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

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DOI:
10.33612/diss.172561514

Publication date:
2021

CHAPTER 1

General Introduction

Asthma

Asthma is a chronic inflammatory disease broadly characterised by symptoms such as coughing, wheezing, and tightness of chest. These symptoms are caused by aberrant airway remodelling and reversible airway obstruction and hyperresponsiveness of the airways to inhaled allergens like house dust mite (HDM) and grass or tree pollen or to environmental stimuli like tobacco smoke and air pollution. Around 1000 people die every day due to asthma and more than 300 million are currently suffering from the disease worldwide (1). Allergen-induced asthma or atopic asthma is the most common type of asthma, described by elevated serum Immunoglobulin (Ig) E, T helper 2 (Th2) cell mediated airway inflammation and airway remodelling. Th2 cells release cytokines IL-4, IL-5, IL-9 and IL-13 that induce IgE production by B-lymphocytes, eosinophilic infiltration into the airways and goblet cell hyperplasia with excessive mucus production (2). IgE antibodies bind to mast cells, which can trigger the release of histamine, leukotrienes, and prostaglandins after IgE crosslinking, resulting in airway obstruction due to excessive smooth muscle contraction, mucosal swelling, and mucus production (2). Early childhood exposure to allergens resulting in allergic sensitisation increases the risk of atopic asthma. Other risk factors for asthma include increased viral infections during early childhood, exposure to tobacco smoke and air pollution (3). Non-allergic or non-atopic asthma is a less common type of asthma, which is frequently characterized by the infiltration and activation of neutrophils (4). Although the exact pathophysiology is not clear, the neutrophilic inflammation may be triggered by the release of cytokines from Th1 and Th17 cells or type-3 innate lymphoid cells (ILC3) upon exposure to non-allergic chemicals, including cigarette smoke and air pollutants, or microbes (5). Other endotypes of asthma include nonallergic eosinophilic asthma, which may be driven by type-2 innate lymphocytes (ILC2), and paucigranulocytic asthma, without apparent neutrophilia and eosinophilia (2).

Airway epithelium in asthma

Inhaled allergens first come in contact with the airway epithelium, which acts as a primary line of defence against environmental insults. In addition to acting as a physical barrier, the airway epithelium is a critical part of the innate immune system executing functions involved in host defence against pathogens through mucociliary clearance and release of anti-microbial peptides, pro-inflammatory cytokines, and interferons (6). The airway epithelial barrier is a pseudostratified layer consisting of different types of epithelial cells; basal cells,

ciliated cells, club cells and goblet cells making up the majority. Basal cells serve as progenitors, which can differentiate into secretory club cells, which can further differentiate into mucus producing goblet cells or mucus clearing ciliated cells (7). Ciliated cells express motile cilia on their apical surface, which through coordinated directional beating, promote clearance of mucus plugs (8), in addition to sensing and responding to mechanical and chemical stimuli (9). Club cells produce distinctive proteins such as club cell secretory protein (CCSP) and other surfactants (surfactant proteins A, B, and D) that contribute to the mucus layer covering the airway epithelium (10). Goblet cells produce mucus, which consists of water, polypeptides, enzymes, and high molecular weight glycoproteins called mucins. So far, at least 20 mucins have been identified in humans, which fall into two main categories: membrane-bound (cell surface) mucins and secreted mucins (11). Membrane bound mucins, like MUC1 and MUC4, consist of specific domains which can interact with signalling pathways and also play a key role in cell-cell and cell matrix interactions (12), while MUC2 provides a protective and lubricating coating of airway epithelium against particles and infectious agents (13). Secreted mucins including MUC5AC and MUC5B constitute the bulk of mucus and are responsible for the gel forming properties of the mucous layer. The mucus layer in the airways is important for maintaining homeostasis, airway defence and mucociliary clearance. In humans, MUC5AC is exclusively expressed in goblet cells, while MUC5B is secreted by submucosal glands as well as goblet cells (14). In mice, the submucosal glands predominantly express Muc5b compared to distal small airway epithelial cells, whereas both proximal and small airway epithelial cells express low levels of Muc5ac (15).

In addition to the environmental factors, genetic factors also contribute to asthma susceptibility (16). Genome-wide association studies (GWAS) have identified a large number of asthma risk genes including IL1RL1, IL33, TSLP, CDHR3 and PDCH1 that are important for airway epithelial function, which are discussed in detail in chapter 2 (2). The asthmatic airway epithelium is characterised by an increase in the number of the goblet cells, termed goblet cell metaplasia, resulting in increased mucus production. While mucus plugs in asthma patients contain both MUC5AC and MUC5B (17), MUC5AC is specifically upregulated in the airway epithelial cells of asthma patients (18). As described in chapter 2 (2), the asthmatic airway epithelium may be more susceptible to damage upon exposure to allergens like HDM, resulting in the loss of adherens junction protein E-cadherin and the disruption of epithelial

