Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY

EAACI

Allergy

REVIEW ARTICLE

Transforming growth factor-beta 1 pathways in inflammatory airway diseases

Y. Yang[1], N. Zhang[2,3], F. Lan[2,3], K. Van Crombruggen[2,3], L. Fang[1], G. Hu[1], S. Hong[1] & C. Bachert[2,3]

[1]Department of Oto-Rhino-Laryngology, The First Affiliated Hospital, Chongqing Medical University, Chongqing, China; [2]Upper Airway Research Laboratory, Department of Oto-Rhino-Laryngology, Ghent University, Ghent, Belgium; [3]Division of Nose, Throat and Ear Diseases, Clintec, Karolinska Institute, Stockholm, Sweden

To cite this article: Yang Y, Zhang N, Lan F, Van Crombruggen K, Fang L, Hu G, Hong S, Bachert C. Transforming growth factor-beta 1 pathways in inflammatory airway diseases. *Allergy* 2014; **69**: 699–707.

Keywords
airway diseases; influencing factors; oxidative stress; smoking; transforming growth factor-beta.

Correspondence
Yucheng Yang, Department of Oto-Rhino-Laryngology, The First Affiliated Hospital, Chongqing Medical University, Youyi Road 1, Yuzhong District, 400016 Chongqing, China.
Tel.: +86 23 89012981
Fax: +86 23 89012981
E-mail: yychxh@163.com

Accepted for publication 26 February 2014

DOI:10.1111/all.12403

Edited by: Douglas Robinson

Abstract

Transforming growth factor-beta 1 (TGF-β1) has been reported being involved in the remodeling and immunosuppression processes of inflammatory airway diseases; understanding the regulation of TGF-β1 is therefore a key to unravel the pathomechanisms of these diseases. This review briefly summarizes the current knowledge on the influencing factors for driving TGF-β1 and its regulatory pathways in inflammatory airway diseases and discusses possible therapeutic approaches to TGF-β1 control. The factors include smoking and oxidative stress, prostaglandins (PGs), leukotrienes (LTs), bradykinin (BK), and microRNAs (miRs). Based on the summary, new innovative treatment strategies may be developed for inflammatory airway diseases with an impaired expression of TGF-β1.

The transforming growth factor-beta (TGF-β) superfamily is critically involved in embryonic development, organogenesis, and tissue homeostasis. It acts as multifunctional regulators of cell growth and differentiation and consists of more than 40 members, mainly including TGF-βs, bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs), activins, and inhibins. Between these superfamily members, there is a complex network of regulatory mechanisms (1, 2). Possessing immunomodulatory and fibrogenic characteristics, TGF-β1 is a pleiotropic and multifunctional cytokine secreted from various types of cells, such as endothelial, epithelial and smooth muscle cells, as well as fibroblasts and most immune system cells.

Transforming growth factor-beta 1 regulation is unique because it is targeted to the extracellular matrix as a biologically inactive complex, which consists of a mature 25-kDa polypeptide dimer (TGF-β), a latency-associated protein (LAP), and a latent TGF-β-binding protein (LTBP). Active TGF-β is released from the latent complex that was activated via cleavage by proteases and other molecules. The activated TGF-β then becomes a ligand for TGF-β type I and II receptors, leading to receptor Smad (R-Smad) phosphorylation, which subsequently binds to the common-partner Smad (co-Smad), and becoming Smad complexes. The Smad complexes translocate into the nucleus and combine with several transcription factors, thereby becoming a transcriptional active complex that activates target genes transcription. In

Abbreviations
AHR, airway hyper-responsiveness; BAMBI, BMP and activin membrane-bound inhibitor; BK, bradykinin; BMP, bone morphogenetic protein; CCL2/MCP-1, chemokine (CC motif) ligand 2/monocyte chemotactic protein-1; COPD, chronic obstructive pulmonary disease; CRS, chronic rhinosinusitis; CysLT, cysteinyl leukotriene; FoxP3, forkhead box P3; LAP, latency-associated protein; LTBP, latent TGF-β-binding protein; miR, microRNA; PG, prostaglandin; RORc, RAR-related orphan receptor C; TGF-β1, transforming growth factor-beta 1.

contrast, inhibitory Smads (I-Smad) Smad 6 and Smad 7 compete with R-Smads for receptor binding, thereby blocking signal transductions. Non-Smad TGF-β1 signal transducers are thought to serve as nodes for cross-talk with other major signaling pathways and quantitatively regulate Smad signaling pathways (1). In immunology, TGF-β1 regulates cellular proliferation, differentiation, and other cellular functions for a variety of cell types, especially regulatory T (Treg) cells. Transforming growth factor-beta 1 is required for CD 4 (+) CD 25 (+) Treg *in vivo* expansion and suppressive capacity (3, 4). Moreover, TGF-β1 has profound regulatory effects on many developmental and physiological processes. These important effects have been demonstrated by TGF-β1-deficient mice which died of a multiorgan autoimmune inflammatory disease a few weeks after birth (5). In general, TGF-β1 could be physiologically understood as a counter-regulatory cytokine to relieve inflammation and initiate repair processes and fibrosis formation.

Transforming growth factor-beta 1 is characterized by control of inflammation, mucus hypersecretion, and excessive fibrosis of airway tissues in chronic airway diseases, such as chronic rhinosinusitis (CRS), asthma, and chronic obstructive pulmonary disease (COPD; 6–8).

With persistent inflammation and remodeling of nasal and paranasal mucosa, CRS is currently classified into two major subgroups: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP; 9). One of our previous studies indicated that there were high TGF-β1 protein levels, increased TGF-β receptor I expression, a large number of phosphorylated Smad2 (pSmad2)-positive cells, and excessive collagen production in CRSsNP, whereas low TGF-β1 protein concentrations, decreased expression of TGF-β receptor II, small number of pSmad2-positive cells, and lack of collagen were determined in CRSwNP. Enhanced TGF-β1 signaling in CRSsNP and decreased TGF-β1 signaling in CRSwNP reflected different remodeling patterns, fibrosis, and edema, thereby indicating that TGF-β1 was significantly involved in the upper airway remodeling process (8, 10).

Asthma is a chronic inflammatory disease involving many inflammatory cells and inflammatory mediators, which leads to persistent chronic airway inflammation and remodeling (11). With pathological development, TGF-β1 is implicated in most of the cellular processes toward airway remodeling, subepithelial fibrosis, airway smooth muscle remodeling, epithelial changes, and microvascular changes in asthmatic patients (7, 12).

Chronic obstructive pulmonary disease is the fourth leading cause of death in developed countries and is characterized by irreversible expiratory airflow limitation due to the small airway disease and emphysema, including airway inflammation with increased mucus production, airway wall remodeling, peribronchiolar fibrosis, and destruction of the alveolar architecture (13). Studies revealed that increased TGF-β1 expression was found in COPD lungs and primary cells, such as epithelial cells, macrophages, or fibroblasts isolated from COPD specimens, suggesting great impact of TGF-β1 signaling on the COPD development and progression (2).

