Reviewer’s report

Title: Epigenetic inactivation of the NORE1A gene correlates with malignant progression of colorectal tumors

Version: 1 Date: 14 August 2010

Reviewer: Hiroyuki Yamamoto

Reviewer’s report:

This study is interesting. The following issues need to be addressed.

Major Compulsory Revisions

1. Epigenetic inactivation and its reactivation by 5-aza-dc has been already reported in SW48 colon cancer cell line (Ref. 15). More importantly, Akino et al. have also reported NORE1A methylation in colon cancer cell lines and colorectal cancer tissues (Gastroenterology 2005;129:156–169). The latter paper is not cited. These results should be described and discussed in detail in the manuscript.

2. Epigenetic inactivation of RASSF1A and RASSF2 has been reported in colorectal cancer. Because only NORE1A was analyzed in this study, the relationship between NORE1A inactivation and inactivation of other RASSF family is unclear. This should be clarified.

3. It would be interesting to analyze the relationship between NORE1A inactivation and KRAS status.

4. Abnormal reduction of NORE1A was correlated with advanced stage. In the abstract, the authors conclude that epigenetic inactivation of NORE1A might be implicated in the malignant progression of colorectal tumors. However, in the last paragraph of discussion, the authors conclude that epigenetic inactivation of NORE1A might be implicated in the development and/or early progression of colorectal tumors. This needs to be better clarified.

5. The authors arbitrarily defined a value less than a half of normal mean (0.64) as abnormally low level. The criterion for the reduction of NORE1A expression in 80 matched tissue sets needs to be described.

6. Regarding the reactivation experiments shown in Figure 3c, did the authors confirm significant demethylation in these cell lines treated with 5-aza-dC? Are there any reasonable reasons why the authors did experiments at a single concentration of 5-aza-dC and a single point after treatment? It would be interesting to analyze the effects of trichostain A (TSA) treatment and those of the combination of 5-aza-dC and TSA.

7. Correlation of NORE1A methylation with inactivation of expression in clinical samples should be presented at protein levels as well by using western blotting...
and/or immunohistochemistry.

Discretionary Revision

1. Functional studies such as growth, apoptosis, and colony formation assay would strengthen the conclusion.

**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.