Migration of Airway Smooth Muscle Cells

William T. Gerthoffer

Department of Pharmacology, University of Nevada School of Medicine, Reno, Nevada

Migration of smooth muscle cells is a process fundamental to development of hollow organs, including blood vessels and the airways. Migration is also thought to be part of the response to tissue injury. It has also been suggested to contribute to airways remodeling triggered by chronic inflammation. In both nonmuscle and smooth muscle cells numerous external signaling molecules and internal signal transduction pathways contribute to cell migration. The review includes evidence for the functional significance of airway smooth muscle migration, a summary of promigratory and antimigratory agents, and summaries of important signaling pathways mediating migration. Important signaling pathways and effector proteins described include small G proteins, phosphatidylinositol 3-kinases (PI3-K), Rho activated protein kinase (ROCK), p21-activated protein kinases (PAK), Src family tyrosine kinases, and mitogen-activated protein kinases (MAPK). These signaling modules control multiple critical effector proteins including actin nucleating, capping and severing proteins, myosin motors, and proteins that remodel microtubules. Actin filament remodeling, focal contact remodeling and propulsive force of molecular motors are all coordinated to move cells along gradients of chemical cues, matrix adhesiveness, or matrix stiffness. Airway smooth muscle cell migration can be modulated *in vitro* by drugs commonly used in pulmonary medicine including β -adrenergic agonists and corticosteroids. Future studies of airway smooth muscle cell migration may uncover novel targets for drugs aimed at modifying airway remodeling.

Keywords: asthma; actin; cytokines; cytoskeleton; signal transduction

Migration of smooth muscle cells occurs during tube formation of hollow organs including blood vessels, the airways and the gastrointestinal system. During lung development migration and differentiation of airway smooth muscle (ASM), precursor cells may be orchestrated by autocrine and paracrine factors that promote maturation of airway wall (1). Addition of ASM cells to the developing airway wall may be a result of both proliferation and cell migration. In the mature lung, autocrine and paracrine production of inflammatory mediators triggers airway wall remodeling involving both smooth muscle hypertrophy and hyperplasia. Increased cell number in muscle bundles may be due to immigration of smooth muscle precursor cells from beyond the muscle or migration of proliferating cells within the muscle bundles. One structural study of biopsies from asthmatic lungs suggests lung myofibroblasts migrate in response to allergen challenge (2). Myofibroblasts from the lamina propria are proposed to differentiate to smooth muscle-like cells based on increased expression of smooth muscle-restricted contractile proteins. Another source of muscle cells may be circulating CD34+ progenitor cells (fibrocytes) that are thought to migrate into airway smooth muscle bundles from the circulation (3). The latter observation is consistent with recent views of atherogenesis, being due in part

to migration of CD34+ progenitors into the tunica media of arteries (4). Migration of myofibroblasts and/or smooth muscle progenitor cells is hypothesized to be part of a response of mesenchymal cells in the lung to injury perpetrated by inflammation (5).

A central question for studies of airway smooth muscle cell migration is whether tube formation during development, wall thickening, and epithelial-mesenchymal transformation all require smooth muscle cell migration. An argument can be made for cell migration during tube formation based on analogous events in vascular development. Fundamental cellular events are highly conserved during tube formation from *Drosophila* to humans (6), but there are few studies directly addressing the phenomenon of ASM migration *in vivo* during lung development or airway remodeling. Some interesting questions that need to be tested critically are whether differentiated smooth muscle cells migrate in response to cues such as inflammation or lung injury, and whether this recapitulates events that occurred during development (7). Despite a lack of critical tests of the cell biology of migration *in vivo*, significant recent progress has been made in mechanistic studies in cultured ASM cells. The review will summarize some of the steps known to be important in cell migration in general and for smooth muscle cell migration in particular. I will summarize evidence defining necessary signaling pathways and effector molecules, and assess the effects of important drugs used in pulmonary medicine.

CELLULAR MECHANISMS OF MIGRATION

Cell migration is initiated or enhanced by activation of receptors that trigger remodeling of the cytoskeleton and reordering of subcellular organelles (Figure 1). Relevant receptors include a wide variety of G protein-coupled receptors (GPCR), receptor tyrosine kinases (RTK), and matrix adhesive proteins, particularly integrins. Actin polymerization is a proximal event that extends the leading edge of a motile cell toward the stimulus (Figure 1A). Focal contacts assemble immediately behind the leading edge, thus enhancing attachment of the cell membrane to the extracellular matrix (Figure 1B). Myosin motors associated with actin filaments in the body of the cell generate force that moves the cell forward while the cytoskeleton remodels and focal contacts at the rear of the cell detach. All subcellular structures that are tethered by adaptor proteins and motors move along with the remodeling cytoskeleton. Time-lapsed images of cells in culture show that motile cells can be stimulated to move about randomly in the absence of any chemical gradient (chemokinesis), they may follow chemical concentration gradients (chemotaxis), or they may follow paths of varying matrix adhesiveness (haptotaxis). Many studies of cell migration seek to define the signals and signal transduction pathways promoting migration, as well as the cellular machinery that causes movement. Excellent reviews published by members of the Cell Migration Consortium (www.cellmigration.org) have provided the framework for this review. The reader is also referred to a methods study by Goncharova and coworkers for technical details of ASM cell migration methods (8).

Cytoskeletal Proteins and the Physics of Cell Motility

Table 1 is a summary of promigratory and antimigratory agents that modify airway smooth muscle cell motility *in vitro*. Each

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Correspondence and requests for reprints should be addressed to William T. Gerthoffer, Ph.D., Department of Biochemistry, University of South Alabama, Mobile, AL 36688. E-mail: wgerthoffer@usouthal.edu

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Figure 1. Schematic model illustrating the prominent features of a migrating cell. The leading edge of the cell is represented by the cross-hatched region on the right. *Inset A:* The leading edge is a site of rapid actin polymerization, depolymerization, and filament branching. Actin nucleating proteins (mDia1, mDia2, VASP) promote filament formation at the plus (barbed) end. G-actin monomers are added by the action of profilin. Actin filaments are severed by gelsolin and depolymerized by cofilin. Actin branching is regulated by small G proteins acting on WAVE, WASP, and proteins of the ARP2/3 complex. The stiffness of the actin gel and traction forces on the matrix are controlled in part by myosin II motor

proteins that are regulated by activation of multiple kinases (MLCK, PAK, ROCK) and myosin light chain phosphatase (MLCP). *Inset B:* Within the leading edge are nascent focal contacts (red bars) that form to transiently attach the cell to the matrix. Focal contact components include integrins, adaptor proteins (talin, vinculin, tensin, paxillin), regulatory proteins (Src, CAS, FAK), and proteins controlling myosin II activation (MLCK, PAK, MLCP and ROCK). As the cell migrates, nascent focal contacts mature and move toward the rear of cell. Focal contacts at the rear of the cell (red bars on the left) are disassembled as the cell advances. Disassembly requires the action of multiprotein complexes that depend on microtubules (gray filaments) emanating from the microtubule organizing center (MTOC). Reprinted by permission from Reference 74.

