International Journal of Biochemistry and Cell Biology 112 (2019) 1–7

Contents lists available at ScienceDirect

International Journal of Biochemistry and Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Medicine in focus

Extracellular regulation of airway smooth muscle contraction

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ARTICLE INFO

Keywords:
Airway hyperresponsiveness
Bronchodilators
Spasmogens

ABSTRACT

The molecular mechanisms governing the contraction of airway smooth muscle have always been at the forefront of asthma research. New extracellular molecules affecting the contraction of airway smooth muscle are steadily being discovered. Although interesting, this is disconcerting for researchers trying to find a mend for the significant part of asthma symptoms caused by contraction. Additional efforts are being deployed to understand the intracellular signaling pathways leading to contraction. The goal being to find common pathways that are essential to convey the contractile signal emanating from any single or combination of extracellular molecules. Not only these pathways exist and their details are being slowly unveiled, but some carry the signal inside-out to interact back with extracellular molecules. These latter represent targets with promising therapeutic potential, not only because they are molecules downstream of pathways essential for contraction but also because their extracellular location makes them readily accessible by inhaled drugs.

1. Introduction

Airway smooth muscle (ASM) is a tissue for which the physiological function is uncertain but that contributes significantly to respiratory symptoms in asthma. Understanding the molecular mechanisms regulating the contraction of ASM is thus of utmost importance.

The first section of this article describes the uncontested role of ASM in asthma pathogenesis. The second section reviews the newly identified extracellular molecules and receptors capable of contracting, relaxing or modulating ASM contraction. The third section expands on recently discovered contractile signaling pathways in the intracellular space that link back to components in the extracellular space. Finally, the fourth section briefly discusses about the therapeutic potential of these latest breakthroughs.

2. Pathogenesis

Asthma is a lung disorder characterizes by variable airflow obstruction. The symptoms include wheezing, thoracic oppression, respiratory distress and cough. One of the most objective criteria to diagnose asthma is the presence of reversible airway obstruction, which is defined as an increase in forced expiratory volume in 1 s (FEV_1) of more than 200 ml and greater than 12% of the predicted value following the inhalation of a drug that relaxes the ASM (*i.e.*, a bronchodilator such as salbutamol). This feature testifies that ASM contributes to asthma symptoms. However, the assortment of extracellular molecules leading to excessive ASM contraction in asthma is complex, heterogenous and unstable. This not only means that a great number of molecules may contribute to ASM contraction in each patient but that the molecules involved are likely to be variable between patients, as well as to change

Abbreviations: Abi1, Abl interactor 1; ADAM33, A disintegrin and metalloproteinase 33; AHR, airway hyperresponsiveness; ArfGAPs, ADP-ribosylation factor GTPase-activating proteins; Arp2/3, Actin-related proteins 2/3; ASM, airway smooth muscle; BPIFA1, Bacterial permeability family member A1; Ca^{2+}, calcium; c-Abl, Abelson tyrosine kinase; CAS, Crk-associated substrates; CaSR, Ca^{2+}-sensing receptor; Cdc42GAP, Cdc42 GTPase-activating protein; CTTN, Cortactin; DOCK, Dedicator of cytokinesis; DPA, docosahexaenoic acid; ECM, extracellular matrix; ELMO, Engulfment and cell motility protein; FAK, Focal adhesion kinase; F-actin, actin filament; FEV_1, forced expiratory volume in 1 second; FFAR1, Free fatty acid receptor 1; G-actin, globular (monomeric) actin; GEF, guanine nucleotide exchange factor; GIT, G-protein-coupled receptor kinase-interacting protein; GMF-γ, Glia maturation factor-; GPCR, G protein-couple receptor; GTPase, guanosine triphosphatase; HDAC8, Histone deacetylase 8; IL, interleukin; ILK, Integrin-linked kinase; Mfge8, Milk fat globule-EGF factor 8; MLC, Myosin light chain; N-WASp, Neuronal Wiskott-Aldrich syndrome protein; NMII, Non-muscle myosin II; NMHCII, Non-muscle myosin II heavy chain; NM MLC, Non-muscle myosin light chain; Pak, p21-activated kinase; PIP2, phosphatidylinositol 4,5-biphosphate; PIP5K1, Phosphatidylinositol 4-phosphate 5-kinase 1; PINCH, Particularly interesting new cysteine-histidine-rich protein; PIX, Pak-interacting exchange factor; Plk1, Polo-like kinase 1; Pfn-1, Profilin-1; PP1, Protein phosphatase 1; SLK, Ste20-like kinase; ROCK, Rho-associated coiled-coil containing protein kinase; SSAT, Spermidine/spermine N-acetyltransferase; TMEM16A, Transmembrane protein 16A; TNFα, Tumor necrosis factor; VASP, Vasodilator-stimulated phosphoprotein; WTN-5A, Wingless-integrase-1 family member 5A