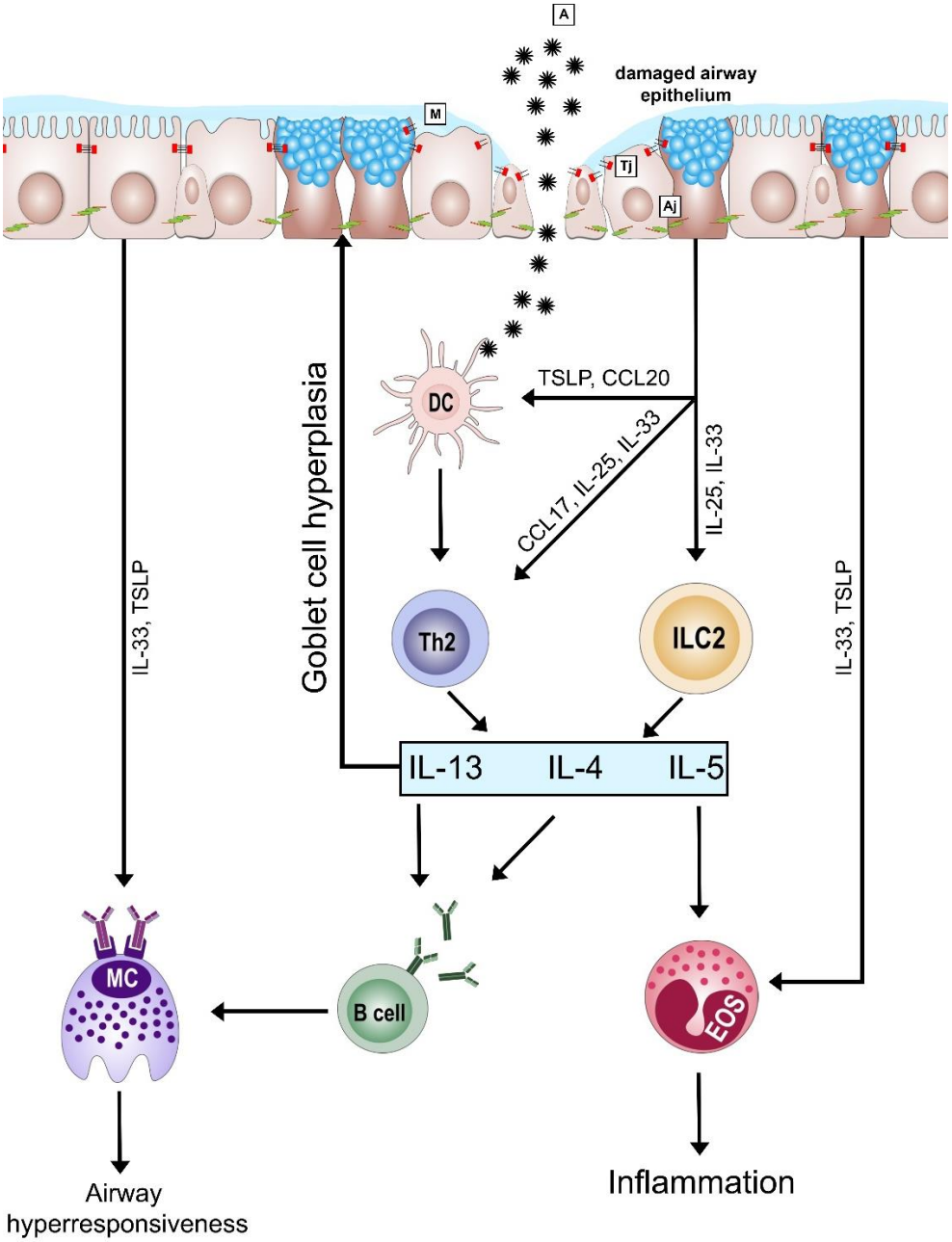

Figure 1: Airway epithelium in asthma. Exposure to allergens (A) causes damage to the airway epithelial barrier characterised by the loss of adherens junctions (Aj) and tight junctions (Tj) and release of pro-inflammatory cytokines and chemokines. DCs activate the Th2 cells and ILC2 cells which further promote the infiltration of eosinophils (Eos) and IgE production by B cells. IgE antibodies bind to mast cells (MC), which trigger release of histamines, leukotrienes and prostaglandins, resulting in airway hyperresponsiveness.

barrier function (19). Of interest, deletion of E-cadherin in all the airway epithelial cells of mice resulted in specific loss of ciliated cells, accompanied by spontaneous infiltration of eosinophils and DCs and goblet cell metaplasia (20). The asthmatic airway epithelium has also been shown to express increased levels of basal cell markers cytokeratin-5, -14 and p63, which indicates an undifferentiated epithelium that is unable to repair and regenerate upon damage, inflicted by environmental factors such as inhaled allergens or viruses, to form a functionally intact barrier (16). The airway epithelial cells, upon exposure to HDM, release several pro-inflammatory cytokines including IL-6, IL-8, Chemokine (C-C motif) ligand 20 (CCL20), CCL17, thymic stromal lymphopoietin (TSLP), IL-25, IL-33, and granulocyte-macrophage colony-stimulating factor (GM-CSF) (2). These can attract and/or activate cells from the innate and adaptive immune system like dendritic cells (DCs) and innate lymphoid cells (ILCs), which further activate T-helper 2 (Th2) cells (Figure 1).