Figure 2. Signaling pathways that regulate actin polymerization and myosin II motors in smooth muscle cell migration. Activation of G protein-coupled receptors (GPCR) and receptor tyrosine kinases (RTK) initiates activation of parallel signaling cascades that culminate in actin filament remodeling, and changes matrix adhesiveness and regulation of myosin II motors that generate traction force. Immediate post-receptor events include activation of trimeric G proteins, Src family tyrosine kinases, phospholipase C (PLC) and PIP2, PI3-kinases (PI3-K), and increased Ca^{2+} . Multiple small G proteins (RhoA, Rac, Cdc42) and calmodulin (CaM) then activate downstream targets that are shown here in darker shades of red. Some targets are effector proteins that regulate actin polymerization including the formins (mDia1 and mDia2), WAVE and WASP, and the ARP2/3 complex. Other targets include members of the MAP kinase family (p38 MAPK and ERK), Rho kinases (ROCK), and p21-activated protein kinases (PAK). The signaling kinases phosphorylate other protein kinases (MAPKAPK, LIMK) or phosphatases (MLCP) to regulate effector proteins (dark blue ovals) that control actin polymerization and traction forces generated by myosin II. Most of the schematic is organized as sets of parallel linear signaling cascades, which is an oversimplification for the sake of clarity. Pathway convergence and crosstalk are known to occur between the pathways shown. Regulation of MLCK is a good example where

both positive and negative inputs are integrated to determine the level of myosin II regulatory light chain phosphorylation and traction force. Reprinted by permission from Reference 74.

TABLE 1. SUMMARY OF AGENTS THAT MODULATE ASM CELL MIGRATION

PROMIGRATORY AGENTS
Growth Factors and Cytokines
BFGF (80)
CC Chemokine ligand 19 (CCL19) (81)
IL-1 β (31)
IL-8 (82)
Leukotriene E4 (34)
PDGF (31, 64, 83)
TGF β 1 (31)
Extracellular Matrix
Collagens I, III, V (34)
Fibronectin (34)
Laminin (34)
Integrins α 5, α V (34)
Other Promigratory Agents
Cyclodextrin (62)
Lysophosphatidic acid (39)
Thrombin (21)
Urokinase plasminogen activator (29, 71)
ANTIMIGRATORY AGENTS
β -adrenergic Agonists and the PKA pathway
Dibutyl cAMP (39)
Formoterol (29)
Forskolin (80)
Cilomolast (80)
Salmeterol (80)
Theophylline (39)
Immunomodulating Drugs
Fluticasone (80)
Pyrimidine synthesis inhibitor, FK778 (84)
Sirolimus (84)
Protease Inhibitors
4-(2-Aminoethyl) benzenesulfonylfluoride HCl (AEBSF) (21)
Iloprost (21)
Prinomastat (20)
TIMPs 1-4 (21)
Protein Kinase and Phosphatase Inhibitors
LY294002 (34, 65)
PP1 (16)
PD98059 (31)
SB203580 (31, 34)
U-0126 (29, 65)
Vanadate (62)
Y27632 (34, 39)
Other Antimigratory Agents
Pertussis toxin (29)
Prostaglandin E2 (34)
Retinoic acid (65)
SB649146 (SP-1 inverse agonist) (83)

agent activates signal transduction cascades that in other cell system are known to cause remodeling of the cytoskeleton, to activate motor proteins, or to alter adhesiveness of the cell to the extracellular matrix. These critical biochemical processes are summarized schematically in Figure 1. There are many actin-associated proteins that coordinate actin polymerization and depolymerization. Some of the best-defined regulatory proteins are shown in Figure 1A. After receptor activation Ca^{2+} , phosphatidylinositol 4,5 bis phosphate (PIP₂), and small G proteins activate multiple signaling cascades, illustrated in Figure 2. Platelet-derived growth factor (PDGF) is a particularly well-studied example of a promigratory signaling molecule. In vascular smooth muscle (VSM) cells the β isoform of PDGF receptor (PDGFR- β) is coupled via phosphatidylinositol 3-kinases (PI3-K) and phospholipase C γ to changes in myoplasmic calcium, hydrolysis of PIP₂, and activation of mitogen-activated protein kinases (MAPK) (9). These signaling intermediates in concert with small G proteins trigger nucleation of F-actin by actin-related protein

(ARP) 2/3 complex at the minus end and uncapping of plus ends by dissociation of actin-capping proteins (Figure 1A). The formins (mDia 1 and 2) promote extension of new actin filaments acting in concert with profilin. Profilin liberated from membrane phospholipid binding sites in response to increased PIP₂ increases nucleotide exchange on G-actin, which increases actin polymerization. mDia1 is activated by RhoA, and mDia2 is activated by Cdc42. Small G proteins also promote filament branching by activating WASP-family verprolin-homologous protein (WAVE) complex and Wiskott-Aldrich syndrome protein (WASP) respectively. WAVE and WASP proteins in turn activate components of the ARP2/3 complex, thus increasing nucleation and F-actin branching. In addition to nucleation and branching, productive actin remodeling also requires filament severing and actin depolymerization. Gelsolin is a severing protein activated by increased myoplasmic Ca^{2+} concentrations. Gelsolin promotes actin nucleation when PIP₂ releases gelsolin from plus ends of F-actin. During migration, filament growth is balanced by filament depolymerization, which is enhanced at the minus end by cofilin. Cofilin serves to limit the length of filaments and to turnover existing filaments. The processes described above act together to produce sufficient force to extend the leading edge of the cell toward the stimulus (10).

During the initial stages of actin polymerization and lamellipodial extension, nascent focal contacts form between the cell membrane and the extracellular matrix (Figure 1B). These structures provide adhesion of lamellipodia to the substrate for propulsion of the cell. At the trailing edge of the cell, mature focal contacts must be disassembled for the cell to release from the matrix and move toward a chemotactic stimulus. This model of focal contacts is based on extensive studies in nonmuscle cells (11, 12). Some of the components of focal contacts have been described in ASM cells and tissues, including adapter proteins paxillin (13, 14), vinculin (15), and talin (13). Several important regulatory proteins have also been investigated in ASM, including focal adhesion kinase (FAK) (13–15) and Src (16–18). Formation and degradation of focal contacts occurs dynamically during migration, and focal adhesion kinase is a critical regulator of these processes. Multiple signaling kinases associate with focal contacts in nonmuscle and smooth muscle cells, including Src family members, FAK, phosphoinositide-dependent kinase 1 (PDK1), MAPK, and PI3-K. Phosphorylation of focal contact components, including FAK, paxillin, and talin, has been observed during ASM contraction (19, 14), or after mechanical strain of cultured cells (13). Aside from two reports of Src phosphorylation and activation during migration (16, 17), there are few detailed studies of focal contact regulation in migrating ASM cells. Proteolysis of focal contact proteins by metalloproteinases is thought to be necessary for turnover of mature focal contacts at the trailing edge. Several recent studies of migrating ASM cells found that up-regulation of MMPs 1, 2, and 3 correlated with increased migration, and that tissue inhibitors of metalloproteinases (TIMPs) and chemical protease inhibitors reduced migration (20, 21). The important characteristics of nascent focal contacts at the cell's leading edge include rapid assembly, a sufficient duration to transmit traction forces to the matrix, and then trafficking to the center of the cell. Nascent focal contacts then turnover or mature to become more stable focal contacts. Stable focal contacts at the rear of the cell must eventually disassemble for the cell to move forward. One interesting question is what are the spatial and temporal features of proteins in nascent focal contacts in smooth muscle, and are they sensitive to inflammation, mechanical strain, or the differentiation state of airway smooth muscle cells? Presumably there are many features of the focal contacts similar to those of migrating non-muscle cells. However, identifying unique protein components

or signaling processes in airway smooth muscle would be a significant advance that might suggest novel targets for inhibiting airway remodeling.

