McDuffie *et al. Respiratory Research* (2024) 25:295
https://doi.org/10.1186/s12931-024-02920-0

Respiratory Research

REVIEW Open Access

$G_{12/13}$ signaling in asthma

Elizabeth L. McDuffie[1], Reynold A. Panettieri Jr.[2] and Charles P. Scott[1*]

Abstract
Shortening of airway smooth muscle and bronchoconstriction are pathognomonic for asthma. Airway shortening occurs through calcium-dependent activation of myosin light chain kinase, and RhoA-dependent calcium sensitization, which inhibits myosin light chain phosphatase. The mechanism through which pro-contractile stimuli activate calcium sensitization is poorly understood. Our review of the literature suggests that pro-contractile G protein coupled receptors likely signal through $G_{12/13}$ to activate RhoA and mediate calcium sensitization. This hypothesis is consistent with the effects of pro-contractile agonists on RhoA and Rho kinase activation, actin polymerization and myosin light chain phosphorylation. Recognizing the likely role of $G_{12/13}$ signaling in the pathophysiology of asthma rationalizes the effects of pro-contractile stimuli on airway hyperresponsiveness, immune activation and airway remodeling, and suggests new approaches for asthma treatment.

Keywords Airway hyperresponsiveness, Airway remodeling, Anticholinergic agents, Asthma, Bronchoconstriction, Calcium sensitization, $G_{12/13}$, Inflammation, Muscarinic 3 acetylcholine receptor, RhoA

Background

G Protein Coupled Receptors (GPCRs) comprise the largest family of cell surface receptors in the human genome (>800 members) [1–3] and coordinate physiological responses to everything from photons to proteins. In contrast to this diversity of receptors and ligands, the heterotrimeric G proteins that mediate intracellular signaling downstream of GPCR activation seem deceptively simple: sixteen Gα subunits that fall into four mechanistic subfamilies (G_s, $G_{q/11}$, $G_{i/o}$ and $G_{12/13}$) and five Gβ subunits that form obligate heterodimers with one of twelve Gγ subunits [4]. GPCR signaling is typically described as resulting from specific coupling between a GPCR and a particular G protein, but most GPCRs couple to multiple G proteins [5]. The structural determinants of G protein selection by GPCRs are not well understood [6, 7]. Differential G protein recruitment (G protein bias) is often governed by ligand binding [8, 9] with signaling outcomes determined by the ensemble of G proteins that are activated. Elucidating the structural and mechanistic basis of G protein bias and using it as a tool for improving drug properties is the focus of considerable research [10–12].

Ligand binding to GPCRs promotes conformational changes that catalyze guanine nucleotide exchange within the α subunits of associated heterotrimeric G proteins [13]. GTP binding dissociates G protein heterotrimers, revealing protein interaction sites that mediate downstream signaling [14]. Although both Gα and Gβγ complexes contribute to signaling downstream of GPCR activation, signaling pathways are typically categorized by their Gα subunit. Activation of G_s stimulates adenylyl cyclase, which increases the cytosolic concentration of cyclic AMP (cAMP) thereby activating protein kinase A (PKA), while activation of $G_{i/o}$ inhibits adenylyl cyclase [15]. Activation of $G_{q/11}$ stimulates phospholipase Cβ (PLCβ), which generates inositol trisphosphate (IP3) and

*Correspondence:
Charles P. Scott
charles.scott@jefferson.edu
[1]Department of Biochemistry and Molecular Biology, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA, USA
[2]Rutgers Institute for Translational Medicine and Science, Child Health Institute, Rutgers University, New Brunswick, NJ, USA

diacylglycerol (DAG), thereby promoting intracellular calcium flux and activation of protein kinase C (PKC) [16].

In 1991, the $G_{12/13}$ subfamily was identified as the fourth class of Gα subunits. This subfamily consists of G_{12} and G_{13} subunits, which share 67% amino acid sequence homology [17]. Unlike other Gα protein subunit subfamilies, activated G_{12} and G_{13} do not regulate enzymes that produce small molecule second messengers such as cAMP, IP3 or Ca^{2+}. Instead, GTP-bound $G_{12/13}$ subfamily members bind to and regulate the Ras homology (Rho) family of guanine nucleotide exchange factors (RhoGEFs) [18, 19]. RhoGEF complexes activate small Rho GTPases, such as RhoA, which play critical roles in regulating cytoskeletal dynamics [20]. Rho GTPases also stimulate numerous downstream signaling pathways through activation of Rho kinase (ROCK), Lim kinase (LIMK) and c-Jun NH_2-terminal kinase (JNK; see Fig. 1) [21]. Deactivation of $G_{12/13}$ occurs through hydrolysis of GTP to GDP via their intrinsic GTPase activity, which can be accelerated by GTPase-activating proteins (GAPs) including regulators of G protein signaling (RGS) domains of RhoGEF family members [22]. $G_{12/13}$ couple to more than 30 GPCRs, including angiotensin II receptors, cysteinyl leukotriene receptors (CysLTR), histamine (H) receptors, lysophosphatidic acid (LPA) receptors, protease-activated receptors (PAR), sphingosine-1-phosphate (S1P) receptors, and thromboxane A2 receptors [5].

Asthma is an obstructive airway disease characterized by airway hyperresponsiveness (AHR), inflammation and airway remodeling (AR). GPCRs play critical roles in regulating bronchomotor tone, and, as such, are the targets of numerous therapeutics that are used to treat asthma and other obstructive pulmonary diseases. GPCRs coupled to G_s, such as the β_2 adrenergic receptor (β_2AR), regulate relaxation of airway smooth muscle, while GPCRs coupled to $G_{q/11}$, such as histamine, leukotriene and muscarinic acetylcholine receptors, promote airway constriction. Notably, $G_{12/13}$ couples to many $G_{q/11}$ coupled receptors in vascular and airway smooth muscle [5], and the RhoA signaling pathway downstream of $G_{12/13}$ activation potentiates smooth muscle contraction [21]. Inflammation plays a fundamental role in the etiology of asthma, thus biologics and corticosteroids are mainstays of therapy. $G_{12/13}$ dependent signaling plays an

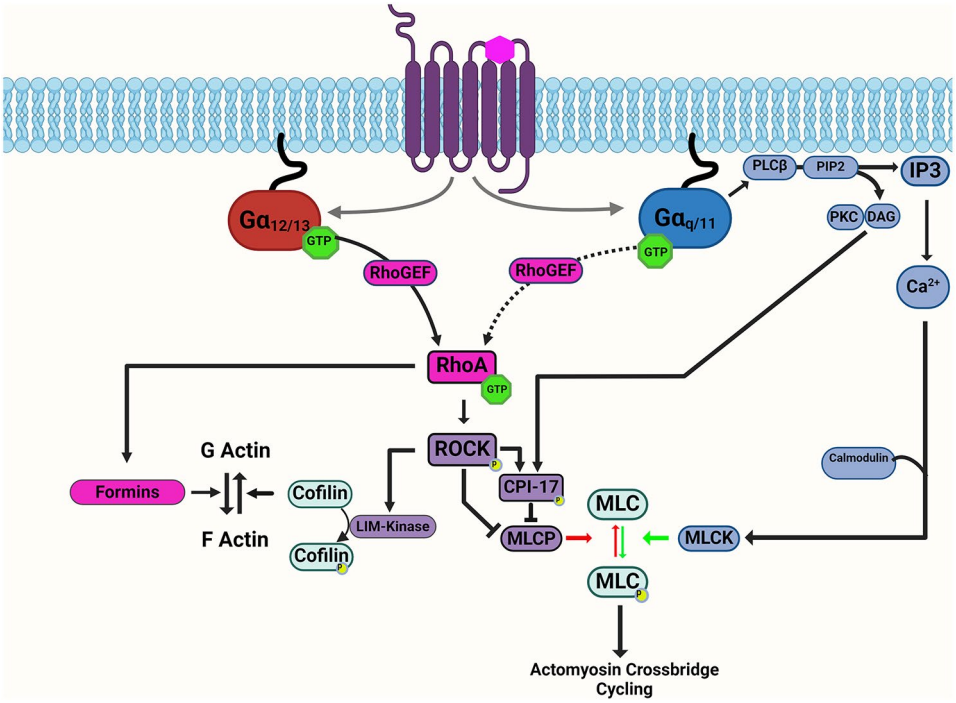

Fig. 1 G protein signaling in smooth muscle contraction. Two G protein signaling pathways contribute to airway smooth muscle contraction. Activation of $G_{q/11}$ upon receptor-dependent guanine nucleotide exchange stimulates the calcium-dependent contractile pathway, whereby GTP-bound $G_{q/11}$ allosterically activates PLCβ-dependent hydrolysis of phosphoinositide bisphosphate (PIP2) into IP3 and DAG, which promote intracellular calcium flux, thereby activating MLCK-dependent phosphorylation of myosin light chain and actomyosin cross-bridge cycling (blue nodes on right side of figure). Calcium sensitization (fuchsia and violet nodes in the center and left of the figure) is mediated by GTP-bound RhoA, which can be generated downstream of activation of either $G_{q/11}$ or $G_{12/13}$, although $G_{12/13}$ are more potent activators [29]. PKC phosphorylation of CPI-17 promotes inhibition of myosin light chain phosphatase, which increases net MLC phosphorylation. GTP-bound RhoA stimulates actin polymerization through activation of formins (fuchsia nodes) and ROCK activity (violet nodes), which inhibit both filament severing and MLCP. ROCK also phosphorylates CPI-17, which further inhibits MLCP. (Created with BioRender.com)

important role in lymphocyte activation and migration. Finally, airway thickening and angiogenesis are stimulated in patients with asthma. Increased expression of $G_{12/13}$ has been reported in animal models of asthma [23], numerous proliferative pathways (mitogen-activated protein kinase (MAPK) [24], Hippo [25], non-receptor tyrosine kinases [26]) are downstream of $G_{12/13}$ activation, and G_{13} has been implicated in both airway smooth muscle (ASM) proliferation and angiogenesis in a variety of physiological and pathological settings, including development and cancer [27, 28].

As the most potent upstream modulator of RhoA [29], $G_{12/13}$ and its downstream signaling pathway constitutes an intriguing target with the potential to impact myriad aspects of the pathophysiology of asthma by inhibiting AHR, inflammation and AR. Since there are limited publications on $G_{12/13}$ signaling in ASM and asthma, each section of this review summarizes relevant findings from non-muscle tissues first and concludes with smooth muscle or ASM data where available. The preponderance of the evidence suggests that targeting pathways that activate the $G_{12/13}$ subfamily and its downstream signaling axis may provide a complementary therapeutic approach for managing asthma and other obstructive pulmonary diseases.

G protein stimulation of myosin light chain phosphorylation regulates smooth muscle contraction and airway hyperresponsiveness

Airway smooth muscle shortening induces narrowing of airways, evoking wheezing, difficulty breathing and chest tightness, which, when coupled with mucus buildup and airway inflammation, is characteristic of asthma [30]. Bronchoconstriction is thought to be mediated by agonist binding to $G_{q/11}$ coupled receptors. The guanine exchange activity of agonist-bound GPCRs enables GTP-bound $G_{q/11}$ to allosterically activate PLCβ-dependent production of IP3 and DAG, thereby stimulating IP3 receptor-dependent Ca^{2+} release from the sarcoplasmic reticulum and PKC-dependent activation of Ca^{2+} channels, which cooperate to increase the concentration of calcium in the cytosol (blue nodes in Fig. 1). Cytosolic Ca^{2+} binding to calmodulin evokes allosteric activation of myosin light chain kinase (MLCK), which phosphorylates the 20 kDa light chain of myosin to promote actomyosin cross-bridge cycling and muscle contraction [31, 32]. Calcium-bound calmodulin in smooth muscle also activates calmodulin-dependent kinase II, which cooperates with PKC to relieve tonic inhibition of the interaction between myosin and actin by calponin [33]. Interestingly, however, the $G_{q/11}$ depsipeptide inhibitor FR900359 (FR) failed to inhibit carbachol-dependent contraction of mouse precision-cut lung slices at 30 nM concentration [34]. A higher concentration of FR (1 μM) only partially inhibited carbachol-dependent contraction of human precision-cut lung slices [35] suggesting that actomyosin cross-bridge cycling still occurs even when $G_{q/11}$-stimulated calcium flux is fully inhibited. If $G_{q/11}$-dependent Ca^{2+} flux is insufficient for actomyosin cross-bridge cycling, what regulates the effects of acetylcholine on smooth muscle tone?

