Reviewer’s report

Title: Diet Folate, DNA Methylation and Genetic Polymorphisms of MTHFR C677T in Association with the Prognosis of Esophageal Squamous Cell Carcinoma

Version: 1 Date: 15 December 2010

Reviewer: Karen Curtin

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General comments:
The research adds to the body of literature in esophageal cancer by addressing dietary and genetic factors and prognosis in the lesser-studied squamous cell carcinoma in a Chinese population. The abstract and manuscript are clearly and concisely written; however, there are minor typographical and grammatical errors and awkward language throughout the manuscript that need attention from the authors (see minor issues section). These can easily be addressed by a careful review of English language usage and making minor corrections.

Major compulsory revisions: None; the methods are appropriate, and generally defined in sufficient detail and the discussion and conclusions are adequately supported by the results. The references acknowledge the body of work for which their study is a relevant addition. However, several minor essential revisions are in order, as detailed below.

Minor essential revisions:
1. Experiments section of Methods and throughout, human gene names should appear in italicized font; also include the dbSNP reference SNP id number (rs#) for the C677T polymorphism.

2. In the Statistical Analysis paragraph of the Methods, the authors state they examined differences between subjects in folate intake categories and survival using Kaplan-Meier curves and Log-rank test. Were the K-M curves/survival times significantly different in Figure 1 between high, moderate, and low intake based on the log-rank test? Please state in the results. It appears survival may be significantly higher for those with high intake compared to moderate and low, but that moderate did not differ significantly from low; the authors might consider comparing high intake to moderate and low intake combined.

3. In the Methods and/or Results, the authors need to describe in more detail how aberrant DNA methylation was determined.

4. Discussion section: Is it possible that those patients in the highest category of intake also exhibited other healthy behaviors (no tobacco use, no or low alcohol); do the aHRs in Table 4 differ substantively if the hazard ratios are also adjusted for alcohol or tobacco use?

5. The authors should tone down their assertion, which the data from a small
study provide suggestive evidence of but do not clearly support, that “This finding indicates that the role of genetic variants in the carcinogenesis depends on the dose of folate intake” to more clearly state that ESCC prognosis may be influenced by folate intake in relation to MTHFR C677T genotype.

6. Cut-points and units (e.g. micrograms) for tertile of folate intake should be explicitly stated in the Results or alternately a footnote in Table 4. Also, please state explicitly if there is a government-mandated folate fortification of processed foods similar to the United States.

Discretionary revisions:

1. In the Experiments section of the Methods, please describe the methylation at the promoter regions in more detail, e.g. CpG island methylation.

2. In the Statistical Analysis section, the authors define time of survival as time between diagnosis and death; was this death from all causes, or primary cause of death Esophageal cancer? Please explicitly state and which and if all cause mortality, indicate if any patients died of a primary cause other than cancer.

3. Results section, paragraph 2, rather than describe single or unmarried patients as “sole patients,” it might be clearer to describe them as “Those patients divorced or living alone.”

4. Results section, paragraph 2, authors state that median survival was >4.59 years for high folate consumption. Does the > sign indicate the majority these patients were alive at the end of the follow up period, given the maximum follow up was 4.66 years.

5. How do the authors see their findings influencing future research, i.e. a randomized clinical trial of folate supplementation in pre/post-esophagectomy patients? It would strengthen the manuscript if the authors more fully address the impact of their findings in terms of future studies, either planned or suggested.

Minor issues not for publication: Authors should correct numerous minor typographical and grammatical errors throughout their manuscript. Some examples: In the Background, paragraph 2 “More and more evidences have indicated.” should read “More and more evidence has indicated.”. Methods paragraph, “All patients were followed by staffs of Yangzhong Cancer Research Institute.” should read “All patients were followed by staff of Yangzhong Cancer.”. Results, paragraph 1, “Among 125 ESCC patients, 5 were loss to follow up.” should read “.lost to follow up.”. Discussion, last paragraph, “This finding indicates that the role genetic variants.” should read “.role of genetic variants.”.

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests:

I declare I have no competing interests.