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Emerging targets for novel therapy of asthma

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Abstract

Significant advances in understanding the cell and molecular biology of inflammation and airway smooth muscle (ASM) contractility have identified several potential novel targets for therapies of asthma. New agents targeting G-protein coupled receptors (GPCR) including bitter taste receptors (TAS2R) agonists and prostaglandin EP4 receptor agonists elicit airway smooth muscle relaxation. The cAMP/PKA pathway continues to be a promising drug target with the emergence of new PDE inhibitors and a novel PKA target protein, HSP20, which mediates smooth muscle relaxation via actin depolymerization. Smooth muscle relaxation can also be elicited by inhibitors of the RhoA/Rho kinase pathway via inhibition of myosin light chain phosphorylation and actin depolymerization. Targeting epigenetic processes that control chromatin remodeling and RNA-induced gene silencing in airway cells also holds great potential for novel asthma therapy. Further investigation may identify agents that inhibit smooth muscle contraction and/or restrain or reverse obstructive remodeling of the airways.

Introduction - Rationale for new asthma therapies

Asthma is a complex syndrome characterized by reversible airways obstruction resulting from allergen exposure and other triggers releasing multiple bronchoconstricting mediators that stimulate airway muscle to contract, thereby further narrowing airways that are already partially occluded by mucous and edema. Symptoms of dyspnea, coughing, exaggerated airway narrowing and wheezing typically accompany the characteristic chronic airway wall inflammation of asthma. Acute bronchoconstriction episodes are suppressed with beta-2 adrenoceptor agonists (e.g., albuterol) that elicit cAMP-dependent smooth muscle relaxation and bronchodilation. Combinations of Inhaled corticosteroids plus or minus a long acting beta agonist (LABA) are used to prevent the inflammatory response as well as to produce long lasting bronchodilation. Other bronchodilators used in asthma therapy include long acting muscarinic agonists (LAMA), leukotriene antagonists, and theophylline, which can be

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used in combination with corticosteroids and LABA to enhance bronchodilation and improve symptomatic relief.

Asthma attacks can occur over periods of many years, which creates additional therapeutic challenges. Chronic insult with allergens or other triggers results in a vicious cycle of bronchoconstriction, leukocyte infiltration, airways inflammation, and pathological remodeling of the airways. Long term structural airway alteration involves multiple cell types and is characterized by subepithelial fibrosis, edema, infiltration of leukocytes, and smooth muscle hypertrophy and hyperplasia. This leads to non-reversible obstruction of airflow causing chronic symptoms and, in rare cases, death. Until the recent advent of bronchial thermoplasty, which ablates some of the overabundant airway smooth muscle, long term remodeling has been untreatable. However, there are several areas of lung research that suggest new targets might emerge for drugs that circumvent some of the current limitations of asthma therapy that include tachyphylaxis to beta adrenergic agonists, corticosteroid insensitivity, off-target effects of corticosteroids, and improvement of effective treatments to reverse obstructive airway remodeling. Several recent reviews summarize advances in asthma and COPD therapies [1, 2, 3, 4] including novel cytokine-directed therapy [5, 6], which will inform the reader of current concepts in those fields. Here, we focus on emerging mechanisms of GPCR and cAMP-dependent bronchodilation, biochemical mechanisms regulating contraction and the actin cytoskeleton, and epigenetic events that might be suitable targets for anti-remodeling therapy. Most of the studies cited are in the pre-clinical experimental phase; some might develop into new avenues for translational studies in animal models and humans.

Novel G-protein-coupled receptor pathways: Bitter taste and EP4 receptors

Recent work on GPCRs in airway smooth muscle shows that several previously uncharacterized signaling pathways can elicit bronchodilation (Figure 1). Bitter taste receptor (eg. TAS2R) agonists cause hyperpolarization of ASM and reduce calcium levels near the plasma membrane thus eliciting bronchodilation [7]. Bitter taste agonists may act through activation of BK channels, but the necessity of BK activation has been challenged [8]. Interestingly activation of bitter taste receptors elicits bronchodilation even in the presence of beta receptor desensitization [9] indicating that they might be useful in patients in whom beta receptor tachyphylaxis occurs. However, bitter taste receptors undergo homologous desensitization which suggests chronic monotherapy with bitter taste agonists may suffer the same limitation as beta adrenergic agonists [10]. In addition, relatively low potency of current agents and the issue of lung-restricted delivery to avoid off-target effects are potential problems that remain to be solved.

