Mechanisms of Vascular Smooth Muscle Cell Migration

William T. Gerthoffer

Abstract—Smooth muscle cell migration occurs during vascular development, in response to vascular injury, and during atherogenesis. Many proximal signals and signal transduction pathways activated during migration have been identified, as well as components of the cellular machinery that affect cell movement. In this review, a summary of promigratory and antimigratory molecules belonging to diverse chemical and functional families is presented, along with a summary of key signaling events mediating migration. Extracellular molecules that modulate migration include small biogenic amines, peptide growth factors, cytokines, extracellular matrix components, and drugs used in cardiovascular medicine. Promigratory stimuli activate signal transduction cascades that trigger remodeling of the cytoskeleton, change the adhesiveness of the cell to the matrix, and activate motor proteins. This review focuses on the signaling pathways and effector proteins regulated by promigratory and antimigratory molecules. Prominent pathways include phosphatidylinositol 3-kinases, calcium-dependent protein kinases, Rho-activated protein kinase, p21-activated protein kinases, LIM kinase, and mitogen-activated protein kinases. Important downstream targets include myosin II motors, actin capping and severing proteins, formins, profilin, cofilin, and the actin-related protein-2/3 complex. Actin filament remodeling, focal contact remodeling, and molecular motors are coordinated to cause cells to migrate along gradients of chemical cues, matrix adhesiveness, or matrix stiffness. The result is recruitment of cells to areas where the vessel wall is being remodeled. Vessel wall remodeling can be antagonized by common cardiovascular drugs that act in part by inhibiting vascular smooth muscle cell migration. Several therapeutically important drugs act by inhibiting cell cycle progression, which may reduce the population of migrating cells. (Circ Res. 2007;100:607-621.)

Key Words: actin • cytoskeletal dynamics • growth factors • signal transduction • vascular smooth muscle cells
process in vessel wall remodeling has stimulated strong interest in cellular events and molecular mechanisms of VSM cell migration. This review summarizes current views of basic mechanisms of cell migration and reviews studies of VSM cells that identify important proximal signals, signaling pathways, and effector proteins. Because of the scope of the literature, many interesting studies could not be included. The reader is referred to earlier reviews by Schwartz and colleagues and Abedi and Zachary for summaries of the earliest studies of VSM cell migration.

Cell migration in vivo or in vitro begins with stimulation of cell surface receptors that transduce the external signal to a series of coordinated remodeling events that alter the structure of the cytoskeleton (Figure 1). Early signaling events trigger actin polymerization, which causes protrusion of the leading edge of the cell toward a chemotactic stimulus or along a path of varying adhesiveness within the extracellular matrix (Figure 1A). New focal contacts form just behind the leading edge. Key details of signaling and effector molecules regulating actin and focal contact remodeling are shown in the insets. Inset A shows that small G proteins (Rho, Rac, and Cdc42) regulate actin-binding proteins (WAVE, WASP, actin-related protein 2/3 [ARP2/3] complex, mDia, vasodilator-stimulated phosphoprotein [VASP]) to promote actin nucleation and filament extension at the plus (barbed) ends, near the plasma membrane (yellow band). Depolymerization of F-actin at the minus (pointed) end is promoted by cofilin. Gelsolin acts as both a severing and capping protein during actin filament turnover. Actin monomers form a complex with profilin, which favors addition of G-actin at the plus end of the filament. Myosin II, which generates traction forces, is activated by Ca\textsuperscript{2+}/calmodulin (CaM)–MLCK and possibly by PAK. Myosin II is inhibited by myosin light chain phosphatase (MLCP). Inset B shows adaptor, signaling, and myosin motor proteins collecting at new focal contacts (red bars) in the leading edge. The focal contact matures, additional components are added and actin–integrin complexes expand in number. Farther back from the leading edge, microtubules (gray lines) radiate from the microtubule-organizing center (MTOC) to both the front and rear of the cell. Microtubules deliver signaling components to focal contacts at the rear of the cell to promote turnover and decreased adhesiveness as the cell moves forward. The models are based on reviews of nonmuscle cell motility by Horwitz and colleagues and Pollard et al.