E-cadherin/β-catenin interaction in asthma

The airway epithelial cells are mechanically connected through various junctional proteins consisting of adherens junctions (AJs), tight junctions (TJs) and (hemi)desmosomes (2). E-cadherin is a calcium-dependent transmembrane protein, which is an integral part of the adherens junctions that mechanically connect adjacent airway epithelial cells. E-cadherin-based adherens junctions are important for the formation of tight junctions and for the maintenance of epithelial barrier function (21). E-cadherin expression has been shown to be significantly downregulated in the asthmatic airway epithelium (22,23). Loss of E-cadherin in airway epithelial cells resulted in decreased epithelial barrier function and CCL17 expression (21) and may lead to increased levels of CCL20 (19). β-catenin is another subunit of adherens junctions, which connects the intracellular domain of E-cadherin to the actin cytoskeleton, thereby aiding in cell adhesion (24). β-catenin also plays a major role in signal transduction by acting as a key nuclear effector in canonical wingless-related integration site (Wnt) signalling, which is not only essential for embryonic development, but is also important in maintaining homeostasis, cell renewal and regeneration of adult tissues and organs (25). β-catenin protects newly synthesised E-cadherin during its transportation from the endoplasmic reticulum to the cell surface by binding to it and shielding a specific peptide sequence motif, which when recognised by a ubiquitin ligase results in the proteolytic degradation of E-cadherin (26). Conversely, E-cadherin stabilises β-catenin at the cell junction and loss of junctional E-cadherin releases β-catenin into the cytoplasm, where its

