Reviewer’s report

Title: Protocadherin-1 is a glucocorticoid-responsive critical regulator of airway epithelial barrier function

Version: 3 Date: 6 February 2015

Reviewer: Steve Georas

Reviewer’s report:

Kozu et al. studied the expression and function of protocadherin-1 (PCDH1) in bronchial epithelial barrier function. The rationale for these studies is that PCDH1 was recently identified in a genome wide association study of asthma, but its function is not well understood. PCDH1 is a member of the cadherin family, other members of which contribute to adherence junction formation. In this paper, the authors used siRNA knockdown, together with standard assays of barrier function (TEER and permeability) and barrier (immunofluorescence microscopy).

This paper is timely, since dysfunction of the epithelial barrier is increasingly associated with asthma and other diseases, yet the molecular mechanisms involved or not well understood.

The paper is succinct and generally well-written. The results are presented in six main figures, and three supplemental figures. Most studies are conducted in 16HBE bronchial epithelial cells, which are a good model to study junctional structure and function, since these cells express polarized tight adherence junctions (like primary basal cells).

There are several apparently novel findings contained in the paper. First, PCDH1 expression increases during monolayer formation in vitro, and co-localizes with E-cadherin (e.g. in adherens junction). PCDH1 knock-down decreased TEER and increased permeability, indicative of barrier dysfunction. Third, PCDH1 knock-down is associated with dysregulated formation of junctional structures in general (Ecad and ZO1) as determined by immunofluorescence (Fig 4A), without apparently affecting their overall protein expression (supplementary figure). Fourth, dexamethasone appears to boost barrier integrity in a PCDH1 dependent manner (figure 5).

This manuscript is timely and straightforward. I have a few suggestions for minor revisions that should improve the impact of the paper.

1. In order to infer mechanism, we need to be sure the PCDH1 knock-down does not interfere with expression of related family members. Consequently, please provide statistical analysis of the Western blots of PCDH1 and E-cadherin levels following PKDH1 siRNA transfection (Fig 2C and Supplementary Figure 3).

2. The legend to Figure 6 does not correspond to the panels shown, and is confusing. Please define what “EDC” stands for (Fig. 6D). The distinction
between “inflamed” and “non-inflamed” sections of lung tissues is important but seems arbitrary: please clarify how this was done objectively. I was not clear if the authors observed a difference in PKDH1 expression between lung tissue samples from asthmatic vs. healthy control subjects. Given variability in TJ/AJ expression, I would be surprised if there were substantial differences between groups given the relatively small numbers of subjects studied (9 subjects per group), but this should be stated clearly.

3. Since PKDH1 knock-down apparently disturbs other junctional structures, it remains to be seen whether this molecule itself contributes to barrier integrity directly (e.g. via cell-cell homotypic interactions) or indirectly (e.g. via stabilizing other AJ or TJ components). Please make this clear in the Discussion.

4. Another area of potential interest is the role of PKDH1 in respiratory virus mediated junctional dysfunction. Some studies alluding to the role of viruses in barrier dysfunction would seem indicated in the Discussion (e.g. reviewed in PMID 25085341).

Minor issues

1. Please confirm that the difference between 124 and 122 eosinophils (/mcl) was statistically significant (p<0.03) in Table 1.
2. Supplemental Figure 2A was jumbled after downloading.

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