Reviewer's report

Title: Identification of Achaete-scute complex-like 1 (ASCL1) target genes and evaluation of DKK1 and TPH1 expression in pancreatic endocrine tumours

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Reviewer: Herbert Chen

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This is an interesting manuscript which focuses on the role of ASCL1 in pancreatic neuroendocrine tumors. They treat BON1 cells with siRNA against ASCL1 and measure changes in expression patterns of several genes. 158 target genes were identified. Some of them were increased in expression some decreased in expression. In addition they studied 22 tumors by immunochemistry for ASCL1 and DKK1 and characterized their expression patterns. They conclude that the numbers of genes with importance for pancreatic endocrine tumourigenesis have been identified are downstream of ASCL1. They conclude that ASCL1 negatively regulated the Wnt tumor antagonist DKK1.

This study is from a well experienced group whose bulk of interest has been pancreatic endocrine cancers. The role of ASCL1 and pancreatic endocrine tumors continues to grow. While ASCL1 clearly has a defined role in small cell lung cancer and medullary thyroid cancer, and carcinoid its role in pancreatic and endocrine tumors is not as well defined. This is true especially because other basic-HLH transcription factors such as Neurogenin-3 may also play a role. The current study is well done. I have some questions for the authors.

1. Do the authors have any indication what the roles of Neurogenin-3 in relation to ASCL1 are in pancreatic neuroendocrine cancers. Most of previous studies regarding developmental biology have studied Neurogenin-3 as well.

2. Previous studies have shown that TPH1 is downstream of ASCL1. Specifically induction of notch1 reduces ASCL1 and decreases TPH1 mIRNA by RNase protection. However, in their study they found an increase in TPH1. How did the authors reconcile this difference?

3. Table 1(B) reveals Western Blot for ASCL1. The bands are very, very faint and hard to interpret. Furthermore, they only show one of the ASCL1 siRNA treatments. Could the authors provide both treatments?

4. In figure 2 (A) the authors provide a mRNA expression patterns various targets. Do have the authors have any expression profiles by Western blot. Particularly it would be important to know if the degree of ASCL1 reduction protein by siRNA corresponds to direct suppression of these factors.

5. Have the authors studied serotonin production after ASCL1 siRNA treatment?
Again this is a very interesting study which has information to contribute to the literature with these issued addressed.

**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:**

no competing interests