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https://doi.org/10.1016/j.biocel.2019.04.012
Received 8 February 2019; Received in revised form 25 April 2019; Accepted 26 April 2019
Available online 28 April 2019
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over time within each patient.

3. Extracellular molecules controlling ASM contraction

The search for extracellular molecules affecting the contraction of ASM is very active. Overall, hundreds of molecules are directly or indirectly involved (Bosse, 2012). Apart from the classical bronchodilators (*e.g.*, prostaglandin E_2 and nitric oxide) and spasmogens (*e.g.*, acetylcholine and histamine), new molecules affecting ASM contraction are steadily being discovered. Due to space constraint, this article can only report on few recent examples.

The Bacterial permeability family member A1 (BPIFA), an antimicrobial protein secreted by the airway epithelium, attenuates ASM contraction (Wu et al., 2017). Resolvin D_2, a D-series resolvin derived from the ω-3 docosahexaenoic acid, prevents the development of ASM hypercontractility elicited by either leukotriene D_4 and Tumor necrosis factor α (TNFα) (Khaddaj-Mallat et al., 2016). Leptin, a hormone controlling energy intake, acts through the cholinergic neurons in the central nervous system to decrease parasympathetic efferences and the release of acetylcholine in the lungs, thereby alleviating muscarinic 3 receptor-mediated ASM contraction (Arteaga-Solis et al., 2013). S100A8 also decreases ASM contraction (Xu et al., 2017). Contrastingly, the epithelium-derived Neuropeptide Y (Li et al., 2016c), Wingless-integrase-1 family member 5 A (WTN-5 A) (Koopmans et al., 2016) and high concentration of carbon dioxide (*i.e.*, hypercapnia) (Shigemura et al., 2018) promote airway hyperresponsiveness (AHR) by increasing the contractile capacity of ASM.

Many receptors were also recently shown to influence ASM contraction. Toll-like receptor-7, an intracellular receptor that specifically recognizes single-stranded bacterial and viral RNAs, relaxes ASM indirectly by stimulating the synthesis of nitric oxide from the nerves (Drake et al., 2013). The ionotropic N-methyl-D-aspartate receptors either contract ASM directly in healthy conditions or relax ASM indirectly through the release of bronchodilating prostaglandins and nitric oxide in inflammatory conditions (Anaparti et al., 2016). Latrophilin receptors, a class of G protein-coupled receptors (GPCR), are overexpressed in asthma and cause ASM contraction either directly or indirectly through the release of nerve-derived acetylcholine (Faiz et al., 2017). The activation of the Free fatty acid receptor 1 (FFAR1) by oleic and linoleic acids potentiates acetylcholine-induced contraction and decreases the relaxing potency of isoproterenol (Mizuta et al., 2015). The Ca^{2+}-sensing receptor (CaSR) contributes to the potentiation of ASM contraction elicited by either spermine or a rise in the extracellular concentration of Ca^{2+}, as well as to *in vivo* AHR elicited by either poly-L-arginine or allergic lung inflammation (Yarova et al., 2015). The Transmembrane protein 16 A (TMEM16 A), a Ca^{2+}-activated chloride channel, potentiates the contraction induced by many spasmogens (Wang et al., 2018a, 2018b). CD148, a receptor-like protein tyrosine phosphatase, also contributes to AHR by increasing the contractile capacity of ASM (Katsumoto et al., 2013).

