Reviewer’s report

Title: Promoter methylation correlates with reduced ndrg2 expression in advanced colon tumour stage

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Reviewer: Yutaka Kondo

Reviewer’s report:

In the current manuscript, Piepoli et al studied DNA methylation targets in colon cancers (CRCs) using microarray based approach. Authors tried to revise the manuscript. However, there are still major issues pertaining to methodology and interpretation. In addition, several sentences in discussion are not accurately described. Further revision is required for warranted publication.

Specific criticisms follow below.

Major Compulsory Revisions:

1. Study is still confusing. Authors used the same data set of microarray, which they have published previously (Ancona, N et al BMC Bioinformatics 2006). Did authors perform additional microarray assay? If not, “Gene expression analysis” in the Materials and methods and “Gene expression profile data analysis” in the Results should be removed. Indeed, the content of the paragraph in the current manuscript (page 9) is very similar to that of their previous manuscript (Ancona, N et al BMC Bioinformatics 2006).

2. The criteria for up regulation (1.65-fold) by 5-Aza (page 6) is not clear. Is there evidence if this small range of up-regulation reflects de-methylation of CpG promoter region?

3. Primer location of MSP and BSA relative to transcription start site might be indicated as a diagram.

4. Fig. 1 is confusing. The legend says, “Expression profile value (shown in the logarithmic scale) of seventeen genes ..... and normalized to housekeeping genes in normal and tumour tissues from the same individual with CRC.” However, 21 genes were illustrated. Data of normal and tumor tissues could not be found. What expression profile value means? Authors interpreted this data and commented seven genes were under expressed. How variable the expression level of each gene in normal tissues? How we compare the expression level between base line (normal tissue) and cancerous tissues.

5. In Fig.2, it is not clear whether the genes they listed are silenced or downregulated at the point of non-treatment. Indeed, expression of ABCA8 is 0.035 and of NDRG2 is 0.112 in supplementary table 1. Authors should compare the expression level of these genes in CRC cell lines with normal cell control at first. In addition, characters in Fig. 2 (HCT116 and SW480) are still too small to read.
6. It has been reported that 5-Aza does not only induce DNA demethylation but also increase gene expression without promoter methylation. Therefore, their strategy to identify the genes regulated by DNA methylation in CRC is not rational. Indeed, they could identify only one convincing gene, NDRG2, using this method, although more than 500 genes were known to be methylated in CRCs. The author’s conclusion that “these findings highlight the utility of combining genomic, epigenetic, and expression data to identify tumour biomarkers” is not well justified.

7. In Fig. 3 and 4, how many clones were sequenced in BSA? At least 5-10 sequences of each sample should be aligned and illustrated in supplemental figures.

8. In Fig. 4, it is not well described how to calculate and summarize the methylation data of BSA in 30 CRC samples.

9. Gel image of MSP should be shown.

10. There are many incorrect sentences in discussion.

   “The most important epigenetic mechanism is represented by the DNA methylation”, Why authors could say “the most important”? how about chromatin remodeling or histone modifications?

   “reversible conversion of cytosine”, generally DNA methylation on CpG islands is irreversible, which can be demethylated by small chemicals, such as 5-Aza or 5-Aza-dC.

   “This chemical modification may occur everywhere in the DNA molecule” is not well accepted idea.

   These are only examples, the other incorrect sentences were found through the discussion. In addition, all of these sentences are not referenced.

   In the end, methodology, writing and drawing of conclusions leaves much to be desired.

Minor Essential Revisions:
1. In the abstract, there is no Methods paragraph.
2. All the abbreviation through the text should be spelled out.

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

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.