Endogenously produced prostaglandin E2 relaxes airway smooth muscle via cAMP-dependent mechanisms, and so limits the effects of bronchoconstrictors (Figure 1). Initial clinical trials of a selective EP2 receptor agonist were disappointing in that it was not effective in treating asthma [11]. However, recent studies of EP2 and EP4 selective agonists and antagonists in isolated human bronchial smooth muscle preparations showed the EP4 subtype mediates relaxation by PGE2 [12]. This supports EP4-selective agonists as candidates for further development of novel bronchodilators [13]. Since there are no trials yet of EP4 receptor agonists in humans EP4 agonists must be considered interesting agents in the early proof of principle stage of development.

Interest in developing EP4 agonists derives from the fact that they act on airway smooth muscle by increasing cAMP to elicit smooth muscle relaxation. Another approach to elevating cAMP is to inhibit phosphodiesterases. There are several new isoform-directed phosphodiesterase inhibitors [2, 14] being developed as novel asthma therapies. Like the

EP4 agonists most data on new PDE inhibitors are from cellular and tissue-level studies, and human trials are still on the horizon. Interest in the functional differences in PDE isoforms and new insights into how cAMP might act in various cellular compartments in smooth muscle suggests that there are undiscovered mechanisms that might be exploited to elicit bronchodilation and perhaps modify airways remodeling via inhibition of smooth muscle cell proliferation and cell migration.

Phosphorylation of HSP20 as a novel cAMP-dependent bronchodilation mechanism

One of the emerging mechanisms of cAMP-dependent airway smooth muscle relaxation is phosphorylation of the small heat shock protein, HSP20 (HSPB1) (reviewed by [15]). Figure 2 is a model summarizing two proposed molecular mechanisms of bronchodilation – depolymerization of F-actin and inhibition of actin and myosin crossbridge formation. Both mechanisms are thought to be regulated by phosphorylation of Ser16 of HSP20 by PKA during smooth muscle relaxation induced by vasodilators and bronchodilators [16, 17].

Inhibition of actin-myosin crossbridge formation was proposed to be due to a troponin I like effect [18, 19, 20], and actin depolymerization is thought to be due to activation of cofilin [21]. HSP20 may well have both effects, acting in parallel as shown in Figure 2. Evidence for this pathway includes: 1. HSP20 is phosphorylated in airway smooth muscle cells and tissues by agents that increase cAMP [22, 20], 2. cell permeant HSP20 phosphopeptide mimic decreases cofilin phosphorylation [22], 3. cell permeant HSP20 phosphopeptide mimic [22] and a full length phospho-HSP20 protein [20] both disrupt F-actin filaments in airway smooth muscle cells and tissues, and 4. a small molecule inhibitor of HSP20 binding to 14-3-3 proteins inhibits airway smooth muscle cell and tissue contraction [23]. As such, HSP20 phosphorylation is an emerging cAMP-dependent mechanism of airway smooth muscle relaxation that is an attractive target for further development of a new class of bronchodilators. Discovery of additional small molecule mimics of HSP20 function might provide a therapeutic strategy to bypass insensitivity to beta-adrenergic agonists.

Rho/Rho kinase pathway

Activation of the Rho/Rho kinase pathway has multiple effects on airway smooth muscle contraction, proliferation and cell migration. Contraction in response to neurotransmitters and other bronchoconstrictors is enhanced by activation of Rho kinases which inhibit myosin light chain phosphatase resulting in “calcium sensitization” [24, 25]. A Rho kinase inhibitor produces relaxation by “calcium desensitization” [24]. Figure 2 shows that myosin light chain phosphatase is a target of the Rho/ROCK pathway. Inhibition of Rho kinases reduces myosin light chain phosphorylation thus producing relaxation. Rho kinases also phosphorylate a wide variety of cytoskeletal substrates including vimentin, ezrin, radixin, moesin (ERM) and LIM kinase (not shown). Phosphorylation of multiple Rho kinase substrates mediate actin cytoskeletal remodeling necessary for cell contraction, cell migration, proliferation and differentiation in smooth muscle cells. Thus far cell and animal studies of Rho kinase inhibitors suggest they might be effective bronchodilators, but human studies have not been conducted [26, 27, 28]). This may be due to the fact that zsystemically administered Rho kinase inhibitors are hypotensive agents, which would be an unwanted off-target effect in normotensive patients. Development of lung-restricted delivery methods could make these agents more useful as bronchodilators which, like HSP20 targeted drugs, are not limited by receptor desensitization.

