The role of E-cadherin/β-catenin signalling in the development of an asthmatic airway epithelial phenotype

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CHAPTER 7

Summary, Discussion & Future Perspectives

Summary

The airway epithelium in asthma is vulnerable to damage and has a compromised barrier function associated with reduced expression of the adherens junction protein E-cadherin. Exposure of the asthmatic airway epithelium to inhaled allergens like house dust mite (HDM) results in increased damage to the epithelial barrier, characterised by a further reduction in E-cadherin and increased release of pro-inflammatory chemokines like C-C Motif Chemokine Ligand (CCL) 17, CCL20, pro-inflammatory cytokines like granulocyte-macrophage colony-stimulating factor (GM-CSF) and alarmins such as thymic stromal lymphopoietin (TSLP), interleukin (IL)-25 and IL-33. These pro-inflammatory mediators attract and/or activate type -2 innate lymphoid cells (ILC2), tissue resident macrophages and dendritic cells (DCs), which activate Th2 cells through antigen presentation. Activated ILC2s and Th2 cells release cytokines IL-4, IL-5, and IL-13 that induce IgE production by B-lymphocytes, eosinophilic infiltration into the airways and excessive mucus production respectively (1). The damaged airway epithelium initiates a remodelling and repair response mediated by growth factors like transforming growth factor (TGF)-β, epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) (2). The expression of higher levels of basal cell markers cytokeratin-5, -14 and p63 in the asthmatic airway epithelium indicates an undifferentiated epithelial phenotype that upon damage is unable to repair and regenerate a functionally intact barrier (3). The loss of E-cadherin, e.g., upon exposure to allergens, results in the release of its intracellular binding partner β-catenin, which not only aids in cell adhesion, but also functions as a transcription factor, regulating the expression of mesenchymal genes. These genes encode proteins including fibronectin, EGF receptor (EGFR) and VEGF, which may contribute to airway wall remodelling (3,4). β-catenin mediated transcriptional activation is achieved through its interaction with various transcriptional co-activators including CREB binding protein (CBP) and p300, resulting in cell proliferation and cell differentiation respectively (5). It remains unknown whether the transcriptional activity of β-catenin upon release from the junctional protein complexes contributes to the changes observed in the airway epithelium in asthma patients, such as relative abundance of basal, undifferentiated cells.

We hypothesised that upon loss of E-cadherin and release of β-catenin from adherens junctions, the subsequent activation of/increased β-catenin/CBP signalling contributes to the asthmatic airway epithelial phenotype.

To investigate this, we addressed the following questions:

- Does β-catenin/CBP signalling promote epithelial barrier (dys)function and pro-inflammatory response in airway epithelial cells upon damage?
- Does the loss of β-catenin from the E-cadherin-mediated adherens junctions in lung epithelial cells increase the susceptibility of the airway epithelium to HDM-mediated effects in mice?
- Does interaction of β-catenin to CBP instead of p300 lead to activation of pathways involved in goblet cell differentiation? If so, is there any crosstalk with the regulation of MUC5AC by the Notch pathway?
- Does β-catenin/CBP pathway enhance airway inflammation, goblet cell metaplasia and airway hyperresponsiveness in mice?

To address these questions, we used the small molecule inhibitor ICG-001 to specifically block β-catenin/CBP signalling. In **chapter 3**, we show that HDM treatment in primary airway epithelial cells (PAECs) resulted in significant increase in the release of chemokine CCL20, which was suppressed by ICG-001. In addition, we observed that ICG-001 improved the barrier function of PAECs at baseline and upon wounding. Overall, we demonstrate that increased β-catenin/CBP signalling disrupts epithelial barrier function and promotes pro-inflammatory activity in PAECs. In **chapter 4**, we explored the effects of loss of E-cadherin-mediated adherens junctions on the susceptibility of the airway epithelium to HDM using Cre-Lox mediated E-cadherin knock-out mice models. We have previously shown that surfactant protein C (SP-C)-driven knock-out of E-cadherin during embryonic development of mice resulted in enlarged alveolar airspaces resembling emphysema (6). Since asthma is mainly a disease of the airways, specific knockout of E-cadherin in airway epithelial cells may be a more relevant model to study the contribution of E-cadherin loss on airway epithelial integrity in asthma, as observed upon HDM exposure. We generated three different models - knock-out of E-cadherin in SP-C expressing cells during embryonic development (model 1), CCSP expressing cells during embryonic development (model 2) and CCSP expressing cells after birth (model 3). In all the three models, we observed that the loss of E-cadherin resulted in denudation of airway epithelial cells along with spontaneous inflammation mediated/featured by the increase of eosinophils in lung. Loss of E-cadherin also led to increased Th2 cell numbers in lung tissue in model 1, but not in models 2 and 3. Loss of E-cadherin did not affect the HDM-mediated effects in all three models. In

