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β-Catenin as a regulator and therapeutic target for asthmatic airway remodeling

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Abstract

Introduction: Pathological alteration in the airway structure, termed as airway remodeling, is a hallmark feature of individuals with asthma and has been described to negatively impact lung function in asthmatics. Recent studies have raised considerable interest in the regulatory role of β-catenin in remodeling of asthmatic airways. The WNT/β-catenin signaling pathway is the key to normal lung development and tightly coordinates the maintenance of tissue homeostasis under steady-state conditions. Several studies indicate the crucial role for β-catenin signaling in airway remodeling in asthma and suggest that this pathway may be activated by both the growth factors and mechanical stimuli such as bronchoconstriction.

Areas covered: In this review, we discuss recent literature regarding the mechanisms of β-catenin signaling activation and its mechanistic role in asthmatic airway remodeling. Further, we discuss the possibilities of therapeutic targeting of β-catenin.

Expert opinion: The aberrant activation of β-catenin signaling by both WNT-dependent and -independent mechanisms in asthmatic airways plays a key role in remodeling of the airways, including cell proliferation, differentiation, tissue repair and extracellular matrix production. These findings are interesting from both a mechanistic and therapeutic perspective, as several drug classes have now been described that target β-catenin signaling directly.

1. Introduction

Airway structure and function are considerably altered in individuals with severe asthma, and several pathological features of the asthmatic airway have been described that negatively impact airway narrowing [1]. These include basement membrane thickening, subepithelial fibrosis, airway neovascularization and mucus cell hyperplasia. Probably the most notable structural alteration in the asthmatic airway wall however is the marked increase in smooth muscle mass [2]. Airway smooth muscle hypertrophy is a pathological feature more prominent in individuals with asthma compared to individuals with chronic obstructive pulmonary disease (COPD) and can be quite profound as about 3.5-fold increases in airway smooth muscle content have been reported in severe asthma patients [3]. Increased airway smooth muscle mass is considered a major contributor to airflow limitation in asthma and negatively impacts bronchial hyperresponsiveness and the bronchodilatory response to a deep breath [2]. The increased airway smooth muscle mass is characterized by both cellular hyperplasia and hypertrophy [4].

Several factors have been described to regulate airway smooth muscle thickening and other features of airway remodeling in asthma. Inflammatory cells directly interact with resident airway structural cells and secrete pro-inflammatory mediators and growth factors that induce proliferation, matrix protein secretion and differentiation [5,6]. Probably one of the best characterized growth factors involved in airway remodeling is TGF-β, which is secreted by airway epithelial cells, airway smooth muscle cells and eosinophils and is known to promote matrix protein production, contractile protein expression and proliferation of...
airway smooth muscle cells and airway fibroblasts [7]. In addition, matrix proteins play direct regulatory roles in airway remodeling by regulating the phenotype and function of airway smooth muscle cells and fibroblasts. A complete survey of the mechanisms that regulate airway remodeling in asthma is beyond the scope of this review and has been reviewed extensively elsewhere [2].

Airway remodeling, including airway smooth muscle thickening is correlated with asthma severity, whereas age and duration of the disease have limited impact on the nature and extent of airway smooth muscle thickening [8]. This suggests that smooth muscle thickening is a determinant rather than a cause of disease severity that may be present in individuals with asthma already early on in the disease. Recent studies support this view and indicate that preschool wheezers who develop asthma at the age of 8 have increased airway smooth muscle mass already at preschool age [9]. In fact, accumulating evidence suggests that asthmatic airway remodeling already occurs in early childhood, often precedes the onset of symptoms and determines the deficit in lung function later in life [10,11]. Epidemiological studies have indicated that early life exposures to environmental factors such as smoke, increase the risk of developing asthma and airway hyperresponsiveness [12]. Animal studies further indicate that maternal smoke exposure during pregnancy is sufficient to trigger airway smooth muscle thickening in the offspring [13]. These changes are associated with the differential expression of developmental pathways such as the WNT/β-catenin signaling pathway [14]. In addition, developmental pathways including the WNT/β-catenin pathway are known to play key roles in tissue repair and remodeling later in life [15]. These and other studies have raised considerable interest in the regulatory role of WNT/β-catenin signaling in remodeling. In this review, the role of β-catenin, both as an effector of WNT signaling and as an effector of TGF-β signaling will be reviewed in the context of asthmatic airway remodeling.