$G_{12/13}$ activates the RhoA/ROCK signaling pathway to regulate actin polymerization and inhibit MLCP

The interaction between myosin and actin in smooth muscle is regulated through phosphorylation of myosin light chain, and by maintaining a large pool of depolymerized actin [36]. Both myosin light chain phosphorylation and actin polymerization are necessary for smooth muscle contraction (Fig. 1) [37, 38].

The actin cytoskeleton is a highly dynamic structure that is regulated by polymerization and depolymerization processes interconverting globular (G) and filamentous (F) actin. This mechanism is particularly true in smooth muscle, where the ratio of F actin to G actin is three to four times less than what is observed in cardiac and skeletal muscle [36]. The Rho family of small GTPases -- Cdc42, Rac1, and RhoA -- regulate a diverse array of cytoskeletal dynamics [39, 40]. GTP-bound RhoA activates formins, which stimulate actin polymerization (fuchsia nodes in Fig. 1) [37]. Since each myosin head interacts with two actin monomers in F actin, polymerization of actin is essential for actinomyosin complex assembly and muscle contraction [41]. Microinjection of activated G_{12} or G_{13} into Swiss-3T3 cells is sufficient to induce actin polymerization in a RhoA-dependent manner [42].

GTP-bound RhoA also activates ROCK, which phosphorylates the myosin targeting subunit (MYPT1) of myosin light chain phosphatase (MLCP). MYPT1 phosphorylation inhibits MLCP binding to myosin light chain (MLC), thereby increasing net MLC phosphorylation (this is referred to as calcium sensitization – see violet nodes in Fig. 1) [43]. ROCK therefore acts synergistically with MLCK to prolong and strengthen muscle contraction [21]. Both ROCK and PKC phosphorylate and activate CPI-17, a smooth muscle specific MLCP inhibitor, which enhances the pro-contractile effect [44]. Polymerized actin is further stabilized through inhibitory phosphorylation of cofilin by LIMK [45], which is activated upon phosphorylation by ROCK. Cofilin phosphorylation impairs binding to F actin [46] thereby inhibiting filament severing and actin depolymerization [47, 48]. In tracheal smooth muscle, acetylcholine-induced stiffness is independent of MLC phosphorylation and calcium flux, but dependent on ROCK [49]. ROCK inhibition blocks contraction of airway smooth muscle both in vitro and in vivo [50–52].

Small GTPases of the Ras superfamily, such as RhoA, must be activated by guanine nucleotide exchange factors to stimulate downstream signaling. RhoGEFs can be activated by either GTP-bound $G_{q/11}$ or $G_{12/13}$, but GTP-bound $G_{12/13}$ are the more potent activators [29]. RhoA and other Ras family members are also regulated by guanine nucleotide dissociation inhibitors (GDIs). GDIs prevent small GTPases from participating in nucleotide exchange, holding them in an inactive state and in some cases blocking them from trafficking to the membrane [53, 54]. In vascular smooth muscle, constitutively activated G_{12} or G_{13} promote vasoconstriction, which can be inhibited by botulinum C3 toxin or ROCK inhibition, while dominant-negative forms of G_{12} or G_{13} inhibit vasoconstriction [55]. Similarly, conditional knockdown of $G_{12/13}$ or leukemia-associated RhoGEF (LARG) normalized age-related hypertension in mice [56], and blocked salt-induced hypertension [57], while loss of either $G_{12/13}$ or the smooth muscle specific RhoGEF, ARHGEF12, in small arteries induced loss of RhoA activation and vasodilation [58]. In a rat model, chronic administration of angiotensin II resulted in increased expression of G_{12} and elevated blood pressure, while co-administration of GNA12 antisense lowered mean blood pressure [59].

$G_{12/13}$ couples to several pro-contractile GPCRs in airway smooth muscle cells (H1, CysLTR2, PAR 1 & 2) [5], and G_{12} and G_{13} are overexpressed in AHR rats compared to healthy animals [23]. Controversy exists in the optical biosensor literature concerning whether muscarinic 3 acetylcholine receptors (M3R) interact with $G_{12/13}$ [5, 8, 34], but functional assays suggest interactions between M3R and $G_{12/13}$ in both human embryonic kidney (HEK293) and human airway smooth muscle (HASM) cells. M3R-dependent activation of G_{12} in HEK293 cells stimulates phospholipase D activity [60, 61]. Knockdown of GNA13 in primary bronchial smooth muscle cells blocked methacholine-induced phosphorylation of myosin light chain [62]. In HASM cells, interaction between M3R and G_{12} was confirmed by co-immunoprecipitation, siRNA knockdown of GNA12 reduced phosphorylation of MYPT1 and MLC, expression of a G_{12} inhibitor (p115RhoGEF-RGS) suppressed carbachol-mediated HASM cell contraction, and RhoA inhibition induced dilation of human precision cut lung slices [63]. Collectively, these data suggest that $G_{12/13}$ couples to most therapeutically relevant pro-contractile GPCRs in both HEK293 and HASM cells.

Levels of TGF-β1, a cytokine that regulates extracellular matrix formation, cell growth, inflammation, and the epithelial-mesenchymal transition (EMT) are increased in airways of patients with severe asthma [64]. Published research suggests that $G_{12/13}$ regulates TGF-β1 expression, although the specifics of the regulatory mechanism are unclear. In HASM cells, pretreatment with TGF-β1 increases RhoA translocation via p115RhoGEF. In $G_{12/13}$ deficient cells, TGF-β1 expression was restored after transfection with constitutively active $G_{12/13}$ QL mutants, suggesting that $G_{12/13}$ play a role in modulating TGF-β1 expression levels [65]. TGF-β1 increases bronchomotor tone and enhances carbachol- and histamine-induced excitation-contraction coupling in human airway smooth muscle and precision cut lung slices, respectively. In isolated HASM cells, methacholine-induced cytoskeletal stiffness was increased after TGF-β1 pre-treatment in a dose-dependent manner. Inhibition of ROCK attenuated TGF-β1 induced single-cell contraction, while having no impact on intracellular calcium release [64]. These data indicate that TGF-β1-mediated physiological effects in HASM occur in a $G_{12/13}$-dependent manner.

In summary, smooth muscle contraction is regulated by $G_{q/11}$ dependent activation of MLCK, which is enhanced by ROCK dependent inhibition of MLCP, and by RhoA-dependent actin polymerization, which is stimulated more robustly by activated $G_{12/13}$ than by activated $G_{q/11}$. The pro-contractile and AHR-inducing effects of TGF-β1 also appear to be regulated by $G_{12/13}$ signaling. The complex interplay between the $G_{q/11}$ and $G_{12/13}$ signaling pathways leads to impaired muscle shortening when RhoA/ROCK activation is compromised, and in elevated resting tone unless both pathways to RhoA/ROCK activation are inhibited.

$G_{12/13}$ signaling regulates immune activation and infiltration

Asthma is a chronic disease mediated by immune infiltration in which both symptoms (e.g., mucus production) and pathology (AHR, AR) are driven by inflammatory processes [66]. Asthma patients display heterogeneous clinical phenotypes, in part due to the spectrum of immune mechanisms that are dysregulated in patients with asthma. In approximately half of all patients, asthma is characterized by type 2 immune response and sensitization to allergens [67]. Immune responses in these patients result from production of IgE and Th2-associated cytokines (IL4, IL5, IL13), which stimulate dendritic and mast cell activation, eosinophil invasion and mucus production [68]. As such, these patients are often responsive to inhaled corticosteroids and biologics that antagonize Th2-associated cytokine signaling. In contrast, patients with minimal production of Th2-associated cytokines are often poorly responsive to inhaled corticosteroids. Inflammation in these patients is driven by macrophages, neutrophils and cytokines like TGF-β, IL-1β and IL-6 [69].

G_{12} and G_{13} have been implicated in various aspects of immune cell activation and trafficking (Fig. 2). S1P is released by mast cells in response to IgE stimulation. S1P receptors on mature dendritic cells coordinate expression

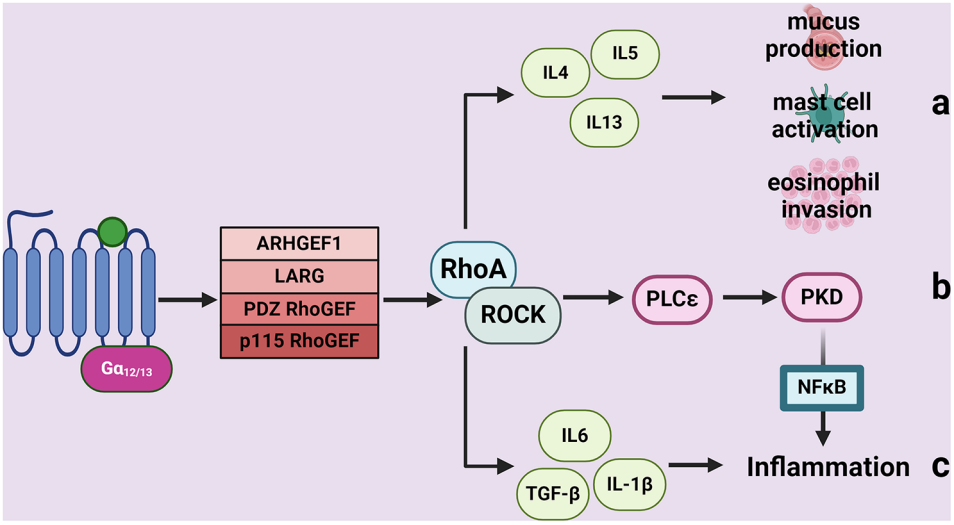

Fig. 2 $G_{12/13}$ signaling in inflammation. $G_{12/13}$-dependent activation of RhoA and ROCK promotes inflammation in both Th2-high (**a**) and Th2-low asthma (**c**). $G_{12/13}$ can also stimulate NFκB-dependent production of inflammatory cytokines (**b**). (Created with BioRender.com)

of Th2 cytokines in a $G_{12/13}$-dependent manner (Fig. 2a) [70, 71], and both S1P concentration and GNA12 expression predict asthma control [72]. In the central nervous system, S1P activates receptors on astrocytes that signal via $G_{12/13}$, RhoA, phospholipase C-epsilon and protein kinase D to stimulate NFκB dependent transcription of inflammatory genes (Fig. 2b) [73]. Biased inhibition of $G_{12/13}$ signaling downstream of PAR2 activation blocks inflammation in vivo [12]. Diminished follicular helper T (Tfh) response was observed in a T-cell specific $G\alpha_{13}$ deficient mouse model, and G_{13} knock-out Tfh cells showed an inability to transduce signal through RhoA mediated ROCK activation, highlighting the regulatory role of the G_{13}-Rho-Rock signaling axis in Thf cell differentiation [74].

The expression level of activating protein 1 (AP-1), a transcription factor that is necessary for the proliferation and differentiation of Th2 cells, was reduced in $G_{12/13}$ knockdown cells [62]. Myeloid-specific knockdown of $G_{12/13}$ results in increased expression of anti-inflammatory genes in macrophages, which protect against atherosclerosis in wild-type mice [75]. GNA12 regulates C5a-mediated migration in macrophages, indicating an anti-inflammatory role for GNA12 in inflammatory bowel disease [76, 77]. Loss of RGS-containing RhoGEFs such as ARHGEF1, LARG, PRG or p115 that activate RhoA and ROCK downstream of $G_{12/13}$ compromise immune function (Fig. 2c) [78, 79]. G_{13} knockdown in microglial cells inhibits LPS-induced activation and Rac-dependent migration [80]. In platelets and leukocytes, outside-in signaling between integrins and G_{13} regulates Rho-dependent, pro-inflammatory secretion and thrombosis [81–84]. $G_{12/13}$ are also implicated in cytokine signaling and inflammation in prostate cancer [85] and mediate renal ischemia-reperfusion injury [86–88].

