Reviewer's report

Title: Association of diminished expression of RASSF1A with promoter methylation in primary gastric cancer

Version: Date: 6 April 2007

Reviewer: Simo Schwartz Jr

Reviewer's report:

General

Previous data has already shown that RASSF1A is inactivated mainly by promoter methylation in several human tumors, among them, gastric cancer (GC). Although no reports are centered in GC from Chinese origin, there are no strong basis to assume that significant differences concerning RASSF1A will characterize these tumors, so the results reported here are as expected. Some major revisions are advised.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. Authors use competitive RTPCR for analyzing mRNA levels of RASSF1A. Reactions done however are cycled 35 times, which is most likely enough for reaching saturation of the amplification. This could mislead the final results and therefore, authors should provide alternative quantitative evidences that mRNA measurements are accurate, such as real time PCR. In particular when same authors compare these levels with protein levels (fold-time changes).

2. It is not advised to compare in terms of fold-times changes mRNA levels with protein levels because methodologies used for determining those levels are based in different technologies (PCR versus western).

3. Authors should at least add the real percentages of tumors with and without expression of RASSF1A for comparison purposes along the text, and it will be most advisable to add a table with the whole data obtained from the analyzed tumors: mRNA levels, protein levels and methylation status for each tumor, so readers will be able to see that tumors with methylation also show lower mRNA and lower protein levels.

4. All data should be normalized. For instance figures 1 and 2, showing mRNA and protein levels for comparison purposes (i.e. considering the levels obtained in the normal tissue as 1). It is a bit confusing to state (pp 8) that "tumors show a significant lower expression of 78.6% than normal tissues". It is advised to normalize data and to state that expression in tumors is 22% of the expression of a normal tissue (as example).

5. Authors also found methylation in a high percentage of normal tissues. Data from other authors have not found methylation however. This might rise questions of whether data from the MSP is indeed targeting the right CpG islands. It is most advised to add the sequences of the bisulfite treated samples in order to show how authors distinguish between hypermethylated and unmethylated sequences. This is important since authors argue about haploinsufficiency of RASSF1A.

6. It is also advised to join results and discussion sections to make the report more attractive.

7. Some sentences are too speculative such as "the much greater..additional mechanisms regulating RASSF1A synthesis and degradation" (in pp8) and should be deleted. Also, this sentence is repeated in the results section. Authors should avoid repetitions and too speculative sentences along the manuscript. For instance, in pp9, the second paragraph comments on data related to tumor stage and differentiation but same authors acknowledge that the size of the samples is too limited and results do not reach significance at all, as it is also showed in table 1. Therefore this comments are not relevant nor based over enough evidences and should be avoided.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. Last paragraph of pp9 should be moved to the introduction section.

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major
compulsory revisions

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.