2. **β-catenin: signaling and activation**

As a membrane-bound protein, β-catenin constitutes a key component of adherens junctions where it interacts with the cadherins and connects them to the cytoskeleton [16]. In addition to its role in adherens junctions, cytosolic and nuclear β-catenin is the central mediator of the canonical WNT signaling pathway where it functions as the transcriptional co-activator for WNT-responsive genes [16,17]. The regulation of the membrane-bound pool of β-catenin is still unclear but it does communicate with the cytosolic pool of β-catenin [16]. Under steady-state conditions, the amount of cytosolic β-catenin is tightly regulated by a multiprotein destruction complex comprising glycogen synthase kinase (GSK)-3β, adenomatous polyposis coli (APC), axin and casein kinase 1 (CK1). In the absence of WNT ligands, the destruction complex constitutively phosphorylates β-catenin marking it for proteasome-mediated degradation, thereby maintaining low levels of β-catenin in the cytosol. Rescuing β-catenin from this destructive phosphorylation by WNT ligands constitutes a key step in β-catenin stabilization and nuclear translocation. WNT ligands bind to frizzled (FZD) receptors which associate with lipoprotein receptor-related protein (LRP)-5/6 co-receptors, leading to the recruitment of the dishevelled adaptor protein, which regulates downstream signaling effectors [17]. This sequesters the GSK-3β-containing
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destruction complex to the membrane by facilitating the interaction between the destruction complex and the cytosolic domain of LRP-5/6 co-receptors in a dishevelled-dependent process [17,18]. This change in the compartmentalization of the destruction complex from cytosol to the membrane rescues β-catenin from GSK-3β-mediated phosphorylation and allows cytosolic accumulation of β-catenin [17,18]. In addition to the membrane localization, WNT ligands can also induce uptake of GSK-3β in the multivesicular bodies, rescuing β-catenin from destruction [19]. The activation of canonical β-catenin signaling by WNT ligands is depicted in Figure 1.

In addition to WNT ligands, several growth factors that bind to and activate receptor tyrosine and serine/threonine kinase receptors (e.g. platelet-derived growth factor [PDGF], TGF-β) can stabilize β-catenin and activate β-catenin-dependent processes. Of note, the WNT-independent activation of β-catenin by these growth factors differs substantially from WNT-induced β-catenin stabilization. Growth factors stabilize β-catenin by directly targeting GSK-3 by phosphorylation. The most observed among them is the phosphorylation at serine 21 of the GSK-3α isoform and serine 9 of the GSK-3β isoform by the phosphoinositide-3-kinase/Akt (PI3K/Akt) pathway [20]. Phosphorylation of GSK-3β inhibits its activation, allowing β-catenin stabilization. Numerous kinases other than Akt, including protein kinase C (PKC) and integrin linked kinase can also phospho-modify and inhibit GSK-3 [20] leading to β-catenin stabilization and signaling. In addition to modulating GSK-3 activity, growth factors can also directly phosphorylate β-catenin in an Akt- or c-Jun N-terminal kinase (JNK)-dependent manner leading to its stabilization and/or activation of β-catenin signaling [17]. The activation of β-catenin signaling by growth factors is summarized in Figure 2.

Asthmatic airways present an extremely heterogeneous environment with a variety of cytokines, growth factors and inflammatory mediators that activate β-catenin signaling. Mitogens such as PDGF stabilize β-catenin and promote its nuclear localization via GSK-3β inactivation in airway smooth muscle cells [21] whereas TNF-α increases cytosolic abundance of β-catenin in human bronchial epithelial cells [22]. In addition to rescuing β-catenin from degradation via GSK-3β inactivation in airway smooth muscle cells and lung fibroblasts [23,24], TGF-β also up regulates β-catenin transcription via the Ras/MEK pathway in airway smooth muscle cells [25]. Similarly, epidermal growth factor (EGF) induces nuclear translocation of β-catenin in human lung epithelial cancer cells lines [26] whereas fibroblast growth factor (FGF)-10 can activate β-catenin signaling in lung epithelium, presumably, by an activating phosphorylation at serine 552 [27]. Interestingly, β-catenin can also be activated by scratching-induced and PKC-mediated inactivation of GSK-3β in bronchial epithelial cells showing a mechanical stimulus-dependent influence on β-catenin signaling [28]. Considering the role of these growth factors in airway remodeling, β-catenin stabilization and activation could be a critical process in this pathological phenomenon.

