Pathobiology of airway smooth muscle remodeling

Christopher A. Febres-Aldana [1] .
Ruth Fernández-Ruiz [2] .
Fabiola A. Placeres-Uray [3] .
Ramona González de Alfonzo [4] .
Marcelo J. Alfonzo [5] .
Itala A. Lippo de Becemberg [6] .

[1]Sección de Biomembranas, Instituto de Medicina Experimental (IME), Facultad de Medicina, Universidad Central de Venezuela, Caracas, Venezuela. chrisceo.gene@gmail.com
[2]Sección de Biomembranas, Instituto de Medicina Experimental (IME), Facultad de Medicina, Universidad Central de Venezuela, Caracas, Venezuela. ruthfernandezruiz@hotmail.com
[3]Sección de Biomembranas, Instituto de Medicina Experimental (IME), Facultad de Medicina, Universidad Central de Venezuela, Caracas, Venezuela. fapuray@hotmail.com
[4]Sección de Biomembranas, Instituto de Medicina Experimental (IME), Facultad de Medicina, Universidad Central de Venezuela, Caracas, Venezuela. alfonzoram@hotmail.com
[5]Sección de Biomembranas, Instituto de Medicina Experimental (IME), Facultad de Medicina, Universidad Central de Venezuela, Caracas, Venezuela. hmag5@hotmail.com
[6]Sección de Biomembranas, Instituto de Medicina Experimental (IME), Facultad de Medicina, Universidad Central de Venezuela, Caracas, Venezuela. italalippo@gmail.com

Correspondencia: Instituto de Medicina Tropical - Facultad de Medicina - Universidad Central de Venezuela.

Consignado el 07 de Mayo del 2015 a la Revista Vitae Academia Biomédica Digital.

RESUMEN

El mecanismo primario de asma y EBPOC involucra una excesiva contracción de las vías aéreas (VA), cuya severidad se encuentra en relación a inflamación crónica. Evidencia reciente sugiere que las células de músculo liso de las vías aéreas (MLVA) poseen elevada plasticidad celular que puede contribuir en inflamación, resultando en su engrosamiento mediante hiperplasia y/o hipertrofia. La interacción MLVA-microambiente tisular es la base para hiperreactividad y remodelado tisular, con contribuciones importantes de virus y mediadores químicos, especialmente acetilcolina. Esta revisión abarca la fisiopatología del remodelado del MLVA en relación a fenotipos graves de enfermedades inflamatorias bronquiales. Un análisis in silico de hibridación entre secuencias de ARN humano y virales fue realizado, obteniendo datos para apoyar una hipótesis de 'hit and run'. Como una propuesta de integración, se resumen los últimos hallazgos moleculares con una perspectiva que ayude al establecimiento de fundamentos para investigaciones futuras y la comprensión de las vías de señalización que regulan la biología del MLVA.

PALABRAS CLAVE: Asma, EPOC, musculo liso bronquial, receptores muscarínicos, virus ARN

PATOBIOLOGÍA DEL REMODELADO DE LAS VÍAS AÉREAS

SUMMARY

The primary mechanism of morbidity and mortality in asthma and COPD is excessive airway narrowing, which severity is based on chronic inflammation. New evidence suggests airway smooth muscle (ASM) cells show extraordinary cellular plasticity that may contribute to airway inflammation, ensuing ASM thickening by either hyperplasia and/or hypertrophy. Tissue microenvironment-ASM interaction is a complex crosstalk that supports hyperresponsiveness and tissue remodeling, with major contributions of viruses and chemical mediators, especially acetylcholine. This review addresses the ASM pathology in relation with severe phenotypes of airway inflammatory diseases. An in silico analysis of hybridization between human and viral RNA strands was performed, obtaining data to support a 'hit and run' hypothesis. As an integrative proposal, we summarized the last molecular findings in this field with a perspective that helps to set the stage for future research toward understanding the signaling pathways regulating ASM biology.

KEY WORDS: Asthma, COPD, airway smooth muscle, muscarinic receptors, RNA virus

PATHOBIOLOGY OF AIRWAY SMOOTH MUSCLE REMODELING

INTRODUCTION

Asthma and chronic obstructive pulmonary disease (COPD) comprise chronic inflammatory disorders characterized by airway hyperresponsiveness and airflow obstruction that can fluctuate over time. They have an increasing economic burden, not to mention their associated disabilities and fatal outcomes. Despite of outnumbered research, we still have not completely understood the big picture of their natural history. Quite a lot of evidence arising from epidemiological, clinical, pathological, and molecular studies, have only provided a short view of the pathobiological mechanisms generating those diseases. The development of

better functional assessment techniques along with a wider availability of new biomarkers allowed to recognize that inflammatory airway diseases, especially asthma, involve multiple subphenotypes that differ in clinical severity, histopathology, response to therapy, and long-term outcome. In consequence, heterogeneous groups have been identified, which are likely originated from a unique genetic background/ environment combination.

Severe phenotypes of airway diseases that course with airway hyperreactivity, implicate airflow obstruction that is either irreversible or only partially reversible. Their severe nature apparently is due to longstanding inflammation in the airways. In this setting, injury cyclicity, age, genetic factors, and previous tissue history induce structural changes; a phenomenon commonly coined as 'airway remodeling'. Airway remodeling is assumed to result in severe phenotypes. However, several clinical and animal studies indicate that the relationship between inflammation, remodeling, and hyperresponsiveness is complex, and still not completely understood. Considering that airway hyperreactivity results in an abnormal airway tone, smooth muscle thickening is thought as the main substrate of abnormal airway mechanics. This has been deeply explored; hence, considerable data is available. In this review, we gathered the last experimental findings in this field to formulate a model of airway smooth muscle (ASM) remodeling that fits into the natural history of common airway diseases, mainly focusing on molecular mechanisms.

I. Overview of airway smooth muscle remodeling

Growing evidence supports various pathophysiological mechanisms of airway diseases, including structural changes seen on severe asthma, COPD, chronic bronchitis, cystic fibrosis, and bronchiectasis[1-4]. Thus, airway remodeling has been defined by modifications in the composition, amount, and organization of local cells, including epithelium, glands, blood vessels, extracellular matrix (ECM), and smooth muscle (see Fig. 1).

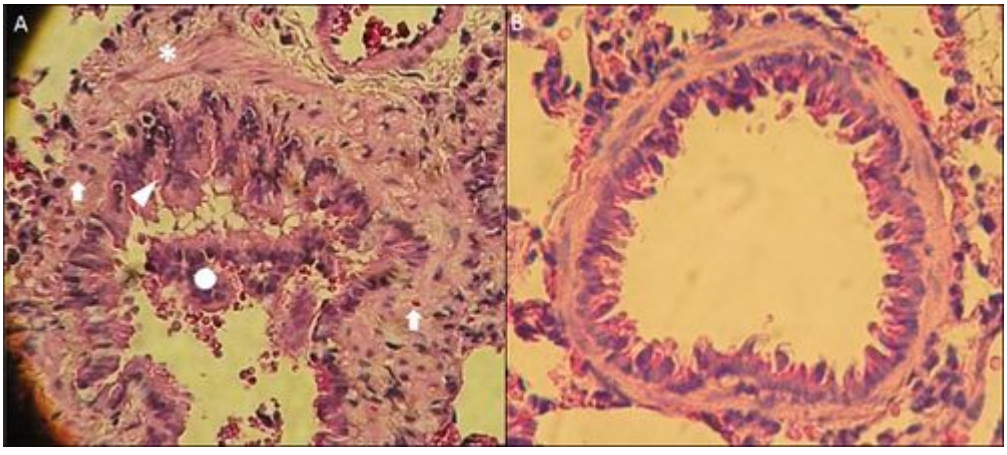


Figure 1. Histopathology of small airways in OVA-sensitized Rats. A bronchiole from OVA-sensitized rat(A) in comparison to a normal bronchiole from saline-nebulized rat(B). Tissue shows remodeling features, such as: epithelial hyperplasia (arrowhead), ASM thickening (asterisk), lymphocytic/eosinophilic inflammation (arrows), and luminal exudate (circle), compare with the normal airway. Lung samples were extracted from rats after a protocol previously described(66). Magnification 200X, Hematoxylin-eosin staining.

Interaction of genetic and environmental factors evolve into assorted outcomes after injury. Many models[5] and clinical studies have shown that symptoms and functional findings are caused by three interconnected factors: 1) chronic inflammation, 2) airway hyperresponsiveness (AHR), and 3) tissue remodeling. Notwithstanding, a major concern in this field is that there is no clear chronologic and quantitative relationships. A current perspective considers that unbalanced immune responses to external factors, such as allergens in asthma, sets up a harmful microenvironment of cyclic injury and repair, which leads to abnormal structure and function[6, 7]. Furthermore, a recent study suggests that chronic mechanical stress resulting from bronchoconstriction *per se* may also lead to remodeling without inflammation[8]. Despite of airway remodeling could be disorder-specific, the airway structure may play a common role to airway narrowing and airflow limitation carrying out poorly reversible airway obstruction.

Evidence of ASM thickening in Asthma and COPD

Asthma and COPD are well-differentiated clinical entities with some overlapping syndromes in the middle[3, 9]. They share some features, especially at pathological level, including: epithelial hyperplasia and dysfunction, subepithelial fibrosis, increased myofibroblasts, increased vascularization, abnormal neurite branching, and dense ASM layers[2, 7, 10]; highlighting that a greater basement membrane thickness as well as smooth muscle hyperplasia are commonest seen on asthma[10, 11]. Their deepness frequently wedges with clinical expression and severity[12, 13]. A study that evaluated bronchial wall thickness by high resolution computed tomography in mild-to-moderate asthma and COPD revealed the airway diameter and thickness were similar[14], but asthma still has received more attention respect to ASM remodeling. Increased ASM mass could be attributable to hyperplasia, hypertrophy or both. Under physiological conditions, ASM located in the central and peripheral airways are bands that wrap up around the airways in a helical pattern. Its thickness, relative to the diameter of the airway lumen, increases towards the periphery, but in absolute terms, the amount is less in the peripheral airways[15]. A morphometric study indicates that the bronchial smooth muscle mass of patients suffering of fatal asthma was twice than non-asthmatics[16]. A major concern of this proposal is the ECM volume was not measured. To solve it, a recent study showed ASM hypertrophy in the large airways in both nonfatal and fatal asthma, but hyperplasia was only seen in large and small airways in fatal cases. Both groups were associated with an absolute increase in ECM[17]. Some degree of airway wall thickening was regularly detected in asthmatics of all severities with predominance in severe cases[18]. The occurrence of remodeling does not seem to depend on the inflammatory response subtype; since, airway structure does not differ between asthmatics with eosinophilia and those without[19]. Contrasting results debate the importance of ASM remodeling because it is not always found in asthma, hence, no differences in averageof smooth musclecell cross-sectional area[20].

There is less evidence supporting ASM thickening in COPD than asthma. Obliteration and fibrosis of the alveolar wall, mucous gland hypertrophy, and goblet cell hyperplasia are well-

known pathological features of COPD[21]. Increased ASM thickness has been found as compared to control, but lesser than asthmatics[15]. Functional implications have been shown, as it correlates with the airway obstruction degree[22]. Additionally, ASM mass and adventitia increased together by 50% in severe COPD affecting the small airway physiology[23]. Biopsy studies from large airways reported no increase in ASM; moreover, smooth muscle protein isoforms were not increased, but there was a slight increment in myosin light chain kinase (MLCK) without changing the myosin light chain phosphorylation[24]. Conflicting results showed that remodeling may occur in the central airways by greater ECM protein deposition and increased ASM[25].

This data points out that asthma and COPD could progress with variable degree of ASM remodeling, but no direct evidence has been obtained supporting reversibility. A murine model of asthma suggests that after allergen cessation, the goblet hyperplasia and collagen deposition resolved first and then lymphocytic infiltration along with ASM thickening[26]. This brings up an open question whether in human diseases a complete removal of the tissue hazard can be accompanied by spontaneous resolution of airway remodeling.

Inflammatory Microenvironment Orchestrates ASM Remodeling

Airway remodeling is associated with longstanding inflammation. Interleukin (IL)-1β, IL-6, and Tumor Necrosis Factor-α (TNF-α) as well as growth factors such as Platelet-Derived Growth Factor (PDGF), Epidermal Growth Factor (EGF), Insulin-like Growth Factor (IGF), and Transforming Growth Factor-β (TGF-β), have pleiotropic effects; however, specific immune responses portray distinctive pathologic features[7, 27]. T cell reactions cover a wide spectrum of divergent cytokine networks. In asthma, for example, clinical phenotypes match inflammatory profiles: I-type hypersensitivity reaction (IgE-dependent), Th2 predominant inflammation, and non-Th2 associated response[28]. However, a common feature seen in all cases is the ASM thickening (see Fig. 2).

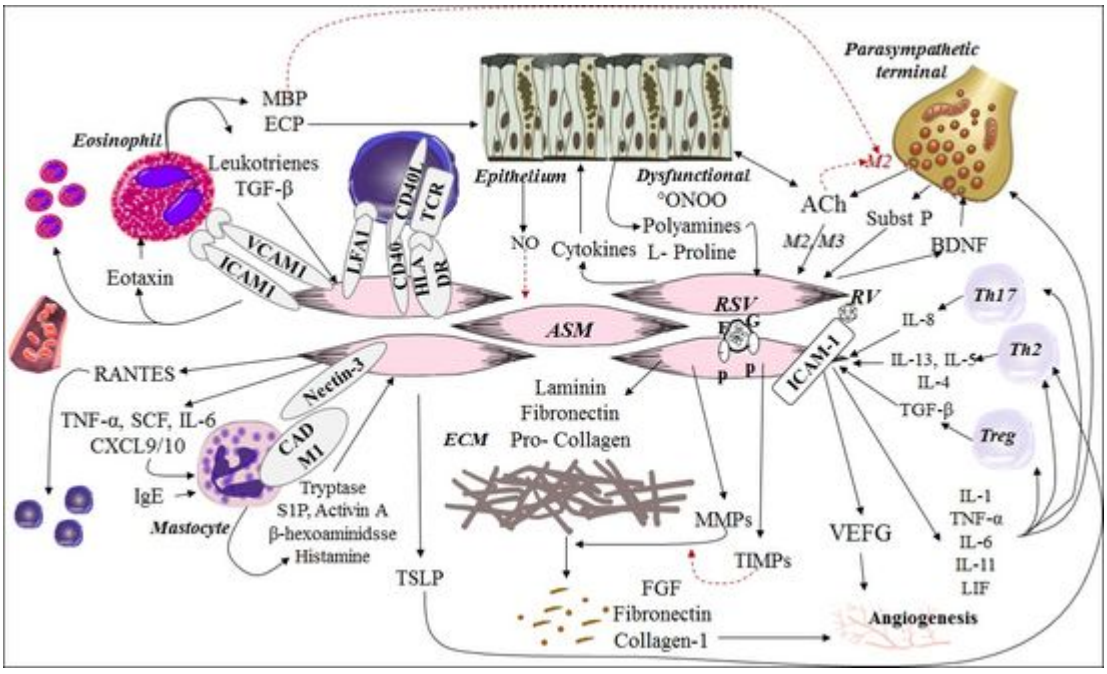


Figure 2. Crosstalk between ASM with cellular and non-cellular components of the airways during inflammation. (See the text for explanation).

Eosinophils are the most prominent inflammatory cells in the airways of asthmatics[21]. In the course of hypersensitivity reactions, eosinophils localize in close relation to ASM. For instance, small airways contain eosinophils in their outer portion (between ASM and alveolar attachment), whereas in the large airways they are present predominantly in their inner portion (between ASM and basement membrane)[29]. This eosinophil-ASM relationship can enhance cell proliferation by cysteinyl leukotriene secretion[30]. Eosinophil homing in the airways depends on Th2 cytokines and eotaxin. Adhesion to ASM is mediated by cell adhesion molecules (ICAM-1, VCAM-1) that are constitutively expressed and also upregulated. ASM cells (ASMC)-derived cytokines could promote eosinophil differentiation, perpetuating the burden of eosinophils into ASM bundles[31].

In a similar way, mastocyte infiltration is prominent in ASM bundles[32]. Mastocyte migration and adhesion potentiate tissue remodeling because histamine, tryptase, activin A, sphingosine 1-phosphate (S1P), β- hexosaminidase, and TNF-α, stimulate many ASMC functions[33]. Despite these mediators can stimulate cell proliferation, mast cell seems not to be relevant for neither proliferation or survival[34]. On the other hand, mastocyte can induce the thymic stromal lymphopoietin (TSLP) in ASM that is highly determinant of Th2 polarization[35]. Mast cell placement, proliferation and survival into the ASM could occur through allergen-independent mechanisms[36].

Neutrophilic inflammation also occurs in severe asthma and COPD. Even though, clinical phenotypes that course with predominant eosinophilic inflammation lead to ASM thickening in both large and small airways, the neutrophil infiltration is almost restricted to concurrent small airway remodeling[37]. A histopathological study of children with fatal untreated respiratory syncytial virus (RSV) infection showed vascular leakage and neutrophil recruitment into the submuscular layer, smooth muscle, and airway epithelium, resembling fatal cases of obstructive diseases[38]. IL-8 has been implicated, and it seems to be secreted by Th17 cells[39]. Nowadays, it is known that Th9, Th22, and Th25 cells also modulate countless aspects of airway immunity[40]. Nonetheless, the most significant cytokine expression in asthma includes IL-4, IL-9, IL-13, eotaxin and RANTES. This profile correspond to an upregulated Th2 reaction[41]. $CD4^+$ T cells transfer from ovalbumin (OVA)-sensitized rats to non-sensitized rats (adoptive lymphocyte transfer), showed that an specific subset of Th2 cells drove airway remodeling in non-sensitized rats after few OVA challenges[42]. However, evidence from animal models and humans indicate that Th2 hypothesis is an incomplete explanation for asthma pathogenesis, as allergic and nonallergic types are pathologically indistinguishable[43]. It has also been reported that airway epithelial cells in asthmatics upregulate the EGF receptor (EGFR) expression, a receptor tyrosine kinase (RTK), even in absence of significant eosinophilic inflammation[44]. A recent study demonstrated subepithelial fibrosis in severe asthmatics without evidence of Th2 inflammation[45]. Depletion of $CD4^+$ cells, previous to chronic OVA challenge, significantly reduced peribronchial inflammation but did not

completely reverse ASM thickening[46]. Although Th2 cytokines have pro-remodeling actions *in vitro*, controversial results have been found as IL-5 and IL-13 do not increase ASMC proliferation, but they induce phenotypic switching[47, 48]; and IL-4 inhibits ASMC replication[49]. These studies suggest that remodeling can also occur independently of Th2 inflammation and other factors are needed.

COPD is also accompanied by airway inflammation that is different from asthma, but ASM remodeling still occur[21]. Chronic inflammation induced by chronic cigarette smoking consists of neutrophil, macrophage, B cell, and $CD8^+$ T cell recruitment, and it worsens as disease severity increases[50]. T-lymphocytes and macrophages are the predominant cells, being $CD8^+$ T-lymphocyte infiltration the most remarkable feature in both large and small airways, and there is also absence of significant eosinophilic inflammation[51]. Although, $Foxp3^+$ regulatory T cells play a role in fibrogenesis, there is no predominant T $CD4^+$ subset. This supports the concept that cyclic events of cytokine and growth factor surges could be the main drivers regardless of the etiology and immune polarization.

An intricate network underlies the ASM and its surroundings, not only immune cells but also neural parasympathetic endings, mesenchymal cells, ECM, and epithelium (see Fig.2). For example, ASM activation by proinflammatory cytokines and substance P can induce the brain-derived neurotrophic factor (BDNF) expression for spatial coordination of neuronal branching[52], and vascular endothelial growth factor (VEGF) for control of angiogenesis to assure adequate perfusion, the latter have an important repercussion on vascular leakage and vasogenic edema during fatal asthma[53]. Neuronal development also would coordinate spatial distribution of ASM, because substance P induces both migration and proliferation[54]. Nevertheless, the airway epithelium could have a greater contribution due to its plasticity and inflammatory properties. Dysfunctional epithelial cells release growth factors, as well as, acetylcholine (ACh) and leukotrienes that could contribute to ASM growth, ECM deposition, and angiogenesis[55]. Moreover, the epithelium is an important source of nitric oxide (NO) in the airways, which has relaxing and other anti-remodeling effects. Physiological NO is produced by constitutively expressed neuronal and endothelial NO synthase (n-,e-NOS)[56]. However, cytokines increase inducible NOS (iNOS) and arginase expression. A greater iNOS/ arginase activity decreases L-arginine bioavailability, which generates an uncoupled iNOS that not only synthases NO, but it also produces superoxide and peroxynitrite. These molecules are capable of causing cellular toxicity and promoting AHR[57]. Functional consequences of increased arginase are reinforced by L-arginine transport blockage with eosinophil-derived polycations. L-Ornithine, a product of urea cycle, is a precursor of polyamines and L-proline, both involved in cell proliferation, collagen synthesis and chromatin remodeling[58]. This exemplifies how noncontractile ASM functions are modulated by many conditions; therefore, the commonest experimental approaches based on univariate analysis can under- or overestimate their contribution on smooth muscle processes.