Figure 1. Actin cytoskeleton dynamics and focal contact remodeling at the leading edge of a migrating cell. Cross-hatched areas at the leading edge (right) indicate areas of high actin polymerization and depolymerization. In this area, new focal contacts (red bars) are forming in the lamellipodia, just behind the leading edge. Key details of signaling and effector molecules regulating actin and focal contact remodeling are shown in the insets. Inset A shows that small G proteins (Rho, Rac, and Cdc42) regulate actin-binding proteins (WAVE, WASP, actin-related protein 2/3 [ARP2/3] complex, mDia, vasodilator-stimulated phosphoprotein [VASP]) to promote actin nucleation and filament extension at the plus (barbed) ends, near the plasma membrane (yellow band). Depolymerization of F-actin at the minus (pointed) end is promoted by cofilin. Gelsolin acts as both a severing and capping protein during actin filament turnover. Actin monomers form a complex with profilin, which favors addition of G-actin at the plus end of the filament. Myosin II, which generates traction forces, is activated by Ca\textsuperscript{2+}/calmodulin (CaM)–MLCK and possibly by PAK. Myosin II is inhibited by myosin light chain phosphatase (MLCP). Inset B shows adaptor, signaling, and myosin motor proteins collecting at new focal contacts (red bars) in the leading edge. As the focal contact matures, additional components are added and actin–integrin complexes expand in number. Farther back from the leading edge, microtubules (gray lines) radiate from the microtubule-organizing center (MTOC) to both the front and rear of the cell. Microtubules deliver signaling components to focal contacts at the rear of the cell to promote turnover and decreased adhesiveness as the cell moves forward. The models are based on reviews of nonmuscle cell motility by Horwitz and colleagues and Pollard et al.
ness in the extracellular matrix (haptotaxis). Major goals of studies of vascular smooth muscle migration include defining the proximal signals and signal transduction pathways activated as well as defining the components of the cellular machinery that affect cell movement. These studies are part of a large effort focused on mechanisms of migration of leukocytes, fibroblasts, and the amoeboid form of Dictyostelium discoideum. For more detailed analysis of mechanisms of cell migration in nonmuscle cells, the reader is referred to the Cell Migration Consortium web site (http://www.cellmigration.org) and several excellent reviews by members of the consortium.9–11

Methods for Measuring Cell Migration
A variety of in vivo and in vitro approaches are used to define basic mechanisms of cellular migration. Most studies use either microscopic methods to record changes in cell position and shape or a modification of the Boyden chamber to assay movement of cells through a porous membrane. Immunohistochemical studies of vessels from atherosclerotic humans and animal models of vessel injury report a snapshot of smooth muscle cells in the media and intima. In diseased or injured tissues, there are more cells in the intima, attributable in part to cell migration.12 Specific aspects of cell behavior during migration in culture are imaged by phase contrast or fluorescence microscopy and analyzed with image analysis software. Changes in cell shape and position are typically induced in a “wounding” assay, in which part of a monolayer of cells is scraped off of the culture plate and the movement of remaining cells is monitored as the “wound” is refilled with cells. Chemoattractants can be added to the culture dish and chemokinetic effects monitored. A stable gradient of attractant is usually not achieved in this protocol unless the agent is incorporated into agarose gel or some other slow-release formulation.13 High-throughput wound closure assays have been developed for screening large numbers of treatments.14,15 One interesting use of this technology was the identification of a novel Rho-activated protein kinase (ROCK) inhibitor, Rockout, by screening a library of small molecules.14

The Boyden chamber assay is a popular alternate approach to wounding assays. In the Boyden chamber assay, cultured smooth muscle cells are plated on the top surface of a porous membrane (8- to 10-μm pores). The cells migrate to the bottom surface and are stained and counted. Chemokinetic and chemotactic effects of a treatment can be distinguished by “checkerboard” analysis.16 The simplest approach to counting cells on the membrane is to fix and stain the cells to visualize the nucleus. An alternate approach is to extract stain and measure absorbance of the extract.17 Nuclei can also be visualized with fluorescent DNA stains and counted by fluorescence microscopy, or cells detached from the bottom of the membrane and DNA can be detected with a fluorometer or fluorescence plate reader. Results are typically reported as fold change in migration relative to unstimulated cells or as number of cells migrating per microscope field or filter surface area. Boyden chamber assays measure the response of a population of cells to a given treatment or treatments.