Published evidence suggests that $G_{12/13}$ and downstream RhoA/ROCK signaling mediate inflammation in diverse physiological and pathological settings. Inhibiting this pathway may cooperate, or even synergize, with biologics and inhaled corticosteroids in Th2 high asthma, and provide a novel approach to suppress inflammation in patients who are refractory to corticosteroid therapy.

$G_{12/13}$ regulation of airway remodeling and hyperplasia

AR is a hallmark of the pathophysiology of asthma [89]. In airways, hyperproliferation evokes thickening of both smooth muscle and the extracellular matrix, which narrows bronchi and predicts poor patient outcomes [90]. HASM cells from patients with asthma proliferate faster than ASM cells from control patients [91, 92]. Activation of $G_{q/11}$-coupled receptors stimulates MAPK, which promotes proliferation of ASM and contributes to hyperplasia, angiogenesis and tissue remodeling in a wide variety of contexts [93]. Proliferation downstream of the $G_{12/13}$ –RhoA signaling axis is regulated by kinases, pro-proliferative transcription factors, and alterations in gene expression (Fig. 3).

G_{12} was the first Gα subunit that was shown to be capable of oncogenic transformation upon overexpression [94, 95]. Introduction of mutations that inhibit the intrinsic GTPase activity of G_{12} (Q229L) resulted in focus forming activity in NIH-3T3 cells that was similar to the most potent viral oncogenes [95, 96]. Similarly, overexpression of G_{13} is sufficient to transform NIH-3T3 fibroblasts and to promote xenograft growth in nude mice, and the GTPase deficient mutant (Q226L) is a potent

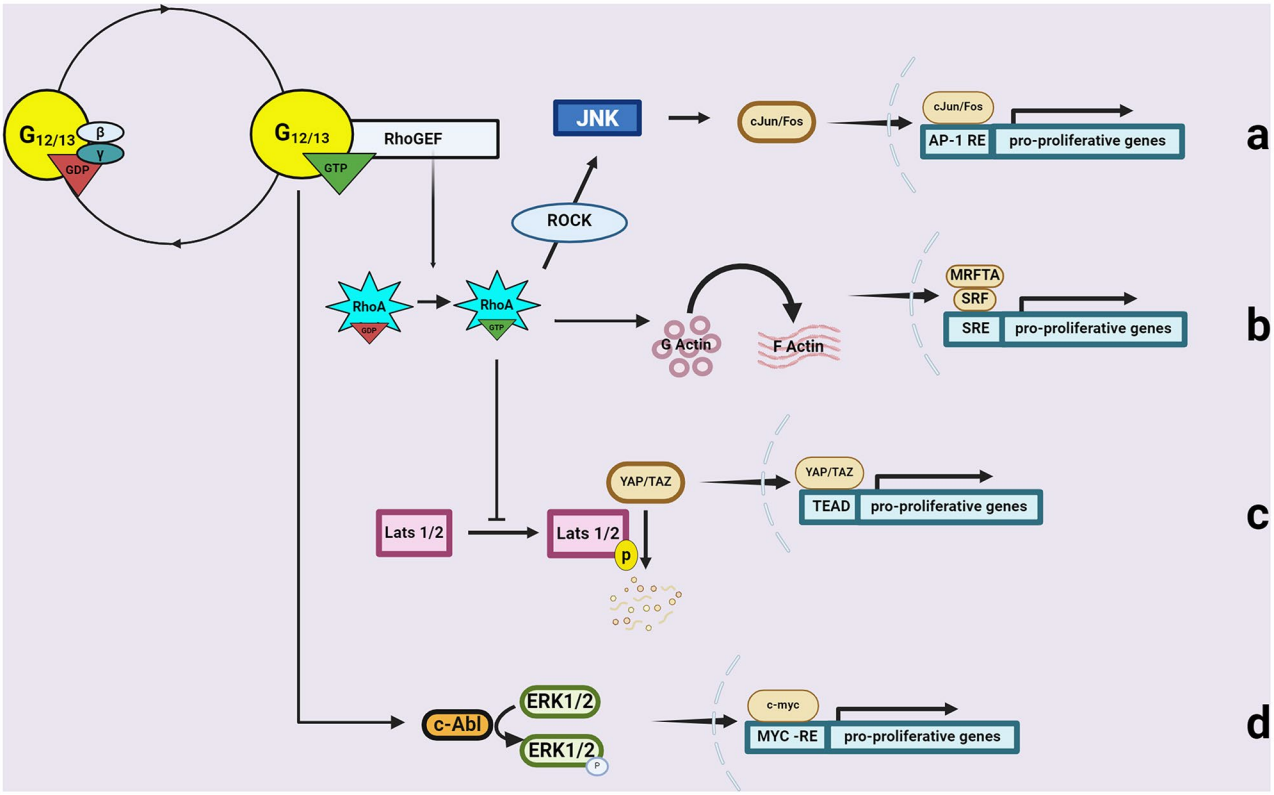

Fig. 3 $G_{12/13}$ signaling in proliferation. $G_{12/13}$ activate RhoGEFs that generate GTP-bound RhoA, activating ROCK and stimulating proliferative gene transcription via AP-1 (**a**) and SRE (**b**) promoters. GTP-bound RhoA inhibits phosphorylation of Lats 1/2, thereby blocking negative regulation of the Hippo pathway (**c**). $G_{12/13}$ bind to c-Abl, affecting ERK-dependent proliferation (**d**). (Created with BioRender.com)

oncogene [96, 97]. These genes, which were cloned from a Ewing sarcoma plasmid library (gep oncogenes), are dysregulated in numerous human cancers (reviewed in [98]). Dysregulation of $G_{12/13}$ signaling has been implicated in cardiac remodeling, angiogenesis and hypertrophy [99–102], in growth factor-stimulated cell migration [103], and in hepatic, cardiac and pulmonary fibrosis [104–106].

The proliferative effects of dysregulated $G_{12/13}$ signaling were originally attributed to activation of the JNK pathway [24]. Expression of constitutively active G_{12} leads to increased JNK activity and c-Jun phosphorylation (Fig. 3a) [107]. $G_{12/13}$-dependent c-fos activation via the serum-response element (SRE) has also been implicated in gep oncogenesis [108]. Serum response factor (SRF) is a transcriptional activator that binds SRE in the promoter region of genes involved in pro-proliferative signaling pathways. Actin polymerization stimulated by RhoGEF/RhoA activation induces translocation of myocardin-related transcription factor A (MRTFA) to the nucleus, where it functions as a co-activator with SRF (Fig. 3b) [109–111]. AP-1 dependent transcription serves as an integrated read-out of the actin polymerization (c-fos/SRE) and ROCK stimulation (JNK/c-Jun) functions of activated RhoA [112]. AP-1 activation stimulates expression of MDM2, which promotes degradation of p53 and FOXO1, thus driving malignancy and EMT [113, 114]. $G_{12/13}$-dependent activation of the JNK pathway also promotes cell migration [78] and invasion [115]. In smooth muscle, S1P dependent activation of $G_{12/13}$ stimulates growth and proliferation in a RhoA and SRE-dependent manner [116].

The Hippo signaling pathway can modulate cell proliferation downstream of $G_{12/13}$ induced RhoA signaling. LPA, S1P, and PAR signaling through $G_{12/13}$ and RhoA inhibits phosphorylation of Lats 1/2, which blocks negative regulation of the oncogenic transcription factors YAP and TAZ (Fig. 3c) [117, 118]. In glioblastoma, S1P/$G_{12/13}$/RhoA-mediated YAP activation promotes cellular invasion and metastasis [110], while LPA activation of $G_{12/13}$ and RhoA is implicated in YAP-mediated hepatocellular carcinoma [119]. In addition to affecting Lats 1/2 phosphorylation, $G_{12/13}$ and RhoA-dependent activation of YAP and TAZ is also significantly regulated by actin polymerization [120]. In vascular smooth muscle cells, knockdown of $G_{12/13}$, RhoA inhibition, and/or disruption of actin cytoskeleton formation all prevented YAP/TAZ activation, thereby inhibiting tissue remodeling [25]. The response of smooth muscle to mechanical stretch is mediated by the interaction between G_{13} and integrins,

which inhibits $G\alpha_{13}$-dependent RhoA activation and YAP/TAZ transcription, thereby preventing proliferation, inflammation, and angiogenesis [84]. G_{13} binding to integrins stimulates the non-receptor tyrosine kinase c-Src, which promotes cell attachment and spreading [121]. In contrast, focal adhesion kinase (FAK) is activated in a G_{13}-dependent fashion downstream of PAR [29], LPA [122], GRP4 [123] and CCK2 [124], causing sustained RhoA activation that promotes cell migration in ovarian, skin and colon cancer.

Abelson tyrosine kinase (c-Abl) is a primary regulator of actin dynamics and contraction in airway smooth muscle [125]. c-Abl inhibition attenuates actin polymerization, but fails to impact pro-contractile myosin phosphorylation [126]. In platelets, proline-rich tyrosine 2 (Pyk2) activation downstream of $G_{12/13}$ regulates shape change. Co-immunoprecipitation studies have shown that G_{13} interacts directly with c-Abl tyrosine kinase in endothelial cells, regulating actin cytoskeletal dynamics and inducing cell remodeling and cell migration [26]. In smooth muscle cells, knockdown of c-Abl inhibited $ERK_{1/2}$ phosphorylation, a key mediator of smooth muscle proliferation (Fig. 3d) [127].

$G_{12/13}$ -mediated signaling through JNK, YAP/TAZ and non-receptor tyrosine kinases is implicated in proliferation, angiogenesis and tissue remodeling in diverse disease pathologies, including vascular and airway smooth muscle hypertrophy. Antagonizing pathways that activate $G_{12/13}$ and its downstream signaling axis may suppress several hallmarks of AR, including increased smooth muscle mass.

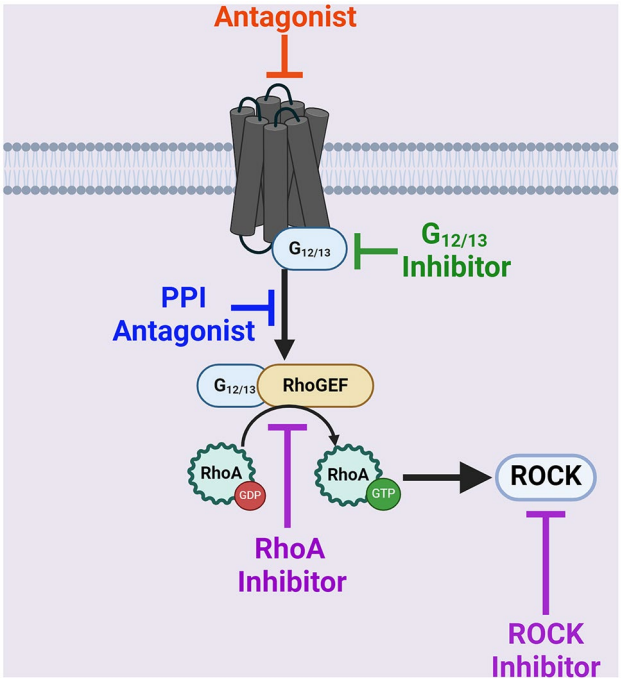

Fig. 4 Targets for regulating $G_{12/13}$ signaling. Hyperactive $G_{12/13}$ signaling in airway smooth muscle can be attenuated by antagonizing upstream receptors (red), by developing direct inhibitors of G_{12}, G_{13} (green) or complexes between $G_{12/13}$ and RhoGEFs (blue), or by inhibiting RhoA or ROCK (purple). (Created with BioRender.com)

$G_{12/13}$ pathway pharmacology

Therapeutic suppression of $G_{12/13}$ signaling can be achieved by balanced or biased antagonism of upstream receptors, direct G protein inactivation, blocking of activation-dependent protein-protein interactions or through inhibition of downstream signaling pathways (Fig. 4).