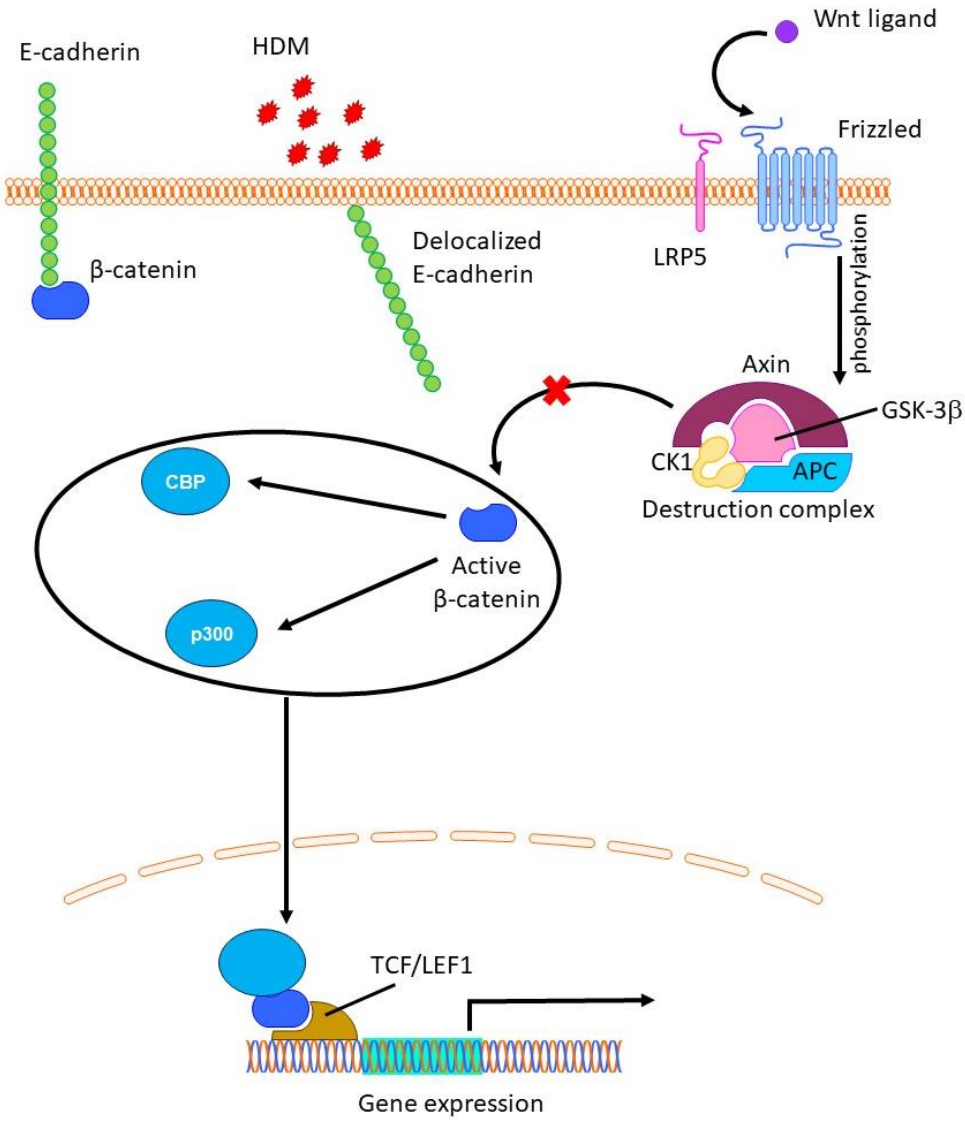

Figure 2: The dual role of β-catenin in cell adhesion and gene regulation. Allergens like HDM delocalise junctional E-cadherin resulting in the release of β-catenin into cytoplasm, where is targeted for proteolytic degradation by the destruction complex. Upon Wnt ligand mediated phosphorylation and inactivation of GSK-3β, the cytosolic β-catenin escapes from the destruction complex and binds to TCF/LEF group of transcriptional factors and other co-activators such as CBP and p300 resulting transcription of target genes.

levels are normally controlled by the 'β-catenin destruction complex' through proteasomal degradation by phosphorylation and ubiquitination. The destruction complex is composed of

proteins axin, adenomatous polyposis coli (APC), casein kinase-1 (CK1) and glycogen synthase kinase (GSK)-3β. Inactivation of GSK-3β, for example by Wnt ligands or growth factors like TGF-β or EGF, prevents the degradation of active (non-phosphorylated) β-catenin resulting in its nuclear translocation and association with DNA-binding transcription factors of the TCF/LEF family (27) (Figure 2). This leads to the transcriptional activation of E-cadherin repressors such as snail and slug as well as mesenchymal markers, including fibronectin, EGFR, and VEGF, which may contribute to airway wall remodelling (16). The loss of epithelial markers like E-cadherin and increase in the expression of mesenchymal proteins like ED-A fibronectin, alpha-smooth muscle actin (α-SMA) and vimentin is known as epithelial-mesenchymal transition (EMT), which has been proposed to play a role in airway remodelling in asthma (28). TGF-β alone, and in combination with HDM disrupted junctional E-cadherin and promoted EMT in airway epithelial cells (29,30). Furthermore, TGF-β signalling has been shown to interact with β-catenin signalling to regulate EMT (31). Besides EMT, a large number of genes involved in cell adhesion, migration, proliferation, differentiation, inflammation and remodelling have been discovered to be regulated by β-catenin, emphasizing its wide spectrum of functional roles (32,33). In addition, enhanced β-catenin activity *in vivo* has been shown to promote goblet cell differentiation (34). This ability of active β-catenin to regulate the expression of several genes is achieved through selectively recruiting and binding to various transcriptional co-activators, including cAMP response element-binding protein (CREB)-binding protein (CBP) and its closely related homolog E1A-binding protein (p300), resulting in transcriptional activation of different subsets of genes (35–37). Small molecule inhibitors ICG-001 and IQ1, which specifically inhibit the β-catenin/CBP and β-catenin/p300 pathways respectively, have been used to obtain insights into the role of these individual pathways (35–37). For instance, β-catenin/CBP interaction results in the expression of cell proliferation genes like *cyclin D1* and *survivin* in colon carcinoma cells (35,36), while β-catenin/p300 interaction induces cell differentiation in embryonic stem cells by inhibiting the expression of Nanog (37). Inhibition of the β-catenin/CBP pathway using ICG-001 prevented smooth muscle remodelling and deposition of extracellular matrix in *in vitro* and *in vivo* models of asthma (38). ICG-001 also inhibited proliferation, migration and EMT in airway epithelial cells (39), and suppressed airway inflammation and goblet cell metaplasia in a toluene diisocyanate mouse model of asthma (40). Furthermore, inhibition of β-catenin downstream activity attenuated airway inflammation, smooth muscle thickness, subepithelial fibrosis, hyperresponsiveness and

goblet cell metaplasia in mouse models of asthma (41). In particular, β-catenin/CBP pathway's role of maintaining the cells in an undifferentiated status may resemble components of the asthmatic phenotype, where the cells are unable to properly form cell junctions, polarise and differentiate. All these studies provide sufficient evidence to support an important role for β-catenin in the development of asthma.

