our lipofection method. Thus, our results indicted that S-phase accumulation was an early event of TS silence in ACC3 cell line [Page 14, line 5 to 16].

2. Our preliminary cDNA microarray in SCC cells data showed that p21 was a primarily down-regulated gene in treatment with TS siRNA transfection. In addition, we found the up-regulation of p21 and wild-type p53 in ACC3 cells transfected with TS siRNA. We consider that the activation of p21 pathway occurred in an early stage of TS silence, because the effect of our transfection was primary and temporal [Page 14, line 13 to 16].

Referee #3 (Dr. Maria Zajac-Kaye)

1. The reviewer kindly recommended the study in several different cell lines such as HTB-41 cells. In the present study, we focused on the efficacy of TS silence in adenoid cystic carcinoma (ACC), because this type of uncommon salivary gland carcinoma is characterized by frequent occurrence of recurrence and distant metastasis and the effective therapy has not been established. However, the available line from ACC is very rare and most experimental investigations have been performed by using ACC3 cells as a representative cell line of adenoid cystic carcinoma [Ref.35/ Page 16, line 7 to 9]. HTB-41 cells were established from mucoepidermoid carcinoma, a different histological type from adenoid cystic carcinoma. Whereas mucoepidermoid carcinoma is originated from excretory ductal epithelium, adenoid cystic carcinoma is believed to occur from terminal (intercalated) epithelium. Chemo-sensitivity has been quite different between these tumors. Mucoepidermoid carcinoma has been known to be relatively effective in conventional chemotherapy such as cisplatin and TS inhibitor. However, the efficacy of TS inhibitors has not been investigated in adenoid cystic carcinoma [Ref. 26]. We further addressed the reason why we focused on adenoid cystic carcinoma and ACC3 cells in the revised manuscript, quoting additional references [Page 12, line 3 to 21].

2. According to the reviewer’s suggestion, we mentioned the result of preliminary experiment with another siRNA for TS gene [Page 13, line 1 to 4]