Reviewer's report

Title: Comprehensive profiling of DNA methylation in colorectal cancer reveals three subgroups with distinct clinicopathological and molecular features

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Reviewer: Takeshi Nagasaka

Reviewer's report:

Ang et al studied a series of 119 colorectal tissues, including 91 cancers and 28 normal colonic mucosa in an attempt to address the association between DNA methylation at global CpG sites and CIMP phenotype. Associations between BRAF/KARS mutations and CIMP-high phenotype have been shown by their CIMP classification that was categorized by unsupervised hierarchical clustering analyzes. The manuscript is well written and shows an important piece of information for DNA methylation at global CpGs in colorectal cancer as well as normal colonic mucosa. However, the authors need to address the following critical issues:

Major Compulsory Revisions:

1. Weisenberger et al showed strong association among BRAF mutation, sporadic MSI-high, and CIMP-high(ref 10). In this study, CIMP-high by the criterion of CIMPW also shows strong association to MSI and BRAF mutation. Thus, the criterion of CIMPW is reproducible and easy to follow. However, the CIMP criterion in this study is not easy to follow. Although the authors have categorized 3 CIMP subgroup, the clustering result shows 4 branches (Figure1). Thus the criterion of CIMP in this study is very obscure, and may be no reproducible. If the other clustering analyzes were used, different results will appear. The authors should address this issue.

2. In addition, Weisenberger et al showed specific association of KRAS mutation with methylation in several genes amongst their large panel of markers studied (ref 10). Nagasaka et al also demonstrated that KRAS mutation may be associated with an increased level of methylation but the genes affected may be distinct from those associated with BRAF mutation (ref 13 and 16). In this study, CIMP-high is strongly associate with both BRAF and KRAS mutation. Is methylation spectrum of BRAF mutant cancer differ from that of KRAS mutant cancer or not ?

Minor Essential Revisions:

1. Page 9, line 13, sentence beginning "Interestingly, the two patients with CIMP-L MSI+ tumors were aged 44 and 60 years, suggesting the underlying cause of the MSI+ phenotype was germline or somatic mutation of the mismatch repair genes rather than hMLH1 methylation". This sentence should be excluded because there is no evidence to say so in this study.
**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.