There are two primary sources of force generation necessary for cell migration: actin polymerization promoting protrusion of the leading edge, and enzymatic activity of myosin motors. Myosin motors produce traction forces transmitted to the extracellular matrix through focal contacts. Smooth muscle myosin II is regulated by Ca^{2+} -calmodulin activation of myosin light chain kinase (MLCK), which phosphorylates the 20-kD regulatory myosin light chains. Ca^{2+} -independent activation of myosin II might also occur, most likely by RhoA activation of Rho-activated protein kinase (ROCK) and direct phosphorylation of myosin regulatory light chains. ROCK phosphorylation of myosin II has been described in nonmuscle cells (22), but there is no direct evidence of this event in airway smooth muscle. Although the basic elements of force-generating mechanisms are known to exist in migrating smooth muscle cells, the details of how myosin motors are regulated during migration and how motors respond to the physical nature of the matrix is poorly defined. Polte and co-workers have shown that decreasing matrix stiffness reduces myosin light chain phosphorylation in cultured vascular smooth muscle cells (23). They also found that inhibiting myosin ATPase with 2,3-butanedione 2-monoxime (BDM) reduced myosin light chain phosphorylation. Conversely, disrupting microtubules with nocodazole increased myosin phosphorylation. One interpretation is that decreased adhesiveness, decreased matrix stiffness, and reduced force from myosin II motors all reduced the prestress on the cytoskeleton. Reduced prestress then inhibited myosin phosphorylation by some undefined biochemical mechanism(s). The results suggest a model for cell migration where myosin II exerts traction force on the matrix, and the matrix in turn modifies phosphorylation and activation of myosin II as a function of matrix stiffness. This begs several interesting questions relevant to airway remodeling. Does inflammation influence migration of smooth muscle cells and myofibroblasts directly through increased contractile tone and thus prestress, and also because the composition and the compliance of the extracellular matrix changes? While there are not direct studies of tone and migration in asthma, work by Parameswaran and colleagues demonstrating promigratory influences of collagen, elastin and laminin on ASM cell migration is consistent with the second hypothesis (16, 24).

Although actin polymerization and focal contact remodeling have garnered much attention in studies of cell migration, it is clear that microtubules are also remodeled. In nonmigrating cells the microtubule organizing center (MTOC) and the nucleus are centered in the cell (Figure 1). During migration the nucleus is relocated toward the trailing edge of the cell in part by Cdc42 regulation of myotonic dystrophy kinase-related Cdc42 binding kinase (MRCK) (25). Nuclear relocation was found to depend on phosphorylation myosin II by MRCK. The necessity for microtubular dynamic instability is suggested by paclitaxel (Taxol) inhibition of VSM cell migration (26). Paclitaxel stabilizes microtubules and prevents or reduces dynamic instability. Microtubules are thought to be important factors in disassembly of stable focal contacts at the rear of migrating cells, which is necessary for disengagement of the trailing edge from the matrix (27). There are no studies in ASM directly testing the relevance of microtubular dynamic instability, but there is indirect evidence of a plausible signaling pathway that might promote dynamic instability necessary for cell polarization. Urokinase stimulates both VSM and ASM cell migration (28, 29). Urokinase induces VSM migration via a pathway including PI3-K γ , Akt, and glycogen synthase kinase 3 β (GSK3 β) (28). GSK3 β has been linked to microtubule function by interacting with the tumor suppressor

adenomatous polyposis coli (APC), which can interact with the plus end of microtubules and regulate cell polarity (30). Further work on microtubular dynamic instability and its role in ASM cell polarity is required to test this hypothesis.

SIGNALS AND SIGNALING CASCADES

Soluble and Solid State Signals

Defining the environmental cues that promote or inhibit motility is a common goal of cell migration studies. There are many promigratory and antimigratory molecules belonging to diverse chemical and functional families including small biogenic amines, peptide growth factors, cytokines, and extracellular matrix components. PDGF was one of the first peptide growth factors used to elicit airway smooth muscle cell migration (31). In addition to soluble promigratory signals like PDGF, the composition and physical nature of the matrix are likely to exert strong influences on cell migration. An early study by Zhang and colleagues identified the laminin β 1 chain as necessary for migration of smooth muscle cells from mouse lung explants (32). Later studies have shown promigratory effects of collagens, fibronectin, and laminin (16); promigratory effects of matrix metalloproteinase activation; and antimigratory effects of TIMPs and chemical protease inhibitors (20, 21). There is evidence matrix composition is altered in individuals with asthma and in cultured cells exposed to proinflammatory agents (33, 24). However, the question of whether matrix composition alters migration *in vivo* has not been addressed directly. *In vitro* migration data are consistent with the notion that ASM cell migration will depend on the combined effects of soluble and matrix-bound signals. These signals probably act primarily from the outside, but may also influence integrin activation from the inside, which alters cell adhesiveness to the matrix.

Table 1 is a summary of soluble and matrix-associated signals that enhance or inhibit ASM cell migration *in vitro*. Most of the promigratory compounds are autocrine or paracrine signaling molecules, several of which are produced in the inflamed airways. The effect on cell motility in most cases is chemotactic, but in other studies synergistic chemokinetic effects have been described (34). Given the very large number of molecules known to mediate cell-cell signaling in the lungs and the recent growing interest in ASM cell migration, this list is likely to grow substantially in the near future. Although the promigratory agents are effective *in vitro*, it is not known if they all have important effects *in vivo*. The necessity of particular signaling molecules or signaling pathways can be tested in knockout and transgenic mouse models. This strategy was used to establish the necessity of PDGF and PDGF receptors in pericyte cell migration during blood vessel development (35).

Signaling Cascades

Figure 2 illustrates some of the most commonly studied pathways mediating cell migration in both smooth muscle and non-muscle cells. It illustrates a central role of pathways modifying the actin cytoskeleton and myosin II motor proteins. The levels of signal transduction are arranged as cascades originating with activation of receptors. Both RTK and GPCR are known to promote cell migration in many cell types. Immediately downstream of receptors there are several critical signaling components including small G proteins (RhoA, Rac, Cdc42), and trimeric G-proteins. Activated G proteins, Ca^{2+} , and phospholipids such as PIP2 activate lipid kinases, Ca^{2+} -dependent protein kinases, ROCK, and MAPK (Figure 2). The protein kinases phosphorylate a variety of substrates, some of which are other protein kinases (e.g., MAPKAP kinase, LIM kinase) or proteins that

regulate the ultimate effectors (e.g., WAVE, WASP). The effector proteins in this scheme ultimately regulate two major functions: actin polymerization and activation of motor proteins. Agents that increase the frequency or amplitude of myoplasmic Ca^{2+} oscillations or increase mean Ca^{2+} concentration will activate MLCK to phosphorylate myosin II, which generates traction forces that move the cell.