Transforming growth factor-beta 1-induced signaling pathways in airway diseases are constantly implicated in complex pathomechanisms and may provide potential therapeutic targets for their treatment (2, 14–16). To date, several reviews have mainly focused on TGF-β1 activation and receptor signaling in airway diseases (7, 8, 14, 17). This review aims to provide the key pathways by which the external environment or microenvironment impacts on the activation/expression of TGF-β1 in inflammatory airway diseases.

Pathways driving TGF-β1

Tobacco smoking and oxidative stress

Oxidative stress is a result of imbalance between toxicant-induced reactive oxygen species (ROS) or reactive nitrogen species (RNS) and the antioxidant defense system. Oxidative stress may initiate and augment airway inflammation and may also be resulted from airway inflammation in a positive feedback loop (18), which has been reported to play a causative role in airway inflammation, such as nasal polyps (NPs), asthma, and COPD (19–21).

In our living environment, any air pollutant or toxicant interfering with the airway can most likely trigger symptoms of airway, in which the role of oxidative stress is gaining increasing scientific attention, especially regarding antioxidant therapies (21). Until now, environmental tobacco smoke has been confirmed as one of the strongest exogenous toxicants for initiating airway diseases and as a leading cause of preventable death in the United States and worldwide, especially leading to airway diseases such as COPD, asthma, lung cancer, and laryngeal cancer (22, 23).

Evidence of a close link between smoking and TGF-β1 expression in airways has emerged from studies, as tobacco smoking induced the release of various inflammatory mediators and growth factors, including TGF-β1, epidermal growth factor receptor (EGFR), IL-1, IL-8, and granulocyte colony-stimulating factor (G-CSF), through the oxidative stress pathway. Transforming growth factor-beta 1 influencing factors related to tobacco smoking and oxidative stress is shown in Fig. 1.

Studies have shown that ROS could activate TGF-β1 either directly or indirectly by activating proteases (24). In the meantime, TGF-β1 induces ROS production as part of its signal transduction pathway through the superoxide free radical-producing nicotinamide adenine dinucleotide phosphate (NADPH) oxidase catalytic subunit NOX4 (25). Compared with the control nasal mucosa, the oxidative stress marker malondialdehyde, NOX1, and NOX4 increase, whereas the antioxidant superoxide dismutase and nitric oxide (NO) decrease in CRSwNP tissues (26). A recent GA (2) LEN network research in 19 European centers with postal questionnaires has shown that CRS is more common in smokers than in nonsmokers (OR 1.7: 95% CI 1.6–1.9), suggesting that smoking cessation may be an important therapeutic option for CRS patients (27). Another study has analyzed the effects of cigarette smoke (CS) on upper and lower airways in mice exposed to mainstream CS and

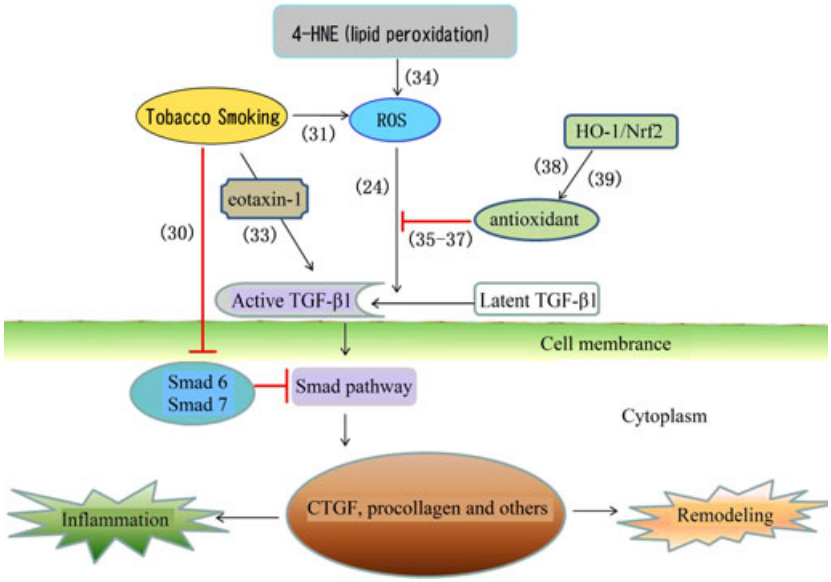

Figure 1 Scheme of tobacco smoking and oxidative stress pathways driving transforming growth factor-beta 1 (TGF-β1). The numbers beside the arrows indicate corresponding references. 4-HNE, 4-hydroxy-2-nonenal; CTGF, connective tissue growth factor; HO-1, heme oxygenase-1; Nrf2, NF-E2-related factor 2; TGF-β1, transforming growth factor-beta 1.

revealed that the expression of forkhead box P3 (FoxP3), RAR-related orphan receptor C (RORc), and interleukin-17 (IL-17) in nose and lungs is changed, with lower TGF-β1 levels at 24 weeks in noses but higher TGF-β1 levels at 4 weeks in lungs. The results indicate that CS-induced inflammation may be differently regulated in the upper and lower airways in mice; the role of TGF-β1 in upper airways remains to be elucidated (28).

In addition, studies on lung tissues of cigarette smokers have consistently demonstrated the existence of TGF-β1 in small airway epithelial cells and airway wall cells via the assessment of the mRNA levels or protein expression. Also in cultured small airway epithelial cells, the mRNA levels of TGF-β1 have been found significantly higher in the cells derived from the smoking group and patients with COPD than in those obtained from nonsmokers, indicating a positive correlation between the smoking history (pack-years) and the degree of small airway obstruction in smokers and patients with COPD (29). The inhibitory factors Smad6 and Smad7 were significantly down-regulated in COPD patients by cigarette smoke and decreased in airway epithelial cells, which may partially explain the TGF-β1-mediated effect on COPD (30).

