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

**Version:** 1 **Date:** 30 March 2009

**Reviewer:** Eric K Nakakura

**Reviewer's report:**

**Major Compulsory Revisions**

1) On line 239, the authors state that Tph1 expression was increased in Ascl1 siRNA transfected cells as anticipated. However, based on previously published work, which they referenced (JCEM 90: 4350-6, 2005), one would anticipate Tph1 expression would be decreased in Ascl1 siRNA transfected cells. The reason is that Notch signaling inhibits Ascl1 expression, serotonin production, and Tph1 expression. Because this is an unexpected finding, it is necessary to verify the microarray data. Tph1 expression should be evaluated using qRT-PCR and Western blot analysis (or immunofluorescent analysis) in Ascl1 siRNA transfected BON cells. To date, the published literature suggests Ascl1 is a pro-endocrine transcription factor.

2) In Figure 1, Ascl1 siRNA reduced Syn1 expression in BON cells. However, in the Table 2, Syn1 expression is not listed as being decreased in BON cells treated with Ascl1 siRNA. The again raises questions as to the validity of their microarray findings.

3) Because of these discrepancies, I have significant concerns regarding the validity of their microarray findings. It is very important for the authors to validate with qRT-PCR and Western blot analysis (or immunofluorescent analysis) their microarray findings in BON cells treated with Ascl1 siRNA looking at numerous putative target genes suggested by the microarray data.

4) The immunohistochemical analysis of Ascl1, Dkk1, and Tph1 expression in pancreatic endocrine tumors does not show any convincing expression patterns. It would be helpful to evaluate their expression in PET samples using qRT-PCR. Also, since no clear patterns are apparent, increasing the sample size might be useful to detect relative expression patterns among these genes.

5) Since the function of Ascl1 has already been evaluated in other cell lines, such as lung cancer and medullary thyroid cancer, their study would be bolstered by assays of BON cells after Ascl1 knockdown, such as proliferation rate, growth in soft agar, flow cytometry, and chromatin immunoprecipitation.
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 interest.