Apart from the Wnt/β-catenin signalling, dysregulation of Notch signalling has also been shown to be linked to the pathogenesis of asthma and other lung diseases (42). Notch is a highly conserved transmembrane receptor protein that plays a key role in cell development, proliferation, differentiation, determination of cell fate and maintenance of homeostasis. So far, four different Notch receptors – Notch 1, 2, 3 and 4 have been identified in humans along with their various ligands that belong to the delta like protein 1 (DLL1), DLL3, DLL4 and Jagged 1 (JAG1), JAG2 protein families (43). Both the Notch receptors and their ligands consist of an extracellular domain, a transmembrane domain, and an intracellular domain. The ligand-mediated activation of the Notch family of receptors induces a series of proteolytic cleavages, which initiate cell signalling by the release of the notch intracellular domain (NCID) into the cytoplasm. The free NCID translocates to the nucleus and interacts with DNA-binding protein CBF1–Suppressor of Hairless–LAG1 (CSL; also known as RBPJ) and the co-activator Mastermind-like transcriptional co-activator 1 (MAML1), resulting in the transcription of target genes (43). Although the mechanisms by which Notch signalling modulates epithelial homeostasis and responses to environmental insults are not completely understood, various Notch target genes are differently expressed in healthy and asthmatic airway epithelium (44,45). Activation of Notch 1 enhances the expression of the transcription SAM pointed domain-containing ETS transcription factor (SPDEF), which increases the expression of MUC5AC in airway epithelial cells, resulting in goblet cell hyperplasia (46). The Notch signalling pathway also plays a crucial role in controlling the fate of airway epithelial cells upon injury (44). Of interest, Notch signalling has been shown to interact with the Wnt/β-catenin signalling pathway during development and in diseased conditions such as in different types of cancers (47–50). Although both β-catenin and Notch regulate similar cellular processes in the airway epithelium, it is not completely clear to what extent these two pathways overlap and contribute to the epithelial abnormalities observed in asthma.

Small Molecule Inhibitors

Small molecule inhibitors have been increasingly used in the last two decades, not only for gaining a deeper understanding of various signalling pathways and their role in cellular functions, but also as therapeutic strategies for various diseases (51–54). In order to investigate the role of β-catenin/CBP, β-catenin/p300, and Notch signalling, we have used three small molecule inhibitors Indocyanine Green-001 (ICG-001), IQ-1, and Dibenzazepine (DBZ), respectively. The molecular structures of the three inhibitors are shown in Figure 3. Detailed information on the function, specificity, and mechanism of these inhibitors are discussed below.

ICG-001 is a small molecule inhibitor that blocks β-catenin/T cell factor (TCF) downstream signalling. In 2004, Emami and colleagues discovered that ICG-001 specifically binds to CBP, which blocks the interaction of β-catenin and CBP, thereby specifically inhibiting the β-catenin/CBP signalling in human colon carcinoma cell lines (35). Although CBP shares close homology with the co-activator p300 (63%), it has been confirmed that ICG-001 does not inhibit the interaction of β-catenin and p300 (35). In fact, ICG-001 has been shown to promote the binding of β-catenin to p300, at the expense of β-catenin/CBP interaction (35). Furthermore, ICG-001 did not affect the protein expression levels of β-catenin, CBP and p300, which makes ICG-001 highly effective in the inhibition of β-catenin/CBP signalling (35). Using ICG-001, β-catenin/CBP signalling has been shown to specifically regulate the expression of genes such as *Cyclin D1* and *Survivin* (35,36). ICG-001 has been shown to inhibit cell proliferation in many types of cancer cells, thereby supporting its use in anti-cancer treatments (55–58).

IQ-1 is also a small molecule inhibitor which blocks the downstream signalling of the β-catenin/TCF pathway. IQ-1 was discovered by Miyabayashi and colleagues in 2007, who showed that it specifically binds to the PR72/130 subunit of protein phosphatase 2 (PP2A), resulting in decreased binding of β-catenin to p300, thereby inhibiting the β-catenin /p300 signalling in embryonic stem cells (37). It has also been confirmed that IQ-1 does not inhibit the interaction of β-catenin and CBP (37). Moreover, IQ-1 has been showed to enhance the binding of β-catenin to CBP, while having no effect on the protein expression of p300 (37). IQ-1 has been shown to maintain embryonic stem cells in an undifferentiated state by specifically regulating the expression of *Nanog* (37).