Small G Proteins and ROCK

The small G proteins (Ras, Rho, Rac, Cdc42) are very early elements in signaling pathways that promote cell migration (Figure 2). Both RTKs and GPCRs activate several small G proteins via regulation of guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). There are a few studies of GEFs in VSM cell migration (36, 37). However, the GEFs and GAPs that regulate small G protein function in ASM cell migration are undefined. In general, activation of small G proteins activates downstream protein kinases that phosphorylate effector proteins regulating actin, microtubule, or intermediate filament function. Rho A activates ROCK in ASM tissues (38), and the Rho kinase inhibitor Y-27632 blocks ASM cell migration (34, 39). ROCK phosphorylates the myosin-binding subunit of myosin light chain phosphatase (MLCP), thus reducing phosphatase activity (40). Rho-ROCK regulation of MLCP is a major mechanism of agonist-induced calcium sensitization of smooth muscle contraction that probably also regulates cell migration. ROCK can also directly phosphorylate myosin light chains *in vitro* at Ser19, and this has been proposed as a contributor to calcium-independent contraction of smooth muscles (41). In cultured 3T3 fibroblasts, ROCK directly phosphorylates myosin II in the center of the cells, but not myosin II near the cortex (22). Myosin II phosphorylation might be regulated somewhat differently in stationary, nonproliferating contractile cells in a smooth muscle bundle compared with isolated, proliferating, migratory cells. It seems possible that ROCK might replace or act together with MLCK when phosphorylating myosin II in a migrating cell. ROCK also regulates actin polymerization by activation of LIM kinase, which phosphorylates cofilin. Activation of LIM kinase will reduce cofilin-mediated actin depolymerization, thus favoring increased F-actin. There are dynamic changes in LIM kinase levels and cofilin expression in vascular smooth muscle during arteriogenesis and experimental inflammation (42, 43). However, the significance of a ROCK/LIMK/cofilin signaling cascade in ASM cell migration has not been addressed.

Rac family members (Rac1 and Rac2) are small G proteins known to mediate cell migration in nonmuscle and smooth muscle cells. Rac family members are known to be activated in proliferating ASM smooth muscles (44). In VSM cells Rac1 and Rac2 mediate PDGF-stimulated migration (45). Rac2 is induced by cytokines in VSM, interacts with a cytokine-inducible scaffold protein called AIF-1, and overexpression of Rac2 is sufficient to increase cell migration (46). Rac1 appears to be negatively regulated by the Slit2 axon guidance protein in VSM cells (47). This suggests the hypothesis that Slit guidance proteins contribute to tube formation in smooth muscle organs in some manner similar to axon guidance in the developing nervous system. Whether this is important in the developing airways, and whether migration of ASM smooth muscle cells or smooth muscle progenitors depend on Rac family activation is unknown. It is also not clear what effector proteins are downstream of Rac or Cdc42 in ASM. In VSM and nonmuscle cells, Rac1 and Rac2 both activate p21-activated protein kinases (PAKs) and WAVE. WAVE regulates the ARP2/3 complex to promote actin polymerization (Figures 1 and 2). The roles of Rac- and Cdc42-regulated signaling systems remain to be carefully defined in

ASM migration, but likely downstream effectors include the p21-activated protein kinases.

PAKs

PAKs are serine/threonine protein kinases activated by small G proteins that phosphorylate targets known to regulate cell migration. Several PAK isoforms (PAKs 1, 2, and 3) are expressed in ASM, and overexpression of a kinase-inactive mutant PAK1 has profound inhibitory effects on both random cell movement and PDGF-stimulated chemotaxis of human ASM cells (48). Targets of PAK that regulate cell motility include other protein kinases, intermediate filaments, actin-binding proteins, and myosin II. LIM kinase is a well-known substrate of PAK that phosphorylates cofilin (49). Unphosphorylated cofilin binds actin and promotes actin depolymerization. Phosphorylation of cofilin disrupts binding of cofilin and actin, allowing actin polymerization. PAK directly phosphorylates two other actin binding proteins: cortactin and caldesmon. Actin branching may be regulated by phosphorylation of cortactin in smooth muscle cells (50). PAK phosphorylation of h-caldesmon activates smooth muscle actomyosin, thus increasing muscle contraction (51, 52). However, in ASM cells in culture l-caldesmon is the predominant caldesmon isoform expressed. PAK phosphorylation of l-caldesmon is necessary for actin remodeling and migration of Chinese Hamster Ovary cells (53), but it is not clear if PAK also phosphorylates l-caldesmon in migrating ASM cells. In addition to modifying actin-binding proteins, PAK may regulate activation of actomyosin indirectly or directly. PAK phosphorylation of MLCK inhibits myosin phosphorylation (54), which would be expected to reduce actomyosin activation and cell migration. In contrast, direct phosphorylation of myosin II by PAK promotes actin remodeling and contraction of endothelial cells (55). Therefore, one might predict that PAKs would activate actomyosin and perhaps promote ASM cell migration. It is not clear if opposing effects of PAK on smooth muscle actomyosin occur simultaneously, or if one mechanism predominates. Inhibition of ASM migration by kinase-inactive PAK1 is more consistent with PAK inhibiting myosin motor function, reducing actin polymerization and blocking the effects of HSP27 (48). It is possible that PAK exerts different effects depending on the phenotype of the cell (contractile versus proliferating and migratory), and the nature of the stimulus.

In addition to important effects on the actin cytoskeleton and myosin motor proteins, PAK might also modify microtubule and intermediate filament structure during smooth muscle cell migration. Rac1 activation of PAK might stabilize microtubules by inhibiting Op18, a microtubule destabilizing factor (56). Intermediate filament stability may be reduced by PAK phosphorylation of vimentin (57, 58). Although kinase-inactive PAK inhibits ASM smooth muscle cell migration, it is not clear which of the various biochemical targets of PAK are necessary for ASM cell migration. Nor is it clear how PAK enzyme activity and chaperone functions are controlled in space and time to promote coordinated movement. There is little known of the various adapters and regulators of PAK known to contribute to spatial and temporal control in nonmuscle cells (e.g., Nck, PIX, and paxillin kinase linker).

Src Family Tyrosine Kinases

Src family nonreceptor tyrosine kinases and PI3-Kinases are upstream signal transduction elements activated by RTK as well as by GPCR (Figure 2). Airway smooth muscle cells express multiple Src family members including Src, Fyn, Lyn, and Yes (59). Src family members are implicated in ASM migration by reports of phosphorylation of Src during migration, inhibition of

migration by PP1 and inhibition of migration by kinase-inactive Src (16, 17, 60). In pulmonary artery smooth muscle cells Src activity is not necessary for cell attachment, but cell spreading and chemotaxis both depend on Src activity and PI-3 kinase activity (61). A role for Src and FAK in urokinase-induced migration is suggested by the effect of vanadate (a phosphatase inhibitor) to enhance phosphorylation of focal contact proteins (62). Carlin and coworkers suggested activation of uPAR receptors leads to activation of phosphatases that modify FAK and focal contact proteins resulting in turnover of focal contacts and increased cell motility. Other pathways downstream of Src include the extracellular signal-regulated kinase (ERK) MAPK signaling cascade and its downstream targets (described below). Establishing the substrates for Src family tyrosine kinases is complicated by redundancy in Src signaling. There are multiple Src family members expressed in smooth muscles with similar target phosphorylation sites and probably overlapping sets of substrates. Using only chemical inhibitors to identify Src family functions is prone to error because chemical Src inhibitors are not highly selective for nonreceptor tyrosine kinase family members. In other cell signaling systems cells from single and multiple Src family knockout mice have been used to address this problem (63). Mammalian expression vectors can also be used in rescue strategies in cell cultures from knockout animals to allow critical hypothesis testing.