ASMC are multifunctional

The relevance of ASMCs in pulmonary diseases has been recognized since the last century. The consensus until a few years ago was to consider them just as effectors. However, far from their abilities to contract and relax, ASMCs proliferate, migrate, secrete chemokines/cytokines, and express surface receptors for cell adhesion and leukocyte activation, having a crucial role in airway dysfunction[59]. A concept of plasticity emerged when those functions were associated with specific circumstances and required wide adjustment in gene expression[60, 61]. ASM hypertrophy and/or hyperplasia involve not only outer cell influences, but also ASMC reactions with paracrine/autocrine properties[7, 62]. Quite a lot of molecules could coordinate this loop, such as: growth factors, cytokines, chemokines, ECM molecules, G protein-coupled receptor (GPCR) agonists, natriuretic peptides (NPs), NO, and others[63-66].

Several *in vitro* synthetic functions have been shown. Also, ASM in mild asthmatics has constitutive staining for RANTES[67]. Further cytokines secreted by ASM include IL-1β and IL-6 family cytokines, such as leukemia inhibitory factor (LIF) and IL-11[68]. These have deeper effects on recruitment, proliferation, and differentiation of eosinophils, mastocytes, T cells, and B cells, establishing a bidirectional regulatory network. Mainly, a $CD4^+$ T cell- myocyte crosstalk through direct contact has shown to be determinant of ASM remodeling[42]. Airway homing of T cells is CCL5 or RANTES-guided, which is released by ASMCs. Likewise, strong adhesion between these two cell types has also been described[69]. Remarkably, even though ASMCs are not usually thought as antigen-presenting cells, evidence supports the expression of major histocompatibility complex class (MHC) II molecules making them capable of antigen presentation. Moreover, ASMCs express the cell adhesion molecules (CAMs)/costimulatory molecules, CD40, CD40L, CD80, CD86, ICAM-1 (CD54), VCAM-1 and LFA-1 (CD11a/CD18)[70]. The CD44-dependent T cell adhesion to ASMCs is not only significant to exchange inflammatory signals, but also to induce ASM hyperplasia through RTK activation[71]. This cooperative signaling mediates proasthmatic-like changes in ASM responsiveness, and denotes a potential mechanism of remodeling.

In the airway, a net of collagenous and noncollagenous proteins influences cellular behaviors. ECM components include collagens, fibronectin, members of the matrix metalloproteinase (MMP) family, as well as their inhibitors (TIMP)[59]. After serum stimulation, ASMCs were found to generate elastin, laminin-β1,-2, and -γ1, thrombospondin, collagen-I-V, and decorin[72]. In addition to promoting ECM deposition, ASMCs are capable to affect its degradation. Human ASMCs release progelatinase A (MMP-2 precursor) and, after TNF-α stimulation, gelatinase B (MMP-9)[73]. MMP production suggests that ASM contributes to ECM turnover, and subsequently the airway remodeling, because inhibition of the autocrine-derived MMP-2 has antiproliferative effects on ASMC culture[74]. Therefore, ECM degradation could be essential for ASM phenotypic modulation, being degradation of the pericellular collagen fibrils a requirement to allow cell division[75]. Serum levels of TIMP-1 and MMP-9 are raised in both asthma and COPD, supporting a straight relationship between clinical expression and tissue remodeling. The MMP-9/TIMP-1 ratio and periostin levels could be consider biomarkers of active disease[76]. Cyclic inflammation/ repair simultaneously occur to cyclic ECM degradation and deposition, which could be a critical phase in ASM thickening.

Crosstalk between ASM Remodeling and Hyperresponsiveness

Airway narrowing and abnormal muscle relaxation are the hallmarks of asthma, COPD, and bronchitis. Multiple mechanisms have been proposed to explain the AHR, like increased vagal tone, cytokine-potentiated increment of free intracellular calcium, increased MLCK activity, and activation of the procontractile Rho kinase pathway[77]. All of them have in common that could hasten the shortening velocity. Therefore, even though remodeling can be triggered by hypersensitivity reactions, infections, environmental pollutants, and developmental abnormalities, AHR could be just generated by unbalanced responses to contractile vs relaxing factors[78]. The role of ASM remodeling as a substrate of AHR was uncertain because functional abnormalities can be seen without changes in the bronchial smooth muscle mass[79]. However, increasingly data supports a role in severe AHR phenotypes, and irreversible or partially reversible airflow obstruction[43]. The structure determines both passive tone and active responses to agonist stimulation. ASM remodeling involves phenotypic changes that enhance its thickening, and during this process a decline in force induced by repetitive length changes is seen, but then it rapidly adapts and recovers its ability to generate force. In this way, higher passive stiffness could contribute to increased AHR by attenuating the extent of ASM length fluctuations during tidal breathing, i.e., ASMCs adapt by assuming a shorter resting length while retaining its ability to generate force[27]. For that reason, after induced bronchoconstriction, deep inspiration causes airways of asthmatic individuals to dilate transiently.

Expression of immunomodulatory molecules by ASMCs can delay inflammation resolution and lead to aberrant healing, which is a potential mechanism of AHR[78]. The change in the ASMC population compromises an increase of synthetic properties, which can modulate the contractile mass. Particularly, if it is considered that the whole ASM is coupled by gap junctions, and the calcium dynamic differs between ASMC phenotypes. Propagation of wave-like calcium currents from modulated ASMC to contractile ASM would hypothetically affect not only contractile functions but also noncontractile activities, as discussed in following sections. Other noncontractile elements, including excessive ECM content, may lead to nonreversible airway obstruction by reducing airway distensibility[80]. Whether increased ASM supports abnormal reactions to agonists or makes the airway stiffer, it definitely provides an exceptional substrate for AHR in a framework of progression and severity, at least for asthma. In fatal asthma, airway wall thickness is increased around 50-230%, while in nonfatal asthma it ranges from 25 to 150%, most studies pointing out hyperplasia over hypertrophy as the predominant mechanism[11-13, 43].

ASMC PLASTICITY: ORIGINS AND PHENOTYPES

The ASM thickening has been studied in many animals and human models, wherein ASMC cultures have provided some ideas about pathways underlying the origin of hyperplasia and hypertrophy. Once ASMC populations were characterized *in vivo* and *in vitro*, heterogeneous

subgroups with distinctive phenotypes were identified. A wide range of functions depend on culture conditions[60]. Manipulating such environments allowed comprehension of the rules for phenotypic transition[61, 81, 82]. Hence, ASMC could be sorted into three categories: 1) contractile (c-ASMC), 2) synthetic/proliferative (s/p-ASMC), and 3) hypercontractile (h-ASMC) (see Table 1). Also, a few switching routes have been described, where *modulation* means a shift from contractile to synthetic/proliferative, and *maturation* is the inverse transition. Turning into hypercontractile is also possible, and some authors have speculated about its irreversibility; however, *in vitro* ASMCs can tolerate cyclic phenotypic adjustments. An important aspect is that modulation and maturation exemplify an adaptation model to tissue microenvironment fluctuations, events that could take place *in vivo* and drive critical phases of airway remodeling. Accordingly, transition from native c-ASMC to s/p-ASMC would be the initial step, then, replication of s/p-ASMC would warrant smooth muscle hyperplasia, and finally aberrant differentiation from either s/p-ASMC or c-ASMC to h-ASMC would cause muscle hypertrophy. How these phenotypical modifications fit in the natural history of airway diseases is a matter of debate.

Phenotypic Markers

Smooth muscle has typical features in primary cultures (see Table 1, Fig. 3). A long cellular body, central nucleus, few granulations, and cytoplasmic inclusions (3A), a confluent monolayer with "hill and valley" aspect (**3C**), and shrunk reaction to contractile agonists define smooth muscle cells[83]. However, each ASMC subpopulation has specific characteristics. For example, the synthetic/proliferative phenotype has satellite flattened shape with multiple extensions (3B), a high number of organelles for protein and lipid synthesis, abundant mitochondria, a higher proliferative response, decreased contractile proteins, shutdown of responses to contractile agonists, and secretion of growth factors, collagen, cytokines, bradykinin, and eotaxin[81,84]. Furthermore, modulated ASMCs show increased protein expression of fetal and non-muscle isoforms. Synthetic and proliferative functions do not correspond to different traits. Indeed, the cell distribution with synthetic activities could vary between 20 to 60%, and almost half of replicating ASMCs produce cytokines. Also, secretion can be done by non-replicating cells[81]. On the other hand, the contractile phenotype is associated with a decreased number of synthetic organelles, a stronger response to contractile agonists, increased expression of contractile and structural proteins, and an increased M3/M2 muscarinic receptor expression rate[84-86]. Other markers including Ca^{2+} profiles[87], miRNA expression[88], and transcription factors expression[89] have been useful for phenotype distinction.

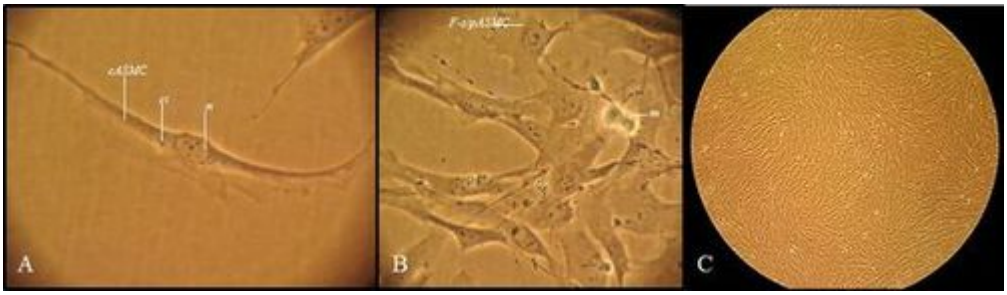


Figure 3. Primary cultured of Rat ASMCs. Cells were obtained by enzymatic digestion of rat trachea and cultured in supplemented medium as previously described(122) . (A) Contractile phenotype, (ci) cytoplasmic inclusions, (n) cell nucleus. (B) When the cell population underwent to growth some cells adopted a myofibroblast-like morphology (F-s/pASMC), (m) mitosis. (C) Cell confluence of 80-90%, cell population readopted contractile morphology with a hill and valley array. Magnification 400X A, B, 100X C.

Table 1. Characterization and Biomarkers of ASMC Phenotypes

Phenotype	Contractile	Synthetic/ Proliferative	Hypercontractile
Function	Regulation of airway resistance	Immunomodulation ECM Turnover Chemotaxis ASM hyperplasia AHR	ASM hypertrophy AHR
Features in primary culture			
Induction	Maturation -High density -Serum starvation	Modulation - Low density - Mitogens	Cyclic exposure to: - Mitogens, TGF-β
Proliferative rate	Slow	Fast	--
Morphology			
Shape	Elongated-spindle	Satellite Flattened	Elongated-spindle
Myofilaments	+	+/-	+++
Contractile proteins/cell size ratio	↔	↓	↔
Mitochondria	+	+++	++
SR/ Golgi apparatus	+	+++	++
Caveolae	+ (> 150.000)	-	+++
Gap junctions	+++	+	+++
Biomechanics			
V_o/V_{max}	Normal	↓	↑↑↑
Contribution to airway resistance	Reversible (normal contraction-relaxation)	-- (affect dynamics of contractile mass)	↑↑↑: ↓ relaxation
Response to agonists	+	+/-	+++: AHR
Biomarkers			
Contractile Proteins			
sm-α-actin	++	+++	++
sm-MHC	++	+/-	++
Structural Proteins			
non-sm-actin (β/γ)	+/-	+++	++
non-sm-MHC	+/-	+++	?
vimentin	+	+++	?
smoothelin-A	++	+/-	?
SM-22	++	+/-	?
Regulatory Proteins			
MLCK	++	+/-	++++
***h*-Caldesmon**	++	+/-	?
***l*-Caldesmon**	+/-	++	?
Calponin	++	+/-	?
Caveolin	+++	+/-	?
Desmin	++	+/-	?
PKC (α/βI/βII)	+/-	+++	?
CD44	+/-	+++	?
M_3AchR	++	+/-	+++
ET_A receptor	++	+++	?
ET_B receptor	++	+/-	?
Distrofin	++	+/-	?
Integrin $\alpha_7\beta_1$	++	+/-	?
Cathepsin k	+	++	?
FSP	++	+	?
AKR1C3	-	-	-
Calcium Dynamics	Sparks	Waves	Sparks

++: expressed; +++: highly expressed; ++++: very highly expressed; +/-: poorly expressed; V_o: velocity of shortening; V_{max}: maximum shortening capacity. ?: unknown, ↑: increased, ↓: decreased, *h*: heavy form, *l*: light form, FSP: fibroblast surface protein, AKR1C3: Aldo-keto reductase 1C3 (specific marker of mesenchymal cells)

Evidence of *in vivo* Plasticity

Several findings support *in vivo* occurrence of ASMC plasticity, especially in asthma. Plasticity is a universal property of primary ASMC cultures derived from both healthy and diseased humans, and healthy and sensitized animals. Immunohistochemistry to identify contractile proteins is highly variable as well, which can reflect a broad heterogeneity of myocytes in the normal airway that is maintained in cell culture, as demonstrated by a divergent proliferative capacity[90]. If functional features are compared, healthy or control vs asthmatic or sensitized groups, significant differences can be found. ASMCs from asthmatics or sensitized animals show more proliferative and synthetic capabilities than their physiologic counterparts, findings that are preserved despite tissue dissolution follow by cell culture[62, 66, 89, 91-93]. Abnormal ASMCs could not only resemble s/p-ASMCs or arise from c-ASMCs, but also have distinctive features such as: abnormal protein synthesis[94], expression of odd transcription factors isoforms with a lack of response to glucocorticoids[91], increased mitochondrial biogenesis and activity[93], abnormal calcium dynamics[92], increased CysLTR-1 leukotriene receptor expression[55], increased activity of promitogenic pathways[95], and declined of antiproliferative pathways[66]. In consequence, an increased ASM mass may be explained by *intrinsic* alterations in pathological ASMCs that facilitate their proliferative and secretory activities. Asthmatic ASM produces more proinflammatory, proangiogenic, and proremodeling factors, including eotaxin, VEGF, and connective tissue growth factor (CTGF), and fewer antimitogenic factors, such as E2- type prostaglandin (PGE2)[59]. These would reflect deeper differences in cell populations that constitute the ASM under pathological settings, and they would likely originate from comparable modulation and maturation events on native ASMCs.

Many questions arise from the alterations observed on asthmatic or sensitized cultured ASMCs. Based on the plasticity phenomena, any transformation of *in vivo* ASMC phenotype which persistence depends on tissue microenvironment should not be seen *in vitro* because the phenotype will adjust to culture conditions. Although, cited studies do not precise whether those pathologic features are irreversible along culture passages, persistence of functional abnormalities after tissue fragmentation and culturing suggests that these cells underwent through a dysfunctional route of phenotypic modulation, which could be at least partially irreversible. In view of that, epigenetic mechanisms could be a suitable explanation to such phenotypic switch. For example, eotaxin hypersecretion by ASMCs has been related to histone H4 lysine 5 and lysine 12 acetylation at the eotaxin promoter induced by TNF-α[96]. Other synthetic activities, such as VEGF hypersecretion, were due to a loss of a repression complex, in which a differential histone H3 lysine 9 methylation modulating Sp1 and RNA polymerase II binding to the VEGF promoter was implicated[97]. Binding of serum response factor (SRF), a transcription factor that controls phenotypic stability, to DNA is associated with post-transcriptional histone modifications including di-methylation of lysine residues 4 and 79 on histone H3, acetylation of lysine 9 on histone 3 and acetylation of histone H4. Histone deacetylases (HDACs) have also been implicated in regulating smooth muscle replication because the HDAC inhibitor TSA can prevent cell proliferation[88]. In *in vivo*, the valproic acid (HDAC inhibitor) did not affect inflammation induced by OVA challenges, but notably reduced the airway thickening including the ASM with blunting of AHR[98]. In addition to HDAC modifications, DNA methylation can generate a specific long-term *signature*. For example,

expression of IL-13 in the airways ensued significant changes in methylation of 177 genes, most of which were associated with a Th2 signature over resident cells[99]. Using methylated DNAimmunoprecipitation-next generation sequencing (MeDIP-seq), it was determined that airway remodeling and AHR in house- dust- or mite-sensitized rats are related to specific methylation patterns at several TGF-β signaling-related genes[100], explaining the longevity of abnormal pro-fibrotic responses of local cells during inflammation and phenotypic persistence after tissue extraction. Unfortunately, there is currently no direct evidence of epigenetic regulation of ASMC proliferation. Moreover, whether or not DNA methylation or histone acetylation can influence phenotypic switching have to be determined as well. The contribution of miRNAs will be discussed in following sections.

Potential Sources of ASMCs

The *in vivo* source of ASMCs under pathological conditions is unclear. ASM may originate from increased proliferation or prolong survival of preexisting smooth muscle with proliferative and/or contractile phenotype; however they could also arise from other cell lines that could migrate into the bundles and then differentiate into ASMCs (see Fig.4). Remarkably, other airway cells may undergo to phenotypic modulation that is characterized by α-sm-actin expression and development of organelles for synthetic functions. Accordingly, mesenchyme such as fibroblasts may generate myofibroblasts, whose classical phenotypic markers are indistinguishable from s/p-ASMCs[101]. This fact allowed researchers to postulate a spectrum of mesenchymal plasticity (fibroblasts ↔ myofibroblasts ↔ ASMCs)[102]. However, it does not undermine experimental findings obtained with *in vitro* systems, as a high proportion (~60%) of primary airway mesenchymal cultures truly correspond to primary smooth muscle[60].

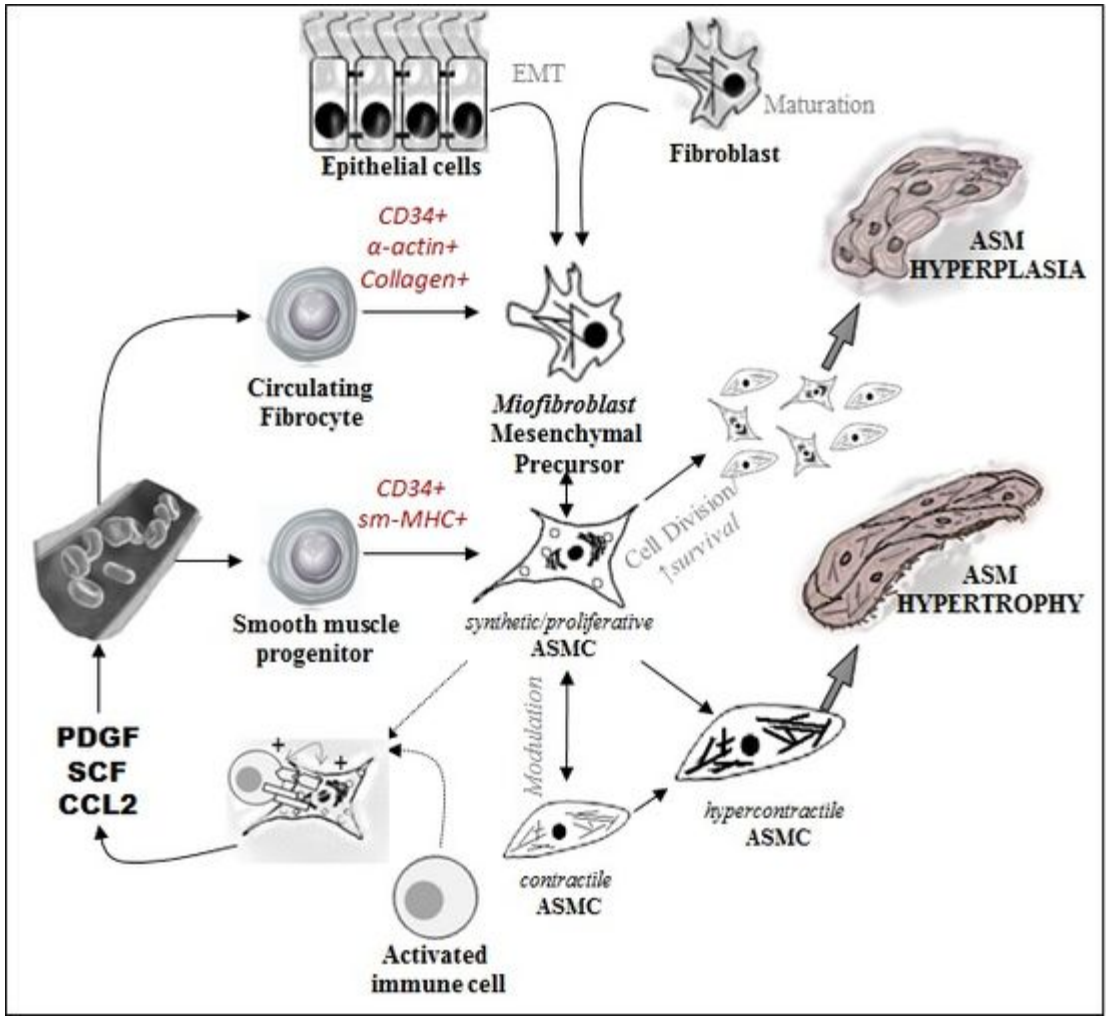


Figure 4. Potential sources of ASMC precursors in the origin of ASM thickening. (See the text for explanation).

