Reviewer 2 v.2

Comments to the Author

Aim of the presented work was to investigate the impact of beta-catenin/TGFbeta1 signaling in an asthma rat model. The authors focused their research on inflammatory conditions and remodeling processes in the rat lungs. By inhibiting beta-catenin signaling with ICG001 they observed a reduced inflammation (e.g. IL17, Eosinophils) and an effect on TGFbeta1 expression. Furthermore, they saw a positive effect on the main mechanisms of airway remodeling (e.g. alpha-smooth-muscle mass, EMT). The treatment of rats with ICG001 was compared to untreated and Budesonid treated controls. Effects of Budesonid and ICG001 were mainly similar.

The current manuscript is comprehensibly and clearly written. The authors of this study focused on an important and not completely solved scientific question regarding the modulation of beta-catenin signaling and the role of TGFbeta1 for the treatment of bronchial asthma.

Nevertheless, there are some major and minor issues which have to be solved before a publication could be recommended. Especially regarding missing information's about the airway inflammation and the allergic phenotype of the rats. Also, the authors did not discussed already published work which showed adverse effects of beta-catenin signaling in allergic diseases.

Major points:

1. Results: The classical OVA-Alum Asthma model induces a typical Th2 dominated phenotype. One of the major results of this manuscript is the downregulation of inflammatory signals shown in HE staining's, Eosinophil numbers and IL17, IL10, IL35 expression. Only eosinophil numbers are specific for type 2 asthma, but you observed only ~25% eosinophils (4 we model 16 total, 4 Eos) of the total induced inflammatory cells (neg vs pos control). In contrast IL17 is associated with neutrophilic asthma, but those numbers are not counted. Please include type 2 specific analysis like IL4/IL5/IL13 levels and sensitization controls like OVA specific Ig levels. If possible, neutrophilic numbers and a differentiation of t cell subtypes in the lung/BAL would be beneficial. Otherwise, the main statement about the anti-inflammatory action must be toned down.

2. Results: In cell culture experiments (fig.3/4) you have used control cells, TGFbeta stimulated, and TGFbeta+ICG001 treated groups. A control of beta-catenin activation is missing. That TGFbeta1 induces beta-catenin is not shown in you experiments. Please include a group with uncontroversial beta-catenin induction (e.g. Wnt1) +/- ICG001. Or, proof the beta-catenin induction by TGFbeta in you cells adequately.

3. Discussion: If Wnt/Beta-catenin does have a pro- or anti-inflammatory role in lungs is still under controversial discussion. Current literature showing proinflammatory roles of Beta-Catenin are well discussed in this article (Kwak 2015, Kumawat 2014). In asthma there are several publications showing anti-inflammatory effects due to Beta-Catenin activation (e.g. Reuter 2014, Bao 2007, Chae 2016, Trischler 2016). Unfortunately, the authors did not mention or discuss the opposite results.

Minor:

1. E-cadherin, alphaSMA, Fn are adequate markers to analyze airway remodeling in a complex in vivo model and additional to histological techniques. These markers are not sufficient to analyze EMT mechanisms in cell culture. Please include Snail, Slug, Claudins or others.

2. The same insufficient controls in Fig. 2F. Snail and MMP-7 are very weak target genes of beta catenin signaling. Please include Axin2, MMP-9 and/or NKD1 to show the higher beta-catenin activity in sensitized rats.

3. If rats develop arrhythmia or other severe symptoms (227-232). So, the model seems to be very strong. None of the observed symptoms were counted or analyzed. Please discuss the strength of the model and include measurements like lung function.

4. RPM is a centrifuge-specific value and should declared as RCF

5. Line 69-71: That TGFbeta induces Wnt/Beta-Catenin has only been shown in a very artificial model including radiation induced fibrosis. Please confirm this with further literature or perform own experiences.

6. Line 116 Typo: of ⬚ after

7. Line 170 Please indicate which cell lysis buffer you have used

8. The quality of pictures in Fig. 4 is very bad