PI3-K

PI3-K, like Src family tyrosine kinases, are early signaling components in pathways controlling multiple processes in smooth muscles. ASM proliferation, contraction, and cell motility are all mediated in part by PI3-K activation. Downstream targets include Akt, S6 kinase, ERK, and p38 MAPK cascades. The PI3-K inhibitor LY-294002 reduces migration of ASM cells, and overexpression of active PI3-K enhances migration (64). Compatible results were obtained in a study in which retinoic acid inhibited PDGF-induced ASM cell migration and inhibited PI3-K signaling (65). There are currently few details of what aspects of cell attachment, spreading or migration are affected in ASM by PI3-K. Regulation of lamellipodia formation is a likely downstream event that would be consistent with the obligatory role of PI3-K in VSM cell spreading (61, 66).

MAP Kinases

Several mechanistic studies demonstrate the necessity of MAPKs or chemotaxis of ASM cells. Both chemical protein kinase inhibitors and expression of mutant protein kinases have been used to address the role of ERK and p38 MAPKs in ASM migration. The small molecule inhibitors are quite effective in cell cultures and reasonably selective for the target kinases (67). There are also effective molecular tools used to express kinase-inactive or constitutively active kinases. Evidence for ERKs regulating ASM migration is limited to antagonism of PDGF-induced migration by MEK1 inhibitors such as PD98059 (31) or U0126 (65). Despite extensive use of chemical and molecular tools in correlative studies of smooth muscle cell migration, it is not entirely clear how ERK activation promotes smooth muscle cell motility. ERKs might act directly in the short term by phosphorylating targets that enhance remodeling of the actin cytoskeleton including h-caldesmon, l-caldesmon, focal adhesion kinase, and calpain. Phosphorylation of l-caldesmon at MAPK consensus sites is necessary for stress fiber remodeling during fibroblast cell spreading and migration (68). L-caldesmon is phosphorylated at the same MAPK consensus sites during VSM migration (69). ERKs might also enhance myosin II activity by phosphorylating and activating MLCK (70). Although

these are all plausible mechanisms, there is relatively little data in ASM on how ERKs might promote cell migration.

In contrast to ERK MAPKs, there is some clear mechanistic insight into how the p38 MAPK cascade regulates ASM cell migration. One of the earliest studies of ASM cell migration established the necessity of a signaling pathway composed of MAP kinase kinase 6b (MKK6), p38 MAPKs, MAP kinase activated protein kinase2 (MAPKAPK), and HSP27 for migration of human ASM cells (31). This pathway regulates migration of several cell types including endothelial cells, macrophages, and breast cancer cells. In addition to regulating HSP27 and actin remodeling, there is evidence that l-caldesmon might be a substrate for p38 MAPKs in migrating ASM cells (71). Phosphorylation of l-caldesmon by ERK, p38 MAPK or cyclin-dependent protein kinases might favor disassembly and turnover of stress fibers and activation of myosin II and therefore promote cell migration.

p38MAPKs are activated in ASM by a variety of stimuli, some of which are promigratory, but others are not (e.g., sodium arsenite). This suggests that p38 MAPK activation is necessary but clearly not the sole pathway activated during migration. As shown in Figure 2, there are many parallel, redundant signaling pathways regulating cell migration. Furthermore, both p38 MAPK and ERK MAPK pathways control expression of genes coding for proteins that directly or indirectly influence cell migration. For example, expression of matrix proteins and matrix metalloproteinases that influence cell migration depends in part on MAPK activity (72). Therefore the role of MAPKs in ASM migration and airway wall remodeling might be a function of effects on actin remodeling as well as transcription of genes and translation of proteins regulated by MAPKs in ASM (73).

MODULATION OF ASM CELL MIGRATION BY DRUGS

If ASM cell migration is a contributing factor to lung pathology then targeting mechanisms that regulate migration is a valid therapeutic option. Defining novel drug targets is frequently cited as an important motivation for investigating basic mechanisms of smooth muscle cell migration. This is certainly true in the literature on VSM cell migration, and there is some reason for optimism. Several important drugs used in cardiovascular medicine reduce atherogenesis and ameliorate the sequelae of vascular injury by inhibiting VSM proliferation and cell migration (74). The statins and drugs incorporated into vascular stents (rapamycin and taxol) are good examples of agents that inhibit proliferation, reduce cell migration, and effectively inhibit vascular wall thickening (75, 26). Inhibiting wall thickening is an emerging role of statins for prophylaxis against atherosclerosis. The hypothesis is that long-term low-dose therapy with statins will reduce pathologic vascular wall remodeling, in part by inhibiting VSM migration. The primary therapeutic target is inhibition of cholesterol synthesis, but some benefits may result from inhibiting mevalonate synthesis and isoprenylation reactions that activate small G proteins. Simvastatin also inhibits ASM cell proliferation, possibly by the same mechanism (76). The small G proteins all participate at multiple steps in cell migration (see Figures 1 and 2). Whether such an effect occurs in the airways is an interesting question given the extensive clinical experience with chronic statin therapy in cardiovascular medicine.

Important drugs used in pulmonary medicine also have profound antimigratory effects (Table 1). β -agonists and other drugs acting via cAMP are antimigratory, as are immunomodulating drugs including glucocorticoids. This raises a number of interesting questions about current asthma therapeutics. In addition to current thinking that corticosteroids and long-acting β -agonists limit matrix synthesis and ASM proliferation, do they

act in part by chronically inhibiting smooth muscle or myofibroblast cell migration? The *in vitro* biochemical data and studies of cell migration summarized in Table 1 are consistent with the hypothesis, but *in vivo* data critically testing this notion are lacking.

Another question arising from the survey of drugs acting on cell migration in Table 1 is: What are plausible future targets for future drug development? MAPKs, Src family tyrosine kinases, PI-3 kinases, and Rho kinases all emerge as potential targets. There are also some very important regulatory proteins not yet established in ASM cell migration, including PTEN; integrin-linked kinase; the WAVE, WASP, ARP 2/3 proteins; and several known regulators of actin filament remodeling. Of the established signaling pathways in Figure 2, MAPKs have been targeted for development of drugs to treat asthma because expression of numerous contractile, proinflammatory, and promigratory signaling proteins depend to varying degrees on MAPK signaling (77). Inhibiting MAPKs inhibits expression of extracellular signals that might trigger cell migration (e.g., PDGF, IL1 β , and IL8). MAPK inhibitors might prove to be beneficial by directly inhibiting cell migration and proliferation, and indirectly by inhibiting proinflammatory gene expression. There is some evidence in animal models of asthma consistent with this view (77), but studies in humans are needed to critically test this strategy.

Rho kinases (ROCK1 and ROCK2) are also interesting targets because Rho kinase inhibitors block VSM and ASM cell migration (34, 39). The Rho kinase inhibitor Fasudil has been used to antagonize cerebral vasospasm and to treat angina pectoris because it antagonizes smooth muscle contraction. Rho kinase inhibitors are effective in reducing airway hyperreactivity in mouse models of asthma (78, 79), but there is no direct evidence yet of clinical benefit in humans. Airway hyperreactivity should be antagonized smooth muscle relaxants and remodeling should in principle be reduced by inhibiting proliferation—both are established roles of Rho kinase in ASM. The significance of inhibiting Rho kinases in cell migration *in vivo* and airway wall remodeling is not yet established. However, Rho kinase inhibitors and other novel protein kinase inhibitors may eventually prove to be beneficial new aspects of asthma therapy. Off-target effects of protein kinase inhibitors are major limitations of these types of drugs, but local delivery of modestly selective inhibitors to the lungs may address this problem somewhat.