Simultaneously, a significant increase in several markers of oxidant burden and signs for disturbed antioxidant/oxidant balance has been reported in lung fibrosis models of patients and animals. Smoke releases active TGF-β1 from recombinant latent TGF-β1 using an oxidant, suggesting that small airway remodeling (SAR) in cigarette smokers may be caused by direct, smoke-mediated, and oxidant-driven induction of growth factor signaling in the airway wall and that SAR does not necessarily require exogenous inflammatory cells (31). In some tests, CS- or antigen-stimulated ROS leads to oxidative damage in bronchial epithelial cells, and prolonged repair responses cause airway remodeling and irreversible airflow limitation. Thioredoxin (TRX) is a redox protein that scavenges ROS, and TRX-overexpressing bronchial epithelial cells attenuate TGF-β1 and activate matrix metallopeptidase 9 (MMP9) expression, preventing airway remodeling from house dust mite (HDM)- or CS-induced inflammation (32). Moreover, chronic co-exposure to tobacco smoke in an asthmatic mice model significantly up-regulated chemokine expression to the airway, such as eotaxin-1 in the airway epithelium with resultant recruitment of cells expressing TGF-β1, which further enhanced airway remodeling (33). 4-Hydroxy-2-nonenal (4-HNE) is a key mediator of oxidant-induced cell signaling and apoptosis, and its modified protein level has been increased in lung tissues of patients with COPD when compared with those of patients without COPD, and a positive correlation has been reported between the 4-HNE adducts and TGF-β1 protein and the mRNA levels in the airway and alveolar epithelium. Using the murine macrophage line J774-A1, 4-HNE can stimulate the binding of AP-1 to DNA and induce TGF-β1 expression, consequently contributing to fibrosis (34).

Additionally, antioxidants have been shown to mitigate airway inflammation or fibrosis by preventing oxidative stress and regulating TGF-β1 in some studies. In an experimental asthma model, treatment with antioxidants like vitamin E, L-arginine, or esculetin (a plant-derived coumarin and immunomodulator) downgrades airway hyper-responsiveness (AHR), Th2 response, OVA-specific IgE, eotaxin, TGF-β1, airway inflammation, and asthmatic features (35–37). In mice with bleomycin-induced pulmonary fibrosis, increased glutathione expression and decreased TGF-β1 expression have been detected in the lungs of the early hemin (HO-1 inducer)

intervention group compared to the control, showing that HO-1 can prevent oxidative stress and pulmonary fibrosis at an early stage (38). Recent studies have suggested that transcriptional induction of several antioxidant genes requires a functional antioxidant response element (ARE) and NF-E2-related factor 2 (Nrf2) plays a crucial role in the induction of these ARE-mediated transcriptional responses. In the lungs of bleomycin-treated Nrf2 −/− mice, increased TGF-β1 levels have been determined, suggesting that Nrf2 confers protection against oxidant-induced fibrosis, at least partially because of its modulation of TGF-mediated effects (39).

Arachidonic acid metabolites

Arachidonic acid metabolites mainly include prostaglandins (PGs; e.g., PGD2, PGE2), leukotrienes (LTs), especially cysteinyl leukotrienes (CysLTs), lipoxins, and thromboxanes (TXAs). These metabolites have regulatory and homeostatic functions and participate in the complicated inflammatory processes of airway eosinophilia, edema, hypersecretion of mucus, remodeling, and AHR observed in patients with airway diseases (40, 41).

Upper airway diseases are linked to the changes in the arachidonic acid cascade. Decreased PGE2 levels, along with increased CysLTs, LTC4 synthase, and 5-lipoxygenase (5-LO) levels, have been detected in CRSwNP tissues from patients especially with aspirin sensitivity (42). Other studies have reported that anti-IgE or Staphylococcus aureus protein A stimulation is able to increase histamine, LTC4/D4/E4, and PGD2 release. It has also been reported that enterotoxin B stimulation induces a significant increase in IL-1β, tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-gamma), IL-5, IL-10, and IL-13 in NPs and inferior turbinates. The increase is significantly higher in the former tissue than in the latter, but TGF-β1 is not induced and its expression only decreased in NP tissues. However, the relationship between Staphylococcus aureus, PGs, LTs, and TGF-β1 in NP tissues needs to be further investigated (43–45).

In lower airways, there are several mechanisms participating in airway inflammation, especially via cytokines such as TGF-β1, IL-10, IL-1β 1, PGs (e.g., PGE2), LTs, and NO, which are interacting with each other (46). *In vitro* experiments indicated that recombinant IL-10 up-regulated TGF-β1 expression in alveolar macrophages, and in contrast, it down-regulated PGE2 production in both lung fibroblasts and macrophages (47). As an antifibrotic PG, PGE2 has been shown to play a beneficial role in fibrotic lung diseases with an increased cyclic adenosine 3′,5′-monophosphate (cAMP), which in turn can inhibit TGF-β-induced myofibroblast differentiation and limit collagen secretion by a Smad-independent signaling (48). This effect of PGE2 could be potentiated by phosphodiesterase 4 (PDE4) inhibitors (49) and restricted by knockdown of PDE4B and PDE4D subtypes (50). In summary, these data suggested that PGE2 signaling might be beneficial in limiting airway fibrosis.

CysLTs and TGF-β1 are both increased in asthmatic airways and may influence the pathophysiology of the airway disease. However, the influence of CysLTs on TGF-β1 has rarely been discussed so far. In human fetal lung (HFL) fibroblasts, TGF-β1 in combination with LTD4 stimulated collagen production and up-regulated CysLT1R expression, and they could further increase collagen expression that was solely induced by TGF-β1. These data revealed that LTD4 increased collagen production via up-regulating the CysLT1R activity, which was induced by TGF-β1. Similarly in eosinophils, TGF-β1 expression was synergistically induced by a combination of LTD4 and IL-5 or granulocyte/macrophage colony-stimulating factor (GM-CSF), although no induction occurred with each single factor. The results suggested that CysLTs stimulated eosinophils to induce TGF-β1 production in allergic inflammation where IL-5 and GM-CSF were abundant and might be involved in the pathogenesis of airway remodeling. Moreover, in HEK293, A549, and NHBE cells transfected with CysLT1R, LTD4 induced TGF-β1 mRNA production in a time- and concentration-dependent manner via a CysLT1-dependent mechanism, indicating that increased expression of CysLTs in asthmatic airways might contribute to AHR in a paracrine loop involving TGF-β1 production in airway epithelial cells (51).

Additionally, in animal models, LT receptor antagonists (e.g., montelukast, pranlukast) could also affect TGF-β1. The TGF-β1 protein levels in lung tissues or bronchoalveolar lavage (BAL) fluids were significantly increased after 7 days of OVA inhalation compared with in control mice, and the TGF-β1 increases were significantly reduced by the administration of montelukast, pranlukast, and anti-IL-11 antibodies. These results implied that CysLT1R antagonists might enhance the features of allergic airway diseases by regulating IL-11 and TGF-β1 expression (52). Furthermore, the montelukast treatment decreased the mRNA levels of IL-6, IL-10, IL-13, and TGF-β1, all of which were increased in bleomycin-induced fibrotic lungs, and modulated the homeostatic balance between CysLT1R and CysLT2R by improving the recovery of CysLT2R mRNA levels (53). In GATA-3-overexpressing mice, montelukast significantly decreased the CysLT and TGF-β levels in lungs and inhibited GATA-3-overexpression-related airway remodeling. Similarly, ONO-4057, an LTB4 receptor antagonist, hindered the development of bleomycin-induced pulmonary fibrosis in mice by decreasing inflammation and altering the TGF-β1, IL-6, IL-13, and IFN-gamma expression (54).