Although interesting, this increasing number of bronchoactive molecules is disconcerting for researchers trying to find a common mend for asthma. The concerted effect of all these molecules in asthma clearly appears pro-contractile, presumably resulting from an increased expression/activity of spasmogens and/or a decreased expression/activity of bronchodilators. However, too many molecules may be involved, which seriously raises doubts about the utility of this growing literature in terms of therapy improvement. A typical historical example is histamine (Larsen, 2001). Histamine is an important mediator of the allergic response and, through the H1 receptor, acts as a powerful spamogen for ASM. Yet, the drugs antagonizing the H1 receptor are generally not effective in asthma and, despite many years of research, H1-antihistamines were never included in treatment guidelines for asthma. A more recent example is Interleukin-13 (IL-13). IL-13 was repeatedly shown to potentiate ASM contraction (Auger et al., 2016). However, several biologics against IL-13 were shown ineffective in asthma (Hanania et al., 2016; Russell et al., 2018). Similar claims can be made for TNF-α (Wenzel et al., 2009) and Interleukin-17 (IL-17) (Busse et al., 2013). Targeting a single molecule in asthma is certainly appealing and compatible with the emerging spirit of promoting precision medicine to address the well-recognized molecular heterogeneity of asthma pathogenesis between patients. The drawback is that, beyond inter-individual variability, there is complexity. The complexity signifies that within any given affected individual, many molecular defects may be contributing. Blocking only one of them is thus expected to benefit, at most, very little (Chang and Bosse, 2016).

4. Inside-out signal required for ASM contraction

Additional efforts are directed towards the understanding of the intracellular pathways leading to ASM contraction. The goal is to identify pathways for which targeting would block contraction irrespective of the dysregulated bronchoactive molecules involved. Conveniently, it turns out that the contractile signals downstream of many cell-surface receptors do converge into common intracellular pathways that are essential for contraction. A typical example is the phosphorylation of the Myosin light chain (MLC) and the subsequent stimulation of the actin-activated ATPase on the myosin head. These downstream steps enable the chemical energy contained in ATP to be transformed into mechanical force and shortening as the myosin heads bound on actin filaments strive to pivot successively, in a process called cross-bridge cycling, to pull the actin filaments.

Ironically, recent discoveries in the field of intracellular signaling bring back to the fore the importance of signaling towards the extracellular space (Fig. 1). Indeed, some intracellular pathways that are essential for contraction elicited by any spasmogen lead to the rearrangement of the cell's cytoskeleton in order to reinforce the connection of the contractile apparatus inside the cell with the extracellular matrix (ECM) and the surrounding cells. The importance of this malleable connection in ASM contraction has been reviewed elsewhere (Tang, 2018; Zhang et al., 2015).

According to our current understanding, three structures are at play, namely focal adhesions, adherens junctions and desmosomes. Whether these structures represent separate entities with distinct function or overlapping structures that continuously rearrange complementarily to fulfill a common function in ASM is currently debatable and addressed below. Regardless, together these structures are considered the system of transmission of ASM (Tang, 2018; Zhang et al., 2015), as they physically link the cell's contractile apparatus to its extracellular surrounding.

Focal adhesions are responsible for cell-ECM connection. They are found at microlocations along the cortical regions of the cells where a variety of ECM components engage with cell-surface integrins and where the cytoplasmic tail of integrins binds, indirectly, to the cell's contractile apparatus. The intracellular side of focal adhesions is actually an evanescent multiprotein module called an adhesome. The assembly of adhesomes relies on a cascade of post-translational molecular events that recruits several scaffolding and enzymatic proteins *vis-à-vis* integrins. Apart from integrins, an amalgam of proteins are involved (Table 1). These proteins interact in a very organized fashion to eventually culminate into the formation of a complex capable of nucleating, polymerizing and branching actin to create *de novo* actin filaments, as well as to elongate and ramify existing ones. This filamentogenesis of actin at the cortical region of the cells ultimately fortifies the links between the plasmalemma and the contractile apparatus and thereby improves the efficiency of the mechanotransduction (Tang, 2018; Zhang et al., 2015). Notably, the phosphorylation of the Non-muscle myosin II heavy chain A and B (NMHCIIA & NMHCIIB) and their assembly into filaments at the cell cortex are also required for adhesome formation, actin filamentogenesis and force development (Zhang and Gunst, 2017).