Humankind has been treating symptoms of asthma with anticholinergic agents for millennia. The Ebers Papyrus (1550 BC) recommends burning of henbane, which produces anticholinergic tropane alkaloids, to "remove phlegm, alleviate coughs and ease breathing" [128]. Extracts from a variety of other nightshade family plants figure prominently in treatment of dyspnea throughout Western medicine [129], including atropine, which was purified from deadly nightshade (*Atropa belladonna*) in 1833 [130], and was used to treat symptoms of asthma until the 1970s, when tropane derivatives with improved side effect profiles became available [131]. Despite (or, perhaps, because of) this long, empirical history of asthma treatment with anticholinergic agents, the effects of muscarinic antagonists have largely been evaluated physiologically rather than pharmacologically. This is particularly true for the effects of muscarinic antagonists on $G_{12/13}$ or RhoA signaling (highlighted in red in Fig. 4). For GPCRs like the M3R that display promiscuous G protein binding, antagonists of one G protein signaling pathway can function as agonists of another [132, 133], or can be biased to prefer one G protein signaling pathway over another [134]. For example, G_{12} biased agonists of the apelin receptor demonstrate sustained cardiac response [135]. The relative importance of $G_{q/11}$ versus $G_{12/13}$ signaling in ASM contraction, the effects of these pathways on therapeutic response to M3R agonists and antagonists, and the utility of modulating G protein signaling bias in obstructive airway diseases remains to be elucidated. Detailed study of the effects of muscarinic antagonists on $G_{q/11}$ versus $G_{12/13}$ signaling may provide insights that can improve disease management for asthma patients, or inform development of novel anticholinergics with enhanced potency, efficacy, and tolerability.

Agonists of the β_2AR, which stimulate PKA-dependent activation of MLCP and smooth muscle relaxation, are the standard of care for asthma patients of every age and every stage of disease. Short-acting beta agonists (SABA) are used symptomatically to relieve acute asthma exacerbations, while long-acting beta agonists (LABA) are used as maintenance therapy by patients with persistent

asthma [136]. Although agonism of relaxation is a common approach to treat acute smooth muscle constriction (e.g., asthma, premature labor), other chronic diseases are often treated with antagonists (e.g., angiotensin converting enzyme inhibition or angiotensin receptor blocking in hypertension [137]; anticholinergics or alpha-blockers in detrussor hyperreflexia [138, 139]). Activated G_S is the least oncogenic Gα subunit [97], and cAMP is generally antiproliferative [140], but chronic, unbiased beta-agonism causes iatrogenic eosinophila, mucus production and MAPK activation [141], which may contribute to the increased risk of fatal asthmatic attacks observed in patients who are treated with LABAs [142, 143]. In principle, antagonism of hyperresponsive constrictive pathways (bronchoprotection) would circumvent the adverse therapeutic effects associated with LABA treatment [144], and anticholinergics are considered safe and effective for the treatment of chronic obstructive pulmonary disease (COPD) [145], but FDA-approved, long-acting muscarinic antagonists (LAMAs) display minimal benefit as add-on treatment for patients whose asthma is not well controlled with standard therapies [146, 147]. Notably, this conclusion is based on acute (bronchoconstriction) rather than chronic (AR) endpoints. Given the chronic activation of oncogenic $G_{q/11}$ and $G_{12/13}$ signaling that is characteristic of AHR, it would be worthwhile to assess whether anticholinergics that are able to suppress both $G_{q/11}$ and $G_{12/13}$ signaling might be effective in reducing AR when co-administered with beta agonists and inhaled corticosteroids.

Unlike bronchorelaxation, which is regulated by a single receptor (β_2AR), multiple receptors can evoke constriction of ASM. Inhibiting the downstream G protein (highlighted in green in Fig. 4) through which multiple bronchoprovocational agents signal might provide a unified mechanism for relieving bronchoconstriction, but there are no FDA approved drugs that target G proteins. Toxins, natural products and their derivatives regulate several subfamilies of G proteins [148–152], but no natural product activators or inhibitors that are specific to $G_{12/13}$ subfamily members have been reported. As essential signaling proteins that are expressed in most tissues, G proteins are typically considered to be poor therapeutic targets, but G_{12} may be an exception. G_{12} knockout mice are viable, fertile and display minimal developmental abnormalities [153]. Nevertheless, G_{12} knockdown inhibits contraction of vascular and airway smooth muscle [59, 63] and GNA12 expression predicts asthma control [72]. Direct antagonism of G_{12} signaling may have considerable therapeutic value.

To transduce downstream signals, $G_{12/13}$ relies on protein-protein interactions (PPIs) with RhoGEFs. The avidity that dominates the free energy of PPIs is difficult to outcompete with the affinity of protein-ligand interactions, which is why PPIs are considered challenging pharmacologic targets [154]. However, a variety of emerging approaches have succeeded in generating small molecules that can disrupt PPIs (highlighted in blue in Fig. 4) [155–157]. A recent study characterized the PPI between G_i and its GEF, Girdin, as a druggable interaction by performing high-throughput screening. The authors identified a small molecule, NF023, that disrupts GEF activity by binding to a site that overlaps with the G_i-Girdin interface but fails to disrupt formation of the inactive Gαβγ heterotrimer [158]. Analysis of the $G_{12/13}$ interactome may reveal druggable PPI interfaces and enable discrimination of the distinct biological functions of G_{12} and G_{13}. Indeed, the G_{13} interactome has already been validated as a therapeutic target. M3mP6, a peptide derived from the G_{13} binding ExE motif of the integrin beta3 cytoplasmic domain, has antithrombotic effects without off-target adverse symptoms. In a mouse model, post-ischemic injection of M3mP6 protected the heart from myocardial ischemia-reperfusion injury [159].

Receptor-activated $G_{12/13}$ signals primarily through the RhoA/ROCK pathway. As such, inhibitors of RhoA or ROCK offer an alternative approach to interdict pro-contractile, pro-inflammatory and/or proliferative $G_{12/13}$-mediated signaling downstream of multiple, pro-contractile GPCRs (highlighted in purple in Fig. 4). Although RhoA inhibitors (including C3 toxin from *Clostridium botulinum*) have been described [160, 161], these agents are used as research tools rather than therapeutics. In contrast, ROCK inhibitors are FDA approved for treatment of open-angle glaucoma and ocular hypertension [162] and for graft versus host disease [163], and the ROCK inhibitor fasudil is approved in Japan for treatment of cerebral vasospasm and subarachnoid hemorrhage. ROCK inhibitors have also been investigated in clinical trials as treatments for a variety of indications, including amyotrophic lateral sclerosis [164], Parkinson's disease [165], solid malignancies [166], psoriasis [167], chronic kidney disease [168] and pulmonary hypertension [169]. Clinical trials in which ROCK inhibitors are administered systemically typically report few significant adverse events [170]. Hypotension and syncope are commonly observed, but these side effects were minimized in pulmonary hypertension trials by delivering the inhibitor (fasudil) to the lung by inhalation [171]. No human clinical trials evaluating the efficacy of ROCK inhibitors for the treatment of asthma have been registered to date, but ROCK inhibitors are effective in reducing inflammation [172, 173] and airway constriction [174, 175] in cellular and animal models of asthma. Clinical trials of ROCK inhibitors as asthma therapeutics are feasible, and their potential benefits to patients are well supported by ample pre-clinical data.

Numerous small molecules can interact with the downstream $G_{12/13}$ signaling pathway to promote bronchorelaxation. PI3K inhibitors promote anti-inflammatory responses in mouse models, reducing mucus production and airway remodeling [176, 177]. Small molecule inhibitors of class I PI3Ks can act as bronchodilators in human airway smooth muscle cells, highlighting this pathway as a promising target for pharmacologic intervention [178, 179]. In mouse asthma models, the selective PI3K p110δ inhibitor IC87114 attenuated AKT phosphorylation and mitigated airway hyperresponsiveness [180]. Inhibiting class I isoforms directly with p110α knockdown, wortmannin, or the small molecule LY294002 resulted in diminished RhoA and MYPT1 activation, which suppressed MLC phosphorylation [181]. These inhibitors also diminished cell depolarization, a common trait of HASM contraction [182], and reduced glucocorticoid insensitivity in patients with severe asthma by restoring HDAC2 or inhibiting the activation of pro-inflammatory transcription factors [183].

Upstream receptors and downstream kinases are the most tractable targets for suppressing chronic $G_{12/13}$ signaling. However, the effects of anticholinergics on $G_{12/13}$ signaling have never been characterized, and the extent to which RhoA/ROCK or PI3K inhibition can mitigate the effects of $G_{12/13}$ pathway activation is unknown.

Conclusions

The canonical model for smooth muscle contraction posits that actomyosin cross-bridge cycling is regulated primarily by calcium-dependent activation of myosin light chain kinase downstream of M3R and $G_{q/11}$. The intuitive satisfaction of this model benefits from the convergence of two conventional wisdoms from muscle and cell physiology: that muscle contraction is calcium dependent; and that kinase activity is induced while phosphatase activity is constitutive. It's unclear whether either of these conventional wisdoms holds in airway smooth muscle. The calcium dependence of cardiac and skeletal muscle contraction is largely due to the need to relieve tonic inhibition of the interaction between myosin and actin by troponin, which isn't expressed in smooth muscle [184]. The need for troponin in cardiac and skeletal muscle and calponin in smooth muscle suggests that the basal activity of MLCK is sufficient to promote constriction in the absence of tonic inhibition. The efficacy of β-agonists in asthma pharmacotherapy, which activate MLCP, indicates that bronchomotor tone may be regulated primarily by phosphatase activity rather than kinase activity. The inability of FR to block M3R-dependent contraction of airway smooth muscle [34, 35] is consistent with this view, and indicates the existence of a signaling pathway besides $G_{q/11}$ that is able to transduce signals from M3R through RhoA to inhibit MLCP and promote parasympathetic actomyosin cross-bridge cycling. The preponderance of the evidence strongly indicates that this critical signaling pathway is $G_{12/13}$ [61–63], although additional experiments to resolve inconsistencies between functional and biosensor assays would be worthwhile.

Pathologic constriction of airway smooth muscle is the defining symptom of asthma [185]. Smooth muscle contraction relies on actin polymerization and phosphorylation of myosin light chain, both of which are mediated by hyperresponsive acetylcholine signaling to RhoA and ROCK [37], likely through $G_{12/13}$ [29]. $G_{12/13}$ signaling also stimulates production of Th2 cytokines [70], which promote airway inflammation, and activates numerous proliferative pathways (JNK [112], Hippo [84], c-Abl [26]) that may drive AR in hyperresponsive airways. The role of $G_{12/13}$ signaling in airway constriction, inflammation and remodeling has been underappreciated despite compelling direct evidence for its significance [63]. Consequently, the effect of antagonists of constrictive and inflammatory pathways on $G_{12/13}$, RhoA and ROCK activation has never been adequately characterized. Therapeutic strategies that antagonize upstream receptors or that inhibit $G_{12/13}$ or its downstream signaling partners should benefit patients with asthma and likely synergize with current asthma treatments.