Moreover, some other LTs were found to regulate and interact with TGF-β1. In macrophages and dendritic cells (DCs), TGF-β1 up-regulated LTA4 hydrolase as well as increased LTB4 formation in the exosomes. IL-13 could induce pulmonary fibrosis partly due to its ability to induce and activate TGF-β1, while this activation was partly 5-LO dependent (55). As TGF-β1 signaling induced LTC4 synthase and led to enhanced LTC4 expression (56), newly formed LTC4 acted as an inducer for p38/MAPK-mediated activating transcription factor 2 (ATF2) transcription of TGF-β1 via CysLT1R (57). Taken together, the cross-talk might be a positive feedback loop via IL-13-triggered TGF-β1 activation. TGF-β1 heightened the LTC4 level, enhanced the LTC4 activity, and augmented TGF-β1 expression in an amplification cycle (Fig. 2). However, additional experiments are required to verify this hypothetical scheme.

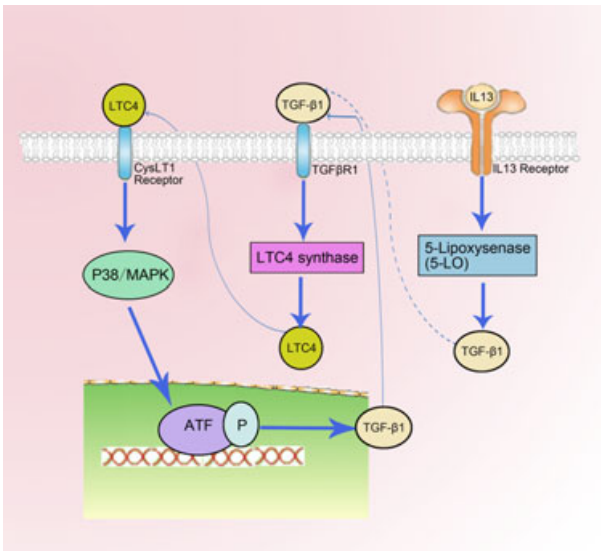

Figure 2 Hypothetical scheme of transforming growth factor-beta 1 (TGF-β1) signaling. After IL-13-induced 5-LO mediated TGF-β1 expression (55), a cycle was triggered in which TGF-β1 induced LTC4 synthase (56) with concomitant LTC4 secretion, followed by up-regulation of TGF-β1 via p38/MAPK/ATF signaling after LTC4 receptor binding (57). ATF, activating transcription factor; CysLT1R, cysteinyl-leukotriene receptor 1; IL-13, interleukin-13; LTC4, leukotriene C4; p38/MAPK, protein 38/mitogen-activated protein kinase; TGF-β1: transforming growth factor-beta 1.

Other pathways

Bradykinin (BK) is a potent inflammatory mediator and its functions are mediated by specific cell surface receptors, which are bradykinin receptor 1 (BR1) and BR2. BK generated in airway secretions is also involved in inflammatory airway diseases.

In upper airways, BK can increase vascular permeability and stimulate glandular secretion and sensory nerves to produce symptoms of rhinorrhea, nasal obstruction, in addition to nasal and throat irritation. These symptoms appear to be mediated by BR2 because BR2 rather than BR1 antagonists abolish rhinorrhea and nasal obstruction (58). An immunohistochemical study of human turbinate mucosa revealed that both BR1 and BR2 were expressed in epithelial cells, submucosal glands, fibroblasts, vascular smooth muscles, vascular endothelial cells, and macrophages. In addition, BR2 expression was demonstrated in peripheral nerve fibers, whereas BR1 expression was not observed in nerves (59). The mRNA expression of BR1 and BR2 in eosinophilic CRSwNP was significantly higher than in normal mucosa (60), suggesting the role of BK and its receptors in the etiology of NPs, where a low expression of TGF-β1 was also detected (61). However, further study is needed to determine whether there is a cross-talk.

In lower airways, BK effectively induced edema and caused bronchoconstriction, and the protein expression of BR1 and BR2 in eosinophils of asthmatic patients was significantly enhanced compared with that of nonasthmatic subjects (62). In cultured human airway smooth muscle (ASM) cells, TGF-β1 increased the mRNA and protein expression of BR2 in a dose (0.5–10 ng/ml)- and time (2–24 h)-dependent manner (63). Additionally, in human lung fibroblasts (HLFs) and A549 cells, BK significantly increased inflammatory cytokines such as TGF-β1, IL-8, G-CSF, monocyte chemotactic protein-1 (MCP-1), and GM-CSF in a time-dependent manner. Moreover, in lung fibroblasts, BK greatly increased alpha-smooth muscle actin at the cellular and molecular levels, which had been entirely blocked by BR2 antagonists. These findings indicated that BK was able to induce fibroblast proliferation and collagen production and BR2 was the responsible receptor for this effect (64). However, in a murine asthma model, the significantly increased airway levels of TGF-β1 and vascular endothelial growth factor (VEGF) did not further rise with the application of the BR2 antagonist (HOE 140) but with that of the BR1 antagonist (R954), which had an inhibitory effect on eosinophils in selected compartments (65). Further study is also required to investigate the counteractions between BK and TGF-β1 in lower airways, as in upper airways.

There are definitely some other factors driving TGF-β1 in airway diseases. Mepacrine is a synthetic antimalarial drug and can reduce arginase and TGF-β1, thereby decreasing the development of subepithelial fibrosis in an OVA-induced asthma mice model (66). Chemokine (CC motif) ligand 2/monocyte chemotactic protein-1 (CCL2/MCP-1) is involved in inflammatory lung disorders and can recruit leukocytes, stimulate histamine or leukotriene release from mast cells or basophils, and induce the fibroblast production of TGF-β1 and procollagen, with enhanced Th2 polarization (67). Hyaluronidases (HYALs) comprise a group of enzymes that degrade hyaluronic acid (HA), and with the HYAL treatment, TGF-β1 production and collagen deposition are decreased in bleomycin-induced lung injury while fibrosis is potently blocked (68). In airway epithelial, Src homology 2 protein tyrosine phosphatase (SHP2), a modulator of TGF-β1 production, appears to modulate the TGF-β1 activity and in turn regulate allergic airway remodeling after allergen provocation in mouse models (69). In summary, there are a large number of factors influencing the TGF-β1 activity, as shown in Fig. 3.

