Reviewer's report

Title: Hypermethylation and Tumor Suppressive Role of Homeodomain Only Protein X Gene in Pancreatic Carcinogenesis

Version: 1  Date: 29 March 2012

Reviewer: Hong Lok Lung

Reviewer's report:

Major Compulsory Revisions:

1. Under the Section "Expression of HOPX transcripts and protein in PC tissues and the corresponding normal pancreas tissues"- can the author use some numbers to demonstrate the association of reduced HOPX expression with hypermethylation? eg. out of how many downregulated cases are also methylated.

2. The method of bisulfite sequencing is needed to be modified before any conclusion about methylation can be made. I suggest to clone the promoter fragment into a vector for each cell lines, and to transform the ligation products and to sequence at least 15 clones for each cell lines. By doing that, whether the CpG sites are “completely” or “partially” can be clearly determined. By the way, why PK59 cells are not included in the sequencing results? Since the HOPX expression is restored after the demethylation treatment in this cell line.

3. Can the authors explain why the HOPX transfectants express a larger protein than the PC cells and primary tumors?

4. The expression levels of HOPX protein in the PANC-1 transfectants are nearly undetectable and so much lower than the MIA Paca2 transfectants by using the V5 tag antibody, why did the authors not to use HOPX antibody?

5. For the functional assays results presented in Fig. 5, not all the clones are included for each assay. Can the authors include the missing clone(s) in each assay?

Minor Essential Revisions

1. The x-axis labels of Figs. 3a, 5c, 5e are unclear, please revise.

2. Some part is missing is Table1.

3. The additional Tables1 and 2 are unclear.

4. Methods-Cell line and tissues Samples-....."All cell lines without MIA Paca2 were maintained in RPMI....." should be replaced with "All cell lines except MIA Paca2 were maintained in RPMI......".

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