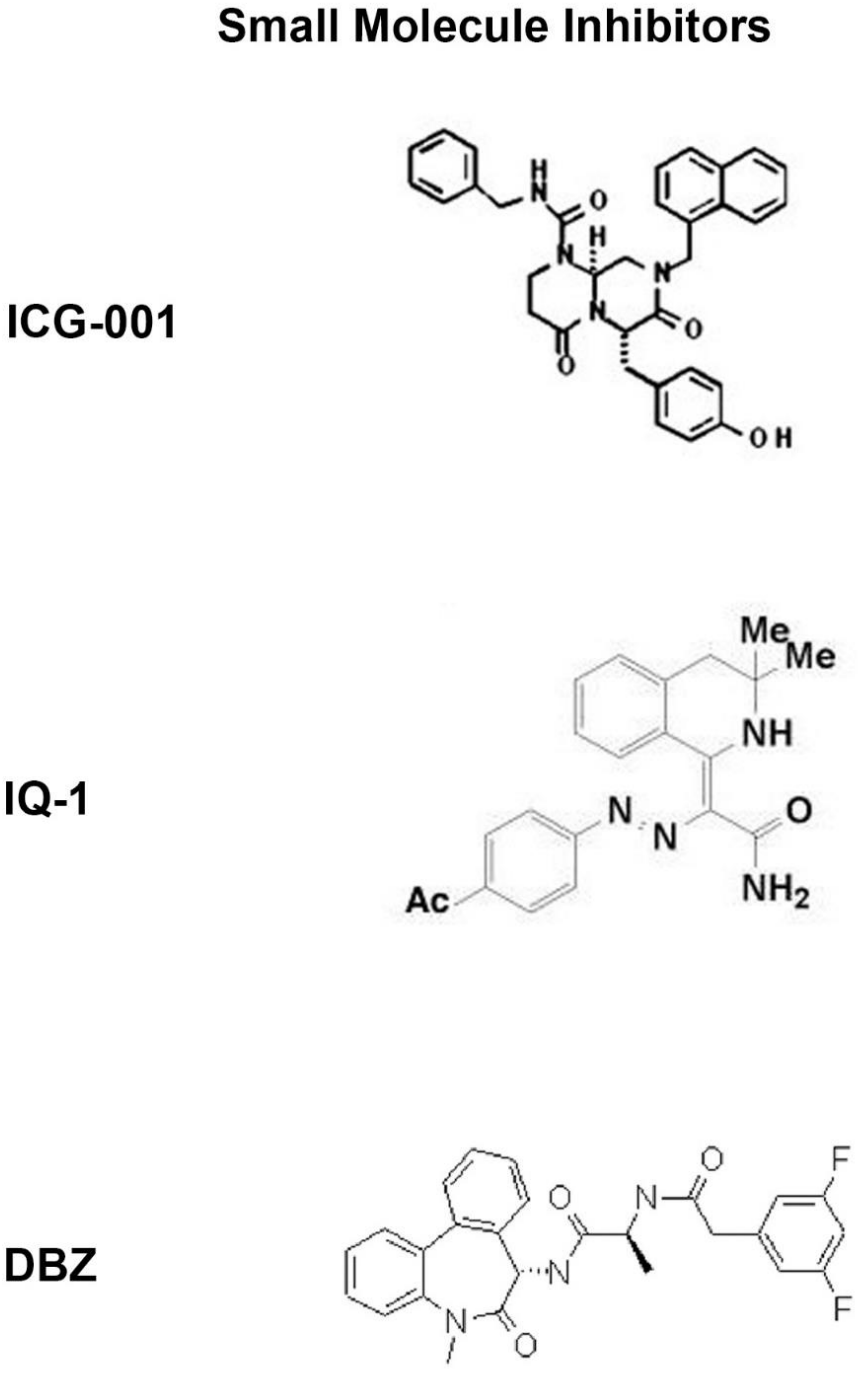

Figure 3: Molecular structures of ICG-001 (35), IQ-1 (37) and DBZ (59).

DBZ (YO-01027) is a γ-secretase inhibitor that blocks the activation of the Notch pathway. Notch is a key substrate of γ-secretase, which mediates the ligand-mediated cleavage and activation of the Notch intracellular domain (60). DBZ blocks γ-secretase, thereby inhibiting the downstream signalling of the Notch pathway (59). γ-secretase also mediates the cleavage of amyloid precursor protein (APP) (61), E-cadherin (62), N- cadherin (63), and CD44 (64). Cleavage of APP results in the generation of toxic amyloid β peptides that are found in Alzheimer's disease, thus alluding the potential of DBZ for use in the treatment of Alzheimer's disease (65).

Mouse models of asthma

Although there are several treatments that are currently in use to control asthma, these suppress symptoms, and do not cure the disease. In addition, some patients do not respond to current asthma medication, and have uncontrolled, often severe disease (66). It is imperative to investigate the molecular mechanisms of asthma in order to develop more effective therapeutic strategies. Most of the *in vitro* systems currently in use lack the complexity to study some of the important phenotypes of asthma such as type-2 immune cell response including eosinophilic inflammation, airway remodelling and airway hyperresponsiveness. Therefore, animal studies such as those using mouse models, which offer a functioning immune system with multiple cell types, are indispensable in providing detailed insights into the cellular and molecular mechanisms useful in understanding the pathophysiology of asthma (67). Since mice do not spontaneously develop manifestations of asthma, models have been developed that depend on intraperitoneal sensitisation with a model allergen such as Ovalbumin (OVA), followed by OVA inhalation challenge to induce allergic airway inflammation that has some overlap with the asthmatic phenotype (68). Although sensitisation with OVA is effective in inducing a strong allergic response, parenteral immunisation protocols do not offer a physiologically accurate model to the inhaled aeroallergens that cause asthma in humans. Especially the role of the (susceptible) airway epithelium in the inception of disease is lost in OVA mouse models. Therefore, improved mouse models have been developed using extracts of aeroallergens such as HDM, grass pollen or cockroach (69). These models depend on sensitisation through the airways, allowing detailed analysis of the role of the airway epithelium and dissection of the genetic susceptibility to asthma conferred by asthma genes expressed in the airway epithelium (69).