CONCLUSIONS

Recent studies of ASM cell migration have defined many important promigratory and antimigratory substances (Table 1). Some mechanistic detail is available suggesting that these agents act on well-established signaling pathways and effector proteins defined in studies on nonmuscle cell motility (Figures 1 and 2). There are, however, important well-known regulators of actin filament remodeling that have not been studied in ASM cell models. There are also few studies identifying novel features of ASM cell migration that might suggest unique targets for new drug development. Further mechanistic studies of cell migration have the potential for identifying such novel targets. While it is likely that cell migration plays an important role in airway development, there is not solid proof that cell migration contributes to airway wall remodeling in asthma. Exactly how hyperplasia of ASM occurs in asthma is unclear. Based on parallels with VSM cell migration and atherosclerosis, ASM cell migration might contribute to hyperplasia. However, it is debatable how much of the increase in muscle mass is due to proliferation and how much is immigration of myofibroblasts, neighboring smooth muscle cells in the bundle, or CD34⁺ cells from the circulation. Defining the relative contributions of re-

sident smooth muscle cells versus immigrating progenitor cells to smooth muscle hyperplasia is an important problem in lung cell biology that is highly relevant to developing new therapeutic approaches to asthma. The increasing use of cell migration as a functional endpoint in lung cell biology indicates many labs are interested in addressing this problem.

Conflict of Interest Statement: W.T.G. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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References

- Shannon JM, Hyatt BA. Epithelial-mesenchymal interactions in the developing lung. *Annu Rev Physiol* 2004;66:625–645.
- Gizycki MJ, Adelroth E, Rogers AV, O'Byrne PM, Jeffery PK. Myofibroblast involvement in the allergen-induced late response in mild atopic asthma. *Am J Respir Cell Mol Biol* 1997;16:664–673.
- Schmidt M, Sun G, Stacey MA, Mori L, Mattoli S. Identification of circulating fibrocytes as precursors of bronchial myofibroblasts in asthma. *J Immunol* 2003;171:380–389.
- Yeh ET, Zhang S, Wu HD, Korbling M, Willerson JT, Estrov Z. Transdifferentiation of human peripheral blood CD34⁺-enriched cell population into cardiomyocytes, endothelial cells, and smooth muscle cells in vivo. *Circulation* 2003;108:2070–2073.
- Holgate ST, Holloway J, Wilson S, Bucchieri F, Puddicombe S, Davies DE. Epithelial-mesenchymal communication in the pathogenesis of chronic asthma. *Proc Am Thorac Soc* 2004;1:93–98.
- Uv A, Cantera R, Samakovlis C. *Drosophila* tracheal morphogenesis: intricate cellular solutions to basic plumbing problems. *Trends Cell Biol* 2003;13:301–309.
- Warburton D, Tefft D, Mailleux A, Bellusci S, Thiery JP, Zhao J, Buckley S, Shi W, Driscoll B. Do lung remodeling, repair, and regeneration recapitulate respiratory ontogeny? *Am J Respir Crit Care Med* 2001;164:S59–S62.
- Goncharova EA, Goncharov DA, Krymskaya VP. Assays for in vitro monitoring of human airway smooth muscle (ASM) and human pulmonary arterial vascular smooth muscle (VSM) cell migration. *Nat Protocols* 2006;1:2933–2939.
- Bornfeldt KE, Raines EW, Skinner MP, Krebs EG, Ross R. Platelet-derived growth factor: distinct signal transduction pathways associated with migration versus proliferation. *Ann N Y Acad Sci* 1995;766:416–430.
- Prass M, Jacobson K, Mogilner A, Radmacher M. Direct measurement of the lamellipodial protrusive force in a migrating cell. *J Cell Biol* 2006;174:767–772.
- Zaidel-Bar R, Cohen M, Addadi L, Geiger B. Hierarchical assembly of cell-matrix adhesion complexes. *Biochem Soc Trans* 2004;32:416–420.
- Vicente-Manzanares M, Webb DJ, Horwitz AR. Cell migration at a glance. *J Cell Sci* 2005;118:4917–4919.
- Smith PG, Garcia R, Kogerman L. Mechanical strain increases protein tyrosine phosphorylation in airway smooth muscle cells. *Exp Cell Res* 1998;239:353–360.
- Tang D, Mehta D, Gunst SJ. Mechanosensitive tyrosine phosphorylation of paxillin and focal adhesion kinase in tracheal smooth muscle. *Am J Physiol* 1999;276:C250–C258.
- Opazo SA, Zhang W, Wu Y, Turner CE, Tang DD, Gunst SJ. Tension development during contractile stimulation of smooth muscle requires recruitment of paxillin and vinculin to the membrane. *Am J Physiol Cell Physiol* 2004;286:C433–C447.
- Parameswaran K, Radford K, Zuo J, Janssen LJ, O'Byrne PM, Cox PG. Extracellular matrix regulates human airway smooth muscle cell migration. *Eur Respir J* 2004;24:545–551.
- Krymskaya VP, Goncharova EA, Ammit AJ, Lim PN, Goncharov DA, Eszterhas A, Panettieri RA Jr. Src is necessary and sufficient for human airway smooth muscle cell proliferation and migration. *FASEB J* 2005;19:428–430.
- Hirshman CA, Zhu D, Pertel T, Panettieri RA, Emala CW. Isoproterenol induces actin depolymerization in human airway smooth muscle cells via activation of an Src kinase and GS. *Am J Physiol Lung Cell Mol Physiol* 2005;288:L924–L931.
- Pavalko FM, Adam LP, Wu MF, Walker TL, Gunst SJ. Phosphorylation of dense-plaque proteins talin and paxillin during tracheal smooth muscle contraction. *Am J Physiol* 1995;268:C563–C571.

