Reviewer's report

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

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Reviewer: Reinhard Dammann

Reviewer's report:

Waraya et al. have analyzed the epigenetic silencing and tumor suppressive role of HOXP in pancreatic cancer (PC). They report frequent hypermethylation of HOXP in pancreatic cancer and overexpression of HOXP reduces the growth of PC cell line. The main weakness of this report is the missing expression of HOXP in normal exocrine pancreatic cells and the lack of expression of HOXP after treatment with epigenetic reactivators (Aza/TSA). This suggests that HOXP inactivation may occur during normal differentiation. Expression of HOXP was only observed in Langerhans islet cells.

Major Compulsory Revisions:

1. Expression of HOXP should be demonstrated in normal cells of the exocrine pancreas.

2. MIA Paca2 exhibits no promoter hypermethylation (Fig. 2A) and protein and RNA expression of HOXP are absent. Why? Thus HOXP silencing in pancreatic cells may occur by an alternative mechanisms. Which?

3. Reexpression of HOXP is absent after AZA and TSA treatment of PK-45H, PK-59, KLM-1 and MIA Paca2. Was the promoter demethylated? The promoter of HOXP in MIA Paca2 is unmethylated and even after Aza treatment no reexpression of HOXP is detected. As already mentioned this suggest that inactivation of HOXP in exocrine pancreatic cells may occurring during normal differentiation. This could be tested. Hypermethylation of the HOXP promoter may be present in pancreatic cancer, however this has no impact on HOXP expression.

4. Ectopic expression of HOXP in pancreatic cancer cells (Fig. 5) does not represent physiological conditions! Cells of the exocrine pancreas do not express HOXP. Tumor suppressive function of HOXP may represent an artifact created by its overexpression.

Minor Essential Revisions:

1. Labels of figures are shifted (Fig. 3A, Fig. 5CE).

2. Figure 1C and Fig. 5A: The first lane is not labeled. Is it a marker or HOXP8?

3. Figure 2B: Reexpression of HOXP is not visible. The authors should perform RT-qPCR.
4. Figure 3B: Protein loading for normal and tumor tissue is completely unequal. Again no expression of HOX PX in normal tissue.

5. Figure 3C: Labels for the second and fourth panels are missing. Same sections as above?

6. Is it not possible to read table 1 and half of the information (reverse primer?) is missing.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**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