Adherens junctions and desmosomes are also microlocated

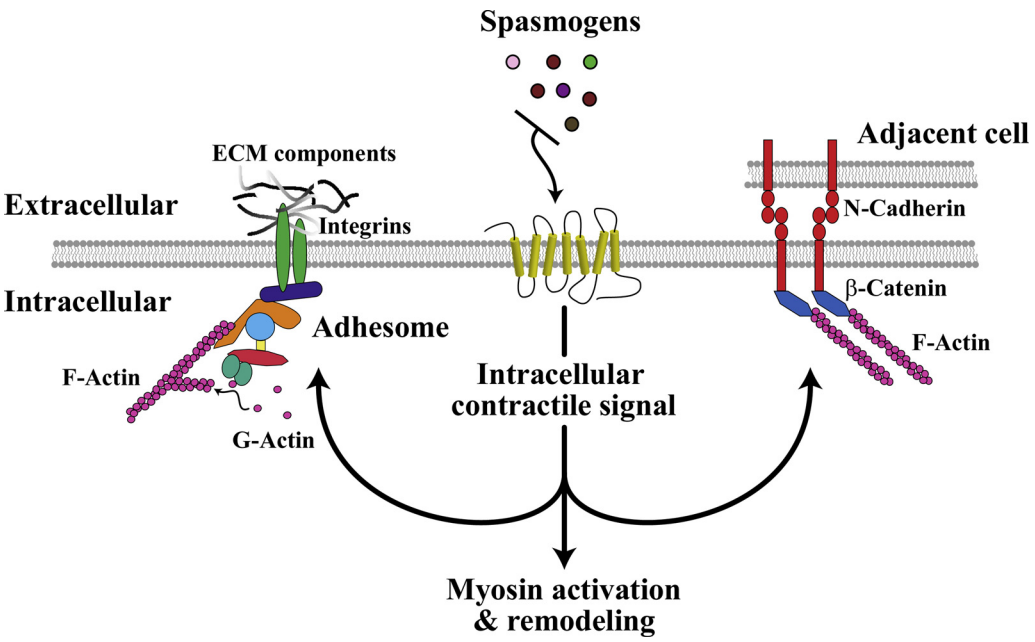

Fig. 1. Inside-out signal required for airway smooth muscle contraction. Cartoons of a focal adhesion and an adherens junction are depicted on the left and right, respectively. The intracellular side of each of these structures is not fixed but rather evanescent. It forms upon contractile activation to ensure proper mechanotranduction. Many other proteins are involved but were not drawn for clarity. As illustrated, several intracellular pathways are required to convey the contractile signal following the binding of a spasmogen onto its cognate cell-surface receptor. One pathway leads to myosin activation and cross-bridge cycling. Another one recruits β-catenins to submembranous regions, which then connect the transmembrane N-cadherins with actin filaments (F-actin) and thereby couple the actin cytoskeletons of two adjacent cells indirectly through adherens junctions. Another pathway instigates a chain of events leading to the formation of submembranous adhesomes *vis-à-vis* integrins; the latter being bound to extracellular matrix components. The adhesomes then catalyse actin filamentogenesis at the cell cortex to generate focal adhesions, which serve as indirect integrin-mediated links between the contractile apparatus and the extracellular matrix. The development of force and shortening in airway smooth muscle thus requires the restructuration of the cortical cell's cytoskeleton in combination with myosin activation. Only together, these processes allow the mechanical work generated by cross-bridges to be transmitted inside the cells and throughout the tissue by the firm attachment of the cell's contractile apparatus with its surrounding.

structures found along the cortical regions of the cells. Instead of connecting cells to ECM like the focal adhesions, they are responsible for cell-cell connection. More precisely, specific transmembrane proteins in both of these structures form extracellular complexes with their counterparts on adjacent cells. While the transmembrane protein involved in adherens junctions is called N-cadherin, the ones involved in desmosomes are called Desmocollin and Desmoglein. Evidently, these cell-cell connections can only serve as force transmission units when they are somehow attached to the contractile apparatus inside the cells. On the intracellular side of adherens junctions, the actin filaments are linked to the cytoplasmic tails of N-cadherin by β-catenin and other linkers. On the intracellular side of desmosomes, the network of vimentin filaments is linked to the cytoplasmic tails of Desmocollin and Desmoglein by Plakophilin, Plakoglobin, Desmoplakin and Desmin. These intracellular links are actually strengthened upon ASM contraction. Indeed, β-catenin is recruited to the cortical regions of the cells and binds to N-cadherin upon ASM contraction (Wang et al., 2015). Similarly, Plakoglobin translocates to the cell periphery and the vimentin network dismantles and undergoes spatial reorientation upon ASM contraction (Tang et al., 2005a; Wang et al., 2006). Interfering with any of these processes decreases ASM force by weakening the intercellular mechanotransmission.