Inside the nucleus, β-catenin partners with various primary and auxiliary proteins including transcription factors, histone modification proteins and chromatin remodeling complexes, to relay the effects of its inducers [16,29]. T-cell factor (TCF)/leukemia enhancer factors (LEF) factors are the principal binding partners for β-catenin in response to canonical WNT
Figure 1. WNT-dependent β-catenin signaling. In the absence of WNT ligands, a destruction complex comprised of Axin, GSK-3β, CK1 and APC captures cytosolic β-catenin and phosphorylates it sequentially via CK1 and GSK-3β activity. The phosphorylated β-catenin is degraded by the ubiquitin-proteasome system. Activation of the FZD and LRP5/6 receptor complex by extracellular WNT ligands leads to sequestration of the β-catenin destruction complex to the membrane receptor complex, primarily mediated by the scaffold protein DVL. This prevents phosphorylation of β-catenin by GSK-3β and CK1 and subsequent proteosomal degradation culminating into the accumulation of cytosolic β-catenin. Unphosphorylated and stabilized β-catenin translocates to the nucleus and activates β-catenin dependent gene transcription. GSK-3β, glycogen synthase kinase-3β; CK1, casein kinase 1; APC, adenomatous polyposis coli; FZD, frizzled; LRP5/6, lipoprotein receptor related protein 5/6; DVL, Dishevelled signaling where they bind the WNT-responsive elements in the promoters and activate target gene transcription. Accordingly, β-catenin-TCF interaction has been observed in airway smooth muscle cells [23] and alveolar [30-32] and bronchial epithelial cells [33] in response to WNTs and various non-WNT signals such as TGF-β. Most of these studies relied on the activation of a TCF-responsive reporter TOP-Flash. Interactions of β-catenin alone or β-catenin/TCF complex with smad3 are also widely reported in airway components [7]. In addition, β-catenin interacts with histone acetyltransferases- CREB-binding protein (CBP)
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Figure 2. WNT-independent β-catenin signaling. Several mechanisms exist that may lead to the activation or inactivation of β-catenin signaling independently of WNT-FZD interaction. Binding of growth factors to their cognate receptors or extracellular matrix/integrin interaction activates intracellular cascades comprised of FAK-dependent or -independent PI3K/Akt signaling or PKC or ILK activation leading to the phosphorylation and inactivation of GSK-3. Other factors may either activate FAK-dependent Grb2/Rac/JNK cascade leading to the direct phosphorylation of cytosolic β-catenin or promote release of Cadherin-bound membrane pool of β-catenin into the cytosol. Stabilized β-catenin, thus, accumulates and translocates to the nucleus, interacting with a broad range of interaction partners to facilitate gene transcription. In addition, growth factors may increase cytosolic β-catenin by promoting β-catenin gene transcription itself. FAK, focal adhesion kinase; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; ILK, integrin-linked kinase; Grb2, Growth factor receptor-bound protein 2; JNK, c-Jun N-terminal kinase.

and p300 in alveolar epithelial [31,34,35] and airway smooth muscle cells (authors' unpublished data). We have recently observed a novel β-catenin-Sp1 interaction in airway smooth muscle cells which is promoted by TGF-β stimulation (Chapter 5). Thus, the
versatility of β-catenin can be attributed to its dynamic association with a broad spectrum of interaction partners which can not only channel β-catenin into a particular response, based on the presence of the array of interacting proteins but can also promote one interaction at the expense of others depending on the cell-stimulus context [34].

3. Functional significance of β-catenin in airway remodeling

β-Catenin participates in a myriad of processes from embryonic development to maintenance of homeostasis in post-natal life. β-Catenin signaling, whether WNT-dependent or independent, is critical for repair and regeneration processes and as such is a major factor in a multitude of pathologies from fibrosis to cancer. Interestingly, increased WNT/β-catenin signaling has been shown in airway smooth muscle cells and airway epithelium in an in vivo model of asthma [36].