In the Boyden chamber assay, the chemotactic gradient diminishes in time as cells migrate through the membrane and onto the bottom surface of the chamber. The method is sensitive and useful for rapidly testing multiple treatments that might alter migration. However, the Boyden chamber is not useful for direct observations of cell shape and position needed for investigating detailed mechanisms of migration. Depending on the design of the experiment, Boyden chamber assays may not distinguish between treatment effects on cell attachment, cell spreading, or cell migration. A Dunn chemotaxis chamber is a modification of a Zigmund chamber that generates a relatively long-lasting gradient and allows imaging of migrating cells.18,19 A long-lived concentration gradient is required to visualize the individual cell trajectories and migration velocities of a slow-moving cell, such as a smooth muscle cell. The Dunn chamber offers a significant advantage in that subtle changes in cell shape and the direction of the motion can be visualized during the experiment.19 A combination of approaches is required to define accurately the effects of promigratory and antimigratory chemicals on cell populations, on individual cell behaviors, and on subcellular structures mediating cell movement.

Mechanisms of Cell Migration
Proximal Signals and Initial Transduction Steps
A common goal of studies of VSM cell migration is to define the environmental cues that promote or inhibit motility. 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. Physical factors including blood flow, shear stress, and matrix stiffness can also influence VSM cell migration.20–22 Peptide growth factors have been studied in some detail since reports in the 1980s and early 1990s indicating that PDGF is a potent, efficacious promigratory stimulus in vivo following vascular injury.23–25 Later studies of PDGF receptor knockout mice suggested that an important role of PDGF was to activate smooth muscle progenitor cells to migrate and form blood vessels.6 Interestingly, the PDGF-AA isoform is antimigratory,27 suggesting the net effect of PDGF in vivo may depend on integration of both short-term promigratory signaling by the BB isoform and antimigratory signaling by the AA isoform. In addition to short-term signaling, PDGF induces longer term sequential activation of other growth factor and cytokine signaling pathways.28 PDGF acts indirectly to induce synthesis of epidermal growth factor and fibroblast growth factor-2, both of which enhance cell migration.29 This is only one example of the well-documented ability of growth factors and cytokines to stimulate their own synthesis by smooth muscles, as well as synthesis of other growth factors and cytokines.30,31 Therefore, both direct and indirect effects of signaling proteins on VSM migration are very likely to occur in vivo during a response to injury or inflammation.

Although PDGF and other signaling proteins controlling VSM migration are soluble, other important proximal signals are presented as part of the extracellular matrix. Early studies by Schwartz and colleagues identified osteopontin as an
TABLE 1. Summary of Agents That Promote VSM Cell Migration

