Reviewer’s report

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

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Reviewer: Yujing Zhang

Reviewer’s report:

Authors of this manuscript (<The silence of MUC2 mRNA induced by promoter hypermethylation associated with HBV in hepatocellular carcinoma>) investigated the methylation status of MUC2 gene and MUC2 mRNA expression in HCC and its relationship to HBV infection. To the best of my knowledge, this is the first paper to report detecting methylation status of MUC2 gene and MUC2 mRNA expression in HCC. It could supply some valuable information on hepatocarcinogenesis.

There are several issues with the manuscript that reduce its potential impact:

1. In Table 1. it shows the correlation between HBV status and MUC2 mRNA levels. But there is no data on the relationship between HBV status and MUC2 promoter hypermethylation shown in Results.

2. Authors tested MCU2 mRNA levels and promoter hypermethylation status in 7721, Huh7 and HepG2 three HCC cell lines, but not show the HBV infection status in those cell lines. So it lacks the data on HCC cell lines to support the point: “HBV could play an important role for the loss of MUC2 gene expression in HCC.”.

3. For detecting MUC2 methylation status by using MSP, did author use positive and negative controls? It didn’t mention in Materials and methods, not show in figures in Results either. For MSP in Figure 2, the marker (bp) also needs to be shown..

4. For detecting MUC2 expression, if author tested its protein levels by using immunohistochemical method or Western blot it could make mRNA levels data more solid.

5. Two references should be cited either in Background or in Discussion:
   A. MUC1, MUC2 and MUC5AC expression in hepatocellular carcinoma with cardiac metastasis. Lin, YS et al. Molecular Medicine Reports.( 2009) 2:291-94..
   B. Differential expression of MUC1, MUC2 and MUC5AC in carcinoma of various sites. Lau, SK et al. American Journal

6. Authors should explain the purpose to run DMSO in Materials and Methods.

The manuscript contains a number of typos/errors that need to be corrected or edited. The followings are few examples:

1. Background page: line 10 from bottom, Cholangiocarcinoma should be cholangiocarcinoma; line 9 from bottom, Extrahepatic should be extrahepatic.

2. Hcc should be HCC (in Abstract page and other pages).

3. In Materials and Methods, under Cell Culture and Treatment, The HCC cancer lines should be HCC cancer cell lines (top line).

4. In Figure 5., G2 should be HepG2.

5. In “Association of MUC2 mRNA with clinicopathologic features (Results), last sentence “This implicated…..” should be “These results implicated…….”

7. Abstract, Materials and Methods and Discussion need to be re-written and English edited.