Potential progenitors also include true multipotent mesenchymal progenitors and stem cells, either located within the airway or derived from peripheral blood. For example, $CD34^{+}$-$CCR7^{+}$-Collagen 1^{+}-sm-α-$actin^{+}$ circulating fibrocytes can migrate towards ASM bundles during inflammatory challenges, and they were unresponsive to the apoptotic effects of glucocorticoids in culture[103]. Fibrocyte migration is directed by the ASM-derived PDGF and CCL2, and at that point its co-locating induces proinflammatory activities in ASMCs[104, 105]. A rare population of $CD34^{+}$-sm-MHC^{+} peripheral mononuclear cells (known as smooth muscle progenitors) has been identified by flow cytometry in OVA-sensitized mice. A similar population seems to generate the smooth muscle in atherosclerosis; however, the study did not precise whether stem cell homing occurred into ASM bundles[106].

The airway epithelium can turn into mesenchymal cells through the epithelial-mesenchymal transition (EMT) route, which has been considered as another source of ASMCs[107], but a linage-tracing study suggests that it may just be a consequence of culture conditions and could not occur *in vivo*[108]. In asthma, epithelial cells show fragileness due to downregulation

of cell adhesion molecules, which makes EMT more likely[109]. EMT is initiated by extracellular signals, such as collagen or hyaluronic acids, and by growth factors like TGF-β and EGF[110]. This process is modulated by bone morphogenesis proteins, and allergen exposure, which amplifies and accelerates it[111]. Hormones have also been associated, since vitamin D attenuates TGF-β-induced expression of EMT markers[112]. Epithelial and mesenchymal cells express both type-1 and type-3 muscarinic receptors (M1, M3)[113]. TGF-β-induced EMT was abolished by muscarinic receptor (mAChR) antagonists and enhanced by acetylcholinesterase (AChE) inhibitors[114]. A positive feedback loop of autocrine and paracrine production of non-neuronal acetylcholine (ACh) and TGF-β orchestrates EMT during chronic inflammation, being a likely source of ASM.

Additionally, an increased number of fibrocytes was observed in the ASM bundles from asthmatics of all severities[115]. However, this study failed to show any link with the lung function, and this location could not be considered abnormal as fibrocytes are normal constituents of ASM bundles under physiological conditions[116]. An increased in mesenchymal cells would be nonspecific and occur in parallel to other cellular changes ongoing in the ASM bundles. Although, many airway cell lines can follow similar modulation pathways as native ASMCs, we focus here on how the behavior and responses of c-ASMC vs s/p-ASMC can explain many abnormal structural and functional features seen on airway diseases.

ACETYLCHOLINE: MORE THAN BRONCHOCONSTRICTION

The parasympathetic network penetrates deeply the airway wall and regulates bronchoconstriction. ACh is the predominant parasympathetic neurotransmitter. Although, ASM express both cholinergic receptors, nicotinic receptors (nAChRs) and mAChRs, the cholinergic effects seem to be mediated by muscarinic activation[117]. In asthma, cutting the parasympathetic supply of the airways (vagotomy) prevents an increased smooth muscle contraction[118], and AHR induced by persistent parasympathetic activation has been shown[119]. The striking role of ACh in airway remodeling was highly commented in a recent article[8], where authors proposed methacholine-induced bronchoconstriction as the main driver of epithelial hyperplasia and subepithelial collagen deposition, independently of inflammation, as chronic stretch and mechanotransduction pathways are well-known mechanisms of muscle differentiation. However, the cited study failed to conclusively demonstrate that only mechanical stress was responsible of all histopathological changes, because they did not assess the non-neuronal and non-contractile effects of cholinergic networks, a non-asthmatic group was not included (taking into account that inflammatory signatures on resident cells change their responses), and ASM layers were not assessed. Nonetheless, despite the limitations, the contribution of ACh in airway remodeling was uncovered.

Two ACh sources have been identified: 1. neural, along parasympathetic fibers from vagal

nerve, and 2. non-neural, from airway epithelium and immune cells. Both are implicated in increased ASM thickening in asthma[120], which was prevented by M3-specific antagonists such as tiotropium bromide[121]. Also, M2 is expressed and its downstream signaling has been related to modulation of some ASMC functions, either promotion or inhibition[122]. ACh, either neuronal or non-neuronal, regulates inflammatory cell responses that may explain the anticholinergic benefitsin asthma and COPD[123]. Collectively, these findings are revealing new therapeutic targets, therefore, we chose the cholinergic signaling pathway to be explored deeply in this review as a prototype of GPCR agonism.

Muscarinic Receptor Signaling

The mAChR family consist of five receptor subtypes that belong to GPCRs. Mammal airways including humans express M1, M2, and M3 subtypes; more precisely, epithelial cells (M1–M4), pulmonary vessel endothelial cells (M1–M5), mesenchymal cells, such as smooth muscle fibers (M2, M3) and fibroblasts (M2> M1> M3> M4), and lung-infiltrating immune cells, such as mononuclear leukocytes (M1–M5) [124]. The M4 mRNA and protein have been reported in rabbit bronchiolar ASM, but not from humans. Although, pharmacological ligand binding studies showed a mixed population M2:M3 in a 4:1 ratio, respectively, a functional dominance of M3 appears to mediate muscarinic effects under physiological circumstances[125]. This predominance could be a consequence of receptor compartmentalization, facilitating or inhibiting signal transduction depending on accessibility to specific transducers or kinetic regulation. M3-induced bronchoconstriction is mainly mediated by a caveolae-dependent system. M2 contribution was mainly uncover in $M3^{-/-}$ knockout mice with cellular caveolae dissolution. Location of M2 and M3 in caveolae is dependent on Caveolin-1 (Cav-1) and Caveolin-3 (Cav-3) expression, respectively[126]. Many processes involve coupling of mAChRs to their cellular effector systems, via heterotrimeric G proteins. These are composed of one α-, β- and γ-subunit and transduction signals depends on both the α-subunit and $\beta\gamma$-subunit. In ASMCs, muscarinic activation is classically considered as the main signal for muscle contraction. However, there is increasing evidence indicating that alterations in its downstream signaling pathways might be responsible for ASM remodeling and AHR[127].

Muscarinic signaling can be divided in pathways for muscle contraction and those with non-contractile effects. In this way, the Gq-coupled M3 activates phospholipase C (PLC), causing hydrolytic conversion of phosphatidylinositol 4,5-biphosphate (PIP2) into inositol 1,4,5-trisphosphate (InsP3) and sn-1,2-diacylglycerol (DAG). InsP3 is involved in Ca^{2+} mobilization from intracellular stores, which generates a rapid and transient increase for muscle contraction, while generated DAG activates PKC with subsequent triggering of mitogen-activated protein kinase (MAPK) signaling for non-contractile effects. Also, MAPK cascade can be activated through direct phosphorylation of Raf-1, independently of PKC[117]. β-arrestins mediate homologous receptor desensitization and endocytosis via clathrin-coated pits of agonist-activated GPCRs. The third intracellular loop (i3) of M3 is required for β-arrestin recruitment after homodimerization (M3/M3) and heterodimerization (M2/M3). Those macrocomplexes not only induce downregulation of mAChRs, but also act as scaffolds for components of the MAPK cascade, facilitating its activation[128]. This is a third mechanism of

M3 signal transduction for cell growth and differentiation.

As a counterpart, Gi/o-coupled M2 contributes to muscle contraction either affecting adenylyl cyclase in an inhibitory manner, or directly enhancing potassium and non-selective ion channels opening, and they both depend on the released βγ dimer. Additionally, M2 modulates the relaxant effects of atrial natriuretic peptide (ANP), as it suppresses the ANP-induced activation of a membrane-spanning guanylyl cyclase via a pertussis toxin (PTX)-sensitive G protein[129]. Since Gi/o is involved in muscarinic-induced actin reorganization, RhoA/Rho-kinase signaling pathway has been related to Ras and phosphoinositide 3-Kinase (PI3-K) activation, contributing to growth factors effects[120].

The complexity of non-canonical muscarinic signaling is illustrated by the ambiguity of their downstream pathways. The cGMP/PKG pathway is a well-documented mediator of muscle relaxation, and it has also anti-remodeling effects. Molecular evidence suggests it could depend on which compartment is activated and signal pattern. Some cascades activated by mAChRs are linked to second messengers such as cGMP by activation of two distinctive guanylyl cyclases. Muscarinic activation of tracheal ASM fragments is associated with contraction, but it also involves the generation of two cGMP signals, at 20-s and 60-s[130]. These signals seem to be essential in reaching the contractile effects of several muscarinic agonists and might be relevant in ASM remodeling. The proposed model[131] for this novel pathway emphasized that 20-s cGMP signal is linked to Gi/o coupled M2 activation, inducing a massive and transient α1β1-NO-soluble guanylyl cyclase (sGC) translocation from cytoplasm to plasma membranes, whereas, the 60-s cGMP signal is associated with a natriuretic peptide receptor (NPR)-GC-B, activated by a Gq16-coupled M3 sensitive to mastoparan. Those signals regulate the muscarinic signal transduction efficacy in response to agonists through phosphorylation events. In this way, M3 phosphorylation by PKG-II may correlate to changes in the receptor affinity to agonists and antagonists. It has been proposed that cGMP induces PKG phosphorylation of the i3 loop that confers a potential feedback mechanism to terminate the cGMP-dependent muscarinic signal transduction cascades at the sarcolemma[132]. Since phosphorylation of the same loop by others serine/threonine kinases downregulates the receptor by endocytosis and G protein uncoupling, indirect observations suggest that PKG-II phosphorylation of M3 induces a mAChR dimer formation. Homodimer formation stabilizes or 'freezes' the M3 population, in a refractory state to agonist activation, and prone to antagonist binding[132]. We speculate in caveolae systems a higher density M3 receptor population would support positive cooperativity through homodimer/heterodimer formation, which could enhance the signal transduction for MAPK activation and subsequent altered gene expression. Thus, cGMP produced in response to muscarinic agonists could be involved in growth promotion instead of classical cell arrest. Relevantly, we demonstrated that this pathway is still functional in sensitized ASMCs, while cGMP cascade induced by NPs and NO was downregulated[131]. A wide-spectrum of outcomes can result from diverse experimental designs. This complicates our understanding of the cholinergic signaling in human airway diseases. A brief scheme that will be discussed herein, includes effects related to remodeling promotion: 1. c-ASMC modulation, 2. s/p-ASMC proliferation synergism, and 3. synergism on h-ASMC induction, and alternatively possible actions for remodeling prevention like decreased s/p-ASMC proliferation. Although, *in vivo* relevance keeps hypothetic, this provide a coherent

and useful picture for planning future research.

MODULATION VS MATURATION

Signaling Pathways Associated with Maturation

Phenotypic switching is regulated by growth factors, GPCR agonists, ECM molecules, and other mediators found in the bronchoalveolar lavage (BAL) of patients with asthma or COPD[133,134]. The contractile phenotype induction is attainable after exposure to either TGF-β, insulin, or laminin[135-138]. Also, maturation can be supported by lacking of mitogens; in primary ASMC cultures (see Fig.3C), high cell confluence lead to cell cycle arrest by cell contact[82, 86]. sm-specific protein expression is enhanced by RhoA/Rho Kinase and/or PI3-K activation. RhoA/Rho kinase promotes actin polymerization as a downstream effector of either GPCR- or RTK-associated pathways (see Fig.5). Subsequent phosphorylation events, RhoK→ phospholipase D (PLD) → LIMK-1→ cofilin could be responsible for G-actin polymerization into F-actin[139]. A decrement in globular actin level releases some proteins into the cytosol, like the transcriptional coactivator megakaryocytic acute leukemia/ megakaryoblastic leukemia-1 (MAL/MKL-1), which can be trafficked into the cell nucleus and make a macrocomplex with both myocardin (other coactivator) and SRF, a transcription factor. As a whole, they bind gene promoters to increase sm-specific gene expression[140, 141]. TGF-β can amplify this pathway for hypercontractile phenotype induction. Type-1 and type-2 TGF-β receptor activation induce phosphorylation and nuclear translocation of Smad proteins that bind to SRF, building up a macrocomplex similar to MAL/MKL-1/myocardin/SRF, with subsequent gene expression modifications[142].

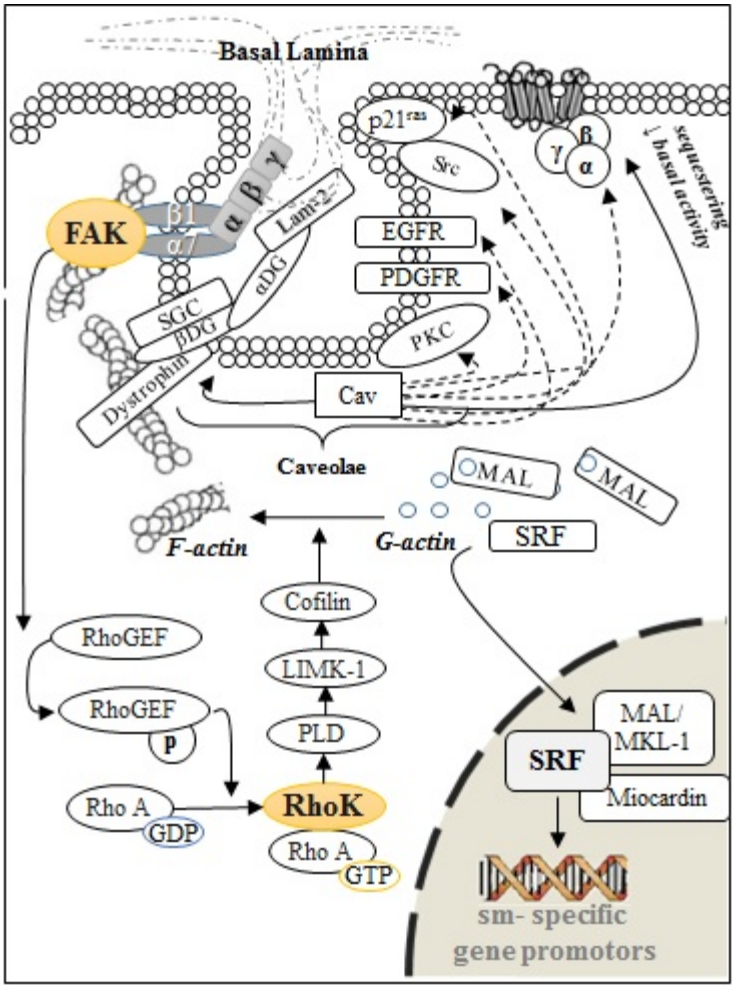


Figure 5. Signaling pathways involve in preserving the ASM in a differentiated state.

The Akt/PI3-Kpathway also affect sm-specific gene expression through the transcription factor FOXO-4[143, 144]. Unphosphorylated FOXO-4 binds myocardin and inhibits its association with SRF. Hence, myocardin is released once PI3-K phosphorylates FOXO-4. However, the reach of this pathway goes far beyond transcriptional regulation. Signaling pathways that converge to ribosomal regulation are needed to complete ASMC maturation, which include effectors such as PI3-K, Akt-1, mammalian target of rapamycin (mTOR), and p70 ribosomal S6 kinase ($p70^{S6K}$). Pharmacologic inhibition of PI3-K and mTOR are enough to prevent $p70^{S6K}$ activation and sm-specific protein accumulation[143]. Moreover, activated mTOR can phosphorylate eIF4 binding protein-1 (4E-BP1), releasing and increasing the eukaryotic initiation factor-4 (eIF4) activity[145], followed by contractile protein accumulation (see **Fig. 6B**).

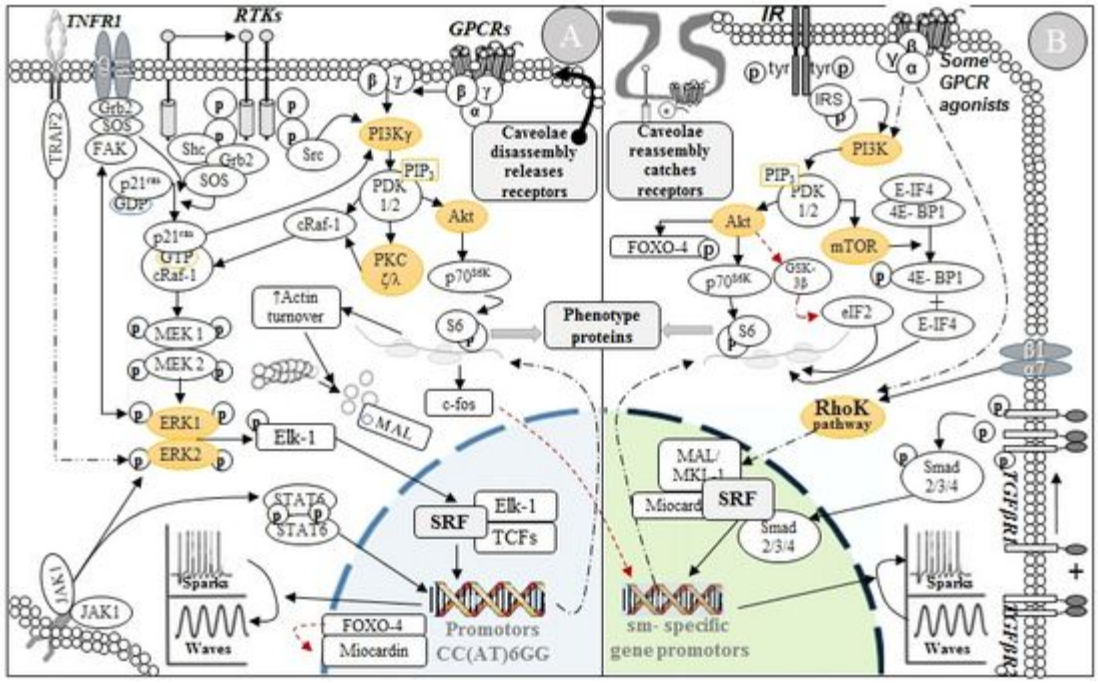


Figure 6. Signaling pathways involve in the control of ASM phenotypes. (A) Modulation, (B) Maturation.

Persistence of the c-ASMC phenotype is highly dependent on caveolae membrane system and cav-1 expression (see Fig. 5)[146]. These flask-shaped structures are classically considered as special compartments for signaling regulation. Cav-1 is located inside those microdomains, being responsible for their formation and maintenance. Interestingly, cav-1 leads to reduced basal activity and sequestration of several receptors and signal transducers related to synthetic/proliferative phenotype induction, such as: PKC, PDGFR, EGFR, Src, and p21ras[147]. Proteins such as α-subunit of G proteins, Rho family members, adenylate cyclase isoforms, 7TM receptors, and others with binding domains to glycosyl- phosphatidylinositol are regulated as well. Caveolae act on behalf of cell adhesion by linking the actin cytoskeleton with the basal lamina, facilitated by laminin-2/α7β1 integrin interaction. The triggered downstream pathway activates a guanine nucleotide exchange factor, RhoGEF, leading to Rho Kinase activation[147]. For this reason, it is possible that mitogen stimulation is not enough to ignite ASMC modulation and cell division, thus caveolae disassembly would be a strict requirement[148].

Laminin is a trimer that bind integrins and non-integrin receptor subtypes, including the dystrophin-glycoprotein complex (DGC). PI3-K inhibition prevents both ASMC maturation and accumulation of DGC proteins, β- and α-DG[149]. Preferential expression of DGC in c-ASMC is related to a tighter regulation of reactions to contractile agonists by caveolae system. There is also evidence suggesting that DGC, through β-DG, influences signal transduction by scaffolding properties and interactions with cav-1[150]. Additionally, basal activity and low grade stimulation of some RTKs, such as the insulin receptor (PI3-K pathway) and GPCRs (Rho Kinase pathway), could have a role in contractile phenotype conservation, especially in confluent cells. These observations acquire more relevance considering that the ASM mass is likely composed of c-ASMC under physiological conditions. In the disease-setting, ECM

component dissolution by MMPs affect the ASMC attachment, shutting down the Rho kinase activity. Unconstrained ASMCs are more susceptible to paracrine influences. Thus, high levels of cytokines and growth factors, and subsequent anomalous repair due to TGF-β, might match with a continuous and dynamic process of phenotypical modulation (contractile → synthetic/ proliferative → hypercontractile/ fibrotic), explaining the ASM thickening and dysfunction.