conclusion, the extent of airway epithelial E-cadherin loss affected the kinetics and magnitude of the airway inflammatory response, although it did not increase the sensitivity to HDM in mice. In **chapter 5**, we investigated the role of β-catenin/CBP signalling and its possible overlap with Notch signalling in promoting goblet cell differentiation. We show that IL-13 significantly increased the expression and production of MUC5AC in primary bronchial epithelial cells (PBECs) differentiated in air-liquid interface (ALI). The IL-13 induced MUC5AC was attenuated by exposure to ICG-001. In addition, we showed that β-catenin/CBP signalling and Notch pathway independently regulate MUC5AC through its transcriptional repressor forkhead box protein A2 (FOXA2) and transcriptional activator SAM pointed domain-containing Ets transcription factor (SPDEF), respectively. Together, we demonstrate that β-catenin/CBP and Notch signalling promote the expression and production of MUC5AC through independent pathways. In **chapter 6**, we aimed to validate the effect of ICG-001 on mucus production *in vivo*. We showed that initial sensitisation of mice, followed by exposure with HDM resulted in increased goblet cell metaplasia, which was significantly attenuated by ICG-001. ICG-001 treatment also significantly decreased the HDM-induced increase in the infiltration of macrophages. HDM exposure increased airway hyperresponsiveness, which was unaffected by ICG-001. Together, we show that β-catenin/CBP signalling promotes goblet cell differentiation, but not HDM-induced airway hyperresponsiveness *in vivo*.

General Discussion

PBECs from asthmatic donors have been shown to be more susceptible to loss of epithelial barrier and release of pro-inflammatory cytokines like CCL20 upon exposure to allergens like HDM compared to the healthy cohort (7). In **chapter 3**, we showed that specific targeting of β-catenin/CBP signalling could play an important role in counteracting the damage-induced disruption of barrier function and increased pro-inflammatory response. Inhibition of β-catenin/CBP signalling in human bronchial epithelial cell line 16HBE using ICG-001 induced a transient increase in epithelial barrier function at baseline and significantly restored the barrier function following injury using thapsigargin – a sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) inhibitor, which we have previously shown to mimic the Ca^{2+}-mediated effects of HDM (7) yet causing a more robust effect. We show that both HDM and thapsigargin induced a significant increase in the release of CCL20 and were both significantly decreased by ICG-001. We have previously shown that the loss of E-cadherin

from the cell membrane leads to increased EGFR activity (8), which may result in elevated Ca^{2+} signalling (9) and subsequent activation of a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) (10,11), a sheddase of CCL20 (12). Of interest, we showed that ICG-001 increased the localisation of junctional E-cadherin and cortical actin. This is in line with the previous studies which showed that ICG-001 partially prevents TGF-β1–induced loss of E-cadherin at cell-cell junctions in PAECs (13) and in lens epithelial cells (14). The dynamic process of actin polymerisation and disassembly has been previously shown to play a key role in the trafficking of E-cadherin to the cell junctions (15), where cortical actin supports the localization of E-cadherin in adherens junctions, which is disrupted upon formation of stress fibres. In support of this notion, blocking of actin polymerisation using cytochalasin D not only resulted in the disruption of E-cadherin-mediated intercellular junctions, but also decreased the epithelial barrier function and increased CCL20 release, which were all partly mitigated by ICG-001. This indicates that ICG-001 prevents the negative downstream effects of disruption of E-cadherin-mediated intercellular contacts by promoting the cortical localization of actin-β-catenin complex. The stabilization of E-cadherin subsequently promotes the barrier function and suppresses the shedding of CCL20. The exact mechanism by which ICG-001 increased junctional localisation of E-cadherin and actin is unclear but is most likely post-transcriptional as these effects were observed within a short time. A possible explanation could be that inhibition of β-catenin/CBP signalling by ICG-001 promotes the stabilisation of E-cadherin/β-catenin binding, which has been recently shown in gastric cancer cells (16), e.g., through cortical localisation of actin. Overall, our findings indicate an important role of β-catenin/CBP signalling in epithelial barrier function and pro-inflammatory responses.

In **chapter 3**, we also show that β-catenin/CBP signalling is involved in promoting proinflammatory activity through release of GM-CSF, which activates the production of granulocytes like eosinophils. In **chapter 4**, we observed spontaneous eosinophilic inflammation in the E-cadherin knockout models, which could be a consequence of increased β-catenin signalling. In contrast to the regulation of junctional E-cadherin by β-catenin/CBP signalling, E-cadherin stabilises β-catenin at the cell junction and loss of junctional E-cadherin may result in increased β-catenin activity (17). Targeted deletion of E-cadherin in most of the lung epithelial cells in mice, which express SP-C during embryonic development, resulted in asthma features like infiltration of eosinophils and dendritic cells (DCs), increased

