Reviewer's report

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

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Reviewer: Nagahide Matsubara

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Major Compulsory Revisions:
First, they performed a gene expression profile assay on both cancer and normal colonic tissues by DNA microarray to choose up- and down-regulated genes. If they really focus on the TSGs, only down-regulated genes have to be further examined. Expression analysis by qPCR or rt-PCR seemed well correlated with the microarray data, however, it is difficult to understand why some of the genes were analyzed in tissue samples and others were analyzed on cell lines to match the result. No gene was analyzed by both methods. It would be better that all genes have to be analyzed on tissue samples compared with the normal counter part.

By the treatment with demethylating agent, 6 genes showed elevation of their mRNA expression. It is interesting that 3 of the constitutively over-expressed genes confirmed by microarray were upregulated by treatment with the demethylating agent. How could it be explained?

Only remaining candidate TSG was the NDRG2 whose expression is possibly controlled by promoter methylation. However, throughout the steps (array analysis, expression analysis or demethylation analysis) to identify NDRG2, it is important that well-known TSGs whose expression is controlled by promoter hypermethylation, such as MGMT or p16, should be assayed in the same experiments as its control. Also, the characteristics of NDRG2 in comparison with the well-known (for example classical CIMP and new CIMP (by Laird et al.)) genes should be evaluated. We would like to know the importance of the NDRG2 in comparison with other important epigenetically controlled genes such as CIMP.

Minor Essential Revisions:
There are so many spelling mistakes especially the name of the genes in the text and figures. HSPH1 vs HPS1, NRDG2 vs NDRG2, HPDG1 vs HPGD etc.

Figure 4, what is (N), (T), (K)?

Discretionary Revisions:
Table 5, frequency of the promoter methylation of MLH1 is 20% in all colorectal cancers. Also the frequency in descending colon (means distal colon including descending and sigmoind?) is 14%, and it is hard to believe because MSI-H cancer, which has promoter methylation of MLH1 unless Lynch syndrome, is
commonly identified in proximal (cicum, ascending and transverse) colon. Methylation of MLH1 matched to the MSI-H?

**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.