Signaling Pathways Associated with Modulation

Transition to a synthetic/proliferative phenotype is enhanced by mitogens such as PDGF, EGF, IGF, fibronectin, collagen-I and -II, bradykinin, GPCR agonists, cigarette smoke extract, lipopolysaccharide (LPS), and reactive oxide species (ROS)[151, 152]. Modulation is quickly reached when *in vitro* ASMCs are not confluent under mitogenic influences (see Fig. 3B), especially fetal bovine serum (FBS) and fetal calf serum (FCS)[86, 122]. Associated pathways converge to increase c-fos (coactivator) expression, which paradoxically needs a prior SRF activation in order to alter gene expression[144]. Nevertheless, this apparent duality could be due to a wide variety of transcriptional coactivators that assertively induce selective gene transcription. Contrasting the SRF-myocardin complex effect, SRF cooperativity with ternary complex factors (TCF) such as Elk-1 affects gene promotors with a CArG (CC(AT)6GG) sequence, inducing cell proliferation instead of maturation. In this way, c-fos upregulation is key to halt contractile gene expression[153]. Furthermore, this coactivator Elk-1 is phosphorylated and activated by the extracellular signal- regulated kinase (ERK)-1 and -2[154]. These kinases along with p38, c-Jun N- terminal kinase (JNK), janus kinases (JAKs), and transcriptional factors, like NFκB and AP-1, could participate in signal delivery to increase synthetic and proliferative activities[154]. Protein synthesis associated with modulation is also favored following S6 ribosomal subunit phosphorylation[138]. Increased cytoskeleton metabolism with actin polymerization blockade generates G-actin accumulation that avoids nuclear translocation of MAL/MKL-1. Also, it has been described that *in vitro* both maturation and modulation are reversible.

ASMC modulation is improved by Th2 cytokines. A mix with TGF-β and leukotriene D4, triggers the expression of 29 transcription factors[155]. IL-13 is relevant to control the expression of aroung 300 locus. Its receptor, the IL-13Rα1/IL-4Rα complex, mediates the phosphorylation of STAT-6, triggering MAPKs for phenotypic modulation[156]. IL-13 can also affect calcium dynamics by upregulation of sarcolipin, which is a transmembrane protein placed at the sarcoplasmic reticulum (SR) that inhibits the sarco/endoplasmic reticulum Ca^{2+}- ATPase (SERCA) activity[157]. Expression of calcium regulatory proteins changes with modulation, therefore, a decrease in voltage-dependent calcium channels, ryanodine receptors, and SERCA2 levels translate into a calcium dynamics dominated by wave-like propagations. This kind of flow contributes to MAPK pathway activation. Ca^{2+} waves also affect the conformational stability of cis elements in 5'untranslated regions (UTRs) of mRNAs and interactions between translational components, regulating protein synthesis[158]. IL-13 signaling is under control of the type-1 suppressor of cytokine signaling (SOCS-1), a protein with chaperone properties. SOCS1 expression is decreased in asthmatic ASMCs and its

inactivation raises synthetic activities when exposure to Th2 cytokines[159]. In summary, diverse signaling pathways are responsible for driving phenotypic modulation of ASMCs (see Fig. 6A).

Muscarinic Activation leads to ASMC modulation

ASM thickening is attainable by persistent muscarinic stimulation[127]. Both pathways, Gi/0 coupled M2 and Gq coupled M3, could generate the activation of MAPK, Rho- kinase, and PI3-K signaling[117]. Moreover, shifting from a synthetic-proliferative to a contractile phenotype is accompanied by a decrease in M2 and a parallel increase in M3 expression[160]. Those observations inquire whether or not cholinergic stimulation may affect phenotypic switching. Accordingly, long-term incubation of rabbit ASMCs with ACh or carbachol (CCh) induced a switch towards s/p-ASMC[161]. Prolonged treatment of bovine ASM strips with the methacholine also diminished contractile protein expression[162]. Transition to a synthetic-proliferative phenotype is characterized by M3 downregulation and blunted contractile responsiveness to cholinergic stimulation[161]. In cited studies, signaling pathways were not evaluated, but considering that cholinergic-induced mitogenesis is related to MAPK activation, it is possible that muscarinic activation allows the nuclear translocation of Elk-1, affecting gene expression linked to the phenotype transition.

Role of non-coding RNAs on Phenotypic Stability

The miRNAs are small noncoding RNAs that have an outstanding participation in gene expression regulation. It makes them excellent candidates to control cell plasticity. Multiple mechanisms are involved in miRNA synthesis and gene regulation, as it was previously described[163]. Shortly, mature miRNA is part of the active RNA- induced silencing complex (RISC) that mediates miRNA/mRNA interaction in a specific fashion. This interaction mostly occurs in the 3'UTR by partial complementarity, thus, miRNAs inhibit elongation during translation, or destabilize mRNA promoting its degradation. In smooth muscle biology, multiple miRNAs regulate cell differentiation and proliferation, under physiological and pathological conditions, especially in vascular smooth muscle, although little is known about ASM (see **Table 2**)[164].

Table 2. Functions of non-coding RNAs in ASM

	RNA (Contribution/ Effect)
Phenotypic Stability	miR-25 (↑modulation) miR-10a (↑maturation, keeps steady state, stem cell differentiation to smooth muscle) miR-26a(↓proliferation, hypercontractile induction) miR-143, miR-145(↑ accumulation contractile proteins)
Inflammatory Response	let7, miR133a(↑RhoA, AHR induction), miR-143 (modulates cell migration), miR-145 (cytoskeletal remodeling, affects podosome formation), miR146a (see table 4), miR 155 (see table 4), miR-708 (negative feedback to TNF-α)
Cell Division	miR-25 (↑proliferation), miR221 (↑proliferation), miR-371-5p, miR-718, miR-1181, miR-1207-5p, miR-1915, miR-3663-3p, LINC00882-002, LINC00883-005, BCYRN1, RP11-46A10.4
Airway Remodeling	miR-16, miR-25(↓matrix protein synthesis), miR26a (airway remodeling by chronic stretch, ↓GSK-3β), miR-133a (↓SRF), miR-146a
Innervation	miR-206 (neuronal branching)

Around 11 miRNAs are upregulated in cytokine-exposed ASMCs. Particularly, miR-25 is significantly modulated after prolonged OVA-challenge[165]. Some cytokines through the ↑miR-25/↓KLF-4 system promote ASMC modulation[166]. The transcription factor kruppel-like factor-4 (KLF-4) represses sm-specific gene expression by recruiting histone H4 deacetylase activity to smooth muscle cell genes, thereby blocking SRF association with methylated histones and CArG box chromatin. Next-generation sequencing identified miR-10a as the most abundant miRNA expressed in primary human ASMCs, accounting for more than 20% of all small RNAs. miR-10a directly suppresses PI3KCA expression and its overexpression reduces ASMC proliferation[167]. TNF-α-induced expression of miR-708 in asthmatic ASMCs is greater than in non-asthmatic. miR-708 decreased JNK, MAPK and Akt phosphorylation and increased MAPK phosphatase-1 (MKP-1) and phosphatase and tension homolog (PTEN)expression. It constitutes a negative feedback for TNF-α signaling downregulation[168]. miR-133a levels were decreased in human ASMCs, along with upregulated RhoA expression during AHR. Those findings were replicated after treatment with IL-13[169]. Sonic hedgehog signaling blocks miR-206 expression to increase the release of BDNF by ASMCs, coordinating branch innervation[52].

A recent study explored the RNA expression profile in cultured ASM[170]. Remarkably, over 200 miRNAs were detected including: miR-371-5p, miR-718, miR-1181, miR-1207-5p, miR-1915, and miR-3663-3p. These miRNAs had been previously related to aberrant proliferation in other cells. Paradoxically, predicted targets cut down gene expression of proteins that are known for remodeling promotion. They also detected a specific long non-coding RNA (lncRNA) profile. lncRNAs have recently emerged as epigenetic tools for gene expression regulation. They can regulate miRNAs as target site decoys, can also directly bind to transcription factors and

participate in assembly of chromatin-modifying complexes as structural components and recruiters of genomic targets[171]. Stimulated human ASMCs expressed 29 lncRNAs, and some of them were previously identified as cell proliferation regulators. Relevantly, an increase in LINC00882-002 and LINC00883-005, and a decrease in BCYRN1 and RP11-46A10.4, could explain why despite of specific miRNAs, a target mRNA transcript is still translated. These lncRNAs could act as 'sponges' for the miRNAs-1207, -150, -940, and -371, blocking the RISC association with the translational machinery. The analysis is more complex if it is considered that a variable expression is seen in each cell cycle phase. In summary, phenotypic switching encompasses responses exquisitely coordinated by multiple signaling pathways, orchestrating gene expression not only at a promotor level, but also involving specific changes in the RNA metabolism.

ASM REMODELING AS A THERAPEUTIC TARGET: EXPERIMENTAL EVIDENCES

Our current failure to treat some phenotypes of severe asthma is a reflection from our poor understanding of its underlying etiology. Classic interventions are directed to exacerbation management and prevention, including mainly bronchodilators and steroids to keep most patients away from flares, but in many cases they are ineffective as shown by clinical trials. Prospective placebo-controlled studies have not shown long-term beneficial effect of steroid treatment for RSV bronchiolitis and subsequent wheezing or asthma[243]. Doubling the dose of inhaled steroids in moderate to severe asthmatics was ineffective in two unrelated studies[280, 281]. Moreover, in adults with persistent asthma even optimal therapy is only able to reduce the frequency of exacerbations by around 40%[282]. In school-age children, moderate doses of inhaled steroids are completely ineffective at reducing exacerbation frequency, duration and severity of wheezing episodes associated with viral infection[283]. Furthermore, 5-day course of oral steroids at the onset of exacerbations in preschool children was ineffective at reducing the duration or exacerbation severity, even in children with systemic eosinophilia[284]. Not to mention the detrimental effects of glucocorticoids, seen on patients who had fatal asthma, in addition to the side effects by a prolonged therapy[285]. The reasons of failure could be secondary to an acquired insensitivity to glucocorticoid actions, which could prevent these drugs from blocking many events that conduce to airway remodeling. A review of the potential mechanisms has been recently published[286]. It is beyond the objectives of this review to analyze all the current recommended asthma and COPD therapies, for which there are available guidelines[287, 288], or to underestimate the corroborated benefits of steroid treatment in the vast majority of asthmatic patients. However, recognizing that common interventions could not affect final outcomes is crucial to find better therapeutic targets. If novel therapies for airway remodeling are developed, it is possible that steroids might be sent apart from the primary treatment of asthma, and COPD progression could be effectively delayed. In this section, we explore some experimental evidence that supports such potential interventions.

Long-Acting Muscarinic Receptor Antagonists

The long-acting muscarinic antagonists (LAMAs), aclidinium, glycopyrronium and tiotropium, bind to human M1-M5 receptors in a concentration-dependent manner, but the highest selectivity is for M3, followed by M2[289]. Blockage of mAChRs has anti-inflammatory and anti-remodeling properties, although, most studies include only tiotropium in their protocols. For example, the anti-inflammatory activity associated with tiotropium on cigarette smoke-induced pulmonary inflammation in mice was related to a dose-dependent reduction of leukotriene-B4, IL-6, chemokines and TNF-α, and also a decreased cell numbers in BAL[290]. In the same way, eosinophil recruitment and AHR in a guinea-pig model of asthma were inhibited by vagal blockade-independent mechanisms[291]. The mechanism of tiotropium was linked to inhibition of TGF-β-induced MAPK signaling and a decreased MMP expression[292]. LAMAs would further affect cell plasticity, as it was demonstrated that aclidinium can inhibit the CCh-, TGF-β-, and cigarette-induced transition from human fibroblast to myofibroblast[293]. Tiotropium also significantly inhibited ASM thickening and Th2 cytokine production by human peripheral blood mononuclear cells in a murine model[294]. The anti-remodeling effect also include decreased sm-MHC expression and decreased isometric relaxation of tracheal strips that were previously exposed to repeated allergen challenge[121]. From the clinical standpoint, in COPD, all three drugs produced significant FEV1 improvement,but only glycopyrronium reduced dyspnea. In severe asthma, only tiotropium has been tested, and it has demonstrated to raise FEV1and decline the risk of exacerbations[289].

Emerging Therapies: Statins, Macrolides, Endothelin Antagonists, Calcium Channel Antagonists and PPARγ Agonists

Statins, through inhibition of 3-hydroxy-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), reduce the synthesis of groups needed for protein isoprenylation, farnesylation and geranylgeranylation, influencing cell signaling. The inhibitory effect ofsimvastatinon FBS-inducedRhoAactivation is antagonized by geranylgeranyl pyrophosphate, but not by farnesyl pyrophosphate. These isoprenoids are required for prenylation of the small G proteinsRhoAand Ras, and it was shown that inhibition ofASMC proliferationbysimvastatinwas due to prevention of geranylgeranylation ofRhoA, but not by farnesylation of Ras[295]. Moreover, lovastatin, isoprenylation inhibitors, or other pharmacological approaches for preventing localization of RhoA in the membrane localization should be considered as a preventiveantiviraltherapyfor selected groups with high risk for severe RSV disease[296]. However, statins are known to decreased cell survival, impacting on signaling that also contributes to bring cells under molecular stress. Autophagy, especially macroautophagy, was discussed as a potential mechanism of phenotypic modulation. Considering that some RNA viruses take advantage of the double-membrane vacuoles, potential improvement of RV replication could be an undesirable effect due to autophagic induction in response to statins[274]. Affecting some pathways associated with maturation can also reinforce ASMC modulation. This may explain why despite the well-stablished anti-inflammatory and pleiotropic effects of statins, clinical trials still failed to show any improvement of inflammatory and functional outcomes in patients with severe asthma[297].

Macrolides are antibiotics that have been widely used in the treatment infectious diseases.

Additionally, immunomodulatory and anti-inflammatory effects have been shown in relation to a suppression of goblet cell hyperplasia and cytokine secretion by regulating the activation of a MAPK/NF-κB pathway[298]. Experimental evidence supports anti-remodeling actions of macrolides, e.g., roxithromycin inhibited ASMC proliferation in a dose-manner dependent. This effect was dependent on the loss of the mitochondrial membrane potential, cytoplasmic accumulation of Cyt c, caspase activation and increasing of $p27^{Kip1}$expression[299]. The same researcher group showed that roxithromycin decreased bronchial wall thickness andASM layer in OVA-sensitized rats, and also downregulated ERK1/2 and upregulated caveolin-1 expression[300].Long-term therapy may improve some functional parameters without affecting clinical outcomes, as showed by a recent metanalysis[301].

Endothelin-1 induces bronchoconstriction, mediates eosinophil recruitment during allergic inflammation, and contributes to airway remodeling by inducing fibroblast and ASMC differentiation and proliferation[302]. Despite of *in vitro* results obtained with endothelin receptor antagonists, such as sitaxsentan or bosentan, a recent small clinical trial did not demonstrate any improvement of functional tests or symptoms in poorly controlled asthma when compared to placebo[303]. On the other hand, calcium channel blockers were classically tested as bronchodilators, but after recent findings of pro-remodeling effects of altered calcium signals, long-term blockade has been proposed. Therefore, gallopamil administration reduces the mitochondrial mass and subsequent ASMC proliferation[304]. A recent doubled-blind randomized clinical trial showed that this calcium channel blocker decreased ASM thickness after 1 year of treatment. Although, there was no immediate clinical improvement during the treatment phase, a significant reduction in asthma exacerbations related to ASM mass reduction was seen during the follow-up[305]. In the same way, the PPAR-γ ligands, rosiglitazone and pioglitazone, have shown to regulate noncontractile and contractile functions of *in vitro* ASMCs, including decreased in proliferation and synthetic activities by increasing heme oxigenase-1 activity, and β2-AR expression that could reduce AHR[306]. However, its benefits in obstructive airway diseases remains to be tested in humans.

Biologic Therapy

Current management of autoimmune diseases and cancer, is based on blocking specific molecular targets through inhibitors and monoclonal antibodies. Unquestionable evidence has been obtained with anti-IgE (omalizumab) for treatment of severe asthma associated with high IgE levels, being included in GINA guidelines. Similar medications have been considered on behalf of their efficacy in chronic inflammatory diseases. A large list, including anti-IL-5 (mepolizumab, reslizumab, enralizaumab), anti-TNF-α (etanercept), anti-IL-4 (pascolizumab, nuvance), anti-IL-4/13 (pitrakinra), anti-IL-9, anti-CD25 (daclizumab), anti-VCAM-1 and anti-TSLP, are now under clinical trials, and some recent publications have shown controversial results[307, 308]. Based on animal models, blocking those pathways results in a reduced airway remodeling via decreased eosinophil, monocyte and T cell recruitment. However, decreasing inflammation was not always correlated with anti-remodeling effects. Clinical studies have not shown consistent results regarding improvement of FEV1, symptom control, and decreased use of short-acting β2-agonists. A common finding is that the number of exacerbations tends

to decrease in the higher-steroid dose groups, without significant clinical efficacy. Whether or not these results were related to prevention of remodeling is unknown, because histopathological assessment were not included in their protocols.

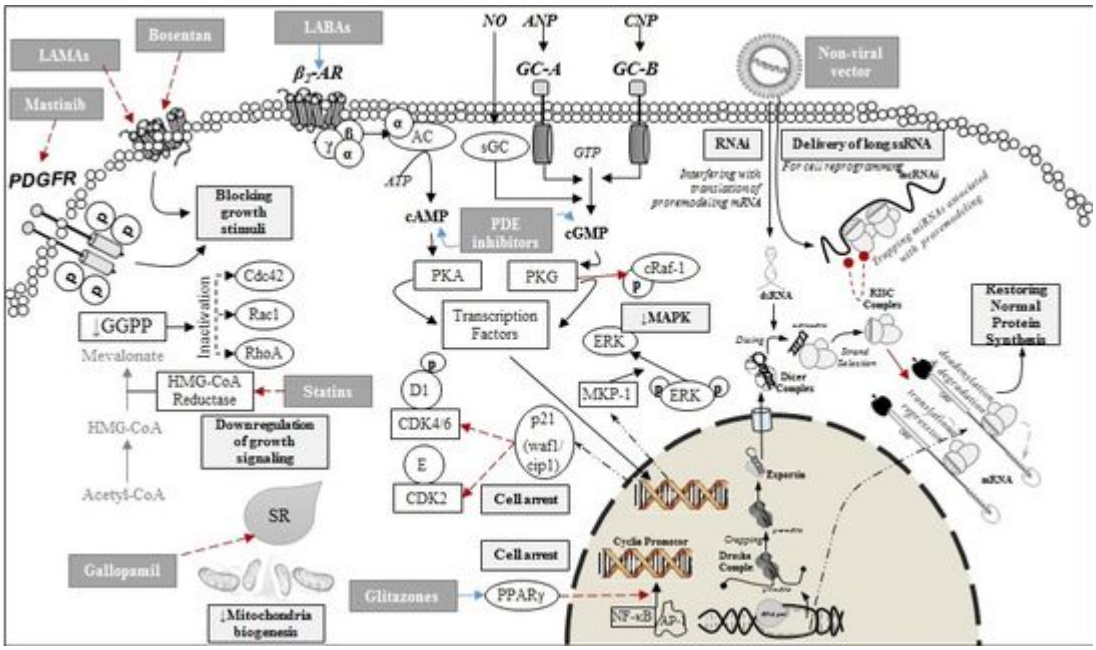
A specific approach is attained with the c-kit/PDGF receptor tyrosine kinase inhibitor mastinib. RTK inhibition by imatinib mesylate decreases collagen deposition, eosinophil infiltration, and ASM thickening in a murine model[309]. Mastinib improved the asthma control score and number of exacerbations[310]. However, no significant improvement in lung function was observed. More randomized clinical trials are needed to precise what biologic therapy is suitable for specific subgroups. New drugs that target specific pathways, such as: antiproteases for modulation of ECM deposition, NFκB inhibitors, PI3K inhibitors, chemokine receptor antagonists, and even old drugs with anti-inflammatory properties, like thalidomide, increase the spectrum of therapeutic interventions[311], but no experimental and clinical research focusing on ASM remodeling have been addressed.

Bronchial Thermoplasty

Reduction of dense ASM using physical forces, like radiofrequency energy, has shown promising results. Bronchial thermoplasty is a FDA-approved bronchoscopy procedure for patients with severe asthma, which delivers high thermal energy to the airway wall to heat and reduce the amount of its cellular components. Although, airways swell on immediate heat administration, this blanching and erythema usually resolved within 1 week, and no long-term adverse effects were noted. In essence, epithelial, blood vessel and nerve injury are follow by tissue regeneration, however for unknown reasons, ASM has demonstrated almost no capacity of regeneration after this procedure, being replaced by connective tissue, instead. Increased airway distensibility, decreased bronchomotor tone both at baseline and in response to increasing doses of methacholine suggest that the AHR reduction correlated well with the degree of ASM reduction, supporting a role of ASM remodeling in humans[312].

Conclusion ASM thickening is a consistent finding in airway remodeling that most likely contribute to the AHR and irreversible or partially reversible airflow obstruction seen on airway diseases, especially those with severe symptoms. Chronic inflammation is a major mechanism of structural transformation occurring at all airway layers. Nevertheless, ASM can also be generated by means of non-inflammatory pathways that would explain the lack of clinical correlation between inflammation and AHR. It remains unclear if ASM thickness depends on resident smooth muscle plasticity, mesenchymal expansion, myofibroblast migration, or stem cell differentiation, all with considerable evidence suggesting a role in this process. A complex molecular network between heterogeneous ASM bundles, containing the contractile, synthetic/proliferative, and hypercontractile phenotypes, and its tissue microenvironment determine the ASM function and outcome after injury, which real contribution can only be estimated by a system biology approach. Cytokines, growth factors, ACh, and viruses seem to have major influences in the genesis of ASM hyperplasia and hypertrophy, particularly, muscarinic signaling has potent effects on the ASMC metabolism regardless of its phenotypic status. Therefore, muscarinic receptors activation catalyzes processes for remodeling modulation, besides its well-known contractile effects, hence, it is an attractive target for long-term pharmacologic blockage by LAMAs. Further understanding of these mediators and the

interaction immune cell-smooth muscle at a epigenetic level could help to identify accurately the pathobiologic mechanisms of abnormal ASM functions and thickening, providing so, specific targets to develop future treatments (see **Fig. 7**) in the advent of gene therapy and nanotechnology[313].



DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

Authors' contributions

C.A.F.A., M.J.A., I.L.B and R.F.R. participated in selection and reviewing of cited literature. C.A.F.A. aimed and wrote the preliminary version of this manuscript, designed tables, performed art-work of figures, and performed the *in silico* analysis for miRNA target and RNA hybridization prediction. F.A.P.U. did the staining technique for figure 1. C.A.F.A., R.F.R., and F.A.P.U. implemented cell culture techniques for figure 3. C.A.F.A, R.F.R, F.A.P.U, R.G.A, M.J.A, I.L.B read, drafted, discussed and approved the final version of this manuscript.

Acknowledgments

Research related to airway smooth muscle biology is supported by grants from the CDCH-UCV, **(PG.09-8629.2013)**. C.A.F.A. and R.F.R. thank to sección de biomembranas for supporting the press release of this thesis. C.A.F.A thanks to Anthony J. Febres-Aldana for assisting in database manipulation and critical review.

REFERENCES

1. Najafi A, Masoudi-Nejad A, Ghanei M, Nourani MR, Moeini A. Pathway reconstruction of

airway remodeling in chronic lung diseases: a systems biology approach. PloS one. 2014;9(6):e100094. Epub 2014/07/01.

2. Skold CM. Remodeling in asthma and COPD--differences and similarities. The clinical respiratory journal. 2010;4 Suppl 1:20-7. Epub 2010/06/04.

3. Jeffery PK. Remodeling and inflammation of bronchi in asthma and chronic obstructive pulmonary disease. Proceedings of the American Thoracic Society. 2004;1(3):176-83. Epub 2005/08/23.

4. Regamey N, Ochs M, Hilliard TN, Muhlfeld C, Cornish N, Fleming L, et al. Increased airway smooth muscle mass in children with asthma, cystic fibrosis, and non-cystic fibrosis bronchiectasis. American journal of respiratory and critical care medicine. 2008;177(8):837-43. Epub 2008/01/26.

5. McAnulty RJ. Models and approaches to understand the role of airway remodelling in disease. Pulmonary pharmacology & therapeutics. 2011;24(5):478-86. Epub 2011/08/10.

6. Halayko AJ, Amrani Y. Mechanisms of inflammation-mediated airway smooth muscle plasticity and airways remodeling in asthma. Respiratory physiology & neurobiology. 2003;137(2-3):209-22. Epub 2003/10/01.

7. Hirota N, Martin JG. Mechanisms of airway remodeling. Chest. 2013;144(3):1026-32. Epub 2013/09/07.

8. Grainge CL, Lau LC, Ward JA, Dulay V, Lahiff G, Wilson S, et al. Effect of bronchoconstriction on airway remodeling in asthma. The New England journal of medicine. 2011;364(21):2006-15. Epub 2011/05/27.

9. de Oca MM, Halbert RJ, Lopez MV, Perez-Padilla R, Talamo C, Moreno D, et al. The chronic bronchitis phenotype in subjects with and without COPD: the PLATINO study. The European respiratory journal. 2012;40(1):28-36. Epub 2012/01/28.

10. Kosciuch J, Krenke R, Gorska K, Baran W, Kujawa M, Hildebrand K, et al. Comparison of airway wall remodeling in asthma and COPD: biopsy findings. Respiratory care. 2012;57(4):557-64. Epub 2011/10/19.

11. Woodruff PG, Dolganov GM, Ferrando RE, Donnelly S, Hays SR, Solberg OD, et al. Hyperplasia of smooth muscle in mild to moderate asthma without changes in cell size or gene expression. American journal of respiratory and critical care medicine. 2004;169(9):1001-6. Epub 2004/01/17.

12. Benayoun L, Druilhe A, Dombret MC, Aubier M, Pretolani M. Airway structural alterations selectively associated with severe asthma. American journal of respiratory and critical care medicine. 2003;167(10):1360-8. Epub 2003/01/18.

13. Pepe C, Foley S, Shannon J, Lemiere C, Olivenstein R, Ernst P, et al. Differences in airway remodeling between subjects with severe and moderate asthma. The Journal of allergy and clinical immunology. 2005;116(3):544-9. Epub 2005/09/15.

14. Gorska K, Krenke R, Kosciuch J, Korczynski P, Zukowska M, Domagala-Kulawik J, et al. Relationship between airway inflammation and remodeling in patients with asthma and chronic obstructive pulmonary disease. European journal of medical research. 2009;14 Suppl 4:90-6. Epub 2010/07/14.

15. Rydell-Tormanen K, Risse PA, Kanabar V, Bagchi R, Czubryt MP, Johnson JR. Smooth muscle in tissue remodeling and hyper-reactivity: airways and arteries. Pulmonary pharmacology & therapeutics. 2013;26(1):13-23. Epub 2012/05/09.

16. Ebina M, Takahashi T, Chiba T, Motomiya M. Cellular hypertrophy and hyperplasia of airway smooth muscles underlying bronchial asthma. A 3-D morphometric study. The American review of respiratory disease. 1993;148(3):720-6. Epub 1993/09/01.

17. James A, Mauad T, Abramson M, Green F. Airway smooth muscle hypertrophy and hyperplasia in asthma. American journal of respiratory and critical care medicine. 2012;186(6):568; author reply 9. Epub 2012/09/18.

18. Witt CA, Sheshadri A, Carlstrom L, Tarsi J, Kozlowski J, Wilson B, et al. Longitudinal changes in airway remodeling and air trapping in severe asthma. Academic radiology. 2014;21(8):986-93. Epub 2014/07/16.

19. Alagha K, Jarjour B, Bommart S, Aviles B, Varrin M, Gamez AS, et al. Persistent severe hypereosinophilic asthma is not associated with airway remodeling. Respiratory medicine. 2015. Epub 2015/01/17.

20. Syyong HT, Pascoe CD, Zhang J, Arsenault BA, Solomon D, Elliott WM, et al. Ultrastructure of Human Tracheal Smooth Muscle from Asthmatic and Non-asthmatic Subjects: Standardized Methods for Comparison. American journal of respiratory cell and molecular biology. 2014. Epub 2014/07/24.

21. Saetta M, Turato G. Airway pathology in asthma. The European respiratory journal Supplement. 2001;34:18s-23s. Epub 2002/10/24.

22. Saetta M, Di Stefano A, Turato G, Facchini FM, Corbino L, Mapp CE, et al. CD8+ T-lymphocytes in peripheral airways of smokers with chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine. 1998;157(3 Pt 1):822-6. Epub 1998/03/28.

23. Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. The New England journal of medicine. 2004;350(26):2645-53. Epub 2004/06/25.

24. Chung KF. The role of airway smooth muscle in the pathogenesis of airway wall remodeling in chronic obstructive pulmonary disease. Proceedings of the American Thoracic Society. 2005;2(4):347-54; discussion 71-2. Epub 2005/11/04.

25. Pini L, Pinelli V, Modina D, Bezzi M, Tiberio L, Tantucci C. Central airways remodeling in COPD patients. International journal of chronic obstructive pulmonary disease. 2014;9:927-32. Epub 2014/09/13.

26. Alrifai M, Marsh LM, Dicke T, Kilic A, Conrad ML, Renz H, et al. Compartmental and temporal dynamics of chronic inflammation and airway remodelling in a chronic asthma mouse model. PloS one. 2014;9(1):e85839. Epub 2014/01/28.

27. Bosse Y, Solomon D, Chin LY, Lian K, Pare PD, Seow CY. Increase in passive stiffness at reduced airway smooth muscle length: potential impact on airway responsiveness. American journal of physiology Lung cellular and molecular physiology. 2010;298(3):L277-87. Epub 2009/12/17.

28. Trejo Bittar HE, Yousem SA, Wenzel SE. Pathobiology of Severe Asthma. Annual review of pathology. 2014. Epub 2014/11/26.

29. Haley KJ, Sunday ME, Wiggs BR, Kozakewich HP, Reilly JJ, Mentzer SJ, et al. Inflammatory cell distribution within and along asthmatic airways. American journal of respiratory and critical care medicine. 1998;158(2):565-72. Epub 1998/08/12.

30. Halwani R, Vazquez-Tello A, Sumi Y, Pureza MA, Bahammam A, Al-Jahdali H, et al. Eosinophils induce airway smooth muscle cell proliferation. Journal of clinical immunology. 2013;33(3):595-604. Epub 2012/11/28.

31. Fanat AI, Thomson JV, Radford K, Nair P, Sehmi R. Human airway smooth muscle promotes eosinophil differentiation. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2009;39(7):1009-17. Epub 2009/05/15.

32. Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. The New England journal of medicine. 2002;346(22):1699-705. Epub 2002/05/31.

33. Okayama Y, Ra C, Saito H. Role of mast cells in airway remodeling. Current opinion in immunology. 2007;19(6):687-93. Epub 2007/09/01.

34. Kaur D, Hollins F, Saunders R, Woodman L, Sutcliffe A, Cruse G, et al. Airway smooth muscle proliferation and survival is not modulated by mast cells. Clinical & Experimental Allergy. 2010;40(2):279-88.

35. Kaur D, Doe C, Woodman L, Wan WY, Sutcliffe A, Hollins F, et al. Mast cell-airway smooth muscle crosstalk: the role of thymic stromal lymphopoietin. Chest. 2012;142(1):76-85. Epub 2011/11/05.

36. Hollins F, Kaur D, Yang W, Cruse G, Saunders R, Sutcliffe A, et al. Human airway smooth muscle promotes human lung mast cell survival, proliferation, and constitutive activation: cooperative roles for CADM1, stem cell factor, and IL-6. Journal of immunology (Baltimore, Md : 1950). 2008;181(4):2772-80. Epub 2008/08/08.

37. Elliot JG, Jones RL, Abramson MJ, Green FH, Mauad T, McKay KO, et al. Distribution of airway smooth muscle remodelling in asthma: Relation to airway inflammation. Respirology (Carlton, Vic). 2015;20(1):66-72. Epub 2014/09/27.

38. Johnson JE, Gonzales RA, Olson SJ, Wright PF, Graham BS. The histopathology of fatal

untreated human respiratory syncytial virus infection. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc. 2007;20(1):108-19. Epub 2006/12/05.

39. Nakagome K, Matsushita S, Nagata M. Neutrophilic inflammation in severe asthma. International archives of allergy and immunology. 2012;158 Suppl 1:96-102. Epub 2012/06/01.

40. Pawankar R, Hayashi M, Yamanishi S, Igarashi T. The paradigm of cytokine networks in allergic airway inflammation. Current opinion in allergy and clinical immunology. 2014. Epub 2014/12/06.

41. Choy DF, Modrek B, Abbas AR, Kummerfeld S, Clark HF, Wu LC, et al. Gene expression patterns of Th2 inflammation and intercellular communication in asthmatic airways. Journal of immunology (Baltimore, Md : 1950). 2011;186(3):1861-9. Epub 2010/12/29.

42. Ramos-Barbon D, Presley JF, Hamid QA, Fixman ED, Martin JG. Antigen-specific CD4+ T cells drive airway smooth muscle remodeling in experimental asthma. The Journal of clinical investigation. 2005;115(6):1580-9. Epub 2005/05/20.

43. Shifren A, Witt C, Christie C, Castro M. Mechanisms of remodeling in asthmatic airways. Journal of allergy. 2012;2012:316049. Epub 2012/02/09.

44. Fedorov IA, Wilson SJ, Davies DE, Holgate ST. Epithelial stress and structural remodelling in childhood asthma. Thorax. 2005;60(5):389-94. Epub 2005/04/30.

45. Bossley CJ, Fleming L, Gupta A, Regamey N, Frith J, Oates T, et al. Pediatric severe asthma is characterized by eosinophilia and remodeling without T(H)2 cytokines. The Journal of allergy and clinical immunology. 2012;129(4):974-82 e13. Epub 2012/03/06.

46. Doherty TA, Soroosh P, Broide DH, Croft M. CD4+ cells are required for chronic eosinophilic lung inflammation but not airway remodeling. American journal of physiology Lung cellular and molecular physiology. 2009;296(2):L229-35. Epub 2008/12/09.

47. Laporte JC, Moore PE, Baraldo S, Jouvin MH, Church TL, Schwartzman IN, et al. Direct effects of interleukin-13 on signaling pathways for physiological responses in cultured human airway smooth muscle cells. American journal of respiratory and critical care medicine. 2001;164(1):141-8. Epub 2001/07/04.

48. Cho JY, Miller M, Baek KJ, Han JW, Nayar J, Lee SY, et al. Inhibition of airway remodeling in IL-5-deficient mice. The Journal of clinical investigation. 2004;113(4):551-60. Epub 2004/02/18.

49. Hawker KM, Johnson PR, Hughes JM, Black JL. Interleukin-4 inhibits mitogen-induced proliferation of human airway smooth muscle cells in culture. The American journal of physiology. 1998;275(3 Pt 1):L469-77. Epub 1998/09/05.

50. Sohal SS, Ward C, Danial W, Wood-Baker R, Walters EH. Recent advances in understanding inflammation and remodeling in the airways in chronic obstructive pulmonary disease. Expert review of respiratory medicine. 2013;7(3):275-88. Epub 2013/06/06.

51. O'Shaughnessy TC, Ansari TW, Barnes NC, Jeffery PK. Inflammation in bronchial biopsies of

subjects with chronic bronchitis: inverse relationship of CD8+ T lymphocytes with FEV1. American journal of respiratory and critical care medicine. 1997;155(3):852-7. Epub 1997/03/01.

52. Radzikinas K, Aven L, Jiang Z, Tran T, Paez-Cortez J, Boppidi K, et al. A Shh/miR-206/BDNF cascade coordinates innervation and formation of airway smooth muscle. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2011;31(43):15407-15. Epub 2011/10/28.

53. Harkness LM, Ashton AW, Burgess JK. Asthma is not only an airway disease, but also a vascular disease. Pharmacology & therapeutics. 2014. Epub 2014/12/03.

54. Li M, Shang YX, Wei B, Yang YG. The effect of substance P on asthmatic rat airway smooth muscle cell proliferation, migration, and cytoplasmic calcium concentration in vitro. Journal of inflammation (London, England). 2011;8(1):18. Epub 2011/07/23.

55. Trian T, Allard B, Dupin I, Carvalho G, Ousova O, Maurat E, et al. House Dust Mites Induce Proliferation of Severe Asthmatic Smooth Muscle Cells via an Epithelium-dependent Pathway. American journal of respiratory and critical care medicine. 2015. Epub 2015/01/09.

56. Ricciardolo FL, Sterk PJ, Gaston B, Folkerts G. Nitric oxide in health and disease of the respiratory system. Physiological reviews. 2004;84(3):731-65. Epub 2004/07/23.

57. Batra J, Chatterjee R, Ghosh B. Inducible nitric oxide synthase (iNOS): role in asthma pathogenesis. Indian journal of biochemistry & biophysics. 2007;44(5):303-9. Epub 2008/03/18.

58. Maarsingh H, Zaagsma J, Meurs H. Arginase: a key enzyme in the pathophysiology of allergic asthma opening novel therapeutic perspectives. British journal of pharmacology. 2009;158(3):652-64. Epub 2009/08/26.

59. Howarth PH, Knox AJ, Amrani Y, Tliba O, Panettieri RA, Jr., Johnson M. Synthetic responses in airway smooth muscle. The Journal of allergy and clinical immunology. 2004;114(2 Suppl):S32-50. Epub 2004/08/17.

60. Singh SR, Billington CK, Sayers I, Hall IP. Clonally expanded human airway smooth muscle cells exhibit morphological and functional heterogeneity. Respiratory research. 2014;15:57. Epub 2014/06/03.

61. Wright DB, Trian T, Siddiqui S, Pascoe CD, Johnson JR, Dekkers BG, et al. Phenotype modulation of airway smooth muscle in asthma. Pulmonary pharmacology & therapeutics. 2013;26(1):42-9. Epub 2012/09/04.

62. Johnson PR, Roth M, Tamm M, Hughes M, Ge Q, King G, et al. Airway smooth muscle cell proliferation is increased in asthma. American journal of respiratory and critical care medicine. 2001;164(3):474-7. Epub 2001/08/14.

63. Gosens R, Meurs H, Bromhaar MM, McKay S, Nelemans SA, Zaagsma J. Functional characterization of serum- and growth factor-induced phenotypic changes in intact bovine tracheal smooth muscle. British journal of pharmacology. 2002;137(4):459-66. Epub

2002/10/03.

64. Deshpande DA, Penn RB. Targeting G protein-coupled receptor signaling in asthma. Cellular signalling. 2006;18(12):2105-20. Epub 2006/07/11.

65. Hamad AM, Johnson SR, Knox AJ. Antiproliferative effects of NO and ANP in cultured human airway smooth muscle. The American journal of physiology. 1999;277(5 Pt 1):L910-8. Epub 1999/11/24.

66. Placeres-Uray F, Gonzalez de Alfonzo R, Lippo de Becemberg I, Alfonzo MJ. Soluble guanylyl cyclase is reduced in airway smooth muscle cells from a murine model of allergic asthma. The World Allergy Organization journal. 2010;3(12):271-6. Epub 2010/12/01.

67. Berkman N, Krishnan VL, Gilbey T, Newton R, O'Connor B, Barnes PJ, et al. Expression of RANTES mRNA and protein in airways of patients with mild asthma. American journal of respiratory and critical care medicine. 1996;154(6 Pt 1):1804-11. Epub 1996/12/01.

68. Lazaar AL, Panettieri RA, Jr. Airway smooth muscle as an immunomodulatory cell: a new target for pharmacotherapy? Current opinion in pharmacology. 2001;1(3):259-64. Epub 2001/11/20.

69. Lazaar AL, Albelda SM, Pilewski JM, Brennan B, Pure E, Panettieri RA, Jr. T lymphocytes adhere to airway smooth muscle cells via integrins and CD44 and induce smooth muscle cell DNA synthesis. The Journal of experimental medicine. 1994;180(3):807-16. Epub 1994/09/01.

70. Hakonarson H, Kim C, Whelan R, Campbell D, Grunstein MM. Bi-directional activation between human airway smooth muscle cells and T lymphocytes: role in induction of altered airway responsiveness. Journal of immunology (Baltimore, Md : 1950). 2001;166(1):293-303. Epub 2000/12/21.

71. Al Heialy S, Risse PA, Zeroual MA, Roman HN, Tsuchiya K, Siddiqui S, et al. T cell-induced airway smooth muscle cell proliferation via the epidermal growth factor receptor. American journal of respiratory cell and molecular biology. 2013;49(4):563-70. Epub 2013/05/10.

72. Johnson PR, Black JL, Carlin S, Ge Q, Underwood PA. The production of extracellular matrix proteins by human passively sensitized airway smooth-muscle cells in culture: the effect of beclomethasone. American journal of respiratory and critical care medicine. 2000;162(6):2145-51. Epub 2000/12/09.

73. Foda HD, George S, Rollo E, Drews M, Conner C, Cao J, et al. Regulation of gelatinases in human airway smooth muscle cells: mechanism of progelatinase A activation. The American journal of physiology. 1999;277(1 Pt 1):L174-82. Epub 1999/07/17.

74. Johnson S, Knox A. Autocrine production of matrix metalloproteinase-2 is required for human airway smooth muscle proliferation. The American journal of physiology. 1999;277(6 Pt 1):L1109-17. Epub 1999/12/22.

75. Schuliga M, Ong SC, Soon L, Zal F, Harris T, Stewart AG. Airway smooth muscle remodels pericellular collagen fibrils: implications for proliferation. American journal of physiology Lung

cellular and molecular physiology. 2010;298(4):L584-92. Epub 2010/01/19.

76. Larocca NE, Moreno D, Garmendia J, De Sanctis JB. Niveles séricos de metaloproteinasa 9 (mmp-9) y del inhibidor tisular de mmp tipo 1 (timp-1) en pacientes venezolanos con asma o con enfermedad pulmonar obstructiva crónica (epoc). Revista de la Facultad de Medicina. 2010;33:6-10.

77. Doeing DC, Solway J. Airway smooth muscle in the pathophysiology and treatment of asthma. Journal of applied physiology (Bethesda, Md : 1985). 2013;114(7):834-43. Epub 2013/01/12.

78. Prakash YS. Airway smooth muscle in airway reactivity and remodeling: what have we learned? American journal of physiology Lung cellular and molecular physiology. 2013;305(12):L912-33. Epub 2013/10/22.

79. Thomson RJ, Bramley AM, Schellenberg RR. Airway muscle stereology: implications for increased shortening in asthma. American journal of respiratory and critical care medicine. 1996;154(3 Pt 1):749-57. Epub 1996/09/01.