release of CCL17 and mucus production (6). In addition, E-cadherin knockout resulted in enlarged alveolar spaces resembling emphysema, which we also observed (in model 1) in **chapter 4**. Though we specifically targeted the deletion of E-cadherin expression in proximal airway epithelial cells using CCSP in model 2 and model 3, we still observed an enlargement of airspaces in these models. The effects on the alveolar epithelium as a result of the deletion of E-cadherin in proximal epithelial cells could be due to the ability of CCSP-expressing club cells to serve as stem/progenitor cells and differentiate into alveolar epithelial cells (18). This underscores the significance of club cells not only in the formation and integrity of airway epithelium, but also contribute to alveolar differentiation during embryonic/postnatal development. In all the three E-cadherin knockout mouse models, we observed that a few epithelial cells in the airways retained the expression of E-cadherin, indicating the presence of epithelial cells which lack the expression of SP-C that include a small subset of basal cells (6), or do not express CCSP such as the non-secretory airway epithelial cells.

Increased numbers of Th2 cells in knockout (Cre^+) mice were observed in lung tissue only in model 1, indicating that the loss of distal epithelial cells may be crucial for induction of Th2 cell inflammation. The eosinophilic inflammation was observed in all the three models, suggesting that the eosinophilic infiltration seen in model 2 and model 3 could be activated by type-2 innate lymphoid cells (ILC2 cells), which also secrete IL-5 that is necessary for the activation of eosinophils (19). Unfortunately, this is difficult to validate as we were unable to detect ILC2 cells in our samples. Furthermore, loss of E-cadherin can increase EGFR signalling, which may also be involved in the observed effects of infiltration of inflammatory cells as we have previously shown that it is involved in production and release of various pro-inflammatory cytokines (8). HDM-induced eosinophilic inflammation in wild type (Cre^-) mice in model 2 and model 3, but not in model 1. This suggests that there may be some intrinsic differences between the SP-C and CCSP breeding lines from which the wild type mice of the three models were derived, resulting in the differences in their susceptibility to HDM. In addition, HDM increased Th2 cells only in the Cre^- mice of model 3, and this was accompanied by a trend towards increase in eosinophils. Though we previously showed that PBECs from asthma patients are more susceptible to the effects of HDM compared to PBECs from heathy donors, HDM did not induce any aggravated responses in Cre^+ mice in any of the three models. This suggests that the loss of E-cadherin is already a potent stimulus that is sufficient to induce pro-inflammatory effects. Exactly to what extent this relates to the

asthmatic airway epithelium, where the expression of E-cadherin has been shown to be significantly downregulated compared to the healthy epithelium (20,21), remains to be established. However, one relevant difference is that in the mouse model we achieve a full loss of E-cadherin when compared to a posttranslational effect on E-cadherin localisation and expression level in the asthmatic airway epithelium.

As E-cadherin stabilises β-catenin at the cell junctions, the lower expression of E-cadherin in PBECs from asthma donors compared to non-asthma donors as previously shown (7), may result in increased β-catenin signalling in PBECs from asthma patients, which was validated in **chapter 5**. We show that activity of β-catenin decreased in the ALI differentiated PBECs from non-asthma donors, but not in asthma donors by quantifying the expression of β-catenin target gene cyclin D1 (*CCND1*). This indicates a prolonged activity of β-catenin in asthma epithelium during differentiation at ALI, which may thus lead to impaired recovery of the epithelial barrier as well as promotion of inflammatory responses and increased mucus production. We also show that the expression of *FOXA2*, a repressor of *MUC5AC* gene, was significantly lower in differentiated PBECs from asthma donors compared to non-asthma donors. This falls in line with the previous study where expression of an activated form of β-catenin in lung cells in mice during development resulted in decreased Foxa2 and goblet cell metaplasia (22). Furthermore, we showed that specific blocking of β-catenin/CBP signalling using ICG-001 attenuated MUC5AC production independent of the presence of IL-13, by increasing the expression of MUC5AC repressor *FOXA2* in differentiated PBECs. In addition, blocking of β-catenin/p300 binding using IQ-1 did not have any effect on the MUC5AC production, which indicates that β-catenin/p300 is not involved in goblet cell differentiation. All these results are supportive of an important role of β-catenin/CBP signalling in epithelial alterations in asthma.