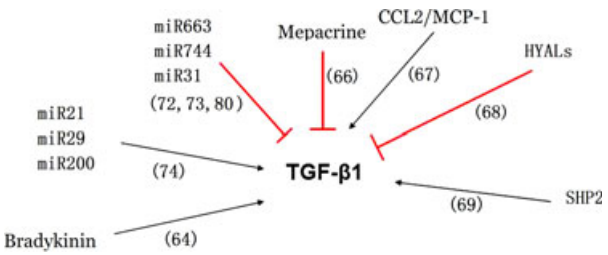

Figure 3 Scheme of transforming growth factor-beta 1 driving factors. The numbers beside the arrows indicate corresponding references. BMP, bone morphogenetic protein; CCL2/MCP-1, chemokine (CC motif) ligand 2/monocyte chemotactic protein-1; HYALs, hyaluronidases; miR, microRNA; SHP2, Src homology 2 protein tyrosine phosphatase.

TGF-β1-related microRNAs

MicroRNAs (miRs) are a class of noncoding RNAs for prompt, endogenous, and post-transcriptional regulation of gene expression and have an emerging role in diverse physiological and pathological processes including inflammatory responses (70). Experimentally validated results suggested that miRs influenced the TGF-β pathway at multiple levels, and moreover, TGF-β signaling itself enhanced the maturation of miRs, resulting in a bidirectional cross-talk (71). A direct action of miRs on the protein synthesis of TGF-β1 was extensive post-transcriptional regulation via its 5′- and 3′-UTRs, leading to the repression of TGF-β1 synthesis by miR-744 (72) and miR-663 (73).

In a bleomycin-induced mouse model of pulmonary fibrosis, dysregulation of several miRs including miR-21, miR-29, and miR-200 was identified (74). MiR-21 was postulated to target the inhibitory Smad7, thus enhancing TGF-β1 signaling by reducing its suppression and increasing promoted miR-21 levels, silenced miR-21 levels attenuated TGF-β1 expression in primary pulmonary fibroblasts, and *in vivo* antagonism of miR-21 decreased the severity of experimental fibrosis in mice (75). Among up-regulated miRs in human idiopathic pulmonary fibrosis (IPF) were miR-21, miR-154 (76), and miR-155 (77), while miR-29 and miR-200 were down-regulated via TGF-β1/Smad3 signaling, correlating with changes in extracellular matrix deposition and remodeling (78, 79). MiR-31 inhibited the profibrotic activity of TGF-β1 in normal lung fibroblasts and diminished the fibrogenic, contractile, and migratory activities of IPF fibroblasts (80).

Apart from lung fibrosis, specific miR profiles have also been identified in allergic inflammatory diseases, such as asthma, allergic rhinitis (AR), and eosinophilic CRSwNP, focusing on the activation of T cells (e.g., miR-21 and miR-146) and regulation of eosinophil development (e.g., miR-21 and miR-223; 81). Subjects with current AR symptoms had increased the levels of miR-155, miR-205, and miR-498, but reduced the levels of let-7e in nasal mucosa (82), and miR-125b was specifically up-regulated in eosinophilic CRSwNP (83). However, their roles and cross-talk with TGF-β1 in regulating key pathogenic mechanisms in allergic inflammation are currently not understood and warrant future study.

Through regulation of TGF-β1 signaling, miRs may be potential targets for the development of novel pathological fibrotic disorder therapies, including pulmonary fibrosis, such as antisense oligonucleotides for antagonizing miRs and use of viral expression systems to overexpress miRs. However, therapeutic miRs are in their infancy, and more research is required to understand the mechanisms of action and roles in pathology (71, 74).

Therapeutic approaches to TGF-β1

Transforming growth factor-beta 1 is generally a key for understanding inflammation and remodeling in inflammatory airway diseases and may provide potential therapeutic targets for their treatment (15). Therapeutic approaches may involve regulation of the TGF-β signaling pathway or its influencing factors. Experimental therapies that aim at the TGF-β1 gene utilize the antisense DNA, DNAzyme, or RNA interference (RNAi), especially in cancer cells (84). Several therapeutic agents including monoclonal antibodies, soluble TGF-β receptors, antisense oligonucleotides, and inhibitors of ALK5 (TGF-β RI) are under investigation. Recent reviews have summarized the current status of anti-TGF-β therapies in clinical trials (85, 86), and further information can be obtained from the website of National Institutes of Health (NIH) http://clinicaltrials.gov.

In addition, more attention should be paid to the driving pathways, such as ceasing smoking and maintaining the balance of oxidative stress and arachidonic acid metabolites. However, further research is necessary to evaluate the therapeutic value of the treatment with the TGF-β1 activity modified for airway diseases.

Conclusions

Transforming growth factor-beta 1 is implicated in remodeling processes and immunosuppression in inflammatory airway diseases; its regulation by other influencing factors is shown to play crucial roles in the development of pathogenic airway tissues. Meanwhile, a constant cross-talk between TGF-β1 and pro- or anti-inflammatory cytokines, such as IFN-γ, IL-17A, IL-10, IL-13, IL-1β, together with other factors produced in the tissue micromilieu, such as activin A, microRNAs, or members of signaling pathways, plays an important role in inflammation regulation and outcome of immune responses (87). To obtain more details about these mechanisms, further analysis is required.

Transforming growth factor-beta 1 might also be considered as a double-edged sword in inflammatory airway diseases like in cancer (18). On the one hand, TGF-β1 is responsible for persistent epithelial activation and structural remodeling; on the other hand, it exerts an immunomodulatory role by inhibiting T-cell activation and down-regulating inflammatory responses. It is perhaps the balance between these two facets, which finally determine the outcome. Unraveling the roles of TGF-β1 driving factors and their signal pathways as well as gaining knowledge about the maintenance of their balance obviously requires a considerable amount of work and will undoubtedly represent a significant challenge in the future.

Acknowledgments

This project was supported by the Fund for Scientific Research Flanders (FWO-Vlaanderen – Projects 3G.0489. 08 to C.B., G.0642.10N to C.B and O.K.) and by the Interuniversity Attraction Poles program (IUAP) – Belgian state – Belgian Science Policy P7/30 and by the foreign training project – the first affiliated hospital, Chongqing Medical University – China (2011) to YY, and by the medical research projects of Chongqing Health Bureau (2012-2-024) – China to YY.

Conflicts of interest

The authors declare that they have no conflicts of interest.