Statins

Rho proteins that couple GPCR activation to Rho kinases and downstream targets are prenylated to enhance their localization to the plasma membrane and allow signaling complexes to form. The prenylation reactions of Rho-family proteins, including farnesylation and geranyl-geranylation, depend on mevalonate produced by 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity. This suggests that Rho-dependent processes in inflammation and airway smooth muscle contraction might be sensitive to statins which inhibit HMG-CoA reductase and reduce prenylation of RhoA (Figure 3). Studies in cells show that simvastatin reduces ASM proliferation and migration [29], ASM contraction [30] and reduces airway hyperresponsiveness in mice [31], [32]. The geranylgeranyltransferase inhibitor, GGTI-2133, inhibits airway hyperreactivity in albumin-sensitized mice [32]. In humans, a small clinical trial failed to show any anti-inflammatory effect of one month treatment with simvastatin [33], but several trials where statins were coadministered with inhaled corticosteroids suggest that statins might provide a modest reduction in inflammation and a modest improvement in asthma symptoms [34]. Statins are an interesting class of drugs approved for other diseases that could potentially be repurposed for therapy of asthma in combination with corticosteroids. It will be important to determine whether chronic treatment of asthma with statins as adjunct therapy would reverse airway wall remodeling and/or potentiate the antiinflammatory effects of inhaled corticosteroids.

Epigenetic reprogramming by histone modifications and microRNAs

a. Pathways controlling transcription have been studied extensively in lung cells to elucidate mechanisms of asthma pathogenesis. In contrast, epigenetic mechanisms controlling protein abundance (Figure 3A) and microRNA-mediated control of translation (Figure 3B) are not as well defined, particularly in airway smooth muscle cells. **Histone deacetylase (HDAC) Modifiers**

Modulation of HDAC activity is a promising strategy for modifying the phenotype of airway cells to reduce contractility, inhibit inflammation or to alter obstructive remodeling (Figure 3A). HDAC2 is downregulated in humans with asthma and COPD [35], which contributes to corticosteroid insensitivity. Therefore, selective upregulation of HDAC2 might be an effective therapy for steroid-resistant asthma. In contrast to the specific effects of HDAC2 the non-selective HDAC inhibitor, Trichostatin A, antagonizes airway smooth muscle contraction with no significant anti-inflammatory effect [36], although a previous study showed anti-inflammatory effects in a sensitized mouse model [37]. The bronchodilating effect of trichostatin A is consistent with beneficial effects in asthmatics of the antiepileptic agent, valproic acid, which is also a broad spectrum HDAC inhibitor [38]. Similar to corticosteroids, HDAC inhibitors are not selective agents for any particular cell type or specific genes. This raises a question of how they might be used chronically to treat asthma without intolerable long term adverse effects. More thorough analysis of the histone code using HDAC isoform selective agents and a careful analysis of off-target effects is warranted. Chromatin structure and gene expression patterns are also controlled by histone methylation and demethylation which may contribute to the pathogenesis and treatment of asthma [39]. The study of agents modifying the various covalent modifications of histones in asthma and in normal airway cells is an emerging topic with potentially high significance in developing novel classes of drugs to treat asthma.

b. MicroRNAs

In airway smooth muscle, miRNAs have been identified that influence contractile protein expression (miR-25, [40]), cell calcium levels (miR-140-3p, [41]), Rho kinase signaling (miR-133a, [42, 43]) and smooth muscle hypertrophy ([44]). Proof of the concept that miRNAs can be targeted for therapy of asthma was provided by studies of miRNAs and inflammation in a sensitized mouse model. Foster and colleagues [45] treated mice sensitized with house dust mite with antisense oligonucleotide against miR-145. They showed anti-miR145 therapy reduced airway hyperreactivity, reduced Th2 cytokine production and diminished tissue eosinophilia. These mouse studies are very promising, but further investigation of additional miRNAs and RNAi-based drugs are needed to establish the efficacy and safety of this new class of modulators of airways structure and function. Successful development of novel miRNA antagonist requires careful consideration of problems with drug delivery to the lung tissues, cell and molecular target specificity and toxicity due to off-target effects. The field is advancing rapidly with reports of positive preclinical efficacy of RNA—based oligonucleotides used in cancer therapy, cardiovascular diseases and inflammatory diseases.