Abbreviations

GPCR	G protein-coupled receptor
cAMP	cyclic AMP
PKA	Protein Kinase A
PLCβ	Phospholipase Cβ
IP3	Inositol Trisphosphate
DAG	Diacylglycerol
PKC	Protein Kinase C
Rho	Ras Homology
GEF	Guanine Exchange Factor
ROCK	Rho-Associated Kinase
LIMK	Lim Domain Kinase
JNK	c-Jun NH_2-terminal kinase
GAP	GTPase Accelerating Protein
RGS	Regulator of G Protein Signaling
CysLTR	Cysteinyl Leukotriene Receptors
H	Histamine
LPA	Lysophosphatidic Acid
PAR	Protease-Activated Receptor
S1P	Sphingosine-1-Phosphate
AHR	Airway Hyperresponsiveness
AR	Airway Remodeling
β_2AR	Beta-2 Adrenergic Receptor
MAPK	Mitogen-Activated Protein Kinase
ASM	Airway Smooth Muscle
MLC	Myosin Light Chain
MLCK	Myosin Light Chain Kinase
FR	$G_{q/11}$-selective depsipeptide inhibitor FR900359
G	Globular
F	Filamentous
MLCP	Myosin Light Chain Phosphatase
MYPT	Myosin Phosphatase Targeting Subunit
GDI	Guanine Nucleotide Dissociation Inhibitor
LARG	Leukemia-Associated RhoGEF
ARHGEF	Rho Guanine Exchange Factor

GNA12	Gene Encoding Guanine Nucleotide Binding Protein Subunit Alpha 12
GNA13	Gene Encoding Guanine Nucleotide Binding Protein Subunit Alpha 13
M3R	Muscarinic 3 Acetylcholine Receptor
HEK	Human Embryonic Kidney
HASM	Human Airway Smooth Muscle
siRNA	Short Interfering RNA
TGF	Tumor Growth Factor
EMT	Epithelial-Mesenchymal Transition
IgE	Immunoglobulin E
IL	Interleukin
Th2	T-helper 2
NFkB	Nuclear Factor kB
Tfh	T Follicular helper
AP-1	Activator Protein 1
PRG	PDZ Domain-Containing RhoGEF
Gep	Gene from Ewing Sarcoma Plasmid Library
SRE	Serum Response Element
SRF	Serum Response Factor
MRTFA	Myocardin-Related Transcription Factor A
MDM2	Mouse Double Minute 2
FOXO1	Forkhead Box Protein O1
Lats1/2	Large Tumor Suppressors 1 & 2
YAP	Yes-Associated Protein
TAZ	Transcriptional Activator with a PDZ Binding Motif
c-SRC	Cellular Rous Sarcoma Virus Homolog
FAK	Focal Adhesion Kinase
GRP	Gastrin-Releasing Peptide
CCK	Cholecystokinin
c-Abl	Cellular Abelson Murine Lymphosarcoma Virus Homolog Tyrosine Kinase
Pyk2	Protein Tyrosine Kinase 2
ERK	Extracellular Signal-Regulated Kinase
SABA	Short-Acting Beta Agonist
LABA	Long-Acting Beta Agonist
COPD	Chronic Obstructive Pulmonary Disease
FDA	Food and Drug Administration
LAMA	Long-Acting Muscarinic Antagonist
PPI	Protein-Protein Interaction
PI3K	Phosphoinositide-3 Kinase
AKT	Protein Kinase B/Gene that Causes Thyomas in the Akr Mouse Strain
HDAC	Histone Deacetylase
PIP2	Phosphoinositide Bisphosphate

Acknowledgements
Publication made possible in part by support from the Thomas Jefferson University Open Access Fund.

Author contributions
ELM wrote the manuscript and generated the figures, with editorial and content suggestions from RAP and CPS.

Funding
This work is supported by NIH grants UL1TR003017 and P01HL114471 (awarded to RAP). ELM is supported by NIH grant P01HL114471-S1.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 14 June 2024 / Accepted: 19 July 2024
Published online: 02 August 2024

References

1. Fredriksson R, Lagerstrom MC, Lundin LG, Schioth HB. The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. Mol Pharmacol. 2003;63(6):1256–72.
2. Pierce KL, Premont RT, Lefkowitz RJ. Seven-transmembrane receptors. Nat Rev Mol Cell Biol. 2002;3(9):639–50.
3. Yang D, Zhou Q, Labroska V, Qin S, Darbalaei S, Wu Y, et al. G protein-coupled receptors: structure- and function-based drug discovery. Signal Transduct Target Ther. 2021;6(1):7.
4. Hurowitz EH, Melnyk JM, Chen YJ, Kouros-Mehr H, Simon MI, Shizuya H. Genomic characterization of the human heterotrimeric G protein alpha, beta, and gamma subunit genes. DNA Res. 2000;7(2):111–20.
5. Hauser AS, Avet C, Normand C, Mancini A, Inoue A, Bouvier M et al. Common coupling map advances GPCR-G protein selectivity. Elife. 2022;11.
6. Okashah N, Wan Q, Ghosh S, Sandhu M, Inoue A, Vaidehi N, et al. Variable G protein determinants of GPCR coupling selectivity. Proc Natl Acad Sci U S A. 2019;116(24):12054–9.
7. Masuho I, Kise R, Gainza P, Von Moo E, Li X, Tany R, et al. Rules and mechanisms governing G protein coupling selectivity of GPCRs. Cell Rep. 2023;42(10):113173.
8. Smith JS, Hilibrand AS, Skiba MA, Dates AN, Calvillo-Miranda VG, Kruse AC. The M3 muscarinic acetylcholine receptor can signal through multiple G protein families. Mol Pharmacol. 2024.
9. Namkung Y, LeGouill C, Kumar S, Cao Y, Teixeira LB, Lukasheva V, et al. Functional selectivity profiling of the angiotensin II type 1 receptor using pathway-wide BRET signaling sensors. Sci Signal. 2018;11:559.
10. Peng X, Yang L, Liu Z, Lou S, Mei S, Li M, et al. Structural basis for recognition of antihistamine drug by human histamine receptor. Nat Commun. 2022;13(1):6105.
11. Höring C, Conrad M, Söldner CA, Wang J, Sticht H, Strasser A, et al. Specific Engineered G Protein Coupling to histamine receptors revealed from Cellular Assay experiments and accelerated Molecular Dynamics simulations. Int J Mol Sci. 2021;22:18.
12. Avet C, Sturino C, Grastilleur S, Gouill CL, Semache M, Gross F, et al. The PAR2 inhibitor I-287 selectively targets Galphaq and Galpha12/13 signaling and has anti-inflammatory effects. Commun Biol. 2020;3(1):719.
13. Weis WI, Kobilka BK. The molecular basis of G protein-coupled receptor activation. Annu Rev Biochem. 2018;87:897–919.
14. Knight KM, Ghosh S, Campbell SL, Lefevre TJ, Olsen RHJ, Smrcka AV, et al. A universal allosteric mechanism for G protein activation. Mol Cell. 2021;81(7):1384–96. e6.
15. Gilman AG. G proteins and dual control of adenylate cyclase. Cell. 1984;36(3):577–9.
16. Rhee SG. Regulation of phosphoinositide-specific phospholipase C. Annu Rev Biochem. 2001;70:281–312.
17. Strathmann MP, Simon MI. G alpha 12 and G alpha 13 subunits define a fourth class of G protein alpha subunits. Proc Natl Acad Sci U S A. 1991;88(13):5582–6.
18. Suzuki N, Nakamura S, Mano H, Kozasa T. Galpha 12 activates rho GTPase through tyrosine-phosphorylated leukemia-associated RhoGEF. Proc Natl Acad Sci U S A. 2003;100(2):733–8.
19. Hart MJ, Jiang X, Kozasa T, Roscoe W, Singer WD, Gilman AG, et al. Direct stimulation of the guanine nucleotide exchange activity of p115 RhoGEF by Galpha13. Science. 1998;280(5372):2112–4.
20. Spiering D, Hodgson L. Dynamics of the rho-family small GTPases in actin regulation and motility. Cell Adh Migr. 2011;5(2):170–80.
21. Somlyo AP, Somlyo AV. Ca2 + sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphatase. Physiol Rev. 2003;83(4):1325–58.
22. Ross EM, Wilkie TM. GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. Annu Rev Biochem. 2000;69:795–827.
23. Chiba Y, Misawa M. Increased expression of G12 and G13 proteins in bronchial smooth muscle of airway hyperresponsive rats. Inflamm Res. 2001;50(6):333–6.
24. Voyno-Yasenetskaya TA, Faure MP, Ahn NG, Bourne HR. Galpha12 and Galpha13 regulate extracellular signal-regulated kinase and c-Jun