Similar to the thin filaments of actin and the intermediate filaments of vimentin, the thick filaments of smooth-muscle myosin also remodel upon contractile activation. In fact, they increase in number but decrease in length, presumably to facilitate the dislocation of myosin units in order to optimize their contact with the restructuring actin cytoskeleton (Liu et al., 2013).

Overall, actin, vimentin and myosin dynamics in conjunction with adhesome formation are newly discovered intracellular processes that are essential for the contraction elicited by any spasmogen. The coordinated fashion whereby they are set in motion following the stimulation of ASM entitles the mechanical force generated by the cross-bridges to be transmitted inside the cells and across the plasmalemma to the ECM and the surrounding cells. A recent study actually suggested that the potentiation of force by Rho-associated coiled-coil containing protein kinase (ROCK) in ASM is not mediated through Ca^{2+} sensitization by the inactivation of MLC phosphatase as it has long been believed, but rather by the activation of p21-activated kinase (Pak) and the ensuing filamentogenesis of actin through the Cdc42/Neuronal Wiskott-Aldrich syndrome protein (N-WASp)/Actin-related proteins 2/3 (Arp2/3) axis (Zhang et al., 2018). Once again, this highlights the importance of this malleable connection in force development.

Whether ASM cells preferentially bind to their surrounding through focal adhesions or adherens junctions is strongly regulated by the stiffness of the ECM (Polio et al., 2018). On a stiff ECM, the cells preferentially form focal adhesions. The total amount of force generated by an ensemble of ASM cells for any given degree of ECM stiffness is also enhanced when the cells connect onto the ECM compared to when they connect to each other (Polio et al., 2018). Therefore, both types of connections may well increase upon ASM contraction, but predominant connections with the ECM through focal adhesions may contribute to AHR. Importantly, in conditions favoring an increased amount of focal adhesions, the cells always display a decreased amount of adherens junctions and *vice-versa* (Polio et al., 2018). This suggests that these structures are truly swapping for reasons yet unknown.

Recent studies also demonstrated that Vimentin phosphorylation by Pak or by the sequential activation of Ste20-like kinase (SLK) and Polo-like kinase 1 (Plk1) leads to Crk-associated substrates (CAS) dissociation from vimentin filaments and its phosphorylation by Abelson tyrosine kinase (c-Abl), which are all necessary steps enabling CAS, as well as c-Abl-phosphorylated and Histone deacetylase 8 (HDAC8)-deacetylated Cortactin (CTTN), to bind with Profilin-1 (Pfn-1) and instigate actin filamentogenesis and ASM contraction (Li et al., 2014, 2016a). These results suggest a direct interplay between the vimentin filaments in desmosomes and the actin filaments in focal adhesions. Their concomitant and mutually regulated remodeling during ASM contraction suggest that they might well be coordinated to fulfill a concerted function.

Regardless of the type and degree of connections, accumulating evidences suggest that disrupting the links between integrins and the ECM decreases ASM contraction by impairing the efficiency of the

Table 1
Proteins involved in the formation of adhesomes upon contractile activation.