References

1. Piek E, Heldin CH, Ten Dijke P. Specificity, diversity, and regulation in TGF-beta superfamily signaling. *FASEB J* 1999;**13**:2105–2124.
2. Konigshoff M, Kneidinger N, Eickelberg O. TGF-beta signaling in COPD: deciphering genetic and cellular susceptibilities for future therapeutic regimen. *Swiss Med Wkly* 2009;**139**:554–563.
3. Huber S, Schramm C, Lehr HA, Mann A, Schmitt S, Becker C et al. Cutting edge: TGF-beta signaling is required for the *in vivo* expansion and immunosuppressive capacity of regulatory CD4 + CD25 + T cells. *J Immunol* 2004;**173**:6526–6531.
4. Van Bruaene N, Perez-Novo CA, Basinski TM, Van Zele T, Holtappels G, De Ruyck N et al. T-cell regulation in chronic paranasal sinus disease. *J Allergy Clin Immunol* 2008;**121**:1435–1441.
5. Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M et al. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature* 1992;**359**:693–699.
6. Sejima T, Holtappels G, Bachert C. The expression of fibrinolytic components in chronic paranasal sinus disease. *Am J Rhinol Allergy* 2011;**25**:1–6.
7. Halwani R, Al-Muhsen S, Al-Jahdali H, Hamid Q. Role of transforming growth factor-beta in airway remodeling in asthma. *Am J Respir Cell Mol Biol* 2011;**44**:127–133.
8. Van Bruaene N, Bachert C. Tissue remodeling in chronic rhinosinusitis. *Curr Opin Allergy Clin Immunol* 2011;**11**:8–11.
9. Bachert C, Van Bruaene N, Toskala E, Zhang N, Olze H, Scadding G et al. Important research questions in allergy and related diseases: 3-chronic rhinosinusitis and nasal polyposis – a GALEN study. *Allergy* 2009;**64**:520–533.
10. Van Zele T, Claeys S, Gevaert P, Van Maele G, Holtappels G, Van Cauwenberge P et al. Differentiation of chronic sinus diseases by measurement of inflammatory mediators. *Allergy* 2006;**61**:1280–1289.
11. Le Bellego F, Perera H, Plante S, Chakir J, Hamid Q, Ludwig MS. Mechanical strain increases cytokine and chemokine production in bronchial fibroblasts from asthmatic patients. *Allergy* 2009;**64**:32–39.
12. Kim ES, Kim SH, Kim KW, Park JW, Kim YS, Sohn MH et al. Basement membrane thickening and clinical features of children with asthma. *Allergy* 2007;**62**:635–640.
13. Hogg JC. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. *Lancet* 2004;**364**:709–721.
14. Yang YC, Zhang N, Van Crombruggen K, Hu GH, Hong SL, Bachert C. Transforming growth factor-beta1 in inflammatory airway disease: a key for understanding inflammation and remodeling. *Allergy* 2012;**67**:1193–1202.
15. Semlali A, Jacques E, Rouabhia M, Milot J, Laviolette M, Chakir J. Regulation of epithelial cell proliferation by bronchial fibroblasts obtained from mild asthmatic subjects. *Allergy* 2010;**65**:1438–1445.
16. Shi LL, Xiong P, Zhang L, Cao PP, Liao B, Lu X et al. Features of airway remodeling in different types of Chinese chronic rhinosinusitis are associated with inflammation patterns. *Allergy* 2013;**68**:101–109.
17. Salib RJ, Howarth PH. Transforming growth factor-beta in allergic inflammatory disease of the upper airways: friend or foe? *Clin Exp Allergy* 2009;**39**:1128–1135.
18. Dut R, Dizdar EA, Birben E, Sackesen C, Soyer OU, Besler T et al. Oxidative stress and its determinants in the airways of children with asthma. *Allergy* 2008;**63**:1605–1609.
19. Noguchi C, Umino T, Miyazaki Y, Jinta T, Usui Y, Yoshizawa Y. TGF-beta and glutathione promote tissue repair in cigarette smoke induced injury. *J Med Dent Sci* 2007;**54**:109–116.
20. Jeanson L, Kelly M, Coste A, Guerrera IC, Fritsch J, Nguyen-Khoa T et al. Oxidative stress induces unfolding protein response and inflammation in nasal polyposis. *Allergy* 2012;**67**:403–412.
21. Chung KF, Marwick JA. Molecular mechanisms of oxidative stress in airways and lungs with reference to asthma and chronic obstructive pulmonary disease. *Ann N Y Acad Sci* 2010;**1203**:85–91.
22. Adcock IM, Caramori G, Barnes PJ. Chronic obstructive pulmonary disease and lung cancer: new molecular insights. *Respiration* 2011;**81**:265–284.
23. Pietinalho A, Pelkonen A, Rytila P. Linkage between smoking and asthma. *Allergy* 2009;**64**:1722–1727.
24. Qi S, den Hartog GJ, Bast A. Superoxide radicals increase transforming growth factor-beta1 and collagen release from human lung fibroblasts via cellular influx through chloride channels. *Toxicol Appl Pharmacol* 2009;**237**:111–118.
25. Koli K, Myllarniemi M, Keski-Oja J, Kinnula VL. Transforming growth factor-beta activation in the lung: focus on fibrosis and reactive oxygen species. *Antioxid Redox Signal* 2008;**10**:333–342.
26. Moon JH, Kim TH, Lee HM, Lee SH, Choe W, Kim HK et al. Overexpression of the superoxide anion and NADPH oxidase isoforms 1 and 4 (NOX1 and NOX4) in allergic nasal mucosa. *Am J Rhinol Allergy* 2009;**23**:370–376.
27. Hastan D, Fokkens WJ, Bachert C, Newson RB, Bislimovska J, Bockelbrink A et al. Chronic rhinosinusitis in Europe–an underestimated disease. A GA(2)LEN study. *Allergy* 2011;**66**:1216–1223.
28. Huvenne W, Perez-Novo CA, Derycke L, De Ruyck N, Krysko O, Maes T et al. Different regulation of cigarette smoke induced inflammation in upper versus lower airways. *Respir Res* 2010;**11**:100.
29. Takizawa H, Tanaka M, Takami K, Ohtoshi T, Ito K, Satoh M et al. Increased expression of transforming growth factor-beta1 in small airway epithelium from tobacco smokers and patients with chronic obstructive pulmonary disease (COPD). *Am J Respir Crit Care Med* 2001;**163**:1476–1483.
30. Springer J, Scholz FR, Peiser C, Groneberg DA, Fischer A. SMAD-signaling in chronic obstructive pulmonary disease: transcriptional down-regulation of inhibitory SMAD 6 and 7 by cigarette smoke. *Biol Chem* 2004;**385**:649–653.
31. Wang RD, Wright JL, Churg A. Transforming growth factor-beta1 drives airway remodeling in cigarette smoke-exposed tracheal explants. *Am J Respir Cell Mol Biol* 2005;**33**:387–393.
32. Chuang CY, Chang CH, Huang YL. Thioredoxin mediates remodeling factors of human bronchial epithelial cells upon interaction with house dust mite-stimulated eosinophils. *Inhal Toxicol* 2009;**21**:153–167.
33. Min MG, Song DJ, Miller M, Cho JY, McElwain S, Ferguson P et al. Coexposure to environmental tobacco smoke increases levels of allergen-induced airway remodeling in mice. *J Immunol* 2007;**178**:5321–5328.
34. Rahman I, van Schadewijk AA, Crowther AJ, Hiemstra PS, Stolk J, MacNee W et al. 4-Hydroxy-2-nonenal, a specific lipid peroxidation product, is elevated in lungs of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2002;**166**:490–495.