Blocking of the Notch pathway using inhibitor DBZ has been shown to suppress the IL-13-induced MUC5AC production in human airway epithelial cells (23). In addition, microarray analysis of IL-13 overexpressing transgenic mice showed increased expression of *Spdef* and *Muc5ac* (24). As β-catenin and Notch signalling pathways were shown to interact during tissue development and in diseases like cancer (26–28), we further investigated if β-catenin/CBP signalling interacts with Notch signalling pathways in regulating MUC5AC. We found that there was no overlap between the β-catenin/CBP and Notch pathways and

both these pathways act independently to regulate *MUC5AC* through *FOXA2* and *SPDEF*, respectively. Alternatively, CCL20, which we showed to be significantly decreased by ICG-001, has been previously shown to induce mucus production in airway epithelial cells (25). Of interest, FOXA2 has been shown to promote the expression of E-cadherin in breast cancer cells (29), while deletion of Foxa2 induced Th2 cell mediated inflammation as well as airspace enlargement in developing lung (30). Recently, CCSP (Scgb1a1) has been identified to be regulated by Foxa2 and *Scgb1a1* knockout mice also exhibited increased airway inflammation and hyperresponsiveness upon exposure to Ovalbumin, compared to the wild-type mice (31). The negative regulation of FOXA2 by β-catenin/CBP signalling, as shown in **chapter 5**, provides valuable insight into the mechanisms of airway mucus production and can be used to effectively develop therapeutics that target these pathways.

In **chapter 6**, we validate the effect of inhibition of β-catenin/CBP signalling on mucus production in mice by showing that ICG-001 mitigates the HDM-induced goblet cell metaplasia and mucus production. However, ICG-001 had no effect on the HDM-induced airway inflammation and airway hyperresponsiveness. This is in apparent contrast to our observations using PBECs cultured *in vitro*, where we show that ICG-001 significantly decreased CCL20 and GM-CSF. Similarly, ICG-001 was previously shown to be able to reduce the airway inflammation toluene diisocyanate mouse model of asthma (32). The conflicting results from different studies could be due to the differences in experimental design such as the initial HDM sensitisation during days 0-2 already stimulating the immune response before the blocking of β-catenin/CBP signalling with ICG-001 later during days 14-17. ICG-01 also prevented the HDM-induced downregulation of E-cadherin expression, further emphasizing on the role of E-cadherin/β-catenin in the development of pro-inflammatory and remodelling phenotypes observed in the asthmatic airway epithelium. In line with our findings, ICG-001 has been shown to reduce the toluene diisocyanate-induced goblet cell metaplasia in mice (32). With excessive mucus production being a common feature of asthma, β-catenin/CBP signalling could be a therapeutic target for designing new drugs.

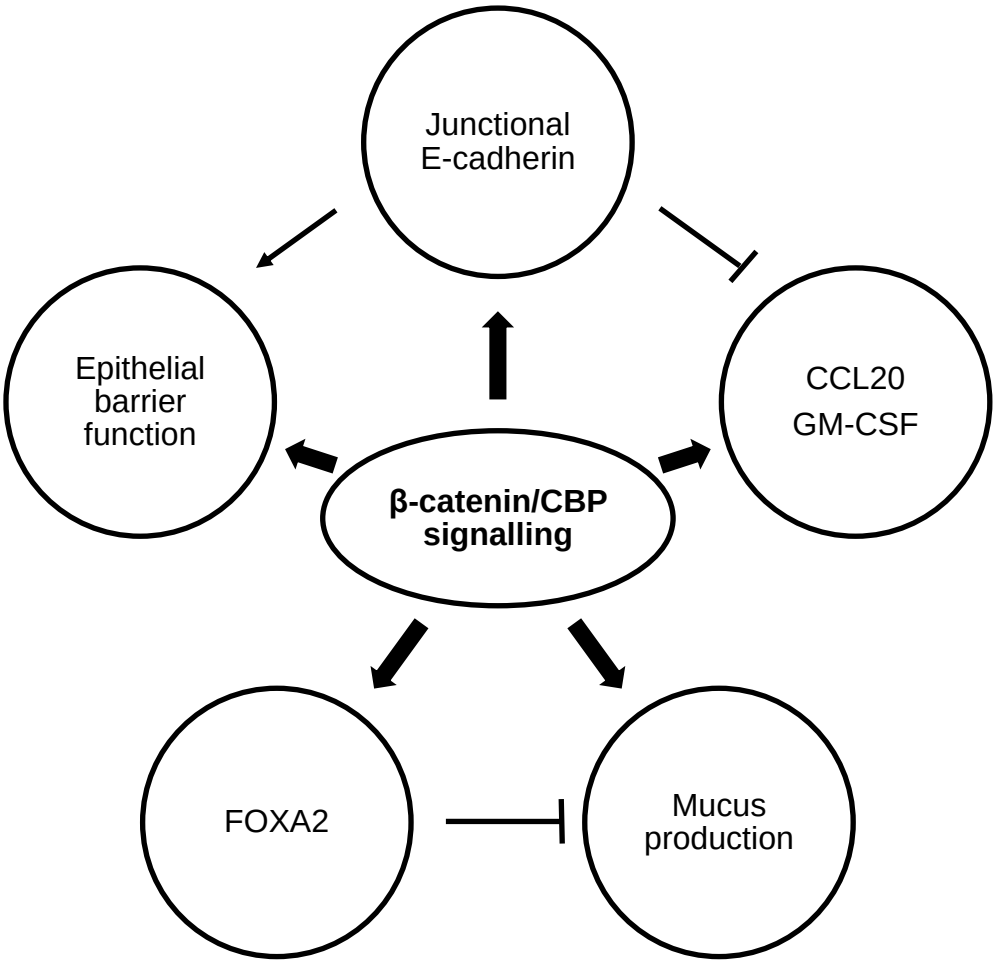

Figure 1: Diverse roles of β-catenin/CBP signalling in promoting the asthmatic airway epithelial phenotype

