Reviewer's report

Title: SMAD4 Loss Triggers the Phenotypic Changes of Pancreatic Ductal Adenocarcinoma Cells

Version: 1 Date: 28 September 2013

Reviewer: Ruben R Plentz

Reviewer's report:

The paper by Chen et al. explored nicely the role of the tumor suppressor gene SMAD4 during the in vitro and in vivo carcinogenesis of pancreatic ductal adenocarcinoma (PDAC). The group used 3 human PDAC cell lines as well as a Xenograft mouse model. SMAD4 was over expressed or silenced by common techniques. Cells were additional treated with different cytostatic drugs and by TGF-Beta-1 and EGFR inhibitors. Importantly, the authors found that the re-expression of SMAD4 does not affect the tumor cell viability, but enhances cell migration in vitro. SMAD4 restoration induced several transcriptional factors and SMAD4 loss increased chemo resistance in vitro. PDAC cells with a loss of SMAD4 showed a decrease of cell viability in response to EGFR inhibitor treatment. The authors conclude that only a sub-group of patients with PDAC will respond to targeted therapies against TGF-Beta and/or EGFR signaling pathways. The study is interesting and novel, but there are still a few questions to be answered prior publication:

Major Compulsory Revisions:

- Figure 1: A: It would be better to show the kDa of the analyzed proteins directly in the figure. B: What is the unit of the y-axis ? C: The labeling of the treatment with TGF-Beta is not perfect (+ vs. -). D: Misspelling of puro (puri).
- Figure 2: Is this a specific effect of PDAC cell lines or do other cells (e.g. colorectal cancer) behave the same ? The labeling of the pictures is not in order. It would be nice to see macroscopic and H&E stained pictures of the Xenograft tumors. It is important to calculate a migration and invasion index. Are the differences significant ? There are no pictures of the invaded and counter-stained cells.
- Figure 3: A: What are the units of the y-axis ? B: It would be better to show the kDa of the analyzed proteins directly in the figure. There is only one picture of beta-actin as a loading control. How can the authors explain the differences between the analyzed stem cell markers, especially CD44 and CD133?
- Figure 4: A: There is only one picture of beta-actin as a loading control. It would be better to show the kDa of the analyzed proteins directly in the figure.
- Figure 5: What was the rationale to use drugs like Cisplatin, etc. ? It would be helpful to integrate light microscope pictures of the untreated and treated cells, perhaps as a new Suppl. Figure.
- Figure 6: It is important to calculate a migration index. Are the differences significant?
- Suppl. Figure 1: There are no scale bars. What is the rational to test these markes by IHC?
- What is already known about therapies with SB231542 and Gefitinib? The results should be integrated in the discussion.

Minor Essential Revisions:
- The introduction is missing some general words about the new therapies with FOLFIRINOX and nab-Paclitaxel.
- The introduction is too long and repeating the goals and reasons of the underlying study.
- In vitro and in vivo has to be italicized throughout the manuscript.
- References 9-11 are not containing data about lung cancer.

**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.