Reviewer's report

**Title:** The silence of MUC2 mRNA induced by promoter hypermethylation associated with HBV in Hepatocellular Carcinoma

**Version:** 2  **Date:** 27 April 2012

**Reviewer:** Norishige Yamada

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The authors aimed to investigate the relationship among expression levels of MUC2 mRNA, methylation status of MUC2 promoter and survival for patients with hepatocellular carcinoma (HCC). From their studies of methylation-specific PCR, quantitative real-time PCR and statistical analysis, they concluded that MUC2 mRNA silenced by promoter methylation is significantly associated with worse survival in HCC. They also found significant correlation among MUC2 expression level, presence of HBV and AFP in HCC patients. The finding in this manuscript is interesting and potentially important. However, there are several points that should be addressed before publication can be recommended.

Major Compulsory Revisions:

1. In figure 2B, No.159 and Non-HCC in No.185, which expressed high levels of MUC2 clearly showed methylation; Non-HCC in No.51 and No.53, which expressed low levels of MUC2 showed unmethylation band. Moreover, methylation band was obtained in almost all samples including Non-HCC. The authors need to show the methylation ratio in each sample. Otherwise it might be difficult to conclude that MUC2 promoter methylation is associated with MUC2 mRNA expression in each sample.

2. In figure 5, AZA+TSA in Huh7 showed methylation regardless of MUC2 mRNA high expression. The authors should speculate on the reason.

Minor Essential Revisions:

3. The authors need to define the name of the company, city, states (in the case of United States), and the nations the authors got the materials from should appear first, followed by the company name, thereafter.

4. Figure 2B was not referred to in the text.

5. The quality of the photograph for bands in Fig.2B is not fine enough.

6. Discussion, line 6 of 2nd paragraph and line 7 of last paragraph: “MUC4” should be “MUC2”.

**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.