In **chapter 6**, we also observed that HDM was effective in eliciting an inflammatory response in the airways involving eosinophils, neutrophils, lymphocytes, and macrophages. This response contrasts the type of airway inflammation induced by HDM in wild type (Cre^-) mice in **chapter 4**, where HDM mainly induced eosinophilic inflammation but did not sigifiantly increase the infiltration of macrophages and neutrophils. The differences in the airway inflammation could be because of the differences in HDM challenge protocol and the composition of HDM extracts used (33). The HDM extracts used in **chapter 4 and chapter 6** were from different manufacturers - Citeq and Greer, respectively. We have previously

shown that the type of inflammation induced in these mouse models depends on the composition of the HDM extract. HDM extract from Greer but not Citeq significantly increased the release of CCL17 and CCL20, which are chemotactic for macrophages and neutrophils. Another explanation for the differences in the effect of HDM on airway inflammation could be due to intrinsic differences between the C57BL/6 and BALB/c strains of mice used in **chapters 4 and 6**, respectively. Indeed, it has been previously shown that the C57BL/6 and BALB/c mice display differential inflammatory responses upon allergen exposure (34). However, in contrast to our findings the allergen-induced inflammatory responses including total leucocytes, eosinophilic, neutrophils and Th2 cytokines IL-4 and IL-13 were shown to be significantly stronger in C57BL/6 mice compared to BALB/c mice (34). Therefore, the higher inflammatory responses we observed in the BALB/c mice of **chapter 6** are likely due to the HDM and the challenge protocol used in this study.

Limitations

Our findings provide novel insights into the mechanisms of β-catenin/CBP signalling, although there were also some limitations in our experimental models. In **chapter 4**, we observed that knockout of E-cadherin resulted in denudation of airway epithelial cells in mice, which made it difficult to validate the increased β-catenin activity in these cells that lack E-cadherin expression. More generally, current *in vitro* models, including those used in **chapter 3** and **chapter 5**, have their limitations in recapitulating the complexity of the 3D structure of the lungs as well as cell composition and the interactions between bronchial epithelial cells and immune cells. The mouse models used in **chapter 4** and **chapter 6** do provide the complexity of the lung, but here the translation from mouse to human findings needs to be considered. There are also intrinsic differences in the composition of the airway epithelium between humans and mice. For example, not all progenitor cells present in the mouse airways have been confirmed in humans (35), which may explain discrepancies in the findings from *in vitro* and *in vivo* studies such as between **chapter 5 and 6**. *In vitro* models using human organoids consisting of various types of epithelial cells or more complex microfluidic models could be useful in representing the structural complexity of the human airways, but here environmental exposures are more challenging. Co-culturing of epithelial cells with specific types of immune cells can also be used to study the inflammatory mechanisms. In addition to the differences *in vitro* and *in vivo* systems, the timing and mode of administration of ICG-001 may explain differences between **chapter 5 and chapter 6**.

Future studies should be focussed on improving the current models by pre-treating the mice before the HDM sensitisation to obtain clarity on the potential of ICG-001 to suppress early HDM-induced changes during the sensitization phase, such as the airway inflammation and airway hyperresponsiveness. Comparison of different modes of administration of ICG-001 (intranasal vs intraperitoneal) is also necessary to understand the best way of delivery of the inhibitor to the airway epithelial cells.

