Reviewer's report

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

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Reviewer: Nagahide Matsubara

Reviewer's report:

In this revised manuscript entitled “Promoter methylation correlates with reduced NDRG2 expression in advanced colon tumor” I found that my previous concerns have been satisfactory addressed in general. However, there are few points that have to be explained before acceptance of this manuscript.

# Major Compulsory Revisions

Since there is no explanation in the text of figure legends, I have no idea which CpG island (I guess each number indicates CpG island) corresponds to the actual CpG islands in the NDRG2 promoter sequence in Figure 3. Through the Figure, only 3 skipping CpG islands (among the whole 16 CpG islands) showed methylation in colorectal cancer compared to the counterpart normal tissue. This is uncommon identified in other typical genes controlled by promoter methylation (usually consecutive methylations are observed in the promoter).

Then, by the quantitative NDRG2 methylation, 22 (73%) of the samples showed increased relative methylation among paired 30 colorectal cancer and counterpart normal tissue, which is fine. Finally, authors performed MSP analysis on the same paired 30 samples and concluded that 27% of colorectal cancers showed methylation and none of the normal tissue showed methylation in NDRGW2 promoter. However, according to the methylation status showed in Figure 3, where only 3 CpG in colorectal cancer were differently methylated compared to the normal tissue, and half of the 16 CpG islands showed methylation in both colorectal cancer and normal tissue samples. Accordingly it is quite hard to understand the result of MSP assay, mainly because of the lack of explanation which CpG islands are assessed by the MSP. Authors should show both in the text and the figure legend (preferably by adding a figure of promoter structure) which CpG island is assessed by the 2 individual MSP assays within the NDRG2 promoter.

# Minor Essential Revisions

There are no figure legends in this manuscript. For example, I could not understand what $2^Dct$ in y-axis mean shown in Figure 2.

Page 10 line 5: According to the Figure one NR3C2 showed expression profile value > 1 in tumor tissue of two (instead of one) patients with CRC.

# Discretionary revisions
Page 11 line 17: hemimethylation usually indicates methylation in one allele. Partial methylation (in opposition to the ‘full methylation’) may be better in this case.

**Level of interest:** An article of importance in its field

**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.