Name	Role in adhesome	Reference
α-actinin	An actin-binding protein that translocates to the plasmalemma and provides structural stability by binding to integrins.	(Zhang and Gunst, 2006)
Abi1 (Abl interactor 1)	An adaptor protein that forms of a trimeric complex with CAS and c-Abl, which is required for its binding and activating effect on N-WASp. Abi also preserves the activation of c-Abl.	(Wang et al., 2013)
Arp2/3 (Actin-related protein 2/3)	A seven-component complex acting as a catalyst for actin polymerization upon binding to the activated (unfolded conformation) form of N-WASp.	(Tang et al., 2005b; Zhang et al., 2005)
c-Abl (Abelson tyrosine kinase)	A non-receptor tyrosine kinase activated by Src-mediated phosphorylation at Y412 that phosphorylates CAS and CTTN, which fosters their association with Pfn-1. C-Abl also activates Abi1 and GMF-γ, which are required for N-WASp- and Arp2/3-dependent polymerization of actin.	(Wang et al., 2014a, b; Wang et al., 2018a, 2018b)
CAS (Crk-associated substrates)	An adaptor protein that is dissociated from vimentin and recruited to the plasmalemma by a mechanism partially dependent on c-Abl, where it forms a complex with c-Abl and Abi1, as well as with Pfn-1 and CTTN.	(Wang et al., 2007, 2013; Wang et al., 2018a, 2018b)
Cdc42	A small GTPase activated by GEFs, such as PIX and DOCK180, that directly binds and catalyzes the activation of N-WASp and regulates its coupling with the Arp2/3 complex.	(Tang and Gunst, 2004; Zhang et al., 2012, 2016)
Cdc42GAP (Cdc42 GTPase-activating protein)	A GTPase regulated by reactive oxygen species that inhibits Cdc42 and the downstream activation of Pak.	(Li et al., 2009)
Cofilin	An actin-severing protein activated by Calcineurin-mediated dephosphorylation at S3 that is involved in actin remodeling.	(Zhao et al., 2008)
CrkII	A Paxillin- and CAS-binding adaptor protein that also interacts and promotes the activation of DOCK180 and N-WASp.	(Tang et al., 2005b; Zhang et al., 2012)
CTTN (Cortactin)	An actin-regulatory protein that is recruited to the plasmalemma with Pfn-1 after its phosphorylation at Y421 by c-Abl.	(Wang et al., 2014a, b; Wang et al., 2018a, 2018b)
DOCK180 (Dedicator of cytokinesis)	A GEF for Cdc42 and Rac that links indirectly to Paxillin through CrkII and that partners with ELMO to catalyze nucleotide exchange.	(Zhang et al., 2012)
ELMO (Engulfment and cell motility protein)	A protein that partners with DOCK180 to catalyze the guanine nucleotide exchange-mediated activation of Cdc42.	(Zhang et al., 2012)
FAK (Focal adhesion kinase)	A kinase recruited to adhesomes via a RhoA-dependent mechanism that phosphorylates Paxillin at specific tyrosine residues (Y31 & 118), which then become docking sites for CrkII.	(Tang and Gunst, 2001; Zhang et al., 2012)
GIT1 & 2 (G-protein-coupled receptor kinase-interacting protein 1 & 2)	An ArfGAPs that regulates Cdc42 by linking Paxillin to PIX.	(Zhang et al., 2012)
GMF-γ (Glia maturation factor-γ)	A suppressor of actin filamentogenesis that undergoes c-Abl-dependent phosphorylation at Y104, which then releases Arp2/3 from GMF-γ and thereby disinhibits actin filamentogenesis.	(Wang et al., 2014a, b)
HDAC8 (Histone deacetylase 8)	An histone deacetylase that interacts and deacetylates the N-WASp-modulating protein CTTN, which increases CTTN binding affinity towards F-actin and thereby promotes actin filamentogenesis.	(Li et al., 2014)
ILK (Integrin-linked kinase)	A serine/threonine kinase recruited to the plasmalemma that associates with β-integrin via Paxillin in combination with its binding partners PINCH and α-parvin.	(Zhang et al., 2007)
Integrins	Complexes of two transmembrane proteins (integrin α & integrin β) that physically link the cell cytoskeleton to the extracellular matrix.	(Zhang and Gunst, 2006)
N-WASp (Neuronal Wiskott-Aldrich syndrome protein)	An initiator of actin filament nucleation recruited to adhesomes through CrkII that couples to Arp2/3 following its activation by Cdc42.	(Tang et al., 2005b; Wang et al., 2013; Zhang et al., 2016, 2005)
NMIIA & B (Non-muscle myosin IIA & IIB)	Motor proteins forming filaments involved in adhesome assembly by translocating cytoskeletal proteins to adhesomes.	(Zhang and Gunst, 2017)
NM MLC (Non-muscle myosin light chain)	A regulatory subunit of NMII that is phosphorylated by RhoA and contributes to the unfolding of the monomeric form of NMHCII into an assembly-competent form, in addition to regulate the myosin motor by enabling actin-activated ATPase on its globular head.	(Zhang and Gunst, 2017)
Pak (p21-activated kinase)	A Cdc42- and ROCK-activated serine-threonine kinase recruited to adhesomes through a RhoA-dependent mechanism that phosphorylates both Paxillin at S273 and Vimentin at S56.	(Wang et al., 2007; Zhang et al., 2018, 2016)
α-parvin	An actin-binding protein forming a complex with ILK and PINCH that is recruited to adhesomes.	(Zhang et al., 2007)
Paxillin	A scaffolding protein recruited to the plasmalemma along with Vinculin. Paxillin then becomes phosphorylated by FAK at Y31 and Y118, as well as by Pak at S273, to subsequently serves as a platform for the recruitment of other regulatory and structural proteins, such as the ILK/PINCH/α-parvin complex and the GEF complexes GIT/PIX and CrkII/DOCK180/ELMO.	(Opazo Saez et al., 2004; Zhang et al., 2012, 2016)
PINCH (Particularly interesting new cysteine-histidine-rich protein)	An adaptor protein forming a constitutive complex with ILK and α-parvin required for the translocation of the complex to adhesomes.	(Zhang et al., 2007)
PIX (Pak-interacting exchange factor)	A GEF binding to GIT1 or GIT2 to activate Cdc42.	(Zhang et al., 2012, 2016)
Plk1 (Polo-like kinase 1)	A serine/threonine kinase phosphorylating Vimentin at S56 following its activation by SLK.	(Li et al., 2016a)
Pfn-1 (Profilin-1)	An actin-binding protein recruited to the plasmalemma that complexes with CTTN and CAS to promote actin filamentogenesis.	(Wang et al., 2014a, b; Wang et al., 2018a, 2018b)
PP1 (Protein phosphatase 1)	A phosphatase interacting and dephosphorylating Vimentin at S56, which entitles CAS to bind Vimentin and inhibit actin polymerization.	(Li et al., 2016b)
SLK (Ste20-like kinase)	A serine/threonine kinase that activates Plk1 by phosphorylating it at T210.	(Li et al., 2016a)
Talin	An adaptor protein recruited to the plasmalemma that is implicated in the physical coupling between the cytoplasmic domain of integrins and F-actin through Vinculin.	(Huang et al., 2014; Opazo Saez et al., 2004)
RhoA		