kinase pathways by different mechanisms in COS-7 cells. J Biol Chem. 1996;271(35):21081–7.
25. Feng X, Liu P, Zhou X, Li MT, Li FL, Wang Z, et al. Thromboxane A2 activates YAP/TAZ protein to induce vascular smooth muscle cell proliferation and Migration. J Biol Chem. 2016;291(36):18947–58.
26. Wang L, Wang D, Xing B, Tan YC, Huang J, Liu B, et al. G-Protein Galpha13 functions with Abl Kinase to regulate actin cytoskeletal reorganization. J Mol Biol. 2017;429(24):3836–49.
27. Zhang Z, Tan X, Luo J, Cui B, Lei S, Si Z, et al. GNA13 promotes tumor growth and angiogenesis by upregulating CXC chemokines via the NF-kappaB signaling pathway in colorectal cancer cells. Cancer Med. 2018;7(11):5611–20.
28. Hot B, Valnohova J, Arthofer E, Simon K, Shin J, Uhlen M, et al. FZD10-Galpha13 signalling axis points to a role of FZD10 in CNS angiogenesis. Cell Signal. 2017;32:93–103.
29. Chikumi H, Fukuhara S, Gutkind JS. Regulation of G protein-linked guanine nucleotide exchange factors for rho, PDZ-RhoGEF, and LARG by tyrosine phosphorylation: evidence of a role for focal adhesion kinase. J Biol Chem. 2002;277(14):12463–73.
30. Prakash YS. Airway smooth muscle in airway reactivity and remodeling: what have we learned? Am J Physiol Lung Cell Mol Physiol. 2013;305(12):L912–33.
31. Billington CK, Penn RB. m3 muscarinic acetylcholine receptor regulation in the airway. Am J Respir Cell Mol Biol. 2002;26(3):269–72.
32. Billington CK, Penn RB. Signaling and regulation of G protein-coupled receptors in airway smooth muscle. Respir Res. 2003;4(1):2.
33. Winder SJ, Walsh MP. Smooth muscle calponin. Inhibition of actomyosin MgATPase and regulation by phosphorylation. J Biol Chem. 1990;265(17):10148–55.
34. Bradley SJ, Wiegman CH, Iglesias MM, Kong KC, Butcher AJ, Plouffe B, et al. Mapping physiological G protein-coupled receptor signaling pathways reveals a role for receptor phosphorylation in airway contraction. Proc Natl Acad Sci U S A. 2016;113(16):4524–9.
35. Carr R 3rd, Koziol-White C, Zhang J, Lam H, An SS, Tall GG, et al. Interdicting Gq activation in Airway Disease by receptor-dependent and receptor-independent mechanisms. Mol Pharmacol. 2016;89(1):94–104.
36. Cipolla MJ, Gokina NI, Osol G. Pressure-induced actin polymerization in vascular smooth muscle as a mechanism underlying myogenic behavior. FASEB J. 2002;16(1):72–6.
37. Zhang W, Du L, Gunst SJ. The effects of the small GTPase RhoA on the muscarinic contraction of airway smooth muscle result from its role in regulating actin polymerization. Am J Physiol Cell Physiol. 2010;299(2):C298–306.
38. An SS, Laudadio RE, Lai J, Rogers RA, Fredberg JJ. Stiffness changes in cultured airway smooth muscle cells. Am J Physiol Cell Physiol. 2002;283(3):C792–801.
39. Nobes CD, Hall A. Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. Cell. 1995;81(1):53–62.
40. Hirshman CA, Emala CW. Actin reorganization in airway smooth muscle cells involves Gq and Gi-2 activation of rho. Am J Physiol. 1999;277(3):L653–61.
41. Fujii T, Namba K. Structure of actomyosin rigour complex at 5.2 Å resolution and insights into the ATPase cycle mechanism. Nat Commun. 2017;8:13969.
42. Gohla A, Harhammer R, Schultz G. The G-protein G13 but not G12 mediates signaling from lysophosphatidic acid receptor via epidermal growth factor receptor to rho. J Biol Chem. 1998;273(8):4653–9.
43. Kitazawa T, Masuo M, Somlyo AP. G protein-mediated inhibition of myosin light-chain phosphatase in vascular smooth muscle. Proc Natl Acad Sci U S A. 1991;88(20):9307–10.
44. Kitazawa T, Eto M, Woodsome TP, Brautigan DL. Agonists trigger G protein-mediated activation of the CPI-17 inhibitor phosphoprotein of myosin light chain phosphatase to enhance vascular smooth muscle contractility. J Biol Chem. 2000;275(14):9897–900.
45. Maekawa M, Ishizaki T, Boku S, Watanabe N, Fujita A, Iwamatsu A, et al. Signaling from rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase. Science. 1999;285(5429):895–8.
46. Agnew BJ, Minamide LS, Bamburg JR. Reactivation of phosphorylated actin depolymerizing factor and identification of the regulatory site. J Biol Chem. 1995;270(29):17582–7.
47. Carlier MF, Laurent V, Santolini J, Melki R, Didry D, Xia GX, et al. Actin depolymerizing factor (ADF/cofilin) enhances the rate of filament turnover: implication in actin-based motility. J Cell Biol. 1997;136(6):1307–22.
48. Kiuchi T, Ohashi K, Kurita S, Mizuno K. Cofilin promotes stimulus-induced lamellipodium formation by generating an abundant supply of actin monomers. J Cell Biol. 2007;177(3):465–76.
49. Lan B, Wang L, Zhang J, Pascoe CD, Norris BA, Liu JC, et al. Rho-kinase mediated cytoskeletal stiffness in skinned smooth muscle. J Appl Physiol (1985). 2013;115(10):1540–52.
50. Iizuka K, Shimizu Y, Tsukagoshi H, Yoshii A, Harada T, Dobashi K, et al. Evaluation of Y-27632, a rho-kinase inhibitor, as a bronchodilator in guinea pigs. Eur J Pharmacol. 2000;406(2):273–9.
51. Iizuka K, Yoshii A, Samizo K, Tsukagoshi H, Ishizuka T, Dobashi K, et al. A major role for the rho-associated coiled coil forming protein kinase in G-protein-mediated Ca2 + sensitization through inhibition of myosin phosphatase in rabbit trachea. Br J Pharmacol. 1999;128(4):925–33.
52. Yoshii A, Iizuka K, Dobashi K, Horie T, Harada T, Nakazawa T, et al. Relaxation of contracted rabbit tracheal and human bronchial smooth muscle by Y-27632 through inhibition of Ca2 + sensitization. Am J Respir Cell Mol Biol. 1999;20(6):1190–200.
53. Fukumoto Y, Kaibuchi K, Hori Y, Fujioka H, Araki S, Ueda T, et al. Molecular cloning and characterization of a novel type of regulatory protein (GDI) for the rho proteins, ras p21-like small GTP-binding proteins. Oncogene. 1990;5(9):1321–8.
54. Ueda T, Kikuchi A, Ohga N, Yamamoto J, Takai Y. Purification and characterization from bovine brain cytosol of a novel regulatory protein inhibiting the dissociation of GDP from and the subsequent binding of GTP to rhoB p20, a ras p21-like GTP-binding protein. J Biol Chem. 1990;265(16):9373–80.
55. Gohla A, Schultz G, Offermanns S. Role for G(12)/G(13) in agonist-induced vascular smooth muscle cell contraction. Circ Res. 2000;87(3):221–7.
56. Wirth A, Wang S, Takefuji M, Tang C, Althoff TF, Schweda F, et al. Age-dependent blood pressure elevation is due to increased vascular smooth muscle tone mediated by G-protein signalling. Cardiovasc Res. 2016;109(1):131–40.
57. Wirth A, Benyo Z, Lukasova M, Leutgeb B, Wettschureck N, Gorbey S, et al. G12-G13-LARG-mediated signaling in vascular smooth muscle is required for salt-induced hypertension. Nat Med. 2008;14(1):64–8.
58. Chennupati R, Wirth A, Favre J, Li R, Bonnavion R, Jin YJ et al. Myogenic vasoconstriction requires G12/G13 and LARG to maintain local and systemic vascular resistance. Elife. 2019;8.
59. Gao J, Denys I, Shahien A, Sutphen J, Kapusta DR. Downregulation of Brain Galpha12 attenuates angiotensin II-Dependent hypertension. Am J Hypertens. 2020;33(2):198–204.
60. Rumenapp U, Asmus M, Schablowski H, Woznicki M, Han L, Jakobs KH, et al. The M3 muscarinic acetylcholine receptor expressed in HEK-293 cells signals to phospholipase D via G12 but not Gq-type G proteins: regulators of G proteins as tools to dissect pertussis toxin-resistant G proteins in receptor-effector coupling. J Biol Chem. 2001;276(4):2474–9.
61. Yuan J, Slice LW, Rozengurt E. Activation of protein kinase D by signaling through rho and the alpha subunit of the heterotrimeric G protein G13. J Biol Chem. 2001;276(42):38619–27.
62. Lee SJ, Lee WH, Ki SH, Kim YM, Lee SJ, Lee CH, et al. Galpha13 regulates methacholine-induced contraction of bronchial smooth muscle via phosphorylation of MLC20. Biochem Pharmacol. 2009;77(9):1497–505.
63. Yoo EJ, Cao G, Koziol-White CJ, Ojiaku CA, Sunder K, Jude JA, et al. Galpha12 facilitates shortening in human airway smooth muscle by modulating phosphoinositide 3-kinase-mediated activation in a RhoA-dependent manner. Br J Pharmacol. 2017;174(23):4383–95.
64. Ojiaku CA, Cao G, Zhu W, Yoo EJ, Shumyatcher M, Himes BE, et al. TGF-beta1 evokes human airway smooth muscle cell shortening and hyperresponsiveness via Smad3. Am J Respir Cell Mol Biol. 2018;58(5):575–84.
65. Shaifta Y, MacKay CE, Irechukwu N, O'Brien KA, Wright DB, Ward JPT, et al. Transforming growth factor-β enhances rho-kinase activity and contraction in airway smooth muscle via the nucleotide exchange factor ARHGEF1. J Physiol. 2018;596(1):47–66.
66. Hammad H, Lambrecht BN. The basic immunology of asthma. Cell. 2021;184(9):2521–2.
67. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med. 2012;18(5):716–25.
68. Deo SS, Mistry KJ, Kakade AM, Niphadkar PV. Role played by Th2 type cytokines in IgE mediated allergy and asthma. Lung India. 2010;27(2):66–71.
69. Whitsett JA, Alenghat T. Respiratory epithelial cells orchestrate pulmonary innate immunity. Nat Immunol. 2015;16(1):27–35.
70. Idzko M, Panther E, Corinti S, Morelli A, Ferrari D, Herouy Y, et al. Sphingosine 1-phosphate induces chemotaxis of immature and modulates cytokine-release in mature human dendritic cells for emergence of Th2 immune responses. FASEB J. 2002;16(6):625–7.
71. Chen H, Chen K, Huang W, Staudt LM, Cyster JG, Li X. Structure of S1PR2-heterotrimeric G13 signaling complex. Sci Adv. 2022;8(13):eabn0067.