Conclusions and future directions

Important advances in anti-inflammatory therapies and G protein coupled receptors mediating bronchodilation should provide new tools to treat problematic asthma patients. However there are still no effective interventions that will reduce airway smooth muscle tone chronically or the smooth muscle mass in extensively remodeled airways of severe asthmatics. To address these problems new drugs targeting previously unrecognized or poorly characterized molecules and processes must be developed. One approach is to develop novel therapies directed at disrupting the actin cytoskeleton and contractile protein assemblies to antagonize smooth muscle contraction. Peptide and small molecule mimics of HSP20 phosphorylation, mechanical perturbations [46] and FGF2 stimulation [47] all do so in cells and tissues but efficacy has not yet been demonstrated in preclinical studies of animal models of asthma. One goal of such drug development studies would be to avoid therapeutic failure due to beta adrenoceptor tachyphylaxis and steroid resistance. Another important approach is to inhibit inflammation and reverse pathological remodeling at multiple steps in multiple cell types, similar to steroid therapy but in a more focused manner. Some of the emerging topics described here – actin cytoskeleton remodeling, the histone code and miRNA regulation of translation offer novel targets that might satisfy the need for effective therapeutic modalities for most or all of the clinically recognized asthma phenotypes.

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Highlights

Bitter taste receptors (TAS2R) and EP4 prostaglandin receptors are emerging targets for new bronchodilators. Heat shock protein HSP20 is a cAMP-dependent inhibitor of bronchoconstriction. Rho kinase signaling is also a promising target for novel bronchodilator therapy. Statins may inhibit small G-protein signaling to promote bronchodilation. New epigenetic targets include histone modifying enzymes and microRNAs.

Figure 1. Proposed mechanisms of smooth muscle relaxation by activation of bitter taste (TAS2R) and prostaglandin E (EP4) receptors in human airway smooth muscle
 TAS2R activation may produce relaxation by activating BK channels to produce hyperpolarization and decreases calcium concentration in limited regions of the cell. Activation of EP2 and EP4 receptors elicit airway smooth relaxation by G_s coupled activation of adenylate cyclase (AC), production of cAMP and activation of protein kinase A (PKA), which phosphorylates multiple substrates to decrease cell calcium concentration. Decreasing calcium reduces activation of myosin light chain kinase (MLCK) thus favoring myosin light chain dephosphorylation by myosin phosphatase (subunits PP1c, MYPT and M20). Dephosphorylation of myosin results in relaxation.

Figure 2. Mechanisms of bronchodilation by phosphorylation of HSP20 and inhibition of Rho kinases

Beta adrenergic agonists act by multiple mechanisms to relax airway smooth muscle including decreasing cell calcium oscillations by reducing calcium entry (not shown), increasing calcium uptake into the sarcoplasmic reticulum (SR), activating myosin phosphatase (subunits PP1c, MYPT, M20) and by phosphorylating HSP20. HSP20 may have multiple effects on the contractile filaments including an indirect effect on activation of the slingshot phosphatase which dephosphorylates cofilin. Dephosphorylated cofilin binds F-actin filaments and promotes depolymerization to G-actin and relaxation. Rho kinase inhibitors enhance the activity of myosin phosphatase (PP1c) to favor dephosphorylated myosin light chains which results in smooth muscle relaxation. Statins inhibit prenylation of small G proteins, including RhoA, which inhibits the Rho/Rho kinase pathway.

A. Histone Code

B. miRNA and anti-miRNA mechanisms

Figure 3. Epigenetic mechanisms that are targets for new drug development in lung diseases
 A. The histone code represented here as histone acetylation (Ac) and methylation (Me) controls condensation and relaxation of chromatin. Relaxed chromatin enhances mRNA synthesis and expression of proteins. Histone modifying enzymes, including histone deacetylases, acetyltransferases, demethylases and methyl transferases are emerging targets for small molecule inhibitors that alter expression of pro-inflammatory genes and genes involved in hyperplasia and hypertrophy of various airway cells.
 B. MicroRNA gene silencing via translational block and mRNA destabilization controls protein abundance. miRNA antagonists inhibit the function of endogenous miRNAs, probably by hybridizing with the endogenous miRNA. This approach is being developed to antagonize protein expression networks that contribute to inflammation and airway hyperreactivity.