80. Ward C, Johns DP, Bish R, Pais M, Reid DW, Ingram C, et al. Reduced airway distensibility, fixed airflow limitation, and airway wall remodeling in asthma. American journal of respiratory and critical care medicine. 2001;164(9):1718-21. Epub 2001/11/24.

81. Sukkar MB, Stanley AJ, Blake AE, Hodgkin PD, Johnson PR, Armour CL, et al. 'Proliferative' and 'synthetic' airway smooth muscle cells are overlapping populations. Immunology and cell biology. 2004;82(5):471-8. Epub 2004/10/14.

82. Ma X, Wang Y, Stephens NL. Serum deprivation induces a unique hypercontractile phenotype of cultured smooth muscle cells. The American journal of physiology. 1998;274(5 Pt 1):C1206-14. Epub 1998/06/05.

83. Chamley-Campbell J, Campbell GR, Ross R. The smooth muscle cell in culture. Physiological reviews. 1979;59(1):1-61. Epub 1979/01/01.

84. Halayko AJ, Salari H, Ma X, Stephens NL. Markers of airway smooth muscle cell phenotype. The American journal of physiology. 1996;270(6 Pt 1):L1040-51. Epub 1996/06/01.

85. Widdop S, Daykin K, Hall IP. Expression of muscarinic M2 receptors in cultured human airway smooth muscle cells. American journal of respiratory cell and molecular biology. 1993;9(5):541-6. Epub 1993/11/01.

86. Halayko AJ, Camoretti-Mercado B, Forsythe SM, Vieira JE, Mitchell RW, Wylam ME, et al. Divergent differentiation paths in airway smooth muscle culture: induction of functionally contractile myocytes. The American journal of physiology. 1999;276(1 Pt 1):L197-206. Epub 1999/01/14.

87. Yu ZH, Wang YX, Song Y, Lu HZ, Hou LN, Cui YY, et al. Up-regulation of KCa3.1 promotes human airway smooth muscle cell phenotypic modulation. Pharmacological research : the official journal of the Italian Pharmacological Society. 2013;77:30-8. Epub 2013/09/24.

88. Clifford RL, Singer CA, John AE. Epigenetics and miRNA emerge as key regulators of smooth muscle cell phenotype and function. Pulmonary pharmacology & therapeutics. 2013;26(1):75-85. Epub 2012/07/18.

89. Borger P, Matsumoto H, Boustany S, Gencay MM, Burgess JK, King GG, et al. Disease-specific expression and regulation of CCAAT/enhancer-binding proteins in asthma and chronic obstructive pulmonary disease. The Journal of allergy and clinical immunology. 2007;119(1):98-105. Epub 2007/01/09.

90. Halayko AJ, Stelmack GL, Yamasaki A, McNeill K, Unruh H, Rector E. Distribution of phenotypically disparate myocyte subpopulations in airway smooth muscle. Canadian journal of physiology and pharmacology. 2005;83(1):104-16. Epub 2005/03/11.

91. Roth M, Johnson PR, Borger P, Bihl MP, Rudiger JJ, King GG, et al. Dysfunctional interaction of C/EBPalpha and the glucocorticoid receptor in asthmatic bronchial smooth-muscle cells. The New England journal of medicine. 2004;351(6):560-74. Epub 2004/08/06.

92. Mahn K, Hirst SJ, Ying S, Holt MR, Lavender P, Ojo OO, et al. Diminished sarco/endoplasmic reticulum Ca2+ ATPase (SERCA) expression contributes to airway remodelling in bronchial asthma. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(26):10775-80. Epub 2009/06/23.

93. Trian T, Benard G, Begueret H, Rossignol R, Girodet PO, Ghosh D, et al. Bronchial smooth muscle remodeling involves calcium-dependent enhanced mitochondrial biogenesis in asthma. The Journal of experimental medicine. 2007;204(13):3173-81. Epub 2007/12/07.

94. Borger P, Miglino N, Baraket M, Black JL, Tamm M, Roth M. Impaired translation of CCAAT/enhancer binding protein alpha mRNA in bronchial smooth muscle cells of asthmatic patients. The Journal of allergy and clinical immunology. 2009;123(3):639-45. Epub 2009/01/06.

95. Chang PJ, Bhavsar PK, Michaeloudes C, Khorasani N, Chung KF. Corticosteroid insensitivity of chemokine expression in airway smooth muscle of patients with severe asthma. The Journal of allergy and clinical immunology. 2012;130(4):877-85 e5. Epub 2012/09/06.

96. Nie M, Knox AJ, Pang L. beta2-Adrenoceptor agonists, like glucocorticoids, repress eotaxin gene transcription by selective inhibition of histone H4 acetylation. Journal of immunology (Baltimore, Md : 1950). 2005;175(1):478-86. Epub 2005/06/24.

97. Clifford RL, John AE, Brightling CE, Knox AJ. Abnormal histone methylation is responsible for increased vascular endothelial growth factor 165a secretion from airway smooth muscle cells in asthma. Journal of immunology (Baltimore, Md : 1950). 2012;189(2):819-31. Epub 2012/06/13.

98. Royce SG, Dang W, Ververis K, De Sampayo N, El-Osta A, Tang ML, et al. Protective effects of valproic acid against airway hyperresponsiveness and airway remodeling in a mouse model of allergic airways disease. Epigenetics : official journal of the DNA Methylation Society. 2011;6(12):1463-70. Epub 2011/12/06.

99. Ooi AT, Ram S, Kuo A, Gilbert JL, Yan W, Pellegrini M, et al. Identification of an interleukin 13-induced epigenetic signature in allergic airway inflammation. American journal of translational research. 2012;4(2):219-28. Epub 2012/05/23.

100. Cheng RY, Shang Y, Limjunyawong N, Dao T, Das S, Rabold R, et al. Alterations of the lung methylome in allergic airway hyper-responsiveness. Environmental and molecular mutagenesis. 2014;55(3):244-55. Epub 2014/01/22.

101. Singh SR, Hall IP. Airway myofibroblasts and their relationship with airway myocytes and fibroblasts. Proceedings of the American Thoracic Society. 2008;5(1):127-32. Epub 2007/12/21.

102. Berair R, Saunders R, Brightling CE. Origins of increased airway smooth muscle mass in asthma. BMC medicine. 2013;11:145. Epub 2013/06/08.

103. Lo CY, Michaeloudes C, Bhavsar PK, Huang CD, Wang CH, Kuo HP, et al. Increased phenotypic differentiation and reduced corticosteroid sensitivity of fibrocytes in severe asthma. The Journal of allergy and clinical immunology. 2014. Epub 2014/12/10.

104. Singh SR, Sutcliffe A, Kaur D, Gupta S, Desai D, Saunders R, et al. CCL2 release by airway smooth muscle is increased in asthma and promotes fibrocyte migration. Allergy. 2014;69(9):1189-97. Epub 2014/06/17.

105. Lin TY, Venkatesan N, Nishioka M, Kyoh S, Al-Alwan L, Baglole CJ, et al. Monocyte-derived fibrocytes induce an inflammatory phenotype in airway smooth muscle cells. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2014;44(11):1347-60. Epub 2014/09/27.

106. Wu Y, Fu H, Yang H, Wang H, Zhang H, Qin D. Smooth muscle progenitor cells involved in the development of airway remodeling in a murine model of asthma. Asian Pacific journal of allergy and immunology / launched by the Allergy and Immunology Society of Thailand. 2014;32(3):203-10. Epub 2014/10/01.

107. Kuroishi S, Suda T, Fujisawa T, Ide K, Inui N, Nakamura Y, et al. Epithelial-mesenchymal transition induced by transforming growth factor-beta1 in mouse tracheal epithelial cells. Respirology (Carlton, Vic). 2009;14(6):828-37. Epub 2009/08/08.

108. Acharya A, Baek ST, Huang G, Eskiocak B, Goetsch S, Sung CY, et al. The bHLH transcription factor Tcf21 is required for lineage-specific EMT of cardiac fibroblast progenitors. Development (Cambridge, England). 2012;139(12):2139-49. Epub 2012/05/11.

109. de Boer WI, Sharma HS, Baelemans SM, Hoogsteden HC, Lambrecht BN, Braunstahl GJ. Altered expression of epithelial junctional proteins in atopic asthma: possible role in inflammation. Canadian journal of physiology and pharmacology. 2008;86(3):105-12. Epub 2008/04/18.

110. Hackett TL, Warner SM, Stefanowicz D, Shaheen F, Pechkovsky DV, Murray LA, et al. Induction of epithelial-mesenchymal transition in primary airway epithelial cells from patients with asthma by transforming growth factor-beta1. American journal of respiratory and critical care medicine. 2009;180(2):122-33. Epub 2009/05/02.

111. Johnson JR, Roos A, Berg T, Nord M, Fuxe J. Chronic respiratory aeroallergen exposure in mice induces epithelial-mesenchymal transition in the large airways. PloS one. 2011;6(1):e16175. Epub 2011/02/02.

112. Fischer KD, Agrawal DK. Vitamin D regulating TGF-ss induced epithelial-mesenchymal transition. Respiratory research. 2014;15(1):146. Epub 2014/11/22.

113. Meurs H, Dekkers BG, Maarsingh H, Halayko AJ, Zaagsma J, Gosens R. Muscarinic receptors on airway mesenchymal cells: novel findings for an ancient target. Pulmonary pharmacology & therapeutics. 2013;26(1):145-55. Epub 2012/07/31.

114. Yang K, Song Y, Tang YB, Xu ZP, Zhou W, Hou LN, et al. mAChRs activation induces epithelial-mesenchymal transition on lung epithelial cells. BMC pulmonary medicine. 2014;14:53. Epub 2014/04/01.

115. Saunders R, Siddiqui S, Kaur D, Doe C, Sutcliffe A, Hollins F, et al. Fibrocyte localization to the airway smooth muscle is a feature of asthma. The Journal of allergy and clinical immunology. 2009;123(2):376-84. Epub 2008/12/17.

116. Kuo KH, Herrera AM, Seow CY. Ultrastructure of airway smooth muscle. Respiratory physiology & neurobiology. 2003;137(2-3):197-208. Epub 2003/10/01.

117. Gosens R, Zaagsma J, Grootte Bromhaar M, Nelemans A, Meurs H. Acetylcholine: a novel regulator of airway smooth muscle remodelling? European journal of pharmacology. 2004;500(1-3):193-201. Epub 2004/10/07.

118. Balogh G, Dimitrov-Szokodi D, Husveti A. Lung denervation in the therapy of intractable bronchial asthma. The Journal of thoracic surgery. 1957;33(2):166-84. Epub 1957/02/01.

119. Bleecker ER. Cholinergic and neurogenic mechanisms in obstructive airways disease. The American journal of medicine. 1986;81(5A):93-102. Epub 1986/11/14.

120. Kistemaker LE, Gosens R. Acetylcholine beyond bronchoconstriction: roles in inflammation and remodeling. Trends in pharmacological sciences. 2014. Epub 2014/12/17.

121. Gosens R, Bos IS, Zaagsma J, Meurs H. Protective effects of tiotropium bromide in the progression of airway smooth muscle remodeling. American journal of respiratory and critical care medicine. 2005;171(10):1096-102. Epub 2005/02/08.

122. Placeres-Uray FA, Febres-Aldana CA, Fernandez-Ruiz R, Gonzalez de Alfonzo R, Lippo de Becemberg IA, Alfonzo MJ. M2 Muscarinic acetylcholine receptor modulates rat airway smooth muscle cell proliferation. The World Allergy Organization journal. 2013;6(1):22. Epub 2014/01/01.

123. Kistemaker LE, Oenema TA, Meurs H, Gosens R. Regulation of airway inflammation and remodeling by muscarinic receptors: perspectives on anticholinergic therapy in asthma and COPD. Life sciences. 2012;91(21-22):1126-33. Epub 2012/03/13.

124. Maeda A, Kubo T, Mishina M, Numa S. Tissue distribution of mRNAs encoding muscarinic acetylcholine receptor subtypes. FEBS letters. 1988;239(2):339-42. Epub 1988/11/07.

125. Lucchesi PA, Scheid CR, Romano FD, Kargacin ME, Mullikin-Kilpatrick D, Yamaguchi H, et al. Ligand binding and G protein coupling of muscarinic receptors in airway smooth muscle. The American journal of physiology. 1990;258(4 Pt 1):C730-8. Epub 1990/04/01.

126. Schlenz H, Kummer W, Jositsch G, Wess J, Krasteva G. Muscarinic receptor-mediated bronchoconstriction is coupled to caveolae in murine airways. American journal of physiology Lung cellular and molecular physiology. 2010;298(5):L626-36. Epub 2009/12/22.

127. Gosens R, Zaagsma J, Meurs H, Halayko AJ. Muscarinic receptor signaling in the pathophysiology of asthma and COPD. Respiratory research. 2006;7:73. Epub 2006/05/11.

128. Novi F, Stanasila L, Giorgi F, Corsini GU, Cotecchia S, Maggio R. Paired activation of two components within muscarinic M3 receptor dimers is required for recruitment of beta-arrestin-1 to the plasma membrane. The Journal of biological chemistry. 2005;280(20):19768-76. Epub 2005/03/17.

129. Nakahara T, Yunoki M, Mitani A, Sakamoto K, Ishii K. Stimulation of muscarinic M2 receptors inhibits atrial natriuretic peptide-mediated relaxation in bovine tracheal smooth muscle. Naunyn-Schmiedeberg's archives of pharmacology. 2002;366(4):376-9. Epub 2002/09/19.

130. Guerra de Gonzalez L, Misle A, Pacheco G, Napoleon de Herrera V, Gonzalez de Alfonzo R, Lippo de Becemberg I, et al. Effects of 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) and Nomega(6)-nitro-L-arginine methyl ester (NAME) on cyclic GMP levels during muscarinic activation of tracheal smooth muscle. Biochemical pharmacology. 1999;58(4):563-9. Epub 1999/07/21.

131. Marcelo J. Alfonzo FP-U, Walid Hassan-Soto, Adolfo Borges, Ramona González de Alfonzo and Itala Lippo de Becemberg. Two Guanylylcyclases Regulate the Muscarinic Activation of Airway Smooth Muscle. In: Sugi H, editor. Current Basic and Pathological Approaches to the Function of Muscle Cells and Tissues - From Molecules to Humans: InTech; 2012.

132. Alfonzo MJ, De Alfonzo RG, Alfonzo-Gonzalez MA, De Becemberg IL. Cyclic GMP regulates M(3)AChR activity at plasma membranes from airway smooth muscle. Molecular membrane biology. 2013;30(8):403-17. Epub 2013/11/02.

133. Naureckas ET, Ndukwu IM, Halayko AJ, Maxwell C, Hershenson MB, Solway J. Bronchoalveolar lavage fluid from asthmatic subjects is mitogenic for human airway smooth muscle. American journal of respiratory and critical care medicine. 1999;160(6):2062-6. Epub 1999/12/10.

134. Tasaka S, Mizoguchi K, Funatsu Y, Namkoong H, Yamasawa W, Ishii M, et al. Cytokine profile of bronchoalveolar lavage fluid in patients with combined pulmonary fibrosis and emphysema. Respirology (Carlton, Vic). 2012;17(5):814-20. Epub 2012/04/21.

135. Tran T, Teoh CM, Tam JK, Qiao Y, Chin CY, Chong OK, et al. Laminin drives survival signals to promote a contractile smooth muscle phenotype and airway hyperreactivity. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2013;27(10):3991-4003. Epub 2013/06/13.

136. Gosens R, Nelemans SA, Hiemstra M, Grootte Bromhaar MM, Meurs H, Zaagsma J. Insulin induces a hypercontractile airway smooth muscle phenotype. European journal of pharmacology. 2003;481(1):125-31. Epub 2003/11/26.

137. Oenema TA, Smit M, Smedinga L, Racke K, Halayko AJ, Meurs H, et al. Muscarinic receptor stimulation augments TGF-beta1-induced contractile protein expression by airway smooth muscle cells. American journal of physiology Lung cellular and molecular physiology. 2012;303(7):L589-97. Epub 2012/08/07.

138. Schaafsma D, McNeill KD, Stelmack GL, Gosens R, Baarsma HA, Dekkers BG, et al. Insulin increases the expression of contractile phenotypic markers in airway smooth muscle. American journal of physiology Cell physiology. 2007;293(1):C429-39. Epub 2007/04/27.

139. Mack CP, Somlyo AV, Hautmann M, Somlyo AP, Owens GK. Smooth muscle differentiation marker gene expression is regulated by RhoA-mediated actin polymerization. The Journal of biological chemistry. 2001;276(1):341-7. Epub 2000/10/18.

140. Wang DZ, Olson EN. Control of smooth muscle development by the myocardin family of transcriptional coactivators. Current opinion in genetics & development. 2004;14(5):558-66. Epub 2004/09/24.

141. Gosens R, Schaafsma D, Nelemans SA, Halayko AJ. Rho-kinase as a drug target for the treatment of airway hyperrespon-siveness in asthma. Mini reviews in medicinal chemistry. 2006;6(3):339-48. Epub 2006/03/07.

142. Goldsmith AM, Bentley JK, Zhou L, Jia Y, Bitar KN, Fingar DC, et al. Transforming growth factor-beta induces airway smooth muscle hypertrophy. American journal of respiratory cell and molecular biology. 2006;34(2):247-54. Epub 2005/10/22.

143. Halayko AJ, Kartha S, Stelmack GL, McConville J, Tam J, Camoretti-Mercado B, et al. Phophatidylinositol-3 kinase/mammalian target of rapamycin/p70S6K regulates contractile protein accumulation in airway myocyte differentiation. American journal of respiratory cell and molecular biology. 2004;31(3):266-75. Epub 2004/04/24.

144. Camoretti-Mercado B, Dulin NO, Solway J. Serum response factor function and dysfunction in smooth muscle. Respiratory physiology & neurobiology. 2003;137(2-3):223-35. Epub 2003/10/01.

145. Zhou L, Goldsmith AM, Bentley JK, Jia Y, Rodriguez ML, Abe MK, et al. 4E-binding protein phosphorylation and eukaryotic initiation factor-4E release are required for airway smooth muscle hypertrophy. American journal of respiratory cell and molecular biology. 2005;33(2):195-202. Epub 2005/05/20.

146. Gosens R, Stelmack GL, Bos ST, Dueck G, Mutawe MM, Schaafsma D, et al. Caveolin-1 is required for contractile phenotype expression by airway smooth muscle cells. Journal of cellular and molecular medicine. 2011;15(11):2430-42. Epub 2011/01/05.

147. Halayko AJ, Tran T, Gosens R. Phenotype and functional plasticity of airway smooth muscle: role of caveolae and caveolins. Proceedings of the American Thoracic Society.

2008;5(1):80-8. Epub 2007/12/21.

148. Gosens R, Stelmack GL, Dueck G, McNeill KD, Yamasaki A, Gerthoffer WT, et al. Role of caveolin-1 in p42/p44 MAP kinase activation and proliferation of human airway smooth muscle. American journal of physiology Lung cellular and molecular physiology. 2006;291(3):L523-34. Epub 2006/04/18.

149. Sharma P, Tran T, Stelmack GL, McNeill K, Gosens R, Mutawe MM, et al. Expression of the dystrophin-glycoprotein complex is a marker for human airway smooth muscle phenotype maturation. American journal of physiology Lung cellular and molecular physiology. 2008;294(1):L57-68. Epub 2007/11/13.

150. Halayko AJ, Stelmack GL. The association of caveolae, actin, and the dystrophin-glycoprotein complex: a role in smooth muscle phenotype and function? Canadian journal of physiology and pharmacology. 2005;83(10):877-91. Epub 2005/12/08.

151. Yang CM, Luo SF, Wang CC, Chiu CT, Chien CS, Lin CC, et al. Tumour necrosis factor-alpha- and interleukin-1beta-stimulated cell proliferation through activation of mitogen-activated protein kinase in canine tracheal smooth muscle cells. British journal of pharmacology. 2000;130(4):891-9. Epub 2000/06/24.

152. Krymskaya VP, Orsini MJ, Eszterhas AJ, Brodbeck KC, Benovic JL, Panettieri RA, Jr., et al. Mechanisms of proliferation synergy by receptor tyrosine kinase and G protein-coupled receptor activation in human airway smooth muscle. American journal of respiratory cell and molecular biology. 2000;23(4):546-54. Epub 2000/10/06.

153. Wang Z, Wang DZ, Hockemeyer D, McAnally J, Nordheim A, Olson EN. Myocardin and ternary complex factors compete for SRF to control smooth muscle gene expression. Nature. 2004;428(6979):185-9. Epub 2004/03/12.

154. Page K, Hershenson MB. Mitogen-activated signaling and cell cycle regulation in airway smooth muscle. Frontiers in bioscience : a journal and virtual library. 2000;5:D258-67. Epub 2000/03/07.

155. Panebra A, Schwarb MR, Glinka CB, Liggett SB. Heterogeneity of transcription factor expression and regulation in human airway epithelial and smooth muscle cells. American journal of physiology Lung cellular and molecular physiology. 2007;293(2):L453-62. Epub 2007/06/15.

156. Woodruff PG. Gene expression in asthmatic airway smooth muscle. Proceedings of the American Thoracic Society. 2008;5(1):113-8. Epub 2007/12/21.

