Reviewer's report

Title: DNA Methylation Subgroups and the CpG Island Methylator Phenotype in Gastric Cancer: A Comprehensive Profiling Approach

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Reviewer: Alfred SL Cheng

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In this study, Loh et al have investigated the methylation status of 60 gastric tumors and paired adjacent tissues using the Illumina GoldenGate Methylation Panel I assay which interrogates 1,421 CpG sites located within 768 cancer-related genes. The result showed that 219 CpG sites in 147 genes were differentially methylated between tumors and matched normal tissues, with HOXA5 and hedgehog signaling being the top-ranked gene and signaling pathway, respectively. Unsupervised clustering and statistical analysis indicated high and low methylation subtypes of gastric cancers, in which female patients were preferentially associated with the former subtype. No significant difference was found in other clinicopathological parameters such as age, metastasis, survival, H. pylori infection.

Major Compulsory Revisions

1. Upon the genome-wide DNA methylation analysis and statistical analysis, the authors identified a number of potentially differentially-methylated genes between gastric tumor and non-tumor samples. However, as mentioned by the authors in Discussion, only one CpG site was evaluated for 70% of these genes. It is therefore important to validate these candidates using an independent and preferentially quantitative DNA methylation assay e.g. pyrosequencing or MethylCap assay. For example, whether the differential methylation of HOXA5 and the 8 genes in the hedgehog signaling pathway (Table 1) can be confirmed?

2. It is now recognized that field effect of DNA methylation occurs in the gastric tissues adjacent to the tumor, even though they look histologically normal. In this study, the authors used tumor-adjacent tissues from the same patients for the DNA methylation analysis and claimed that these are ‘normal gastric tissue’ (line 204 of page 11; line 224 of page 12; line 364 of page 18). Gastric tissues from non-cancer/inflammed patients should be as normal control.

3. The authors described in the abstract and the main text that ‘the existence of a comparable CIMP subtype in gastric cancer has not been clearly established’ because of relatively low interrogated CpG sites and sample size. However, as mentioned in the Discussion by the authors, Kim et al (Cancer Lett 2013) and Zouridis et al (Sci Transl Med 2012) have carried out more comprehensive studies in terms of much more interrogated CpG sites (over 27,000) and larger sample number (over 200). Both studies have revealed the existence of CIMP in subgroups of gastric cancers, which associated with distinct clinicopathological
parameters. What additional information have the authors gained in this study? The new data and insight should be clearly described in the Discussion in comparison with these two studies.

4. Some references are either mis-numbered (e.g. #16 in line 92; #8 in line 109; #28 in line 135; #32 in line 153) or missing (#45-56), which make the manuscript really hard to follow.

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

**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.