3.1 Airway development

β-Catenin activity is essential for proper development and differentiation of airway epithelium and smooth muscle cells. It plays a critical role in the establishment of respiratory progenitors as the conditional inactivation of β-catenin in foregut endoderm in early embryonic development leads to failure of the respiratory development program and absence of lungs and trachea [37] whereas conditional deletion of β-catenin in airway smooth muscle cell precursors impairs their amplification and differentiation and attenuates airway and vascular smooth muscle development [36,38]. Similarly, the presence of canonical WNT signaling antagonist Dickkopf-1 impairs bronchial smooth muscle differentiation in developing lungs [39]. In line with its role in development and differentiation of lungs, β-catenin activity is also required for repair and regeneration of lung tissue following an injury [40]. A deregulated repair response, thus, could lead to an altered differentiation profile of these cells and could contribute to airway remodeling.

It is not yet fully understood whether defects in the WNT/β-catenin pathway during development could underlie the susceptibility to develop asthma later in life, but key findings do provide support for this hypothesis. Animal studies indicate that maternal smoke exposure during pregnancy is sufficient to trigger airway smooth muscle thickening in the offspring [13]. These changes are associated with differential expression of developmental pathways such as the WNT/β-catenin signaling pathway [14]. Further, associations between gene polymorphisms in the WNT signaling pathway (WISP-1 and WIF1) and impaired lung function in asthma exist [41]. Of note, the role of WNT signaling in physiology clearly extends beyond development, and such genetic associations may also reflect a role for WNT signaling in airway remodeling later in life.

3.2 Airway smooth muscle thickening

Airway smooth muscle cell hyperplasia and hypertrophy is an important feature of airway remodeling. WNT/β-catenin signaling has been intrinsically linked to cell proliferation both in the differentiated and stem cell populations. β-Catenin is involved in pro-mitogenic signaling in airway smooth muscle cells [21,25]. PDGF and fetal bovine serum (FBS) induce
airway smooth muscle cell proliferation which is accompanied by increased nuclear abundance of β-catenin. Of note, the proliferative effects of PDGF and FBS were attenuated in the presence of β-catenin specific siRNA, thus underlining the critical role of β-catenin in airway smooth muscle cell proliferation [21]. The proliferative role of β-catenin in airway smooth muscle cells has also been shown in vivo [27,36,38] and activation of β-catenin signaling in the airway smooth muscle in response to the allergen Aspergillus fumigatus has been reported [36]. The pro-fibrotic TGF-β can also induce proliferation in airway smooth muscle cells [42,43]. This proliferative effect of TGF-β has been attributed to MAPK activation [43]. In light of the evidence for TGF-β-induced stabilization and up regulation of β-catenin, which also involves MAPKs [25], it is tempting to speculate that β-catenin mediates the proliferative effects of TGF-β.

3.3 Epithelial repair

In line with its role in proliferation, stabilization and nuclear translocation of β-catenin is observed after injury or insult to airway epithelium. Using an in vitro model for epithelial damage, a study demonstrated scratching-induced activation of β-catenin which, in turn, promoted transcriptional up regulation of cyclin D1 leading to bronchial epithelial cell proliferation and wound closure [28]. c-Myc is a transcription factor involved in the proliferative responses and like cyclin D1, is also a β-catenin target gene. Accordingly, c-Myc upregulation is critical for both bronchial smooth muscle cell and epithelial cell proliferation following an epithelial injury [40]. Interestingly, epithelial damage activates β-catenin signaling in smooth muscle cells inducing their proliferation and release of FGF-10 [27]. This smooth muscle cell-released FGF-10, in turn, activates β-catenin in a WNT-independent and PI3K-dependent manner in epithelial cells and is critical for the proliferation and repair of damaged epithelium [27]. Similarly, in an in vitro model of tracheal epithelial cell differentiation, activation of β-catenin by pharmacological inhibition of GSK-3 leads to increased epithelial cell proliferation. The findings were also extended in an in vivo model where transgenic activation of β-catenin increased the proliferation of lung epithelial basal cells [44].