(*continued on next page*)

Table 1 (*continued*)

Name	Role in adhesome	Reference
	A small GTPase promoting the activation of ROCK, the recruitment of FAK to the plasmalemma, and the formation and activation of NMII filaments.	(Zhang et al., 2010; Zhang and Gunst, 2017)
ROCK (Rho-associated coiled-coil containing protein kinase)	A serine-threonine kinase acting as a RhoA effector that activates Pak.	(Zhang et al., 2018)
VASP (Vasodilator-stimulated phosphoprotein)	A protein phosphorylated at S157 and recruited to the plasmalemma, where it promotes actin filament elongation by oligomerizing with itself, as well as with Vinculin and Pfn-1, via a mechanism dependent on Vinculin phosphorylation at Y1065.	(Wu and Gunst, 2015)
Vinculin	A scaffolding/structural protein that constitutively partners with Paxillin. The complex is recruited to the plasmalemma through a RhoA-dependent mechanism where Vinculin undergoes phosphorylation at Y1065, which enables its unfolding and the subsequent binding of its head and tail domains to integrin-bound Talin and F-actin, respectively.	(Huang et al., 2014, 2011)

mechanotransduction. For example, the cleavage of Fibronectin by Chymase inhibits focal adhesion phosphorylation, ASM adhesion and the enhanced ASM contractility induced by IL-13 through an integrin $\alpha_5\beta_1$-dependent mechanism without altering intracellular mobilization of Ca^{2+} and MLC phosphorylation (Sundaram et al., 2017). The blockade of integrin $\alpha_5\beta_1$ also alleviates AHR in a murine model of asthma and augments the bronchodilator effect of isoproterenol in murine tracheal rings (Sundaram et al., 2017). Another example is Elastase. Elastase degrades many ECM components and decreases the contractility of canine tracheal strips without affecting MLC phosphorylation (Lockett et al., 2018). The effect is associated with the rupture of adhesomes. More specifically, Elastase cleaves Talin, dissociates Talin from Vinculin and decreases phosphorylation of Paxillin, Focal adhesion kinase (FAK) and Vinculin (Lockett et al., 2018). Again, this suggests that the decreased contractility induced by Elastase stems from a defect in force transmission. The results also indicate that disrupting the connection of integrins on the external side of the plasmalemma perturbs the connection on the internal side, suggesting that the connections on either sides of integrins are mutually regulated.