157. Lee JH, Kaminski N, Dolganov G, Grunig G, Koth L, Solomon C, et al. Interleukin-13 induces dramatically different transcriptional programs in three human airway cell types. American journal of respiratory cell and molecular biology. 2001;25(4):474-85. Epub 2001/11/06.

158. Misquitta CM, Chen T, Grover AK. Control of protein expression through mRNA stability in calcium signalling. Cell calcium. 2006;40(4):329-46. Epub 2006/06/13.

159. Fukuyama S, Nakano T, Matsumoto T, Oliver BG, Burgess JK, Moriwaki A, et al. Pulmonary suppressor of cytokine signaling-1 induced by IL-13 regulates allergic asthma phenotype. American journal of respiratory and critical care medicine. 2009;179(11):992-8. Epub 2009/03/21.

160. Gosens R, Nelemans SA, Grootte Bromhaar MM, McKay S, Zaagsma J, Meurs H. Muscarinic M3-receptors mediate cholinergic synergism of mitogenesis in airway smooth muscle. American journal of respiratory cell and molecular biology. 2003;28(2):257-62. Epub 2003/01/24.

161. Stamatiou R, Paraskeva E, Vasilaki A, Mylonis I, Molyvdas PA, Gourgoulianis K, et al. Long-term exposure to muscarinic agonists decreases expression of contractile proteins and responsiveness of rabbit tracheal smooth muscle cells. BMC pulmonary medicine. 2014;14:39. Epub 2014/03/13.

162. Gosens R, Bromhaar MM, Tonkes A, Schaafsma D, Zaagsma J, Nelemans SA, et al. Muscarinic M(3) receptor-dependent regulation of airway smooth muscle contractile phenotype. British journal of pharmacology. 2004;141(6):943-50. Epub 2004/03/03.

163. He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nature reviews Genetics. 2004;5(7):522-31. Epub 2004/06/24.

164. Joshi SR, Comer BS, McLendon JM, Gerthoffer WT. MicroRNA Regulation of Smooth Muscle Phenotype. Molecular and cellular pharmacology. 2012;4(1):1-16. Epub 2012/01/01.

165. Garbacki N, Di Valentin E, Huynh-Thu VA, Geurts P, Irrthum A, Crahay C, et al. MicroRNAs profiling in murine models of acute and chronic asthma: a relationship with mRNAs targets. PloS one. 2011;6(1):e16509. Epub 2011/02/10.

166. Kuhn AR, Schlauch K, Lao R, Halayko AJ, Gerthoffer WT, Singer CA. MicroRNA expression in human airway smooth muscle cells: role of miR-25 in regulation of airway smooth muscle phenotype. American journal of respiratory cell and molecular biology. 2010;42(4):506-13. Epub 2009/06/23.

167. Hu R, Pan W, Fedulov AV, Jester W, Jones MR, Weiss ST, et al. MicroRNA-10a controls airway smooth muscle cell proliferation via direct targeting of the PI3 kinase pathway. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2014;28(5):2347-57. Epub 2014/02/14.

168. Dileepan M, Jude JA, Rao SP, Walseth TF, Panettieri RA, Subramanian S, et al. MicroRNA-708 regulates CD38 expression through signaling pathways JNK MAP kinase and PTEN/AKT in human airway smooth muscle cells. Respiratory research. 2014;15:107. Epub 2014/09/02.

169. Chiba Y, Tanabe M, Goto K, Sakai H, Misawa M. Down-regulation of miR-133a contributes to up-regulation of Rhoa in bronchial smooth muscle cells. American journal of respiratory and critical care medicine. 2009;180(8):713-9. Epub 2009/08/01.

170. Perry MM, Tsitsiou E, Austin PJ, Lindsay MA, Gibeon DS, Adcock IM, et al. Role of non-

coding RNAs in maintaining primary airway smooth muscle cells. Respiratory research. 2014;15:58. Epub 2014/06/03.

171. Hu W, Alvarez-Dominguez JR, Lodish HF. Regulation of mammalian cell differentiation by long non-coding RNAs. EMBO reports. 2012;13(11):971-83. Epub 2012/10/17.

172. Hirst SJ, Martin JG, Bonacci JV, Chan V, Fixman ED, Hamid QA, et al. Proliferative aspects of airway smooth muscle. The Journal of allergy and clinical immunology. 2004;114(2 Suppl):S2-17. Epub 2004/08/17.

173. Redhu NS, Shan L, Al-Subait D, Ashdown HL, Movassagh H, Lamkhioued B, et al. IgE induces proliferation in human airway smooth muscle cells: role of MAPK and STAT3 pathways. Allergy, asthma, and clinical immunology : official journal of the Canadian Society of Allergy and Clinical Immunology. 2013;9(1):41. Epub 2014/02/07.

174. Dekkers BG, Naeimi S, Bos IS, Menzen MH, Halayko AJ, Sadeghi Hashjin G, et al. L-Thyroxine promotes a proliferative airway smooth muscle phenotype in the presence of TGF-beta1. American journal of physiology Lung cellular and molecular physiology. 2014:ajplung 00071 2014. Epub 2014/12/07.

175. Shi R, Chen X, Zhu J, Chen L, Zhu S. [Leptin promotes the proliferation of airway smooth muscle cells and the expressions of HIF-1alpha and NF-kappaB of hypoxic rats]. Xi bao yu fen zi mian yi xue za zhi = Chinese journal of cellular and molecular immunology. 2015;31(1):32-5. Epub 2015/01/13.

176. Florio C, Martin JG, Styhler A, Heisler S. Antiproliferative effect of prostaglandin E2 in cultured guinea pig tracheal smooth muscle cells. The American journal of physiology. 1994;266(2 Pt 1):L131-7. Epub 1994/02/01.

177. Ward JE, Gould H, Harris T, Bonacci JV, Stewart AG. PPAR gamma ligands, 15-deoxy-delta12,14-prostaglandin J2 and rosiglitazone regulate human cultured airway smooth muscle proliferation through different mechanisms. British journal of pharmacology. 2004;141(3):517-25. Epub 2004/01/14.

178. Dekkers BG, Pehlic A, Mariani R, Bos IS, Meurs H, Zaagsma J. Glucocorticosteroids and beta(2)-adrenoceptor agonists synergize to inhibit airway smooth muscle remodeling. The Journal of pharmacology and experimental therapeutics. 2012;342(3):780-7. Epub 2012/06/12.

179. Hamad AM, Clayton A, Islam B, Knox AJ. Guanylyl cyclases, nitric oxide, natriuretic peptides, and airway smooth muscle function. American journal of physiology Lung cellular and molecular physiology. 2003;285(5):L973-83. Epub 2003/10/11.

180. Di Nardo P, Minieri M, Sampaolesi M, Carbone A, Loreni F, Samuel JL, et al. Atrial natriuretic factor (ANF) and ANF receptor C gene expression and localization in the respiratory system: effects induced by hypoxia and hemodynamic overload. Endocrinology. 1996;137(10):4339-50. Epub 1996/10/01.

181. Potter LR, Abbey-Hosch S, Dickey DM. Natriuretic peptides, their receptors, and cyclic guanosine monophosphate-dependent signaling functions. Endocrine reviews.

2006;27(1):47-72. Epub 2005/11/18.

182. Silberbach M, Roberts CT, Jr. Natriuretic peptide signalling: molecular and cellular pathways to growth regulation. Cellular signalling. 2001;13(4):221-31. Epub 2001/04/18.

183. Gosens R, Roscioni SS, Dekkers BG, Pera T, Schmidt M, Schaafsma D, et al. Pharmacology of airway smooth muscle proliferation. European journal of pharmacology. 2008;585(2-3):385-97. Epub 2008/04/18.

184. Lincoln TM, Dey N, Sellak H. Invited review: cGMP-dependent protein kinase signaling mechanisms in smooth muscle: from the regulation of tone to gene expression. Journal of applied physiology (Bethesda, Md : 1985). 2001;91(3):1421-30. Epub 2001/08/18.

185. Patel HJ, Belvisi MG, Donnelly LE, Yacoub MH, Chung KF, Mitchell JA. Constitutive expressions of type I NOS in human airway smooth muscle cells: evidence for an antiproliferative role. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 1999;13(13):1810-6. Epub 1999/10/03.

186. Lucas KA, Pitari GM, Kazerounian S, Ruiz-Stewart I, Park J, Schulz S, et al. Guanylyl cyclases and signaling by cyclic GMP. Pharmacological reviews. 2000;52(3):375-414. Epub 2000/09/08.

187. Papapetropoulos A, Simoes DC, Xanthou G, Roussos C, Gratziou C. Soluble guanylyl cyclase expression is reduced in allergic asthma. American journal of physiology Lung cellular and molecular physiology. 2006;290(1):L179-84. Epub 2005/09/07.

188. Fogli S, Stefanelli F, Picchianti L, Del Re M, Mey V, Bardelli C, et al. Synergistic interaction between PPAR ligands and salbutamol on human bronchial smooth muscle cell proliferation. British journal of pharmacology. 2013;168(1):266-75. Epub 2012/08/29.

189. Foong RE, Shaw NC, Berry LJ, Hart PH, Gorman S, Zosky GR. Vitamin D deficiency causes airway hyperresponsiveness, increases airway smooth muscle mass, and reduces TGF-beta expression in the lungs of female BALB/c mice. Physiological reports. 2014;2(3):e00276. Epub 2014/04/25.

190. Damera G, Fogle HW, Lim P, Goncharova EA, Zhao H, Banerjee A, et al. Vitamin D inhibits growth of human airway smooth muscle cells through growth factor-induced phosphorylation of retinoblastoma protein and checkpoint kinase 1. British journal of pharmacology. 2009;158(6):1429-41. Epub 2009/10/10.

191. Amrani Y, Tliba O, Choubey D, Huang CD, Krymskaya VP, Eszterhas A, et al. IFN-gamma inhibits human airway smooth muscle cell proliferation by modulating the E2F-1/Rb pathway. American journal of physiology Lung cellular and molecular physiology. 2003;284(6):L1063-71. Epub 2003/02/18.

192. Shim JY, Park SW, Kim DS, Shim JW, Jung HL, Park MS. The effect of interleukin-4 and amphiregulin on the proliferation of human airway smooth muscle cells and cytokine release. Journal of Korean medical science. 2008;23(5):857-63. Epub 2008/10/29.

193. He F, Li B, Zhao Z, Zhou Y, Hu G, Zou W, et al. The pro-proliferative effects of nicotine and

its underlying mechanism on rat airway smooth muscle cells. PloS one. 2014;9(4):e93508. Epub 2014/04/03.

194. Kistemaker LE, Bos ST, Mudde WM, Hylkema MN, Hiemstra PS, Wess J, et al. Muscarinic M(3) receptors contribute to allergen-induced airway remodeling in mice. American journal of respiratory cell and molecular biology. 2014;50(4):690-8. Epub 2013/10/26.

195. Ediger TL, Toews ML. Synergistic stimulation of airway smooth muscle cell mitogenesis. The Journal of pharmacology and experimental therapeutics. 2000;294(3):1076-82. Epub 2000/08/17.

196. Patel TB. Single transmembrane spanning heterotrimeric g protein-coupled receptors and their signaling cascades. Pharmacological reviews. 2004;56(3):371-85. Epub 2004/08/20.

197. Conway AM, Rakhit S, Pyne S, Pyne NJ. Platelet-derived-growth-factor stimulation of the p42/p44 mitogen-activated protein kinase pathway in airway smooth muscle: role of pertussis-toxin-sensitive G-proteins, c-Src tyrosine kinases and phosphoinositide 3-kinase. The Biochemical journal. 1999;337 (Pt 2):171-7. Epub 1999/01/12.

198. Oenema TA, Mensink G, Smedinga L, Halayko AJ, Zaagsma J, Meurs H, et al. Cross-talk between transforming growth factor-beta(1) and muscarinic M(2) receptors augments airway smooth muscle proliferation. American journal of respiratory cell and molecular biology. 2013;49(1):18-27. Epub 2013/03/02.

199. Burdon D, Patel R, Challiss RA, Blank JL. Growth inhibition by the muscarinic M(3) acetylcholine receptor: evidence for p21(Cip1/Waf1) involvement in G(1) arrest. The Biochemical journal. 2002;367(Pt 2):549-59. Epub 2002/07/20.

200. Williams CL, Lennon VA. Activation of muscarinic acetylcholine receptors inhibits cell cycle progression of small cell lung carcinoma. Cell regulation. 1991;2(5):373-81. Epub 1991/05/01.

201. Nicke B, Detjen K, Logsdon CD. Muscarinic cholinergic receptors activate both inhibitory and stimulatory growth mechanisms in NIH3T3 cells. The Journal of biological chemistry. 1999;274(31):21701-6. Epub 1999/07/27.

202. Bruges G, Borges A, Sanchez de Villarroel S, Lippo de Becemberg I, Francis de Toba G, Placeres F, et al. Coupling of M3 acetylcholine receptor to Gq16 activates a natriuretic peptide receptor guanylyl cyclase. Journal of receptor and signal transduction research. 2007;27(2-3):189-216. Epub 2007/07/07.

203. Alfonzo MJ, de Aguilar EP, de Murillo AG, de Villarroel SS, de Alfonzo RG, Borges A, et al. Characterization of a G protein-coupled guanylyl cyclase-B receptor from bovine tracheal smooth muscle. Journal of receptor and signal transduction research. 2006;26(4):269-97. Epub 2006/07/05.

204. Uray FP, de Alfonzo RG, de Becemberg IL, Alfonzo MJ. Muscarinic agonists acting through M2 acetylcholine receptors stimulate the migration of an NO-sensitive guanylyl cyclase to the plasma membrane of bovine tracheal smooth muscle. Journal of receptor and signal transduction research. 2010;30(1):10-23. Epub 2009/11/17.

205. Page K, Li J, Hershenson MB. p38 MAP kinase negatively regulates cyclin D1 expression in airway smooth muscle cells. American journal of physiology Lung cellular and molecular physiology. 2001;280(5):L955-64. Epub 2001/04/06.

206. Liu WH, Chen YJ, Cheng TL, Lin SR, Chang LS. Cross talk between p38MAPK and ERK is mediated through MAPK-mediated protein phosphatase 2A catalytic subunit alpha and MAPK phosphatase-1 expression in human leukemia U937 cells. Cellular signalling. 2013;25(9):1845-51. Epub 2013/05/28.

207. Leach K, Simms J, Sexton PM, Christopoulos A. Structure-function studies of muscarinic acetylcholine receptors. Handbook of experimental pharmacology. 2012(208):29-48. Epub 2012/01/10.

208. Robinson SW, Fernandes M, Husi H. Current advances in systems and integrative biology. Computational and structural biotechnology journal. 2014;11(18):35-46. Epub 2014/11/08.

209. Kang JY, Rhee CK, Kim JS, Park CK, Kim SJ, Lee SH, et al. Effect of tiotropium bromide on airway remodeling in a chronic asthma model. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 2012;109(1):29-35. Epub 2012/06/26.

210. Kang JY, Lee SY, Rhee CK, Kim SJ, Kwon SS, Kim YK. Effect of aging on airway remodeling and muscarinic receptors in a murine acute asthma model. Clinical interventions in aging. 2013;8:1393-403. Epub 2013/11/10.

211. Wajant H, Pfizenmaier K, Scheurich P. Tumor necrosis factor signaling. Cell death and differentiation. 2003;10(1):45-65. Epub 2003/03/26.

212. Hamann KJ, Vieira JE, Halayko AJ, Dorscheid D, White SR, Forsythe SM, et al. Fas cross-linking induces apoptosis in human airway smooth muscle cells. American journal of physiology Lung cellular and molecular physiology. 2000;278(3):L618-24. Epub 2000/03/11.

213. Freyer AM, Johnson SR, Hall IP. Effects of growth factors and extracellular matrix on survival of human airway smooth muscle cells. American journal of respiratory cell and molecular biology. 2001;25(5):569-76. Epub 2001/11/20.

214. Oltmanns U, Sukkar MB, Xie S, John M, Chung KF. Induction of human airway smooth muscle apoptosis by neutrophils and neutrophil elastase. American journal of respiratory cell and molecular biology. 2005;32(4):334-41. Epub 2005/01/18.

215. Chang Y, Al-Alwan L, Risse PA, Halayko AJ, Martin JG, Baglole CJ, et al. Th17-associated cytokines promote human airway smooth muscle cell proliferation. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2012;26(12):5152-60. Epub 2012/08/18.

216. Halwani R, Al-Abri J, Beland M, Al-Jahdali H, Halayko AJ, Lee TH, et al. CC and CXC chemokines induce airway smooth muscle proliferation and survival. Journal of immunology (Baltimore, Md : 1950). 2011;186(7):4156-63. Epub 2011/03/04.

217. Zhou D, Zheng X, Wang L, Stelmack G, Halayko AJ, Dorscheid D, et al. Expression and effects of cardiotrophin-1 (CT-1) in human airway smooth muscle cells. British journal of pharmacology. 2003;140(7):1237-44. Epub 2003/11/05.

218. McWhinnie R, Pechkovsky DV, Zhou D, Lane D, Halayko AJ, Knight DA, et al. Endothelin-1 induces hypertrophy and inhibits apoptosis in human airway smooth muscle cells. American journal of physiology Lung cellular and molecular physiology. 2007;292(1):L278-86. Epub 2006/08/22.

219. Zhao LM, Kuang HY, Zhang LX, Wu JZ, Chen XL, Zhang XY, et al. Effect of TRPV1 channel on proliferation and apoptosis of airway smooth muscle cells of rats. Journal of Huazhong University of Science and Technology Medical sciences = Hua zhong ke ji da xue xue bao Yi xue Ying De wen ban = Huazhong keji daxue xuebao Yixue Yingdewen ban. 2014;34(4):504-9. Epub 2014/08/20.

220. Chiba Y, Misawa M. Increased expression of G12 and G13 proteins in bronchial smooth muscle of airway hyperresponsive rats. Inflammation research : official journal of the European Histamine Research Society [et al]. 2001;50(6):333-6. Epub 2001/07/28.

221. Ramji DP, Foka P. CCAAT/enhancer-binding proteins: structure, function and regulation. The Biochemical journal. 2002;365(Pt 3):561-75. Epub 2002/05/15.

222. House SJ, Potier M, Bisaillon J, Singer HA, Trebak M. The non-excitable smooth muscle: calcium signaling and phenotypic switching during vascular disease. Pflugers Archiv : European journal of physiology. 2008;456(5):769-85. Epub 2008/03/28.

223. Mahn K, Ojo OO, Chadwick G, Aaronson PI, Ward JP, Lee TH. Ca(2+) homeostasis and structural and functional remodelling of airway smooth muscle in asthma. Thorax. 2010;65(6):547-52. Epub 2010/06/05.

224. Zhao L, Sullivan MN, Chase M, Gonzales AL, Earley S. Calcineurin/nuclear factor of activated T cells-coupled vanilliod transient receptor potential channel 4 ca2+ sparklets stimulate airway smooth muscle cell proliferation. American journal of respiratory cell and molecular biology. 2014;50(6):1064-75. Epub 2014/01/08.

225. Zou JJ. Role of STIM1/Orai1-mediated store-operated Ca(2)(+) entry in airway smooth muscle cell proliferation.

226. Delmotte P, Sieck GC. Interaction between endoplasmic/sarcoplasmic reticulum stress (ER/SR stress), mitochondrial signaling and Ca regulation in airway smooth muscle (ASM). Canadian journal of physiology and pharmacology. 2014:1-14. Epub 2014/12/17.

227. Roth M, Black JL. An imbalance in C/EBPs and increased mitochondrial activity in asthmatic airway smooth muscle cells: novel targets in asthma therapy? British journal of pharmacology. 2009;157(3):334-41. Epub 2009/04/18.

228. Perry MM, Baker JE, Gibeon DS, Adcock IM, Chung KF. Airway smooth muscle hyperproliferation is regulated by microRNA-221 in severe asthma. American journal of respiratory cell and molecular biology. 2014;50(1):7-17. Epub 2013/08/16.

229. Wang X, El Naqa IM. Prediction of both conserved and nonconserved microRNA targets in animals. Bioinformatics (Oxford, England). 2008;24(3):325-32. Epub 2007/12/01.

230. Comer BS, Camoretti-Mercado B, Kogut PC, Halayko AJ, Solway J, Gerthoffer WT. Cyclooxygenase-2 and MicroRNA-155 Expression are Elevated in Asthmatic Airway Smooth Muscle Cells. American journal of respiratory cell and molecular biology. 2014. Epub 2014/09/03.

231. Comer BS, Camoretti-Mercado B, Kogut PC, Halayko AJ, Solway J, Gerthoffer WT. MicroRNA-146a and microRNA-146b expression and anti-inflammatory function in human airway smooth muscle. American journal of physiology Lung cellular and molecular physiology. 2014;307(9):L727-34. Epub 2014/09/14.

232. Ma L, Brown M, Kogut P, Serban K, Li X, McConville J, et al. Akt activation induces hypertrophy without contractile phenotypic maturation in airway smooth muscle. American journal of physiology Lung cellular and molecular physiology. 2011;300(5):L701-9. Epub 2011/03/08.