3.4 Epithelial-to-mesenchymal transition

The mesenchymal cell population is suggested to constitute a component of remodelled airways in asthma [45]; however, the origin of this mesenchymal cell fraction is not clearly understood. It has been proposed that mesenchymal cells can arise from the epithelial cells and fibroblasts [45]. Epithelial cells can transdifferentiate to lose adhesion and can attain mesenchymal characteristics such as presence of vimentin and α-smooth muscle-actin, enhanced motility and production of extracellular matrix, in a process known as epithelial-to-mesenchymal transition (EMT). TGF-β is considered as a master inducer of EMT in various organs including lung epithelial cells [46] but the function and requirement of β-catenin in TGF-β-induced EMT are not fully clear. TGF-β induces features of EMT in bronchial epithelial cells such as reduced expression of E-cadherin and increased expression of vimentin, α-smooth muscle-actin and fibronectin [47]. Interestingly, the EMT features were augmented by the presence of allergen house dust mite extract [47]. Of note, the TGF-β-induced EMT is accompanied by the activation of β-catenin signaling in the bronchial
epithelial cells which was further enhanced by the presence of house dust mite extract. These observations hold high pathological significance as airway epithelium of asthma patients may undergo repeated aeroallergen exposures which could occur in the presence of increased TGF-β concentrations. Thus, the presence of aeroallergens could precipitate the features of airway remodeling via increased activation of β-catenin and consequently EMT.

Similarly, pharmacological activation of β-catenin in immortalized human bronchial epithelial cells cultured as air-liquid interface model could lead to increased cell proliferation and eventually mesenchymal differentiation of these cells as reflected by the presence of EMT-associated markers such as upregulation of Snail, Slug and cyclin D1 and downregulation of E-cadherin [44]. Transgenic activation of β-catenin in basal cells confirmed these observations as reflected by increased cell mass and increased expression of EMT-associated markers [44]. β-Catenin also induced transient EMT in Clara cells, as measured by the upregulation of Snail [27].

Integrin signaling can also activate β-catenin via α3β1 inducing EMT in response to lung injury [48]. Of note, conditional deletion of α3 integrin in lung epithelium had no effect on TGF-β signaling in alveolar cells but attenuated EMT in response to lung injury [48]. Further, α3 integrin is required for the phosphorylation and release of membrane-bound and E-cadherin-associated β-catenin and its association with phospho-smad2, an interaction with potential implications in β-catenin-induced gene activation leading to EMT and accumulation of myofibroblasts [48,49].

3.5 Myofibroblast differentiation and extracellular matrix production

Altered extracellular matrix profile within and surrounding the smooth muscle bundle is an important feature of airway remodeling in asthma and is believed to be a key participant in the process of airway remodeling [6]. The differentiation of fibroblasts into myofibroblasts may have an important role in extracellular matrix production associated with airway remodeling. WNT-3A induces differentiation of mouse embryonic fibroblasts in a β-catenin and TGF-β-dependent manner as knockdown of either β-catenin or TGF-β attenuates effects of WNT-3A on fibroblasts [50]. Interestingly, β-catenin signaling is required for WNT-3A-induced expression of TGF-β which, in turn, mediates WNT-3A induced differentiation of mouse embryonic fibroblasts into myofibroblasts [50]. Similarly, TGF-β also induced differentiation of primary human lung fibroblasts into myofibroblasts accompanied with the increased expression of α-smooth muscle-actin and extracellular matrix in a β-catenin-dependent manner [24].

Activation of β-catenin signaling mediates TGF-β-induced extracellular matrix expression by airway smooth muscle cells and lung fibroblasts [23,24]. TGF-β induces inactivating phosphorylation at Ser21/Ser9 of GSK-3α/β leading to augmentation of cytosolic and nuclear β-catenin levels in both the airway smooth muscle cells and lung fibroblasts and a concomitant increase in the expression of extracellular matrix proteins [23,24]. Interestingly, the knockdown of β-catenin or pharmacological disruption of β-catenin/TCF signaling by PKF115-584 attenuated TGF-β-induced extracellular matrix expression [23,24]. Interestingly, β-catenin also upregulates WNT-5A expression in airway smooth muscle cells
in response to TGF-β (Chapter 5). Increased WNT-5A expression is observed in airway smooth muscle cells obtained from asthma subjects reflecting the augmented TGF-β concentrations in asthmatic airways (Chapter 3). Of note, WNT-5A has also been shown to mediate TGF-β-induced extracellular matrix production by airway smooth muscle cells (Chapter 3). The aberrant extracellular matrix profile in diseased airways can have profound effect on airway smooth muscle mechanical and functional properties and behaviour [6]. As demonstrated in various studies, collagen I and fibronectin influence airway smooth muscle cell proliferation, survival, migration and contractile phenotype [51,52]. Thus, β-catenin activation contributes in multiple ways to enhance extracellular matrix production in response to TGF-β in the airways and drives airway remodeling.