Here, a word of caution is required though. Undirected disruption of integrin binding on ECM may not necessarily be desirable. Some integrins seem to put a break on ASM contraction. For example, integrin $\alpha_9\beta_1$ suppresses ASM contraction (Chen et al., 2012). The downstream molecular mechanisms by which it occurs is indirect and involves a sequence of events with several intermediates. Briefly, integrin $\alpha_9\beta_1$ co-localizes Phosphatidylinositol 4-phosphate 5-kinase 1γ (PIP5K1γ) and Spermidine/spermine N-acetyltransferase (SSAT). The degradation of higher-order polyamines by SSAT then inhibits PIP5K1γ and consequently reduces the availability of phosphatidylinositol 4,5-biphosphate (PIP2) (Chen et al., 2012). In turn, this inhibits the contraction mediated by the canonical IP_3-Ca^{2+}-MLC axis; as PIP2 is the substrate required for the synthesis of IP_3 by Phospholipase Cβ, the lipid kinase activated by the Gα_q following the binding of the classical spasmogens onto their cognate GPCR. Bottom line, these results suggest that integrins can exert both pro- and anti-contractile effects on ASM.

The full spectrum of endogenous extracellular ligands acting on integrins and their effects on their functions will need to be thoroughly investigated. Milk fat globule-EGF factor 8 (Mfge8) decreases airway responsiveness in mouse models of asthma and prevents the increased ASM contraction induced by IL-13, IL-17 A and TNFα through its RGD integrin-binding site (Kudo et al., 2013). In contrast, the overexpression of A disintegrin and metalloproteinase 33 (ADAM33) increases the contractile capacity of ASM (Duan et al., 2016). The integrin $\alpha_v\beta_6$ also fosters the development of AHR indirectly in a murine model of asthma by activating the latent form of Transforming-growth factor β1, which subsequently downregulates the mouse mast cell protease 4, the murine ortholog of Chymase (Sugimoto et al., 2012). The modulation of ASM contraction by integrins is thus complex. Fine and specific regulations of integrins and ECM components will be needed to acquire the desired response.

5. Therapy

As alluded in the first parts of this article, the molecular etiology of asthma is not only variable between patients, but many molecular defects may be conspiring in a time-varying fashion within any given patient. The problem of inter-individual variability can potentially be solved with precision medicine. However, as stated above, the fact that many molecular defects may be involved within any given patient is a problem difficult to address (Chang and Bosse, 2016). It is even more difficult if the assortment of molecular defects involved is changing over time.

The development of broad-spectrum bronchodilators (*i.e.*, capable of relaxing ASM irrespective of the assortment of spasmogens involved) is more promising. In fact, actively relaxing ASM with β_2-adrenoceptor agonists is convenient and generally effective. However, the β_2-adrenoceptor, like most GPCR, is susceptible to tachyphylaxis. Concerns were also raised regarding their profile of adverse effects. In fact, they are proscribed in monotherapy for the treatment of asthma.

The latest breakthroughs that we have described in this article may open new avenues for the development of improved bronchodilators. As highlighted, the contraction elicited by extracellular molecules relies on common intracellular pathways that signal back towards the cell surface to reinforce the link between the cell's contractile apparatus and the surrounding environment. Accordingly, mounting evidences now suggest that molecular players outside the cells are essential for proper mechanotransduction and, consequently, contraction. This means that not only common downstream targets of contraction exist, but that the drugs needed to block them do not have to enter ASM cells. This newly uncovered inside-out signal thus unveils a new world of possibilities whereby ASM contractility may be regulated by targeting extracellular components. How these possibilities will be exploited to improve treatments that aim to better control the contraction of ASM in asthma will be key.

Acknowledgements

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Canadian Institutes of Health Research (CIHR). YB is supported by a research scholar from FRQS (*Fonds de Recherche du Québec – Santé*).

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