35. Mabalirajan U, Aich J, Leishangthem GD, Sharma SK, Dinda AK, Ghosh B. Effects of vitamin E on mitochondrial dysfunction and asthma features in an experimental allergic murine model. *J Appl Physiol* 2009;**107**:1285–1292.
36. Mabalirajan U, Dinda AK, Sharma SK, Ghosh B. Esculetin restores mitochondrial dysfunction and reduces allergic asthma features in experimental murine model. *J Immunol* 2009;**183**:2059–2067.
37. Mabalirajan U, Ahmad T, Leishangthem GD, Joseph DA, Dinda AK, Agrawal A et al. Beneficial effects of high dose of L-arginine on airway hyperresponsiveness and airway inflammation in a murine model of asthma. *J Allergy Clin Immunol* 2010;**125**:626–635.
38. Jin C, Zhou D, Lv F. Beneficial effects of early (but not late) intervention of heme oxygenase-1 on bleomycin-induced pulmonary fibrosis in mice. *Respir Physiol Neurobiol* 2011;**175**:239–246.
39. Reddy SP. The antioxidant response element and oxidative stress modifiers in airway diseases. *Curr Mol Med* 2008;**8**:376–383.
40. Ortiz JL, Milara J, Lluch J, De Diego A, Sanz C, Cortijo J. Phosphodiesterase-4 inhibition improves corticosteroid insensitivity in pulmonary endothelial cells under oxidative stress. *Allergy* 2013;**68**:64–73.
41. Calabrese C, Triggiani M, Marone G, Mazzarella G. Arachidonic acid metabolism in inflammatory cells of patients with bronchial asthma. *Allergy* 2000;**55** (Suppl 61):27–30.
42. Perez-Novo CA, Watelet JB, Claeys C, Van Cauwenberge P, Bachert C. Prostaglandin, leukotriene, and lipoxin balance in chronic rhinosinusitis with and without nasal polyposis. *J Allergy Clin Immunol* 2005;**115**:1189–1196.
43. Patou J, Holtappels G, Affleck K, Gevaert P, Perez-Novo C, Van Cauwenberge P et al. Enhanced release of IgE-dependent early phase mediators from nasal polyp tissue. *J Inflamm (Lond)* 2009;**6**:11.
44. Patou J, Gevaert P, Van Zele T, Holtappels G, van Cauwenberge P, Bachert C. Staphylococcus aureus enterotoxin B, protein A, and lipoteichoic acid stimulations in nasal polyps. *J Allergy Clin Immunol* 2008;**121**:110–115.
45. Pezato R, Swierczynska-Krepa M, Nizankowska-Mogilnicka E, Derycke L, Bachert C, Perez-Novo CA. Role of imbalance of eicosanoid pathways and staphylococcal superantigens in chronic rhinosinusitis. *Allergy* 2012;**67**:1347–1356.
46. Prado CM, Yano L, Rocha G, Starling CM, Capelozzi VL, Leick-Maldonado EA et al. Effects of inducible nitric oxide synthase inhibition in bronchial vascular remodeling-induced by chronic allergic pulmonary inflammation. *Exp Lung Res* 2011;**37**:259–268.
47. Barbarin V, Arras M, Misson P, Delos M, McGarry B, Phan SH et al. Characterization of the effect of interleukin-10 on silica-induced lung fibrosis in mice. *Am J Respir Cell Mol Biol* 2004;**31**:78–85.
48. Kolodsick JE, Peters-Golden M, Larios J, Toews GB, Thannickal VJ, Moore BB. Prostaglandin E2 inhibits fibroblast to myofibroblast transition via E. prostanoid receptor 2 signaling and cyclic adenosine monophosphate elevation. *Am J Respir Cell Mol Biol* 2003;**29**:537–544.
49. Togo S, Liu X, Wang X, Sugiura H, Kamio K, Kawasaki S et al. PDE4 inhibitors roflumilast and rolipram augment PGE2 inhibition of TGF-{beta}1-stimulated fibroblasts. *Am J Physiol Lung Cell Mol Physiol* 2009;**296**:L959–L969.
50. Selige J, Hatzelmann A, Dunkern T. The differential impact of PDE4 subtypes in human lung fibroblasts on cytokine-induced proliferation and myofibroblast conversion. *J Cell Physiol* 2011;**226**:1970–1980.
51. Bosse Y, Thompson C, McMahon S, Dubois CM, Stankova J, Rola-Pleszczynski M. Leukotriene D4-induced, epithelial cell-derived transforming growth factor beta1 in human bronchial smooth muscle cell proliferation. *Clin Exp Allergy* 2008;**38**:113–121.
52. Lee KS, Kim SR, Park HS, Park SJ, Min KH, Lee KY et al. Cysteinyl leukotriene upregulates IL-11 expression in allergic airway disease of mice. *J Allergy Clin Immunol* 2007;**119**:141–149.
53. Shimbori C, Shiota N, Okunishi H. Effects of montelukast, a cysteinyl-leukotriene type 1 receptor antagonist, on the pathogenesis of bleomycin-induced pulmonary fibrosis in mice. *Eur J Pharmacol* 2011;**650**:424–430.
54. Izumo T, Kondo M, Nagai A. Effects of a leukotriene B4 receptor antagonist on bleomycin-induced pulmonary fibrosis. *Eur Respir J* 2009;**34**:1444–1451.
55. Shim YM, Zhu Z, Zheng T, Lee CG, Homer RJ, Ma B et al. Role of 5-lipoxygenase in IL-13-induced pulmonary inflammation and remodeling. *J Immunol* 2006;**177**:1918–1924.
56. Riddick CA, Serio KJ, Hodulik CR, Ring WL, Regan MS, Bigby TD. TGF-beta increases leukotriene C4 synthase expression in the monocyte-like cell line, THP-1. *J Immunol* 1999;**162**:1101–1107.
57. Perng DW, Wu YC, Chang KT, Wu MT, Chiou YC, Su KC et al. Leukotriene C4 induces TGF-beta1 production in airway epithelium via p38 kinase pathway. *Am J Respir Cell Mol Biol* 2006;**34**:101–107.
58. Austin CE, Foreman JC. A study of the action of bradykinin and bradykinin analogues in the human nasal airway. *J Physiol* 1994;**478**:351–356.
59. Shirasaki H, Kanaizumi E, Himi T. Immunohistochemical localization of the bradykinin B1 and B2 receptors in human nasal mucosa. *Mediators Inflamm* 2009;**2009**:102406.
60. Rostkowska-Nadolska B, Kapral M, Fraczek M, Kowalczyk M, Gawron W, Mazurek U. Transcriptional activity of genes-encoding kinin B1 and B2 receptors and kinin-dependent genes in nasal polyps. *Adv Med Sci* 2009;**54**:211–220.
61. Van Bruaene N, Derycke L, Perez-Novo CA, Gevaert P, Holtappels G, De Ruyck N et al. TGF-beta signaling and collagen deposition in chronic rhinosinusitis. *J Allergy Clin Immunol* 2009;**124**:253–259.
62. Bertram CM, Misso NL, Fogel-Petrovic M, Figueroa CD, Foster PS, Thompson PJ et al. Expression of kinin receptors on eosinophils: comparison of asthmatic patients and healthy subjects. *J Leukoc Biol* 2009;**85**:544–552.
63. Kim JH, Jain D, Tliba O, Yang B, Jester WF Jr, Panettieri RA Jr et al. TGF-beta potentiates airway smooth muscle responsiveness to bradykinin. *Am J Physiol Lung Cell Mol Physiol* 2005;**289**:L511–L520.
64. Vancheri C, Gili E, Failla M, Mastruzzo C, Salinaro ET, Lofurno D et al. Bradykinin differentiates human lung fibroblasts to a myofibroblast phenotype via the B2 receptor. *J Allergy Clin Immunol* 2005;**116**:1242–1248.
65. Vasquez-Pinto LM, Nantel F, Sirois P, Jancar S. Bradykinin B(1) receptor antagonist R954 inhibits eosinophil activation/proliferation/migration and increases TGF-beta and VEGF in a murine model of asthma. *Neuropeptides* 2010;**44**:107–113.
66. Mabalirajan U, Aich J, Agrawal A, Ghosh B. Mepacrine inhibits subepithelial fibrosis by reducing the expression of arginase and TGF-beta1 in an extended subacute mouse model of allergic asthma. *Am J Physiol Lung Cell Mol Physiol* 2009;**297**:L411–L419.
67. Rose CE Jr, Sung SS, Fu SM. Significant involvement of CCL2 (MCP-1) in inflammatory disorders of the lung. *Microcirculation* 2003;**10**:273–288.
68. Bitencourt CS, Pereira PA, Ramos SG, Sampaio SV, Arantes EC, Aronoff DM et al. Hyaluronidase recruits mesenchymal-like cells to the lung and ameliorates fibrosis. *Fibrogenesis Tissue Repair* 2011;**4**:3.
69. Qin XJ, Zhang GS, Zhang X, Qiu ZW, Wang PL, Li YW et al. Protein tyrosine phosphatase SHP2 regulates TGF-beta1 production in airway epithelia and asthmatic airway remodeling in mice. *Allergy* 2012;**67**:1547–1556.
70. Zheng J, Jiang HY, Li J, Tang HC, Zhang XM, Wang XR et al. MicroRNA-23b promotes tolerogenic properties of dendritic cells *in vitro* through inhibiting Notch1/NF-