Thus, β-catenin-dependent processes represent a vicious cycle with significant impact on airway remodeling in asthma. The presence of chronic injury, as in asthma, would lead to persistent activation of β-catenin signaling which would lead to uncontrolled cellular processes such as proliferation, survival, differentiation, EMT, increased contractility and altered and enhanced ECM deposition and hence, airway remodeling.

4. Role of β-catenin in mechanotransduction

There is a growing appreciation that mechanical forces within the airway wall, due to bronchoconstriction and changes in the biomechanical composition of the extracellular matrix, may have an important impact on airway remodeling in asthma [53,54]. For example, mechanical compression of airway epithelial cells during bronchoconstriction leads to TGF-β release, epithelial EGF receptor activation and subepithelial deposition of collagen in mild asthma patients, presumably via mechanisms involving activation of proteases (A disintegrin and metalloproteinases, MMPs) and TGF-β activating integrins (α5β8) [55-58]. Further, recent studies show that the biomechanical properties of the extracellular environment are a crucial determinant of gene expression, proliferation and differentiation of epithelial cells and fibroblasts [59]. These studies have prompted speculations that mechanical forces within the airway wall are an important, perhaps even crucial, mechanism for allergen-induced airway remodeling [54].

β-Catenin could contribute to the mechanical regulation of airway remodeling in several ways. Several studies suggest that mechanical forces such as stretch and compression can activate β-catenin (WNT-independent) signaling in cells. Also, being part of the cadherin/catenin complex at the plasma membrane, β-catenin plays an important role in transducing forces from the cytoskeleton to the extracellular matrix and, therefore, between cells and tissues [60]. Mechanical forces may, therefore, unify the dual cellular role of β-catenin, being a component of the adherens junction as well as a transcriptional co-activator via the mechanisms outlined below.

Cells transmit mechanical forces to neighbouring cells via two main mechanisms- focal adhesions and adherens junctions. Focal adhesions are composed of integrins, which bind to extracellular matrix proteins and are coupled to intracellular signaling involving focal adhesion kinase (FAK) [61]. FAK activation has been linked to β-catenin activation in several ways. Deletion of FAK prevents β-catenin nuclear translocation and gene expression in
keratinocytes [62] and similar regulatory roles of FAK in activating β-catenin signaling have been demonstrated in periodontal ligament cells [63], in breast cancer cells [64], in HEK293 and in COS-7 cells [65]. Likely, this regulatory role of FAK is explained by subsequent PI3K activation, which activates β-catenin by inhibiting GSK-3 [64]. Additionally, FAK can activate JNK via Grb2 and Rac, which also activates β-catenin signaling [65]. A role for Rac1 in shear stress induced β-catenin activation has also been demonstrated in osteoblasts [66]. In the adherens junction, compressive stress can activate β-catenin signaling [67] and the activated β-catenin can team up with other mechanosensitive transcriptional regulators such as TAZ, which play crucial roles in sensing stiffness of the extracellular environment [68]. Of note, β-catenin is also directly involved in the regulation of smooth muscle contraction in the airways, presumably because of its role in linking the cytoskeleton to the N-cadherin/catenin complex in smooth muscle, as β-catenin knock-down considerably reduces contraction in response to methacholine and KCl [69]. Collectively, these data demonstrate that mechanical forces, including bronchoconstriction, cellular compression and the stiffness of the extracellular matrix, can be linked to β-catenin activation via specific intracellular signaling pathways (Figure 2).