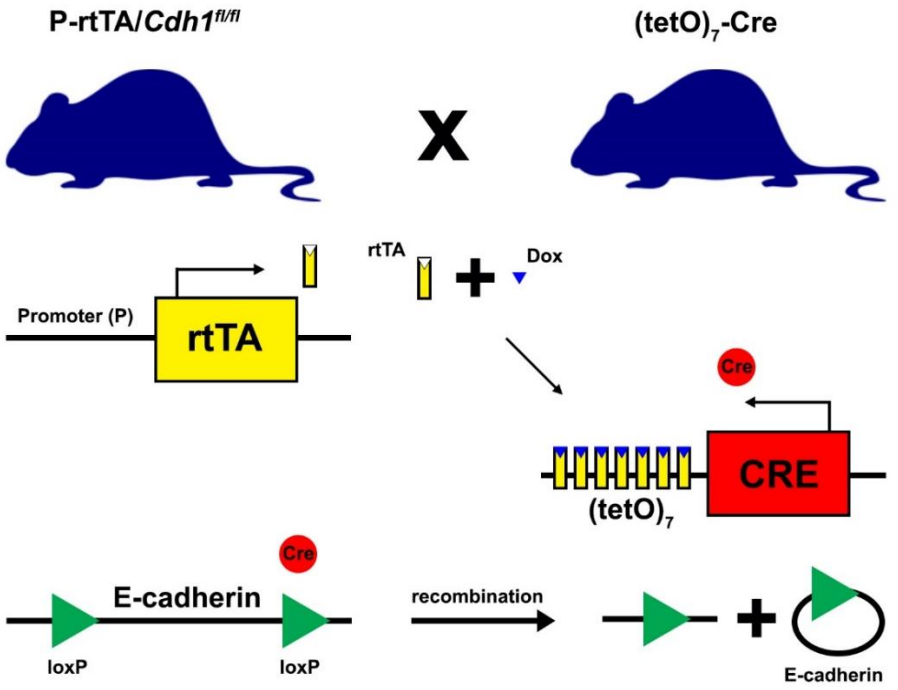

Figure 4: Cyclisation recombinase (Cre) is an enzyme isolated from the P1 bacteriophage, which can perform site specific recombination upon recognition of a pair of specific small 34 base pair (bp) DNA sites called loxP sites (locus of x-over, P1) (68). LoxP sites are also found in P1 bacteriophage flanking both sides of a gene, termed floxed gene. The type of recombination (deletion, inversion, or translocation) carried out by Cre depends on the relative orientation of the loxP sites. If the loxP sites flanking the gene are oriented in the same direction, the Cre recombinase performs an excision of the floxed gene (68,69). The expression of Cre is controlled by using a combination of reverse tetracycline controlled transactivator (rtTA) and tetracycline operon (tetO) system. Insertion of the $tetO_7$ (7 repeats of tetO) sequences upstream of Cre enables its activation through the administration of doxycycline (dox, a tetracycline derivative), indicated as 'Tet-on'. To bind to $tetO_7$, dox also needs to interact with rtTA, whose expression can be targeted to a desired cell type using a specific promoter (P). Upon dox treatment, the E-cadherin gene gets knocked out from the cells expressing promoter (P) in Cre^+ mice due to the activation of Cre, while Cre^- mice function as their respective wild-type controls.

Mouse models have often been used to study the function of individual asthma genes in loss-of-function such as *Il1rl1* knockout mice (70,71) or gain-of-function like IL-13 over-expressing transgenic mice (72,73) models. Though E-cadherin was not identified as a risk allele by GWAS studies, its expression was found to be decreased in asthmatic airway epithelium (22,23). Knock-down of E-cadherin in airway epithelial cells resulted in decreased barrier function and increased pro-inflammatory response (21). Therefore, E-

cadherin knock-out mouse models were developed in order to investigate its role in the pathogenesis of asthma. However, a complete knock-out of E-cadherin in mice has been shown to be lethal (74). Alternatively, E-cadherin can be knocked out using Cre – loxP system in specific subsets of lung epithelial cells driven by promoters including surfactant protein C (SP-C) and club cell secretory protein (CCSP) (Figure 4).

Despite the various uses and applications of mouse models in asthma, they have a few limitations. Mainly, there are species differences in the airway physiology such as disparities in branching pattern and type and location of lung cells between mice and humans, which affect pulmonary responses (67). Due to these differences, some features specific to mice may be observed in mouse models of asthma that are not present in humans. Unlike humans, the inflammation and remodelling in mice are not restricted to the conducting airways and are also observed in the lung parenchyma (75). Furthermore, there is hardly any infiltration of mast cells into the airways of mice (76). Finally, due to the complex and diverse nature of asthma, it is very challenging to develop a mouse model that truly and completely reflects the clinical disease (68). However, we can model specific characteristics of asthma in mice through sensitisation with allergens and/or genetic modification of specific genes that are known to be involved in asthma.