Implications and future perspectives

Though inhaled corticosteroids (ICS) have long been used to control the symptoms of asthma such as airway mucus hypersecretion and airflow obstruction, complications including adrenal suppression, growth suppression, osteoporosis, cataracts, glaucoma, and metabolic disorders may occur with high doses that are required for severe asthmatic patients (36,37). ICS are often supplemented with bronchodilators like long acting β2-agonists (LABAs) or long-acting muscarinic antagonists (LAMAs) to increase their effectiveness (38). Nevertheless, a proportion of the asthmatic patients are resistant to the ICS treatment (39). Therefore, there has been an increasing interest in investigating new signalling mechanisms/pathways involved in the pathogenies of asthma, which could be specifically targeted to cure the disease. Wnt/β-catenin signalling, which plays a key role in embryonic development, tissue regeneration wound repair (40), has been linked to many cancers including breast cancer, colon cancer, colorectal cancer, osteosarcoma, gastric carcinoma, and metabolic disorders such as type II diabetes and obesity, in addition to various other neurological, cardiovascular, and musculoskeletal diseases (41). In recent years, there has been a growing evidence supporting those alterations in Wnt signalling are also associated with chronic lung diseases like chronic obstructive pulmonary disorder (COPD) and asthma (42). Of note, genome wide association studies (GWAS) identify genetic variants mapped to genes involved in Wnt signalling to be associated with asthma susceptibility (43). In addition, increased expression of the canonical Wnt ligand Wnt7a was detected in sputum of asthma patients compared to healthy donors (44), suggesting that Wnt/β-catenin activity may be increased in asthmatic airway epithelium. Increased β-catenin signalling also induced goblet cell metaplasia in mice (22). In fact, inhibition of β-catenin attenuated the airway remodelling in mouse model of allergic asthma (44). In human asthmatic airways, gene expression of Wnt ligands have been either positively (Wnt3A, Wnt5A, Wnt6, and Wnt10A) or negatively (Wnt5B) associated with Th2 inflammation (45). As Wnt/β-catenin signalling can regulate a

diverse range of genes by binding to various co-activators, the genes which are specifically regulated by β-catenin/CBP and β-catenin/300 signalling are currently unknown. One of the genes that we identified to be specifically inhibited by β-catenin/CBP signalling is *FOXA2*, which has been known to negatively regulate Th2 inflammation and airway remodelling in asthma. Future studies should be directed towards identifying more such genes that are regulated by β-catenin/CBP or β-catenin/p300, which may be involved in asthma. Additional studies should also be performed to further investigate the prophylactic effects of ICG-001 on airway inflammation induced by allergens and/or knockout of E-cadherin in mice. It is also important to note that β-catenin/CBP signalling is essential for cell division and inhibiting the pathway can hinder the growth of certain important cell types in lungs. So, studies involving targeted delivery of ICG-001 to desired cell types of lung including goblet cells by using nanoparticles, should be conducted to efficiently mitigate goblet cell metaplasia.

As our findings demonstrate the impact of β-catenin/CBP signalling on the stabilisation of junctional E-cadherin, epithelial barrier function, pro-inflammatory response in airway epithelial cells, goblet cell metaplasia and mucus hypersecretion, we envision the potential of this pathway to be a therapeutic target to asthma. Furthermore, an alternative inhibitor of β-catenin/CBP signalling, PRI-724, has recently finished a phase Ia clinical evaluation on hepatitis C virus (HCV) cirrhosis, where it not only displayed anti-fibrotic effects, but has also been confirmed to be safe for use in humans (46). Finally, we conclude that β-catenin/CBP signalling may play a key role in promoting the asthmatic airway epithelial phenotype through the regulation of airway epithelial barrier integrity, chemokine secretion, goblet cell differentiation and mucus production. Specific inhibition of β-catenin/CBP signalling could be used as a potential alternative therapeutic strategy for asthma.

References

1. Heijink IH, Kuchibhotla V, Roffel MP, Maes T, Knight DA, Sayers I, et al. Epithelial cell dysfunction, a major driver of asthma development. Allergy. 2020 May 27;all.14421.
2. Boxall C, Holgate ST, Davies DE. The contribution of transforming growth factor-beta and epidermal growth factor signalling to airway remodelling in chronic asthma. Eur Respir J. 2006 Jan 1;27(1):208–29.
3. Nawijn MC, Hackett TL, Postma DS, van Oosterhout AJM, Heijink IH. E-cadherin: Gatekeeper of airway mucosa and allergic sensitization. Trends Immunol. 2011;32(6):248–55.