The functional role of mechanically induced β-catenin activation in the airways other than smooth muscle contraction have not yet been demonstrated and it is unclear whether bronchoconstriction regulates airway remodeling in part via the activation of β-catenin signaling. In osteoblasts, however, mechanically activated β-catenin has been linked to actin filament reorganization into stress fibres [70]. Further, β-catenin and TAZ signaling regulate mesenchymal stem cell differentiation [68]. It will be of considerable interest, therefore to investigate the role of mechanically activated β-catenin in airway remodeling in more detail, particularly given the rapidly evolving development of therapeutics directed against β-catenin signaling.

5. β-catenin as a drug target

Significant advances have recently been made in the generation of small molecule inhibitors that target β-catenin directly or indirectly and several of these have shown efficacy in inhibiting fibroproliferative abnormalities such as cancer and fibrosis in animal models. Although most of these inhibitors have not yet been evaluated in models of asthma, several inhibitors that target β-catenin directly have shown efficacy in animal models of fibrosis. We refer to Baarsma et al. for a full review of the availability and use of inhibitors that target the WNT pathway [17].

5.1 Tankyrase inhibitors

Tankyrase 1 and 2 are a class of poly-ADP-ribosylating enzymes that target axin2 for degradation and, therefore, directly interfere with β-catenin stabilization by the destruction complex [71]. Tankyrase inhibitors such as XAV939 were recently described to prevent tankyrase-mediated axin2 degradation leading to stabilization of axin2 and the destruction complex [71,72]. The stabilized destruction complex, thus, considerably reduces active β-catenin signaling. Of note, XAV939 has been evaluated both in vitro and in vivo. XAV939 was shown to inhibit skin fibrosis induced by either bleomycin or adenoviral overexpression
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of TGF-β in mice [73]. Likewise, it was shown to have efficacy against bleomycin-induced pulmonary fibrosis in mice and inhibited TGF-β-induced EMT in vitro [72].

5.2 GSK-3 inhibitors

GSK-3 plays a crucial role in the degradation of β-catenin by the destruction complex. In addition, GSK-3 regulates numerous cellular substrates including transcription factors, kinases and cell cycle regulatory proteins [17,74]. Currently available GSK-3 inhibitors are mostly ATP competitive inhibitors that prevent its kinase activity. Examples include SB216763, CT/CHIR99021, LiCl and TDZD-8 [75]. GSK-3 inhibitors have been evaluated in animal models of asthma, showing inhibition of tissue eosinophilia and airway mucus expression by the GSK-3 inhibitor TDZD-8 [76]. In addition, SB216763 inhibits TGF-β induced myofibroblast differentiation [77], IL-1β induced pro-inflammatory cytokine production by airway smooth muscle [78] and lipopolysaccharide-induced airway fibrosis in guinea pigs [79]. As GSK-3 inhibition would be expected to stabilize β-catenin, these inhibitory effects of GSK-3 inhibition are likely not explained as such, but are more likely the result of phosphorylation of other GSK-3 substrates. Indeed, inhibition of TGF-β induced myofibroblast differentiation by SB216763 is attributed to CREB phosphorylation, which antagonizes Smad signaling [77]. In elastase induced emphysema in mice, on the other hand, LiCl is protected by directly stabilizing β-catenin signaling in alveolar epithelial cells [80]. It appears therefore, that the effects of GSK-3 inhibition are beneficial and protective in several models of lung disease, but the exact contribution of β-catenin signaling to its effects are cell-type and context specific.

5.3 Inhibition of nuclear β-catenin-co-factor interactions

β-Catenin does not directly bind DNA, but instead teams up with transcription factors and co-factors that regulate gene expression. As a result, β-catenin dependent gene expression is highly dependent on the nuclear co-factors it associates with. For example, whereas β-catenin association with Smad2/3 or CBP has been proposed to regulate pro-fibrotic signaling, β-catenin association with p300 may actually promote tissue repair [81]. Recently, small molecule inhibitors have been developed that can target association of β-catenin with specific co-factors. ICG-001, for instance, inhibits the association with CBP and therefore promotes β-catenin binding and signaling through p300 [82]. In contrast, the inhibitor IQ-1 inhibits β-catenin binding to p300 and, therefore, promotes β-catenin binding and signaling through CBP [83]. ICG-001 has been evaluated in animal models of fibrosis and consistently prevents the pro-fibrotic response, in the lungs, the kidney and the skin [34,84,85]. The effects of IQ-1 still require evaluation in animal models of fibrosis. Likewise, the effects of co-factor specific inhibition of β-catenin signaling clearly require future investigation in animal models of asthma.