Scope of the thesis

Asthma is broadly characterised by a chronic type-2 airway inflammation, aberrant airway remodelling and repair along with excessive mucus production in the airways. The asthmatic airway epithelial barrier is frequently damaged and repeated injury by environmental insults such as allergens in combination with impaired repair responses results in the delocalisation of junctional proteins like E-cadherin and β-catenin, as described in chapter 2 (2). Loss of E-cadherin combined with signalling from Wnt or growth factors like EGF and TGF-β enables β-catenin to bind to coactivators such as CBP or p300 and initiate the transcription of various genes involved in cell proliferation and differentiation, respectively. Although β-catenin signalling is important for tissue development and maintenance of homeostasis, aberrant activation of β-catenin signalling through Wnt or other growth factors may contribute to the pathogenesis of asthma (41). Many genes regulated by the Wnt/β-catenin signalling have been documented (33), but little is known about the genes that are specifically regulated by the β-catenin/CBP or β-catenin/p300 pathways and might be dysregulated in asthma. β-

catenin/CBP pathway has been shown to regulate EMT in airway epithelial cells (31,39) and maintain cells in an undifferentiated and proliferative stage (35,36). Since the asthmatic airway epithelium shows features of EMT and exhibits an undifferentiated phenotype, resulting in decreased barrier function, which is accompanied by susceptibility to allergens that cause airway inflammation and mucus hypersecretion (16,28), **we hypothesise that increased β-catenin/CBP signalling contributes to the development of asthma phenotype.**

The scope of this thesis is to investigate the role of abnormal airway epithelial β-catenin signalling in the pathogenesis of asthma, focusing particularly on β-catenin/CBP pathway. By using the small molecule inhibitor ICG-001, we specifically block the binding of CBP to β-catenin to investigate the role of β-catenin/CBP signalling in airway epithelial barrier (dys)function, airway inflammation and mucus production. In **chapter 2**, we review the role of airway epithelial cell dysfunction in the development of asthma. As the loss of E-cadherin weakens airway epithelial barrier function (21) and may lead to higher expression of pro-inflammatory cytokines like CCL20 (19), we hypothesised that downstream β-catenin/CBP signalling specifically leads to loss of epithelial barrier function and increased release of pro-inflammatory cytokines such as CCL20 and GM-CSF. In **chapter 3**, we address this question by investigating the effects of ICG-001 on airway epithelial barrier function and CCL20 production in primary bronchial epithelial cells (PBECs) upon exposure to HDM. Next, we investigated the effects of HDM on an E-cadherin deficient airway epithelium *in vivo*. In **chapter 4**, we hypothesised that loss of E-cadherin in the airway epithelium leads to increased susceptibility to develop inflammation and remodelling of the airways upon exposure to allergens. As a full E-cadherin knock-out is lethal (74), we used Cre-Lox system to restrict the E-cadherin loss to lung epithelial cells. Previously, we showed that knock-out of E-cadherin in mice in cells expressing SP-C during embryonic stage resulted in the denudation of lung epithelial cells both in airways and alveoli (20). In this chapter, we aimed to further limit the knock-out of E-cadherin to cells expressing CCSP during embryonic or post-natal stage and expect to observe a loss of E-cadherin in either airway epithelial cells or club cells respectively, based on the differential expression of CCSP during lung development (77). In these models, we also for the first time evaluate the effect of allergen challenge. To summarise, we created three different E-cadherin knock-out mouse models that are subjected to initial sensitisation and chronic treatment with HDM: Model 1 - Knock-out

of E-cadherin in all the lung epithelial cells, Model 2 - Knock-out of E-cadherin in only airway epithelial cells, and Model 3 - Knock-out of E-cadherin in only club cells. We compared the infiltration of different immune cells in the airways, serum IgE response and mucus production in response to HDM in wild-type and E-cadherin knock-out in the three mouse models.

Activation of β-catenin and Notch signalling has been previously shown to induce goblet cell metaplasia and excessive mucus production in mice (34,78). Although β-catenin and Notch signalling crosstalk has been observed in development and disease (47), it is unclear if the two signalling pathways interact in the airway epithelium to regulate mucus production. We have previously shown that inhibition of β-catenin/CBP signalling has improved epithelial barrier function and reversed EMT in airway epithelial cells (39,79). In **chapter 5**, we hypothesised that β-catenin/CBP signalling regulates goblet cell differentiation through downstream Notch signalling in PBECs cultured in air-liquid interface. First, we assessed the differences in β-catenin and Notch activity in PBECs from asthma and non-asthma donors during development by comparing the expression of β-catenin target gene cyclin D1 (*CCND1*), Notch1 target gene hairy and enhancer of split 1 (*HES1*), and MUC5AC expression and production. Further, we investigated the effect of inhibition of Notch, β-catenin/CBP and β-catenin/p300 signalling using DBZ, ICG-001 and IQ1 respectively on MUC5AC expression and production at baseline and upon stimulation by IL-13. Next, we aimed to validate our findings on the role of β-catenin/CBP signalling on mucus production *in vivo*. In **chapter 6**, we hypothesised that inhibition of β-catenin/CBP signalling attenuates HDM-induced mucus production in mice. We tested this by initially sensitizing the mice with HDM, followed by a chronic HDM treatment with/without ICG-001 and measuring the airway mucus production. In **chapter 7**, we summarise, discuss the relevance of the findings and implications of this thesis, and finally put them into perspective for future studies.

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