4. Yamada S, Pokutta S, Drees F, Weis WI, Nelson WJ. Deconstructing the cadherin-catenin-actin complex. Cell. 2005 Dec 2;123(5):889–901.
5. Valenta T, Hausmann G, Basler K. The many faces and functions of β-catenin. EMBO J. 2012;31(12):2714–36.
6. Post S, Heijink IH, Hesse L, Koo HK, Shaheen F, Fouadi M, et al. Characterization of a lung epithelium specific E-cadherin knock-out model: Implications for obstructive lung pathology. Sci Rep. 2018 Sep 5;8(1):13275.
7. Post S, Nawijn MC, Jonker MR, Kliphuis N, Van Den Berge M, Van Oosterhout AJM, et al. House dust mite-induced calcium signaling instigates epithelial barrier dysfunction and CCL20 production. Allergy Eur J Allergy Clin Immunol. 2013;68(9):1117–25.
8. Heijink IH, Kies PM, Kauffman HF, Postma DS, van Oosterhout AJM, Vellenga E. Down-Regulation of E-Cadherin in Human Bronchial Epithelial Cells Leads to Epidermal Growth Factor Receptor-Dependent Th2 Cell-Promoting Activity. J Immunol. 2007;178(12):7678–85.
9. Moolenaar WH, Aerts RJ, Tertoolen LGJ, De Laat SW. The Epidermal Growth Factor-induced Calcium Signal in A431 Cells. J Biol Chem. 1986;261(1):279–84.
10. Reiss K, Saftig P. The "A Disintegrin And Metalloprotease" (ADAM) family of sheddases: Physiological and cellular functions. Semin Cell Dev Biol. 2009;20:126–37.
11. Nagano O, Murakami D, Hartmann D, De Strooper B, Saftig P, Iwatsubo T, et al. Cell-matrix interaction via CD44 is independently regulated by different metalloproteinases activated in response to extracellular Ca(2+) influx and PKC activation. J Cell Biol. 2004 Jun 21;165(6):893–902.
12. Maretzky T, Reiss K, Ludwig A, Buchholz J, Scholz F, Proksch E, et al. ADAM10 mediates E-cadherin shedding and regulates epithelial cell-cell adhesion, migration, and -catenin translocation. Proc Natl Acad Sci. 2005;102(26):9182–7.
13. Moheimani F, Roth HM, Cross J, Reid AT, Shaheen F, Warner SM, et al. Disruption of β-catenin/CBP signaling inhibits human airway epithelial-mesenchymal transition and repair. Int J Biochem Cell Biol. 2015;68:59–69.
14. Taiyab A, Korol A, Deschamps PA, West-Mays JA. β-Catenin/CBP-Dependent Signaling Regulates TGF-β-Induced Epithelial to Mesenchymal Transition of Lens Epithelial Cells. Invest Ophthalmol Vis Sci. 2016 Oct 1;57(13):5736–47.
15. Luan Le T, Joseph SR, Yap AS, Stow JL, Le T, Luan SR, et al. Protein kinase C regulates endocytosis and recycling of E-cadherin. Am J Physiol Cell Physiol. 2002;283:489–99.
16. Liu Y, Chen H, Zheng P, Zheng Y, Luo Q, Xie G, et al. ICG-001 suppresses growth of gastric cancer cells and reduces chemoresistance of cancer stem cell-like population. J Exp Clin Cancer Res. 2017 Sep 11;36(1).
17. Hinck L, Näthke IS, Papkoff J, Nelson WJ. Dynamics of cadherin/catenin complex formation: Novel protein interactions and pathways of complex assembly. J Cell Biol. 1994 Jun;125(6):1327–40.
18. Zheng D, Soh BS, Yin L, Hu G, Chen Q, Choi H, et al. Differentiation of Club Cells to Alveolar Epithelial Cells in Vitro. Sci Rep. 2017 Jan 27;7(1):1–9.
19. Kabata H, Moro K, Koyasu S, Asano K. Group 2 innate lymphoid cells and asthma. Allergol Int. 2015 Jul 1;64(3):227–34.