20. Hasaneen NA, Zucker S, Cao J, Chiarelli C, Panettieri RA, Foda HD. Cyclic mechanical strain-induced proliferation and migration of human airway smooth muscle cells: role of EMMPRIN and MMPs. *FASEB J* 2005;19:1507-1509.
21. Henderson N, Markwick LJ, Elshaw SR, Freyer AM, Knox AJ, Johnson SR. Collagen I and thrombin activate MMP-2 by MMP-14 dependent and independent pathways: implications for airway smooth muscle migration. *Am J Physiol Lung Cell Mol Physiol* 2006;292:L1030-1038.
22. Totsukawa G, Yamakita Y, Yamashiro S, Hartshorne DJ, Sasaki Y, Matsumura F. Distinct roles of ROCK (Rho-Kinase) and MLCK in spatial regulation of MLC phosphorylation for assembly of stress fibers and focal adhesions in 3T3 fibroblasts. *J Cell Biol* 2000;150:797-806.
23. Polte TR, Eichler GS, Wang N, Ingber DE. Extracellular matrix controls myosin light chain phosphorylation and cell contractility through modulation of cell shape and cytoskeletal prestress. *Am J Physiol Cell Physiol* 2004;286:C518-C528.
24. Parameswaran K, Willems-Widyastuti A, Alagappan VK, Radford K, Kranenburg AR, Sharma HS. Role of extracellular matrix and its regulators in human airway smooth muscle biology. *Cell Biochem Biophys* 2006;44:139-146.
25. Gomes ER, Jani S, Gundersen GG. Nuclear movement regulated by Cdc42, MRCK, myosin, and actin flow establishes MTOC polarization in migrating cells. *Cell* 2005;121:451-463.
26. Sollott SJ, Cheng L, Pauly RR, Jenkins GM, Monticone RE, Kuzuya M, Froehlich JP, Crow MT, Lakatta EG, Rowinsky EK, et al. Taxol inhibits neointimal smooth muscle cell accumulation after angioplasty in the rat. *J Clin Invest* 1995;95:1869-1876.
27. Kaverina I, Krylyshkina O, Small JV. Microtubule targeting of substrate contacts promotes their relaxation and dissociation. *J Cell Biol* 1999;146:1033-1044.
28. Galaria II, Nicholl SM, Roztocil E, Davies MG. Urokinase-induced smooth muscle cell migration requires PI3-K and Akt activation. *J Surg Res* 2005;127:46-52.
29. Carlin SM, Roth M, Black JL. Urokinase potentiates PDGF-induced chemotaxis of human airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2003;284:L1020-L1026.
30. Etienne-Manneville S, Hall A. Cdc42 regulates GSK-3 β and adenomatous polyposis coli to control cell polarity. *Nature* 2003;421:753-756.
31. Hedges JC, Dechert MA, Yamboliev IA, Martin JL, Hickey E, Weber LA, Gerthoffer WT. A role for P38(MAPK)/HSP27 pathway in smooth muscle cell migration. *J Biol Chem* 1999;274:24211-24219.
32. Zhang J, O'Shea S, Liu J, Schuger L. Bronchial smooth muscle hypoplasia in mouse embryonic lungs exposed to a laminin beta1 chain antisense oligonucleotide. *Mech Dev* 1999;89:15-23.
33. Roberts CR, Walker DC, Schellenberg RR. Extracellular matrix. *Clin Allergy Immunol* 2002;16:143-178.
34. Parameswaran K, Cox G, Radford K, Janssen LJ, Sehmi R, O'Byrne PM. Cysteinyl leukotrienes promote human airway smooth muscle migration. *Am J Respir Crit Care Med* 2002;166:738-742.
35. Betsholtz C, Lindblom P, Gerhardt H. Role of pericytes in vascular morphogenesis. *EXS* 2005;94:115-125.
36. Sakata Y, Xiang F, Chen Z, Kiriyama Y, Kamei CN, Simon DI, Chin MT. Transcription factor CHF1/Hey2 regulates neointimal formation in vivo and vascular smooth muscle proliferation and migration in vitro. *Arterioscler Thromb Vasc Biol* 2004;24:2069-2074.
37. Ohtsu H, Mifune M, Frank GD, Saito S, Inagami T, Kim-Mitsuyama S, Takuwa Y, Sasaki T, Rothstein JD, Suzuki H, et al. Signal-crosstalk between Rho/ROCK and c-Jun NH₂-terminal kinase mediates migration of vascular smooth muscle cells stimulated by angiotensin II. *Arterioscler Thromb Vasc Biol* 2005;25:1831-1836.
38. Liu C, Zuo J, Janssen LJ. Regulation of airway smooth muscle RhoA/ROCK activities by cholinergic and bronchodilator stimuli. *Eur Respir J* 2006;28:703-711.
39. Hirakawa M, Karashima Y, Watanabe M, Kimura C, Ito Y, Oike M. Protein kinase A inhibits lysophosphatidic acid-induced migration of airway smooth muscle cells. *J Pharmacol Exp Ther* 2007;322:1102-1108.
40. Fukata Y, Amano M, Kaibuchi K. Rho-Rho-kinase pathway in smooth muscle contraction and cytoskeletal reorganization of non-muscle cells. *Trends Pharmacol Sci* 2001;22:32-39.
41. Van Eyk JE, Arrell DK, Foster DB, Strauss JD, Heinonen TY, Furmaniak-Kazmierczak E, Cote GP, Mak AS. Different molecular mechanisms for Rho family GTPase-dependent, Ca²⁺-independent contraction of smooth muscle. *J Biol Chem* 1998;273:23433-23439.
42. Boengler K, Pipp F, Broich K, Fernandez B, Schaper W, Deindl E. Identification of differentially expressed genes like cofilin2 in growing collateral arteries. *Biochem Biophys Res Commun* 2003;300:751-756.
43. Dai YP, Bongalon S, Tian H, Parks SD, Mutafova-Yambolieva VN, Yamboliev IA. Upregulation of profilin, cofilin-2 and LIMK2 in cultured pulmonary artery smooth muscle cells and in pulmonary arteries of monocrotaline-treated rats. *Vascul Pharmacol* 2006;44:275-282.
44. Page K, Li J, Hodge JA, Liu PT, Vanden Hoek TL, Becker LB, Pestell RG, Rosner MR, Hershenov MB. Characterization of a Rac1 signaling pathway to cyclin D(1) expression in airway smooth muscle cells. *J Biol Chem* 1999;274:22065-22071.
45. Doanes AM, Irani K, Goldschmidt-Clermont PJ, Finkel T. A requirement for Rac1 in the PDGF-stimulated migration of fibroblasts and vascular smooth cells. *Biochem Mol Biol Int* 1998;45:279-287.
46. Tien Y, Autieri MV. Cytokine expression and AIF-1-mediated activation of Rac2 in vascular smooth muscle cells: a role for Rac2 in VSMC activation. *Am J Physiol Cell Physiol* 2006;292:C841-C849.
47. Liu D, Hou J, Hu X, Wang X, Xiao Y, Mou Y, De LH. Neuronal chemorepellent Slit2 inhibits vascular smooth muscle cell migration by suppressing small GTPase Rac1 activation. *Circ Res* 2006;98:480-489.
48. Dechert MA, Holder JM, Gerthoffer WT. P21-activated kinase 1 participates in tracheal smooth muscle cell migration by signaling to P38 Mapk. *Am J Physiol Cell Physiol* 2001;281:C123-C132.
49. Edwards DC, Sanders LC, Bokoch GM, Gill GN. Activation of LIM-kinase by Pak1 couples Rac/Cdc42 GTPase signalling to actin cytoskeletal dynamics. *Nat Cell Biol* 1999;1:253-259.
50. Webb BA, Zhou S, Eves R, Shen L, Jia L, Mak AS. Phosphorylation of cortactin by p21-activated kinase. *Arch Biochem Biophys* 2006;456:183-193.
51. Foster DB, Shen LH, Kelly J, Thibault P, Van Eyk JE, Mak AS. Phosphorylation of caldesmon by p21-activated kinase: implications for the Ca(2+) sensitivity of smooth muscle contraction. *J Biol Chem* 2000;275:1959-1965.
52. McFawn PK, Shen L, Vincent SG, Mak A, Van Eyk JE, Fisher JT. Calcium-independent contraction and sensitization of airway smooth muscle by p21-activated protein kinase. *Am J Physiol Lung Cell Mol Physiol* 2003;284:L863-L870.
53. Eppinga RD, Li Y, Lin JL, Mak AS, Lin JJ. Requirement of reversible caldesmon phosphorylation at p21-activated kinase-responsive sites for lamellipodia extensions during cell migration. *Cell Motil Cytoskeleton* 2006;63:543-562.
54. Wirth A, Schroeter M, Kock-Hauser C, Manser E, Chalovich JM, De Lanerolle P, Pfitzer G. Inhibition of contraction and myosin light chain phosphorylation in guinea-pig smooth muscle by p21-activated kinase 1. *J Physiol* 2003;549:489-500.
55. Chew TL, Masaracchia RA, Goeckeler ZM, Wysolmerski RB. Phosphorylation of non-muscle myosin II regulatory light chain by p21-activated kinase (gamma-PAK). *J Muscle Res Cell Motil* 1998;19:839-854.
56. Wittmann T, Bokoch GM, Waterman-Storer CM. Regulation of microtubule destabilizing activity of Op18/stathmin downstream of Rac1. *J Biol Chem* 2004;279:6196-6203.
57. Ohtakara K, Inada H, Goto H, Taki W, Manser E, Lim L, Izawa I, Inagaki M. p21-activated kinase PAK phosphorylates desmin at sites different from those for Rho-associated kinase. *Biochem Biophys Res Commun* 2000;272:712-716.
58. Li QF, Spinelli AM, Wang R, Anfinogenova Y, Singer HA, Tang DD. Critical role of vimentin phosphorylation at Ser-56 by p21-activated kinase in vimentin cytoskeleton signaling. *J Biol Chem* 2006;281:34716-34724.
59. Pertel T, Zhu D, Panettieri RA, Yamaguchi N, Emala CW, Hirshman CA. Expression and muscarinic receptor coupling of Lyn kinase in cultured human airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2006;290:L492-L500.
60. Parameswaran K, Radford K, Fanat A, Stephen J, Bonnans C, Levy BD, Janssen LJ, Cox PG. Modulation of human airway smooth muscle migration by lipid mediators and Th-2 cytokines. *Am J Respir Cell Mol Biol* 2007;37:240-247.
61. Yamboliev IA, Chen J, Gerthoffer WT. PI 3-kinases and Src kinases regulate spreading and migration of cultured VSMCs. *Am J Physiol Cell Physiol* 2001;281:C709-C718.
62. Carlin SM, Resink TJ, Tamm M, Roth M. Urokinase signal transduction and its role in cell migration. *FASEB J* 2005;19:195-202.
63. Klinghoffer RA, Sachsenmaier C, Cooper JA, Soriano P. Src family kinases are required for integrin but not PDGFR signal transduction. *EMBO J* 1999;18:2459-2471.
64. Irani C, Goncharova EA, Hunter DS, Walker CL, Panettieri RA, Krymskaya VP. Phosphatidylinositol 3-kinase but not tuberlin is required for PDGF-induced cell migration. *Am J Physiol Lung Cell Mol Physiol* 2002;282:L854-L862.