72. McGeachie MJ, Dahlin A, Qiu W, Croteau-Chonka DC, Savage J, Wu AC, et al. The metabolomics of asthma control: a promising link between genetics and disease. Immun Inflamm Dis. 2015;3(3):224–38.
73. Dusaban SS, Purcell NH, Rockenstein E, Masliah E, Cho MK, Smrcka AV, et al. Phospholipase C epsilon links G protein-coupled receptor activation to inflammatory astrocytic responses. Proc Natl Acad Sci U S A. 2013;110(9):3609–14.
74. Kuen DS, Park M, Ryu H, Choi G, Moon YH, Kim JO, et al. Critical regulation of follicular helper T cell differentiation and function by Galpha13 signaling. Proc Natl Acad Sci U S A. 2021;118:43.
75. Grimm M, Tischner D, Troidl K, Albarran Juarez J, Sivaraj KK, Ferreiros Bouzas N, et al. S1P2/G12/13 signaling negatively regulates macrophage activation and indirectly shapes the atheroprotective B1-Cell Population. Arterioscler Thromb Vasc Biol. 2016;36(1):37–48.
76. Yu H, Liu Z. GNA12 regulates C5a-induced migration by downregulating C5aR1-PLCbeta2-PI3K-AKT-ERK1/2 signaling. Biophys Rep. 2023;9(1):33–44.
77. van den Bos E, Ambrosy B, Horsthemke M, Walbaum S, Bachg AC, Wettschureck N, et al. Knockout mouse models reveal the contributions of G protein subunits to complement C5a receptor-mediated chemotaxis. J Biol Chem. 2020;295(22):7726–42.
78. Mikelis CM, Palmby TR, Simaan M, Li W, Szabo R, Lyons R, et al. PDZ-RhoGEF and LARG are essential for embryonic development and provide a link between thrombin and LPA receptors and rho activation. J Biol Chem. 2013;288(17):12232–43.
79. Bouafia A, Lofek S, Bruneau J, Chentout L, Lamrini H, Trinquand A, et al. Loss of ARHGEF1 causes a human primary antibody deficiency. J Clin Invest. 2019;129(3):1047–60.
80. Bettegazzi B, Bellani S, Cattaneo S, Codazzi F, Grohovaz F, Zacchetti D. Galpha13 contributes to LPS-Induced morphological alterations and affects Migration of Microglia. Mol Neurobiol. 2021;58(12):6397–414.
81. Zhang Y, Zhao X, Shen B, Bai Y, Chang C, Stojanovic A, et al. Integrin beta(3) directly inhibits the Galpha(13)-p115RhoGEF interaction to regulate G protein signaling and platelet exocytosis. Nat Commun. 2023;14(1):4966.
82. Kaiser R, Anjum A, Kammerer L, Loew Q, Akhalkatsi A, Rossaro D, et al. Mechanosensing via a GpIIb/Src/14-3-3zeta axis critically regulates platelet migration in vascular inflammation. Blood. 2023;141(24):2973–92.
83. Cheng N, Zhang Y, Delaney MK, Wang C, Bai Y, Skidgel RA, et al. Targeting Galpha13-integrin interaction ameliorates systemic inflammation. Nat Commun. 2021;12(1):3185.
84. Wang L, Luo JY, Li B, Tian XY, Chen LJ, Huang Y, et al. Integrin-YAP/TAZ-JNK cascade mediates atheroprotective effect of unidirectional shear flow. Nature. 2016;540(7634):579–82.
85. Lim WK, Chai X, Ghosh S, Ray D, Wang M, Rasheed SAK, et al. Galpha-13 induces CXC motif chemokine ligand 5 expression in prostate cancer cells by transactivating NF-kappaB. J Biol Chem. 2019;294(48):18192–206.
86. Wang Z, Guan W, Han Y, Ren H, Tang X, Zhang H, et al. Stimulation of dopamine D3 receptor attenuates renal ischemia-reperfusion Injury via increased linkage with Galpha12. Transplantation. 2015;99(11):2274–84.
87. Yu W, Beaudry S, Negoro H, Boucher I, Tran M, Kong T, et al. H2O2 activates G protein, alpha 12 to disrupt the junctional complex and enhance ischemia reperfusion injury. Proc Natl Acad Sci U S A. 2012;109(17):6680–5.
88. Yanamadala V, Negoro H, Gunaratnam L, Kong T, Denker BM. Galpha12 stimulates apoptosis in epithelial cells through JNK1-mediated Bcl-2 degradation and up-regulation of IkappaBalpha. J Biol Chem. 2007;282(33):24352–63.
89. Hough KP, Curtiss ML, Blain TJ, Liu RM, Trevor J, Deshane JS, et al. Airway Remodeling in Asthma. Front Med (Lausanne). 2020;7:191.
90. Ebina M, Takahashi T, Chiba T, Motomiya M. Cellular hypertrophy and hyperplasia of airway smooth muscles underlying bronchial asthma. A 3-D morphometric study. Am Rev Respir Dis. 1993;148(3):720–6.
91. Johnson PR, Roth M, Tamm M, Hughes M, Ge Q, King G, et al. Airway smooth muscle cell proliferation is increased in asthma. Am J Respir Crit Care Med. 2001;164(3):474–7.
92. Cohen L, E X, Tarsi J, Ramkumar T, Horiuchi TK, Cochran R, et al. Epithelial cell proliferation contributes to airway remodeling in severe asthma. Am J Respir Crit Care Med. 2007;176(2):138–45.
93. Hernandez-Lara MA, Yadav SK, Shah SD, Okumura M, Yokoyama Y, Penn RB, et al. Regulation of Airway smooth muscle cell proliferation by Diacylglycerol Kinase: relevance to Airway Remodeling in Asthma. Int J Mol Sci. 2022;23:19.
94. Chan AM, Fleming TP, McGovern ES, Chedid M, Miki T, Aaronson SA. Expression cDNA cloning of a transforming gene encoding the wild-type G alpha 12 gene product. Mol Cell Biol. 1993;13(2):762–8.
95. Xu N, Bradley L, Ambdukar I, Gutkind JS. A mutant alpha subunit of G12 potentiates the eicosanoid pathway and is highly oncogenic in NIH 3T3 cells. Proc Natl Acad Sci U S A. 1993;90(14):6741–5.
96. Voyno-Yasenetskaya TA, Pace AM, Bourne HR. Mutant alpha subunits of G12 and G13 proteins induce neoplastic transformation of Rat-1 fibroblasts. Oncogene. 1994;9(9):2559–65.
97. Xu N, Voyno-Yasenetskaya T, Gutkind JS. Potent transforming activity of the G13 alpha subunit defines a novel family of oncogenes. Biochem Biophys Res Commun. 1994;201(2):603–9.
98. Rasheed SAK, Subramanyan LV, Lim WK, Udayappan UK, Wang M, Casey PJ. The emerging roles of Galpha12/13 proteins on the hallmarks of cancer in solid tumors. Oncogene. 2022;41(2):147–58.
99. Takefuji M, Kruger M, Sivaraj KK, Kaibuchi K, Offermanns S, Wettschureck N. RhoGEF12 controls cardiac remodeling by integrating G protein- and integrin-dependent signaling cascades. J Exp Med. 2013;210(4):665–73.
100. Sivaraj KK, Takefuji M, Schmidt I, Adams RH, Offermanns S, Wettschureck N. G13 controls angiogenesis through regulation of VEGFR-2 expression. Dev Cell. 2013;25(4):427–34.
101. Takefuji M, Wirth A, Lukasova M, Takefuji S, Boettger T, Braun T, et al. G(13)-mediated signaling pathway is required for pressure overload-induced cardiac remodeling and heart failure. Circulation. 2012;126(16):1972–82.
102. Huang J, Qu Q, Dai Y, Ren D, Qian J, Ge J. Detrimental role of PDZ-RhoGEF in Pathological Cardiac Hypertrophy. Hypertension. 2023;80(2):403–15.
103. Shan D, Chen L, Wang D, Tan YC, Gu JL, Huang XY. The G protein G alpha(13) is required for growth factor-induced cell migration. Dev Cell. 2006;10(6):707–18.
104. Kim KM, Han CY, Kim JY, Cho SS, Kim YS, Koo JH, et al. Galpha12 overexpression induced by miR-16 dysregulation contributes to liver fibrosis by promoting autophagy in hepatic stellate cells. J Hepatol. 2018;68(3):493–504.
105. Martin JW, Cavagnini KS, Brawley DN, Berkley CY, Smolski WC, Garcia RD, et al. A Galpha12-specific binding domain in AKAP-Lbc and p114RhoGEF. J Mol Signal. 2016;11:3.
106. Gan X, Wang J, Wang C, Sommer E, Kozasa T, Srinivasula S, et al. PRR5L degradation promotes mTORC2-mediated PKC-delta phosphorylation and cell migration downstream of Galpha12. Nat Cell Biol. 2012;14(7):686–96.
107. Juneja J, Cushman I, Casey PJ. G12 signaling through c-Jun NH2-terminal kinase promotes breast cancer cell invasion. PLoS ONE. 2011;6(11):e26085.
108. Fromm C, Coso OA, Montaner S, Xu N, Gutkind JS. The small GTP-binding protein rho links G protein-coupled receptors and Galpha12 to the serum response element and to cellular transformation. Proc Natl Acad Sci U S A. 1997;94(19):10098–103.
109. Medlin MD, Staus DP, Dubash AD, Taylor JM, Mack CP. Sphingosine 1-phosphate receptor 2 signals through leukemia-associated RhoGEF (LARG), to promote smooth muscle cell differentiation. Arterioscler Thromb Vasc Biol. 2010;30(9):1779–86.
110. Yu OM, Benitez JA, Plouffe SW, Ryback D, Klein A, Smith J, et al. YAP and MRTF-A, transcriptional co-activators of RhoA-mediated gene expression, are critical for glioblastoma tumorigenicity. Oncogene. 2018;37(41):5492–507.
111. Yu OM, Miyamoto S, Brown JH. Myocardin-related transcription factor A and Yes-Associated protein exert Dual Control in G protein-coupled receptor- and RhoA-Mediated transcriptional regulation and cell proliferation. Mol Cell Biol. 2016;36(1):39–49.
112. Marinissen MJ, Chiariello M, Tanos T, Bernard O, Narumiya S, Gutkind JS. The small GTP-binding protein RhoA regulates c-jun by a ROCK-JNK signaling axis. Mol Cell. 2004;14(1):29–41.
113. Jung HS, Seo YR, Yang YM, Koo JH, An J, Lee SJ, et al. Galpha12gep oncogene inhibits FOXO1 in hepatocellular carcinoma as a consequence of miR-135b and miR-194 dysregulation. Cell Signal. 2014;26(7):1456–65.
114. Yang YM, Lee WH, Lee CG, An J, Kim ES, Kim SH, et al. Galpha12 gep oncogene deregulation of p53-responsive microRNAs promotes epithelial-mesenchymal transition of hepatocellular carcinoma. Oncogene. 2015;34(22):2910–21.
115. Yuan B, Cui J, Wang W, Deng K. Galpha12/13 signaling promotes cervical cancer invasion through the RhoA/ROCK-JNK signaling axis. Biochem Biophys Res Commun. 2016;473(4):1240–6.
116. Lockman K, Hinson JS, Medlin MD, Morris D, Taylor JM, Mack CP. Sphingosine 1-phosphate stimulates smooth muscle cell differentiation and proliferation by activating separate serum response factor co-factors. J Biol Chem. 2004;279(41):42422–30.
117. Yu FX, Zhao B, Panupinthu N, Jewell JL, Lian I, Wang LH, et al. Regulation of the Hippo-YAP pathway by G-protein-coupled receptor signaling. Cell. 2012;150(4):780–91.