20. de Boer WI, Sharma HS, Baelemans SMI, Hoogsteden HC, Lambrecht BN, Braunstahl GJ. Altered expression of epithelial junctional proteins in atopic asthma: possible role in inflammation. Can J Physiol Pharmacol. 2008 Mar;86(3):105–12.
21. Hackett T-L, de Bruin HG, Shaheen F, van den Berge M, van Oosterhout AJ, Postma DS, et al. Caveolin-1 Controls Airway Epithelial Barrier Function. Implications for Asthma. Am J Respir Cell Mol Biol. 2013;49(4):662–71.
22. Mucenski ML, Nation JM, Thitoff AR, Besnard V, Xu Y, Wert SE, et al. β-Catenin regulates differentiation of respiratory epithelial cells in vivo. Am J Physiol Cell Mol Physiol. 2005 Dec;289(6):L971–9.
23. Guseh JS, Bores SA, Stanger BZ, Zhou Q, Anderson WJ, Melton DA, et al. Notch signaling promotes airway mucous metaplasia and inhibits alveolar development. Development. 2009 May 15;136(10):1751–9.
24. Kuperman DA, Lewis CC, Woodruff PG, Rodriguez MW, Yee HY, Dolganov GM, et al. Dissecting asthma using focused transgenic modeling and functional genomics. J Allergy Clin Immunol. 2005 Aug 1;116(2):305–11.
25. Faiz A, Weckmann M, Tasena H, Vermeulen CJ, Van Den Berge M, Ten Hacken NHT, et al. Profiling of healthy and asthmatic airway smooth muscle cells following interleukin-1β treatment: A novel role for CCL20 in chronic mucus hypersecretion. Eur Respir J. 2018 Aug 1;52(2).
26. Collu GM, Hidalgo-Sastre A, Brennan K. Wnt-Notch signalling crosstalk in development and disease. Cell Mol Life Sci. 2014 Jun 19;71(18):3553–67.
27. Peignon G, Durand A, Cacheux W, Ayrault O, Terris B, Laurent-Puig P, et al. Complex interplay between β-catenin signalling and Notch effectors in intestinal tumorigenesis. Gut. 2011 Feb 1;60(2):166–76.
28. Wang R, Sun Q, Wang P, Liu M, Xiong S, Luo J, et al. Notch and Wnt/β-catenin signaling pathway play important roles in activating liver cancer stem cells. Oncotarget. 2016;7(5):5754–68.
29. Zhang Z, Yang C, Gao W, Chen T, Qian T, Hu J, et al. FOXA2 attenuates the epithelial to mesenchymal transition by regulating the transcription of E-cadherin and ZEB2 in human breast cancer. Cancer Lett. 2015 Jun 1;361(2):240–50.
30. Chen G, Wan H, Luo F, Zhang L, Xu Y, Lewkowich I, et al. Foxa2 Programs Th2 Cell-Mediated Innate Immunity in the Developing Lung. J Immunol. 2010 Jun 1;184(11):6133–41.
31. Zhu L, An L, Ran D, Lizarraga R, Bondy C, Zhou X, et al. The club cell marker SCGB1A1 downstream of FOXA2 is reduced in asthma. Am J Respir Cell Mol Biol. 2019 Jun 1;60(6):695–704.
32. Yao L, Zhao H, Tang H, Xiong J, Zhao W, Liu L, et al. Blockade of β-catenin signaling attenuates toluene diisocyanate-induced experimental asthma. Allergy. 2017 Apr 1;72(4):579–89.
33. Post S, Nawijn MC, Hackett TL, et al. The composition of house dust mite is critical for mucosal barrier dysfunction and allergic sensitisation. Thorax. 2012;67(6):488-495.
34. Kelada SNP, Wilson MS, Tavarez U, Kubalanza K, Borate B, Whitehead GS, et al. Strain-dependent genomic factors affect allergen-induced airway hyperresponsiveness in mice. Am J Respir Cell Mol Biol. 2011;45(4).
35. Wansleeben C, Barkauskas CE, Rock JR, Hogan BLM. Erratum: Stem cells of the adult lung: Their development and role in homeostasis, regeneration, and disease. Wiley Interdiscip Rev Dev Biol. 2015;4(3).
36. Barnes PJ. Inhaled corticosteroids. Pharmaceuticals. 2010;3(3):514–40.

37. Dahl R. Systemic side effects of inhaled corticosteroids in patients with asthma. Respir Med. 2006 Aug 1;100(8):1307–17.
38. Charriot J, Vachier I, Halimi L, Gamez AS, Boissin C, Salama M, et al. Future treatment for asthma. Eur Respir Rev. 2016 Mar 1;25(139):77–92.
39. Fukunaga K. Corticosteroid Resistance in Asthma. In: Yokoyama A, editor. Advances in Asthma: Pathophysiology, Diagnosis and Treatment. Singapore: Springer Singapore; 2019. p. 53–61.
40. Whyte JL, Smith AA, Helms JA. Wnt signaling and injury repair. Cold Spring Harb Perspect Biol. 2012 Aug;4(8).
41. Ng LF, Kaur P, Bunnag N, Suresh J, Sung ICH, Tan QH, et al. WNT Signaling in Disease. Cells. 2019 Aug 3;8(8).
42. Baarsma HA, Königshoff M. "WNT-er is coming": WNT signalling in chronic lung diseases. Thorax. 2017;72(8):746–59.
43. Barreto-Luis A, Corrales A, Acosta-Herrera M, Gonzalez-Colino C, Cumplido J, Martinez-Tadeo J, et al. A pathway-based association study reveals variants from Wnt signalling genes contributing to asthma susceptibility. Clin Exp Allergy. 2017 May 1;47(5):618–26.
44. Kwak HJ, Park DW, Seo JY, Moon JY, Kim TH, Sohn JW, et al. The Wnt/β-catenin signaling pathway regulates the development of airway remodeling in patients with asthma. Exp Mol Med. 2015 Dec 11;47:e198.
45. Choy DF, Modrek B, Abbas AR, Kummerfeld S, Clark HF, Wu LC, et al. Gene Expression Patterns of Th2 Inflammation and Intercellular Communication in Asthmatic Airways. J Immunol. 2011 Feb 1;186(3):1861–9.
46. Kimura K, Ikoma A, Shibakawa M, Shimoda S, Harada K, Saio M, et al. Safety, Tolerability, and Preliminary Efficacy of the Anti-Fibrotic Small Molecule PRI-724, a CBP/β-Catenin Inhibitor, in Patients with Hepatitis C Virus-related Cirrhosis: A Single-Center, Open-Label, Dose Escalation Phase 1 Trial. EBioMedicine. 2017 Sep 1;23:79–87.