233. Zhou L, Li J, Goldsmith AM, Newcomb DC, Giannola DM, Vosk RG, et al. Human bronchial smooth muscle cell lines show a hypertrophic phenotype typical of severe asthma. American journal of respiratory and critical care medicine. 2004;169(6):703-11. Epub 2003/12/25.

234. Deng H, Hershenson MB, Lei J, Bitar KN, Fingar DC, Solway J, et al. p70 Ribosomal S6 kinase is required for airway smooth muscle cell size enlargement but not increased contractile protein expression. American journal of respiratory cell and molecular biology. 2010;42(6):744-52. Epub 2009/08/04.

235. Deng H, Dokshin GA, Lei J, Goldsmith AM, Bitar KN, Fingar DC, et al. Inhibition of glycogen synthase kinase-3beta is sufficient for airway smooth muscle hypertrophy. The Journal of biological chemistry. 2008;283(15):10198-207. Epub 2008/02/07.

236. Bentley JK, Deng H, Linn MJ, Lei J, Dokshin GA, Fingar DC, et al. Airway smooth muscle hyperplasia and hypertrophy correlate with glycogen synthase kinase-3(beta) phosphorylation in a mouse model of asthma. American journal of physiology Lung cellular and molecular physiology. 2009;296(2):L176-84. Epub 2008/11/18.

237. McKay S, de Jongste JC, Saxena PR, Sharma HS. Angiotensin II induces hypertrophy of human airway smooth muscle cells: expression of transcription factors and transforming growth factor-beta1. American journal of respiratory cell and molecular biology. 1998;18(6):823-33. Epub 1998/06/25.

238. Goncharova EA, Lim PN, Chisolm A, Fogle HW, 3rd, Taylor JH, Goncharov DA, et al. Interferons modulate mitogen-induced protein synthesis in airway smooth muscle. American journal of physiology Lung cellular and molecular physiology. 2010;299(1):L25-35. Epub 2010/04/13.

239. Mohamed JS, Lopez MA, Boriek AM. Mechanical stretch up-regulates microRNA-26a and induces human airway smooth muscle hypertrophy by suppressing glycogen synthase kinase-3beta. The Journal of biological chemistry. 2010;285(38):29336-47. Epub 2010/06/08.

240. Mohamed JS, Hajira A, Li Z, Paulin D, Boriek AM. Desmin regulates airway smooth muscle hypertrophy through early growth-responsive protein-1 and microRNA-26a. The Journal of biological chemistry. 2011;286(50):43394-404. Epub 2011/09/10.

241. Wu P, Dupont WD, Griffin MR, Carroll KN, Mitchel EF, Gebretsadik T, et al. Evidence of a causal role of winter virus infection during infancy in early childhood asthma. American journal of respiratory and critical care medicine. 2008;178(11):1123-9. Epub 2008/09/09.

242. Huckabee MM, Peebles RS, Jr. Novel concepts in virally induced asthma. Clinical and molecular allergy : CMA. 2009;7:2. Epub 2009/01/22.

243. Johnston SL. Overview of virus-induced airway disease. Proceedings of the American Thoracic Society. 2005;2(2):150-6. Epub 2005/08/23.

244. Arden KE, Mackay IM. Human rhinoviruses: coming in from the cold. Genome medicine. 2009;1(4):44. Epub 2009/05/15.

245. Azevedo AM, Durigon EL, Okasima V, Queiroz DA, de Moraes-Vasconcelos D, Duarte AJ, et al. Detection of influenza, parainfluenza, adenovirus and respiratory syncytial virus during asthma attacks in children older than 2 years old. Allergologia et immunopathologia. 2003;31(6):311-7. Epub 2003/12/13.

246. Cubie HA, Duncan LA, Marshall LA, Smith NM. Detection of respiratory syncytial virus nucleic acid in archival postmortem tissue from infants. Pediatric pathology & laboratory medicine : journal of the Society for Pediatric Pathology, affiliated with the International Paediatric Pathology Association. 1997;17(6):927-38. Epub 1997/11/14.

247. Gonzalez-Reyes L, Ruiz-Arguello MB, Garcia-Barreno B, Calder L, Lopez JA, Albar JP, et al. Cleavage of the human respiratory syncytial virus fusion protein at two distinct sites is required for activation of membrane fusion. Proceedings of the National Academy of Sciences of the United States of America. 2001;98(17):9859-64. Epub 2001/08/09.

248. Moore PE, Cunningham G, Calder MM, DeMatteo AD, Jr., Peeples ME, Summar ML, et al. Respiratory syncytial virus infection reduces beta2-adrenergic responses in human airway smooth muscle. American journal of respiratory cell and molecular biology. 2006;35(5):559-64. Epub 2006/06/10.

249. Hakonarson H, Maskeri N, Carter C, Hodinka RL, Campbell D, Grunstein MM. Mechanism of rhinovirus-induced changes in airway smooth muscle responsiveness. The Journal of clinical investigation. 1998;102(9):1732-41. Epub 1998/11/05.

250. Oliver BG, Johnston SL, Baraket M, Burgess JK, King NJ, Roth M, et al. Increased proinflammatory responses from asthmatic human airway smooth muscle cells in response to rhinovirus infection. Respiratory research. 2006;7:71. Epub 2006/05/04.

251. Grunstein MM, Hakonarson H, Whelan R, Yu Z, Grunstein JS, Chuang S. Rhinovirus elicits proasthmatic changes in airway responsiveness independently of viral infection. The Journal of allergy and clinical immunology. 2001;108(6):997-1004. Epub 2001/12/14.

252. Gern JE. Mechanisms of virus-induced asthma. The Journal of pediatrics. 2003;142(2 Suppl):S9-13; discussion S-4. Epub 2003/02/14.

253. Sikkel MB, Quint JK, Mallia P, Wedzicha JA, Johnston SL. Respiratory syncytial virus persistence in chronic obstructive pulmonary disease. The Pediatric infectious disease journal. 2008;27(10 Suppl):S63-70. Epub 2008/10/23.

254. Jafri HS, Chavez-Bueno S, Mejias A, Gomez AM, Rios AM, Nassi SS, et al. Respiratory syncytial virus induces pneumonia, cytokine response, airway obstruction, and chronic inflammatory infiltrates associated with long-term airway hyperresponsiveness in mice. The Journal of infectious diseases. 2004;189(10):1856-65. Epub 2004/05/04.

255. Riedel F, Oberdieck B, Streckert HJ, Philippou S, Krusat T, Marek W. Persistence of airway hyperresponsiveness and viral antigen following respiratory syncytial virus bronchiolitis in young guinea-pigs. The European respiratory journal. 1997;10(3):639-45. Epub 1997/03/01.

256. You D, Becnel D, Wang K, Ripple M, Daly M, Cormier SA. Exposure of neonates to respiratory syncytial virus is critical in determining subsequent airway response in adults. Respiratory research. 2006;7:107. Epub 2006/08/09.

257. Becnel D, You D, Erskin J, Dimina DM, Cormier SA. A role for airway remodeling during respiratory syncytial virus infection. Respiratory research. 2005;6:122. Epub 2005/10/26.

258. Culley FJ, Pollott J, Openshaw PJ. Age at first viral infection determines the pattern of T cell-mediated disease during reinfection in adulthood. The Journal of experimental medicine. 2002;196(10):1381-6. Epub 2002/11/20.

259. Dakhama A, Park JW, Taube C, Joetham A, Balhorn A, Miyahara N, et al. The enhancement or prevention of airway hyperresponsiveness during reinfection with respiratory syncytial virus is critically dependent on the age at first infection and IL-13 production. Journal of immunology (Baltimore, Md : 1950). 2005;175(3):1876-83. Epub 2005/07/22.

260. Tliba O, Amrani Y. Airway smooth muscle cell as an inflammatory cell: lessons learned from interferon signaling pathways. Proceedings of the American Thoracic Society. 2008;5(1):106-12. Epub 2007/12/21.

261. Wark PA, Johnston SL, Bucchieri F, Powell R, Puddicombe S, Laza-Stanca V, et al. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. The Journal of experimental medicine. 2005;201(6):937-47. Epub 2005/03/23.

262. Rochlitzer S, Hoymann HG, Muller M, Braun A. No exacerbation but impaired anti-viral mechanisms in a rhinovirus-chronic allergic asthma mouse model. Clinical science (London, England : 1979). 2014;126(1):55-65. Epub 2013/07/05.

263. Uller L, Leino M, Bedke N, Sammut D, Green B, Lau L, et al. Double-stranded RNA induces disproportionate expression of thymic stromal lymphopoietin versus interferon-beta in bronchial epithelial cells from donors with asthma. Thorax. 2010;65(7):626-32. Epub 2010/07/16.

264. Wu Q, van Dyk LF, Jiang D, Dakhama A, Li L, White SR, et al. Interleukin-1 receptor-associated kinase M (IRAK-M) promotes human rhinovirus infection in lung epithelial cells via the autophagic pathway. Virology. 2013;446(1-2):199-206. Epub 2013/10/01.

265. Papadopoulos NG, Stanciu LA, Papi A, Holgate ST, Johnston SL. A defective type 1 response to rhinovirus in atopic asthma. Thorax. 2002;57(4):328-32. Epub 2002/03/30.

266. Pritchard AL, White OJ, Burel JG, Carroll ML, Phipps S, Upham JW. Asthma is associated with multiple alterations in anti-viral innate signalling pathways. PloS one. 2014;9(9):e106501. Epub 2014/09/10.

267. Tsuchiya K, Isogai S, Tamaoka M, Inase N, Akashi T, Martin JG, et al. Depletion of CD8+ T cells enhances airway remodelling in a rodent model of asthma. Immunology. 2009;126(1):45-54. Epub 2008/06/20.

268. Yewdell JW, Bennink JR. Mechanisms of viral interference with MHC class I antigen processing and presentation. Annual review of cell and developmental biology. 1999;15:579-606. Epub 1999/12/28.

269. Telcian AG, Zdrenghea MT, Caramori G, Laza-Stanca V, Message SD, Kebadze T, et al. Soluble major histocompatibility complex class I-related chain B molecules are increased and correlate with clinical outcomes during rhinovirus infection in healthy subjects. Chest. 2014;146(1):32-40. Epub 2014/02/22.

270. Calven J, Yudina Y, Uller L. Rhinovirus and dsRNA induce RIG-I-like receptors and expression of interferon beta and lambda1 in human bronchial smooth muscle cells. PloS one. 2013;8(4):e62718. Epub 2013/05/10.

271. Takayama S, Tamaoka M, Takayama K, Okayasu K, Tsuchiya K, Miyazaki Y, et al. Synthetic double-stranded RNA enhances airway inflammation and remodelling in a rat model of asthma. Immunology. 2011;134(2):140-50. Epub 2011/09/08.

272. Jackson WT, Giddings TH, Jr., Taylor MP, Mulinyawe S, Rabinovitch M, Kopito RR, et al. Subversion of cellular autophagosomal machinery by RNA viruses. PLoS biology. 2005;3(5):e156. Epub 2005/05/12.

273. Salabei JK, Hill BG. Autophagic regulation of smooth muscle cell biology. Redox biology. 2014;4C:97-103. Epub 2014/12/30.

274. Ghavami S, Mutawe MM, Schaafsma D, Yeganeh B, Unruh H, Klonisch T, et al. Geranylgeranyl transferase 1 modulates autophagy and apoptosis in human airway smooth muscle. American journal of physiology Lung cellular and molecular physiology. 2012;302(4):L420-8. Epub 2011/12/14.

275. Walter MJ, Morton JD, Kajiwara N, Agapov E, Holtzman MJ. Viral induction of a chronic asthma phenotype and genetic segregation from the acute response. The Journal of clinical investigation. 2002;110(2):165-75.

276. Rossi GA, Silvestri M, Colin AA. Respiratory syncytial virus infection of airway cells: Role of

microRNAs. Pediatric pulmonology. 2015. Epub 2015/04/08.

277. Tycowski KT, Guo YE, Lee N, Moss WN, Vallery TK, Xie M, et al. Viral noncoding RNAs: more surprises. Genes & development. 2015;29(6):567-84. Epub 2015/03/21.

278. Baroni D, Arrigo P. MicroRNA Target and Gene Validation in Viruses and Bacteria #. T miRNomics: MicroRNA Biology and Computational Analysis. p. 223-31.

279. Bondanese VP, Francisco-Garcia A, Bedke N, Davies DE, Sanchez-Elsner T. Identification of host miRNAs that may limit human rhinovirus replication. World journal of biological chemistry. 2014;5(4):437-56. Epub 2014/11/27.

280. Harrison TW, Oborne J, Newton S, Tattersfield AE. Doubling the dose of inhaled corticosteroid to prevent asthma exacerbations: randomised controlled trial. Lancet. 2004;363(9405):271-5. Epub 2004/01/31.

281. FitzGerald JM, Becker A, Sears MR, Mink S, Chung K, Lee J. Doubling the dose of budesonide versus maintenance treatment in asthma exacerbations. Thorax. 2004;59(7):550-6. Epub 2004/06/30.

282. Pauwels RA, Pedersen S, Busse WW, Tan WC, Chen YZ, Ohlsson SV, et al. Early intervention with budesonide in mild persistent asthma: a randomised, double-blind trial. Lancet. 2003;361(9363):1071-6. Epub 2003/04/04.

283. Doull IJ, Lampe FC, Smith S, Schreiber J, Freezer NJ, Holgate ST. Effect of inhaled corticosteroids on episodes of wheezing associated with viral infection in school age children: randomised double blind placebo controlled trial. BMJ (Clinical research ed). 1997;315(7112):858-62. Epub 1997/11/14.

284. Oommen A, Lambert PC, Grigg J. Efficacy of a short course of parent-initiated oral prednisolone for viral wheeze in children aged 1-5 years: randomised controlled trial. Lancet. 2003;362(9394):1433-8. Epub 2003/11/07.

285. Haughney J, Price D, Kaplan A, Chrystyn H, Horne R, May N, et al. Achieving asthma control in practice: understanding the reasons for poor control. Respiratory medicine. 2008;102(12):1681-93. Epub 2008/09/26.

286. Matsumura Y. Mechanisms of Reduced Glucocorticoid Sensitivity in Bronchial Asthma. In: Sapey DE, editor. Bronchial Asthma - Emerging Therapeutic Strategies: InTech; 2012.

287. From the Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2014. Available from: http://www.ginasthma.org/.

288. Vestbo J, Hurd SS, Agusti AG, Jones PW, Vogelmeier C, Anzueto A, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. American journal of respiratory and critical care medicine. 2013;187(4):347-65. Epub 2012/08/11.

289. Alagha K, Palot A, Sofalvi T, Pahus L, Gouitaa M, Tummino C, et al. Long-acting muscarinic receptor antagonists for the treatment of chronic airway diseases. Therapeutic advances in

chronic disease. 2014;5(2):85-98. Epub 2014/03/04.

290. Wollin L, Pieper MP. Tiotropium bromide exerts anti-inflammatory activity in a cigarette smoke mouse model of COPD. Pulmonary pharmacology & therapeutics. 2010;23(4):345-54. Epub 2010/04/07.

291. Buels KS, Jacoby DB, Fryer AD. Non-bronchodilating mechanisms of tiotropium prevent airway hyperreactivity in a guinea-pig model of allergic asthma. British journal of pharmacology. 2012;165(5):1501-14. Epub 2011/08/30.

292. Asano K, Shikama Y, Shoji N, Hirano K, Suzaki H, Nakajima H. Tiotropium bromide inhibits TGF-beta-induced MMP production from lung fibroblasts by interfering with Smad and MAPK pathways in vitro. International journal of chronic obstructive pulmonary disease. 2010;5:277-86. Epub 2010/09/22.

293. Milara J, Serrano A, Peiro T, Artigues E, Gavalda A, Miralpeix M, et al. Aclidinium inhibits cigarette smoke-induced lung fibroblast-to-myofibroblast transition. The European respiratory journal. 2013;41(6):1264-74. Epub 2012/09/29.

294. Ohta S, Oda N, Yokoe T, Tanaka A, Yamamoto Y, Watanabe Y, et al. Effect of tiotropium bromide on airway inflammation and remodelling in a mouse model of asthma. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2010;40(8):1266-75. Epub 2010/03/27.

295. Takeda N, Kondo M, Ito S, Ito Y, Shimokata K, Kume H. Role of RhoA inactivation in reduced cell proliferation of human airway smooth muscle by simvastatin. American journal of respiratory cell and molecular biology. 2006;35(6):722-9. Epub 2006/07/22.

296. Gower TL, Graham BS. Antiviral activity of lovastatin against respiratory syncytial virus in vivo and in vitro. Antimicrobial agents and chemotherapy. 2001;45(4):1231-7. Epub 2001/03/21.

297. Zeki AA. Statins and asthma: where we stand, and the next critical steps in research. Current medical research and opinion. 2014;30(6):1051-4. Epub 2014/01/24.

298. Ci X, Chu X, Xu X, Li H, Deng X. Short-term roxithromycin treatment attenuates airway inflammation via MAPK/NF-kappaB activation in a mouse model of allergic asthma. Inflammation research : official journal of the European Histamine Research Society [et al]. 2012;61(7):749-58. Epub 2012/04/07.

299. Dai YR, Wu HY, Wu LQ, Xu H, Yin J, Yan SS, et al. Roxithromycin reduces the viability of cultured airway smooth muscle cells from a rat model of asthma. European review for medical and pharmacological sciences. 2014;18(23):3564-72. Epub 2014/12/24.

300. Wu L, Wang R, Dai Y, Li F, Wu H, Yan S, et al. Roxithromycin suppresses airway remodeling and modulates the expression of caveolin-1 and phospho-p42/p44MAPK in asthmatic rats. International immunopharmacology. 2014;24(2):247-55. Epub 2014/12/07.

301. Tong X, Guo T, Liu S, Peng S, Yan Z, Yang X, et al. Macrolide antibiotics for treatment of

asthma in adults: A meta-analysis of 18 randomized controlled clinical studies. Pulmonary pharmacology & therapeutics. 2014. Epub 2014/09/25.

302. Knobloch J, Lin Y, Konradi J, Jungck D, Behr J, Strauch J, et al. Inflammatory responses of airway smooth muscle cells and effects of endothelin receptor antagonism. American journal of respiratory cell and molecular biology. 2013;49(1):114-27. Epub 2013/04/18.

303. Coyle TB, Metersky ML. The effect of the endothelin-1 receptor antagonist, bosentan, on patients with poorly controlled asthma: a 17-week, double-blind, placebo-controlled crossover pilot study. The Journal of asthma : official journal of the Association for the Care of Asthma. 2013;50(4):433-7. Epub 2013/02/01.

304. Girodet PO, Ozier A, Bara I, Tunon de Lara JM, Marthan R, Berger P. Airway remodeling in asthma: new mechanisms and potential for pharmacological intervention. Pharmacology & therapeutics. 2011;130(3):325-37. Epub 2011/02/22.

305. Girodet PO, Dournes G, Thumerel M, Begueret H, Dos Santos P, Ozier A, et al. Calcium Channel Blocker Reduces Airway Remodeling in Severe Asthma: a Proof-of-concept Study. American journal of respiratory and critical care medicine. 2015. Epub 2015/01/31.

306. Xu J, Zhu YT, Wang GZ, Han D, Wu YY, Zhang DX, et al. The PPARgamma agonist, rosiglitazone, attenuates airway inflammation and remodeling via heme oxygenase-1 in murine model of asthma. Acta pharmacologica Sinica. 2015;36(2):171-8. Epub 2015/01/27.

307. Cho JY. Recent advances in mechanisms and treatments of airway remodeling in asthma: a message from the bench side to the clinic. The Korean journal of internal medicine. 2011;26(4):367-83. Epub 2011/12/30.

308. Lee JH, Sohn JH, Ryu SY, Hong CS, Moon KD, Park JW. A novel human anti-VCAM-1 monoclonal antibody ameliorates airway inflammation and remodelling. Journal of cellular and molecular medicine. 2013;17(10):1271-81. Epub 2013/07/17.

309. Berlin AA, Hogaboam CM, Lukacs NW. Inhibition of SCF attenuates peribronchial remodeling in chronic cockroach allergen-induced asthma. Laboratory investigation; a journal of technical methods and pathology. 2006;86(6):557-65. Epub 2006/04/12.

310. Humbert M, de Blay F, Garcia G, Prud'homme A, Leroyer C, Magnan A, et al. Masitinib, a c-kit/PDGF receptor tyrosine kinase inhibitor, improves disease control in severe corticosteroid-dependent asthmatics. Allergy. 2009;64(8):1194-201. Epub 2009/07/21.

311. Larocca NE, Moreno D, Garmendia JV, De Sanctis JB. New pharmacological treatments for patients with chronic obstructive pulmonary disease (COPD). Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia. 2011;155(1):43-50. Epub 2011/04/09.

312. Sheshadri A, McKenzie M, Castro M. Critical review of bronchial thermoplasty: where should it fit into asthma therapy? Current allergy and asthma reports. 2014;14(11):470. Epub 2014/09/06.

313. Yin H, Kanasty RL, Eltoukhy AA, Vegas AJ, Dorkin JR, Anderson DG. Non-viral vectors for gene-based therapy. Nature reviews Genetics. 2014;15(8):541-55. Epub 2014/07/16.