118. Mo JS, Yu FX, Gong R, Brown JH, Guan KL. Regulation of the Hippo-YAP pathway by protease-activated receptors (PARs). Genes Dev. 2012;26(19):2138–43.
119. He H, Sugiyama A, Snyder NW, Teneche MG, Liu X, Maner-Smith KM, et al. Acyl-CoA thioesterase 12 suppresses YAP-mediated hepatocarcinogenesis by limiting glycerolipid biosynthesis. Cancer Lett. 2023;565:216210.
120. Regue L, Mou F, Avruch J. G protein-coupled receptors engage the mammalian Hippo pathway through F-actin: F-Actin, assembled in response to Galpha12/13 induced RhoA-GTP, promotes dephosphorylation and activation of the YAP oncogene. BioEssays. 2013;35(5):430–5.
121. Gong H, Shen B, Flevaris P, Chow C, Lam SC, Voyno-Yasenetskaya TA, et al. G protein subunit Galpha13 binds to integrin alphaIIbbeta3 and mediates integrin outside-in signaling. Science. 2010;327(5963):340–3.
122. Bian D, Mahanivong C, Yu J, Frisch SM, Pan ZK, Ye RD, et al. The G12/13-RhoA signaling pathway contributes to efficient lysophosphatidic acid-stimulated cell migration. Oncogene. 2006;25(15):2234–44.
123. Justus CR, Yang LV. GPR4 decreases B16F10 melanoma cell spreading and regulates focal adhesion dynamics through the G13/Rho signaling pathway. Exp Cell Res. 2015;334(1):100–13.
124. Masia-Balague M, Izquierdo I, Garrido G, Cordomi A, Perez-Benito L, Miller NL, et al. Gastrin-stimulated Galpha13 activation of rgnef protein (ArhGEF28) in DLD-1 Colon carcinoma cells. J Biol Chem. 2015;290(24):15197–209.
125. Anfinogenova Y, Wang R, Li QF, Spinelli AM, Tang DD. Abl silencing inhibits CAS-mediated process and constriction in resistance arteries. Circ Res. 2007;101(4):420–8.
126. Jia L, Tang DD. Abl activation regulates the dissociation of CAS from cytoskeletal vimentin by modulating CAS phosphorylation in smooth muscle. Am J Physiol Cell Physiol. 2010;299(3):C630–7.
127. Jia L, Wang R, Tang DD. Abl regulates smooth muscle cell proliferation by modulating actin dynamics and ERK1/2 activation. Am J Physiol Cell Physiol. 2012;302(7):C1026–34.
128. Cohen SG. Asthma in antiquity: the Ebers Papyrus. Allergy Proc. 1992;13(3):147–54.
129. Jackson M. Divine stramonium: the rise and fall of smoking for asthma. Med Hist. 2010;54(2):171–94.
130. Mein HFG. Ueber die Darstellung Des Atropins in Weissen Kristallen. Annalen Der Pharmacie. 1833;6(1):67–72.
131. Diamant Z, Boot JD, Virchow JC. Summing up 100 years of asthma. Respir Med. 2007;101(3):378–88.
132. Suen JY, Cotterell A, Lohman RJ, Lim J, Han A, Yau MK, et al. Pathway-selective antagonism of proteinase activated receptor 2. Br J Pharmacol. 2014;171(17):4112–24.
133. Hollenberg MD, Mihara K, Polley D, Suen JY, Han A, Fairlie DP, et al. Biased signalling and proteinase-activated receptors (PARs): targeting inflammatory disease. Br J Pharmacol. 2014;171(5):1180–94.
134. Sedki D, Cho A, Cao Y, Nikolajev L, Atmuri NDP, Lubell WD, et al. Prostaglandin F2alpha and angiotensin II type 1 receptors exhibit differential cognate G protein coupling regulation. J Biol Chem. 2022;298(9):102294.
135. Tran K, Sainsily X, Cote J, Coquerel D, Couvineau P, Saibi S, et al. Size-reduced macrocyclic analogues of [Pyr(1)]-apelin-13 showing negative Galpha12 Bias still produce prolonged Cardiac effects. J Med Chem. 2022;65(1):531–51.
136. Expert Panel Working Group of the National Heart, Prevention Program Coordinating L, Cloutier C, Baptist MM et al. AP,. 2020 Focused Updates to the Asthma Management Guidelines: A Report from the National Asthma Education and Prevention Program Coordinating Committee Expert Panel Working Group. J Allergy Clin Immunol. 2020;146(6):1217-70.
137. Chen R, Suchard MA, Krumholz HM, Schuemie MJ, Shea S, Duke J, et al. Comparative first-line effectiveness and safety of ACE (angiotensin-Converting enzyme) inhibitors and angiotensin receptor blockers: a multinational cohort study. Hypertension. 2021;78(3):591–603.
138. Pang R, Zhou XY, Wang X, Wang B, Yin XL, Bo H, et al. Anticholinergics combined with alpha-blockers for treating lower urinary tract symptoms related to benign prostatic obstruction. Cochrane Database Syst Rev. 2021;2(2):CD012336.
139. Stoniute A, Madhuvrata P, Still M, Barron-Millar E, Nabi G, Omar MI. Oral anticholinergic drugs versus placebo or no treatment for managing overactive bladder syndrome in adults. Cochrane Database Syst Rev. 2023;5(5):CD003781.
140. Schmitt JM, Stork PJ. Cyclic AMP-mediated inhibition of cell growth requires the small G protein Rap1. Mol Cell Biol. 2001;21(11):3671–83.
141. Nguyen LP, Al-Sawalha NA, Parra S, Pokkunuri I, Omoluabi O, Okulate AA, et al. beta(2)-Adrenoceptor signaling in airway epithelial cells promotes eosinophilic inflammation, mucous metaplasia, and airway contractility. Proc Natl Acad Sci U S A. 2017;114(43):E9163–71.
142. Nelson HS, Weiss ST, Bleecker ER, Yancey SW, Dorinsky PM, Group SS. The Salmeterol Multicenter Asthma Research Trial: a comparison of usual pharmacotherapy for asthma or usual pharmacotherapy plus salmeterol. Chest. 2006;129(1):15–26.
143. Salpeter SR, Buckley NS, Ormiston TM, Salpeter EE. Meta-analysis: effect of long-acting beta-agonists on severe asthma exacerbations and asthma-related deaths. Ann Intern Med. 2006;144(12):904–12.
144. Yan D, Hamed O, Joshi T, Mostafa MM, Jamieson KC, Joshi R, et al. Analysis of the Indacaterol-regulated transcriptome in human airway epithelial cells implicates Gene expression changes in the adverse and therapeutic effects of beta(2)-Adrenoceptor agonists. J Pharmacol Exp Ther. 2018;366(1):220–36.
145. Sharafkhaneh A, Majid H, Gross NJ. Safety and tolerability of inhalational anticholinergics in COPD. Drug Healthc Patient Saf. 2013;5:49–55.
146. Westby M, Benson M, Gibson P. Anticholinergic agents for chronic asthma in adults. Cochrane Database Syst Rev. 2004;2004(3):CD003269.
147. Novelli F, Malagrino L, Dente FL, Paggiaro P. Efficacy of anticholinergic drugs in asthma. Expert Rev Respir Med. 2012;6(3):309–19.
148. Vaughan M, Moss J. Mechanism of action of choleragen. J Supramol Struct. 1978;8(4):473–88.
149. Shah BH. Enhanced degradation of stimulatory G-protein (gs alpha) by cholera toxin is mediated by ADP-ribosylation of Gs alpha protein but not by increased cyclic AMP levels. Adv Exp Med Biol. 1997;419:93–7.
150. Murayama T, Ui M. Loss of the inhibitory function of the guanine nucleotide regulatory component of adenylate cyclase due to its ADP ribosylation by islet-activating protein, pertussis toxin, in adipocyte membranes. J Biol Chem. 1983;258(5):3319–26.
151. Takasaki J, Saito T, Taniguchi M, Kawasaki T, Moritani Y, Hayashi K, et al. A novel Galphaq/11-selective inhibitor. J Biol Chem. 2004;279(46):47438–45.
152. Schrage R, Schmitz AL, Gaffal E, Annala S, Kehraus S, Wenzel D, et al. The experimental power of FR900359 to study Gq-regulated biological processes. Nat Commun. 2015;6:10156.
153. Gu JL, Muller S, Mancino V, Offermanns S, Simon MI. Interaction of G alpha(12) with G alpha(13) and G alpha(q) signaling pathways. Proc Natl Acad Sci U S A. 2002;99(14):9352–7.
154. Wang ZZ, Shi XX, Huang GY, Hao GF, Yang GF. Fragment-based drug discovery supports drugging 'undruggable' protein-protein interactions. Trends Biochem Sci. 2023;48(6):539–52.
155. Berg T. Small-molecule inhibitors of protein-protein interactions. Curr Opin Drug Discov Devel. 2008;11(5):666–74.
156. Basse MJ, Betzi S, Bourgeas R, Bouzidi S, Chetrit B, Hamon V, et al. 2P2Idb: a structural database dedicated to orthosteric modulation of protein-protein interactions. Nucleic Acids Res. 2013;41(Database issue):D824–7.
157. Konstantinidou M, Visser EJ, Vandenboorn E, Chen S, Jaishankar P, Overmans M, et al. Structure-based optimization of Covalent, Small-Molecule stabilizers of the 14-3-3σ/ERα protein-protein Interaction from nonselective fragments. J Am Chem Soc. 2023;145(37):20328–43.
158. DiGiacomo V, de Opakua AI, Papakonstantinou MP, Nguyen LT, Merino N, Blanco-Canosa JB, et al. The Gαi-GIV binding interface is a druggable protein-protein interaction. Sci Rep. 2017;7(1):8575.
159. Pang A, Cheng N, Cui Y, Bai Y, Hong Z, Delaney MK et al. High-loading Galpha13-binding EXE peptide nanoparticles prevent thrombosis and protect mice from cardiac ischemia/reperfusion injury. Sci Transl Med. 2020;12(552).
160. Quilliam LA, Lacal JC, Bokoch GM. Identification of rho as a substrate for botulinum toxin C3-catalyzed ADP-ribosylation. FEBS Lett. 1989;247(2):221–6.
161. Shang X, Marchioni F, Evelyn CR, Sipes N, Zhou X, Seibel W, et al. Small-molecule inhibitors targeting G-protein-coupled rho guanine nucleotide exchange factors. Proc Natl Acad Sci U S A. 2013;110(8):3155–60.
162. Pagano L, Lee JW, Posarelli M, Giannaccare G, Kaye S, Borgia A. ROCK inhibitors in corneal diseases and Glaucoma-A Comprehensive Review of these emerging drugs. J Clin Med. 2023;12(21).
163. Ali F, Ilyas A. Belumosudil with ROCK-2 inhibition: chemical and therapeutic development to FDA approval for the treatment of chronic graft-versus-host disease. Curr Res Transl Med. 2022;70(3):103343.
164. Lingor P, Weber M, Camu W, Friede T, Hilgers R, Leha A, et al. ROCK-ALS: protocol for a Randomized, Placebo-Controlled, double-blind phase IIa trial of Safety, Tolerability and Efficacy of the rho kinase (ROCK) inhibitor Fasudil in Amyotrophic lateral sclerosis. Front Neurol. 2019;10:293.
165. Wolff AW, Bidner H, Remane Y, Zimmer J, Aarsland D, Rascol O, et al. Protocol for a randomized, placebo-controlled, double-blind phase IIa study of the safety, tolerability, and symptomatic efficacy of the ROCK-inhibitor Fasudil

in patients with Parkinson's disease (ROCK-PD). Front Aging Neurosci. 2024;16:1308577.
166. McLeod R, Kumar R, Papadatos-Pastos D, Mateo J, Brown JS, Garces AHI, et al. First-in-human study of AT13148, a dual ROCK-AKT inhibitor in patients with solid tumors. Clin Cancer Res. 2020;26(18):4777–84.
167. Zanin-Zhorov A, Weiss JM, Trzeciak A, Chen W, Zhang J, Nyuydzefe MS, et al. Cutting Edge: selective oral ROCK2 inhibitor reduces clinical scores in patients with Psoriasis Vulgaris and normalizes skin Pathology via Concurrent Regulation of IL-17 and IL-10. J Immunol. 2017;198(10):3809–14.
168. Matoba K, Takeda Y, Nagai Y, Sekiguchi K, Yokota T, Utsunomiya K, et al. The Physiology, Pathology, and therapeutic interventions for ROCK isoforms in Diabetic kidney disease. Front Pharmacol. 2020;11:585633.
169. Montagnoli TL, da Silva JS, Sudo SZ, Santos AD, Gomide GF, de Sa MPL et al. ROCK inhibition as potential target for treatment of Pulmonary Hypertension. Cells. 2021;10(7).
170. Barcelo J, Samain R, Sanz-Moreno V. Preclinical to clinical utility of ROCK inhibitors in cancer. Trends Cancer. 2023;9(3):250–63.
171. Gupta V, Gupta N, Shaik IH, Mehvar R, McMurtry IF, Oka M, et al. Liposomal fasudil, a rho-kinase inhibitor, for prolonged pulmonary preferential vasodilation in pulmonary arterial hypertension. J Control Release. 2013;167(2):189–99.
172. Franova S, Molitorisova M, Kalmanova L, Palencarova J, Joskova M, Smiesko L, et al. The anti-asthmatic potential of rho-kinase inhibitor hydroxyfasudil in the model of experimentally induced allergic airway inflammation. Eur J Pharmacol. 2023;938:175450.
173. Zhang W, Li X, Zhang Y. Rho-kinase inhibitor attenuates airway mucus hypersecretion and inflammation partly by downregulation of IL-13 and the JNK1/2-AP1 signaling pathway. Biochem Biophys Res Commun. 2019;516(2):571–7.
174. Wang L, Chitano P, Pare PD, Seow CY. Mechanopharmacology and Synergistic Relaxation of Airway Smooth Muscle. J Eng Sci Med Diagn Ther. 2019;2(1):0110041–110047.
175. Duan Y, Long J, Chen J, Jiang X, Zhu J, Jin Y, et al. Overexpression of soluble ADAM33 promotes a hypercontractile phenotype of the airway smooth muscle cell in rat. Exp Cell Res. 2016;349(1):109–18.
176. Duan W, Aguinaldo Datiles AM, Leung BP, Vlahos CJ, Wong WS. An anti-inflammatory role for a phosphoinositide 3-kinase inhibitor LY294002 in a mouse asthma model. Int Immunopharmacol. 2005;5(3):495–502.
177. Takeda M, Ito W, Tanabe M, Ueki S, Kato H, Kihara J, et al. Allergic airway hyperresponsiveness, inflammation, and remodeling do not develop in phosphoinositide 3-kinase gamma-deficient mice. J Allergy Clin Immunol. 2009;123(4):805–12.
178. Koziol-White CJ, Yoo EJ, Cao G, Zhang J, Papanikolaou E, Pushkarsky I, et al. Inhibition of PI3K promotes dilation of human small airways in a rho kinase-dependent manner. Br J Pharmacol. 2016;173(18):2726–38.
179. McNamara CR, Degterev A. Small-molecule inhibitors of the PI3K signaling network. Future Med Chem. 2011;3(5):549–65.
180. Lee HJ, Lee EJ, Seo M. Galpha12 protects vascular endothelial cells from serum Withdrawal-Induced apoptosis through regulation of miR-155. Yonsei Med J. 2016;57(1):247–53.
181. Huang S, Chen LY, Zuraw BL, Ye RD, Pan ZK. Chemoattractant-stimulated NF-kappaB activation is dependent on the low molecular weight GTPase RhoA. J Biol Chem. 2001;276(44):40977–81.
182. Wang Y, Cheng J, Tandan S, Jiang M, McCloskey DT, Hill JA. Transient-outward K + channel inhibition facilitates L-type Ca2 + current in heart. J Cardiovasc Electrophysiol. 2006;17(3):298–304.
183. Bi J, Min Z, Yuan H, Jiang Z, Mao R, Zhu T, et al. PI3K inhibitor treatment ameliorates the glucocorticoid insensitivity of PBMCs in severe asthma. Clin Transl Med. 2020;9(1):22.
184. Webb RC. Smooth muscle contraction and relaxation. Adv Physiol Educ. 2003;27(1–4):201–6.
185. Keglowich LF, Borger P. The Three A's in asthma - Airway Smooth Muscle, Airway Remodeling & Angiogenesis. Open Respir Med J. 2015;9:70–80.

Publisher's Note