κB signalling pathways. *Allergy* 2012;**67**:362–370.
71. Butz H, Racz K, Hunyady L, Patocs A. Crosstalk between TGF-beta signaling and the microRNA machinery. *Trends Pharmacol Sci* 2012;**33**:382–393.
72. Martin J, Jenkins RH, Bennagi R, Krupa A, Phillips AO, Bowen T et al. Post-transcriptional regulation of Transforming Growth Factor Beta-1 by microRNA-744. *PLoS ONE* 2011;**6**:e25044.
73. Tili E, Michaille JJ, Adair B, Alder H, Limagne E, Taccioli C et al. Resveratrol decreases the levels of miR-155 by upregulating miR-663, a microRNA targeting JunB and JunD. *Carcinogenesis* 2010;**31**:1561–1566.
74. Bowen T, Jenkins RH, Fraser DJ. MicroRNAs, transforming growth factor beta-1, and tissue fibrosis. *J Pathol* 2013;**229**:274–285.
75. Liu G, Friggeri A, Yang Y, Milosevic J, Ding Q, Thannickal VJ et al. miR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. *J Exp Med* 2010;**207**:1589–1597.
76. Milosevic J, Pandit K, Magister M, Rabinovich E, Ellwanger DC, Yu G et al. Profibrotic role of miR-154 in pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2012;**47**:879–887.
77. Pottier N, Maurin T, Chevalier B, Puissegur MP, Lebrigand K, Robbe-Sermesant K et al. Identification of keratinocyte growth factor as a target of microRNA-155 in lung fibroblasts: implication in epithelial-mesenchymal interactions. *PLoS ONE* 2009;**4**:e6718.
78. Yang S, Banerjee S, de Freitas A, Sanders YY, Ding Q, Matalon S et al. Participation of miR-200 in pulmonary fibrosis. *Am J Pathol* 2012;**180**:484–493.
79. Cushing L, Kuang PP, Qian J, Shao F, Wu J, Little F et al. miR-29 is a major regulator of genes associated with pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2011;**45**:287–294.
80. Yang S, Xie N, Cui H, Banerjee S, Abraham E, Thannickal VJ et al. miR-31 is a negative regulator of fibrogenesis and pulmonary fibrosis. *FASEB J* 2012;**26**:3790–3799.
81. Lu TX, Rothenberg ME. Diagnostic, functional, and therapeutic roles of microRNA in allergic diseases. *J Allergy Clin Immunol* 2013;**132**:3–13.
82. Suojalehto H, Toskala E, Kilpeläinen M, Majuri ML, Mitts C, Lindström I et al. MicroRNA profiles in nasal mucosa of patients with allergic and nonallergic rhinitis and asthma. *Int Forum Allergy Rhinol* 2013;**3**:612–620.
83. Zhang XH, Zhang YN, Li HB, Hu CY, Wang N, Cao PP et al. Overexpression of miR-125b, a novel regulator of innate immunity, in eosinophilic chronic rhinosinusitis with nasal polyps. *Am J Respir Crit Care Med* 2012;**185**:140–151.
84. Wang SL, Yao HH, Guo LL, Dong L, Li SG, Gu YP et al. Selection of optimal sites for TGFB1 gene silencing by chitosan-TPP nanoparticle-mediated delivery of shRNA. *Cancer Genet Cytogenet* 2009;**190**:8–14.
85. Santibanez JF, Quintanilla M, Bernabeu C. TGF-beta/TGF-beta receptor system and its role in physiological and pathological conditions. *Clin Sci (Lond)* 2011;**121**:233–251.
86. Akhurst RJ, Hata A. Targeting the TGFbeta signalling pathway in disease. *Nat Rev Drug Discov* 2012;**11**:790–811.
87. Mayer AK, Bartz H, Fey F, Schmidt LM, Dalpke AH. Airway epithelial cells modify immune responses by inducing an anti-inflammatory microenvironment. *Eur J Immunol* 2008;**38**:1689–1699.