65. Day RM, Lee YH, Park AM, Suzuki YJ. Retinoic acid inhibits airway smooth muscle cell migration. *Am J Respir Cell Mol Biol* 2006;34:695–703.
66. Campbell M, Trimble ER. Modification of PI3K- and MAPK-dependent chemotaxis in aortic vascular smooth muscle cells by protein kinase C β 2. *Circ Res* 2005;96:197–206.
67. Bain J, McLauchlan H, Elliott M, Cohen P. The specificities of protein kinase inhibitors: an update. *Biochem J* 2003;371:199–204.
68. Kordowska J, Hetrick T, Adam LP, Wang CL. Phosphorylated L-caldesmon is involved in disassembly of actin stress fibers and postmitotic spreading. *Exp Cell Res* 2006;312:95–110.
69. Yamboliev IA, Gerthoffer WT. Modulatory role of ERK MAPK-caldesmon pathway in PDGF-stimulated migration of cultured pulmonary artery SMCs. *Am J Physiol Cell Physiol* 2001;280:C1680–C1688.
70. Klemke RL, Cai S, Giannini AL, Gallagher PJ, de Lanerolle P, Cheresh DA. Regulation of cell motility by mitogen-activated protein kinase. *J Cell Biol* 1997;137:481–492.
71. Goncharova EA, Vorotnikov AV, Gracheva EO, Wang CL, Panettieri RA Jr, Stepanova VV, Tkachuk VA. Activation of p38 MAP-kinase and caldesmon phosphorylation are essential for urokinase-induced human smooth muscle cell migration. *Biol Chem* 2002;383:115–126.
72. Liang KC, Lee CW, Lin WN, Lin CC, Wu CB, Luo SF, Yang CM. Interleukin-1 β induces MMP-9 expression via p42/p44 MAPK, p38 MAPK, JNK, and nuclear factor- κ B signaling pathways in human tracheal smooth muscle cells. *J Cell Physiol* 2007;211:759–770.
73. Gerthoffer WT, Singer CA. MAPK regulation of gene expression in airway smooth muscle. *Respir Physiol Neurobiol* 2003;137:237–250.
74. Gerthoffer WT. Mechanisms of vascular smooth muscle cell migration. *Circ Res* 2007;100:607–621.
75. Poon M, Marx SO, Gallo R, Badimon JJ, Taubman MB, Marks AR. Rapamycin inhibits vascular smooth muscle cell migration. *J Clin Invest* 1996;98:2277–2283.
76. Takeda N, Kondo M, Ito S, Ito Y, Shimokata K, Kume H. Role of RhoA inactivation in reduced cell proliferation of human airway smooth muscle by simvastatin. *Am J Respir Cell Mol Biol* 2006;35:722–729.
77. Duan W, Wong WS. Targeting mitogen-activated protein kinases for asthma. *Curr Drug Targets* 2006;7:691–698.
78. Henry PJ, Mann TS, Goldie RG. A Rho kinase inhibitor, Y-27632 inhibits pulmonary eosinophilia, bronchoconstriction and airways hyperresponsiveness in allergic mice. *Pulm Pharmacol Ther* 2005;18:67–74.
79. Taki F, Kume H, Kobayashi T, Ohta H, Aratake H, Shimokata K. Effects of Rho-kinase inactivation on eosinophilia and hyper-reactivity in murine airways by allergen challenges. *Clin Exp Allergy* 2007;37:599–607.
80. Goncharova EA, Billington CK, Irani C, Vorotnikov AV, Tkachuk VA, Penn RB, Krymskaya VP, Panettieri RA Jr. Cyclic AMP-mobilizing agents and glucocorticoids modulate human smooth muscle cell migration. *Am J Respir Cell Mol Biol* 2003;29:19–27.
81. Kaur D, Saunders R, Berger P, Siddiqui S, Woodman L, Wardlaw A, Bradding P, Brightling CE. Airway smooth muscle and mast cell-derived CC chemokine ligand 19 mediate airway smooth muscle migration in asthma. *Am J Respir Crit Care Med* 2006;174:1179–1188.
82. Govindaraju V, Michoud MC, Al-Chalabi M, Ferraro P, Powell WS, Martin JG. Interleukin-8: novel roles in human airway smooth muscle cell contraction and migration. *Am J Physiol Cell Physiol* 2006;291:C957–C965.
83. Waters CM, Long J, Gorshkova I, Fujiwara Y, Connell M, Belmonte KE, Tigyi G, Natarajan V, Pyne S, Pyne NJ. Cell migration activated by platelet-derived growth factor receptor is blocked by an inverse agonist of the sphingosine 1-phosphate receptor-1. *FASEB J* 2006;20:509–511.
84. Deuse T, Schrepfer S, Koch-Nolte F, Haddad M, Schafer H, Detter C, Reichenspurner H. Sirolimus and FK778: a comparison of two anti-proliferative immunosuppressants for prevention of experimental obliterative airway disease. *Transpl Int* 2006;19:310–318.