6. Expert opinion

WNT/β-catenin signaling has been widely implicated in health and disease. Although much research effort has been invested in understanding aberrant β-catenin signaling in malignancies and fibrotic disorders, a clear functional significance of β-catenin signaling in
experimental asthma and airway remodeling is not yet available. Importantly, clinical studies scrutinizing the role of β-catenin signaling in asthmatic subjects are also absent. However, evidence from experimental models, as discussed in this review, clearly support a role for β-catenin signaling in asthma. These observations provide the much essential basis to develop our understanding pertaining to the role of β-catenin in asthma and its associated pathological features. Both growth factors and physical forces dominate the asthmatic airway niche. Owing to the interlinking structural and signaling functions, β-catenin could sense the bronchoconstriction-induced mechanical forces and transmit the signals to the nucleus, thereby delivering the effects on airway remodeling. This hypothesis, though highly tempting, is marred by our lack of in-depth understanding of two crucial features of β-catenin signaling- mechanonsensing/transduction by β-catenin and the communication between the structural and signaling function of β-catenin. Further studies are needed to advance our understanding in these areas. Several studies have now been published that report the role of β-catenin signaling in proliferation and differentiation of airway mesenchymal and epithelial cells. Accordingly, β-catenin signaling is critical for promoting tissue repair but can also be profibrotic. The regulatory mechanisms governing this dual role of β-catenin are currently under intense investigation. So far, data suggest that this duality could best be alluded to the cell-specific effects and to the specific recruitment of nuclear cofactors that regulate β-catenin-driven gene expression. It is reasonable to believe that the cell-specific effects of β-catenin could arise from differential expression or availability of the cofactors under steady state or in the presence of a particular stimulus.

The stimulus-specific functional output of β-catenin signaling could, in fact, define the contrasting effects of β-catenin signaling in repair and fibrosis. Whereas WNT-driven β-catenin activation may promote repair in one experimental model, TGF-β-driven β-catenin activation may actually promote fibrosis in another. This TGF-β-driven β-catenin signaling may be entirely WNT-independent. Accordingly, targeting β-catenin signaling in airway disease may be of benefit but an important consideration is the involvement of specific cofactors that are activated by β-catenin in the context of the disease studied. As a result, targeting specific cofactors (such as β-catenin/CBP with ICG-001) may inhibit β-catenin-driven fibrosis, yet preserve β-catenin-driven repair. Fine tuning of β-catenin signaling, thus, defines the difference between health and disease and future studies in this area are likely and warranted.

Thus, investigating the role of WNT/β-catenin and TGF-β/β-catenin signaling in airway remodeling in asthma holds high significance. Equally important is to understand the communication of the membrane-bound and cytosolic pool of β-catenin. Of note, we still await a clear understanding of the checks and balances in β-catenin signaling and a detailed view of its binding partners that will provide the required mechanistic insight into the functioning of β-catenin. Altogether, these studies may open up new therapeutic avenues for the treatment of airway remodeling in asthma.
Declaration of interest

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Chapter 2

References

β-Catenin as a regulator and therapeutic target for asthmatic airway remodeling


* First paper to report the role for βL-catenin in TGF-β1-induced extracellular matrix production in fibroblasts and its association with COPD.


* Reports crucial role for β-catenin in parabronchial smooth muscle cells to initiate airway epithelial repair following an injury.


Chapter 2


* First evidence that co-factor specific beta-catenin inhibition (CBP/β-catenin) by ICG-001 inhibits fibrosis in the lung.


** Provides evidence of WNT/β-catenin signaling activation in mouse model of asthma.


* Reports role for β-catenin in lung progenitor development


* Reports key role for β-catenin in mesenchymal lineage differentiation


β-Catenin as a regulator and therapeutic target for asthmatic airway remodeling


* Reports direct regulation by β-catenin of the mechanosensing transcription factor TAZ and demonstrates its importance in gene expression.
* The first paper to report a role for β-catenin in smooth muscle contraction.
* Elegant study showing the role of pY654 β-catenin in fibrosis and its regulation by tankyrase inhibition.
* First proof of concept showing WNT mediated β-catenin activation may regulate repair of the emphysematous lung.