<table>
<thead>
<tr>
<th>Growth Factors and Cytokines</th>
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<tr>
<td>Angiotensin II77</td>
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<tr>
<td>Basic fibroblast growth factor (bFGF)178</td>
</tr>
<tr>
<td>Heparin-binding epidermal growth factor (HB-EGF)179</td>
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<tr>
<td>Insulin-like growth factor-1 (IGF-1)180</td>
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<tr>
<td>Interleukin-1β (IL-1β)181</td>
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<tr>
<td>Interleukin-6 (IL-6)182</td>
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<tr>
<td>PDGF23</td>
</tr>
<tr>
<td>Transforming growth factor-β1 (TGF-β1)183,184</td>
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<tr>
<td>Tumor necrosis factor-α (TNF-α)185,186</td>
</tr>
<tr>
<td>Thrombin186</td>
</tr>
<tr>
<td>Urokinase plasminogen activator187,188</td>
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<tr>
<td>Vascular endothelial growth factor (VEGF)189,190</td>
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<table>
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<tr>
<th>Extracellular matrix components</th>
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<tr>
<td>Collagen I, IV37</td>
</tr>
<tr>
<td>Collagen VIII191,192</td>
</tr>
<tr>
<td>Fibronectin19</td>
</tr>
<tr>
<td>Hyaluronan193,194</td>
</tr>
<tr>
<td>Laminin77</td>
</tr>
<tr>
<td>Osteopontin32</td>
</tr>
<tr>
<td>Thrombospondin196</td>
</tr>
<tr>
<td>Vitronectin196</td>
</tr>
<tr>
<td>Other molecules</td>
</tr>
<tr>
<td>ATP, UTP197</td>
</tr>
<tr>
<td>Norepinephrine196</td>
</tr>
<tr>
<td>High glucose (25 mmol/L)199</td>
</tr>
<tr>
<td>Histamine199</td>
</tr>
<tr>
<td>Serotonin198</td>
</tr>
<tr>
<td>Sphingosine-1 phosphate (S1P)75,76</td>
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Important matrix component that promotes VSM migration.32 Osteopontin is a matrix glycoprotein containing an RGD (Arg, Gly, Asp) motif in the N-terminal domain that ligates CD44 and several integrins, including αβ3 integrins, in a variety of cell types.33 During atherogenesis or following vascular injury, osteopontin expression increases and is thought to enhance VSM migration via αβ3 integrin signaling.34–36 Similar promigratory effects have been reported for each of the major constituents of the extracellular matrix of the vessel wall, including collagens I and IV and laminin37 (see Table 1). Integrin ligation is necessary for VSM adhesion to the matrix, and it triggers promigratory intracellular signaling cascades from the “outside in.” Details of key signaling cascades are described in more detail below. Integrin activation from “inside out” also occurs by virtue of soluble signals promoting protein and inositol phosphorylation events that regulate formation of nascent focal contacts (see Figure 1B). One important example is activation of the actin-binding protein talin, which alters the cytoplasmic domains of integrins to increase cell adhesiveness.38,39 As in other motile mammalian cells, VSM cell migration depends on integration of both soluble signals acting from the outside in and on changes in matrix attachment via activation of integrins from the inside out.

In addition to having promigratory features, the extracellular matrix can have antimigratory effects. In the normal, uninjured vessel wall, VSM cells are nonmigratory because there is little stimulus to migrate, they are not proliferating, and because the matrix is highly adhesive. Focal adhesions are presumably of the fibrillar type, which are large and relatively stable. In the absence of significant matrix metalloproteinase activity, contractile smooth muscle cells remain spindle shaped and firmly adherent to the matrix. Heparin in the matrix may also strongly inhibit cell motility.40,41 as do tissue inhibitors of metalloproteinases (TIMPs).42,43 Whether VSM cells migrate in vivo and in what direction is likely controlled by promigratory stimuli such as osteopontin balanced by antimigratory influences such as stable focal adhesions, TIMPs, and heparin.

The identity of soluble signals and matrix components that modify vascular smooth muscle migration has been a topic of active research for nearly 20 years, resulting in a voluminous literature. Table 1 summarizes examples of growth factors, cytokines, matrix components, and other molecules that enhance cell migration in vitro. The table is an incomplete list that focuses on extracellular signaling molecules important in normal cardiovascular function, vascular development, or vascular pathology. Table 2 shows a partial list of inhibitory molecules that are either endogenous molecules that regulate cardiovascular function or agents that contribute to vascular pathology, or are cardiovascular drugs. Many of the agents listed are implicated to some extent as paracrine or autocrine signals released in response to injury. Whether these agents all have important effects in vivo is an open question. In most cases, relevant in vivo studies of mouse models in which the signaling protein or its receptor is knocked out are either lacking or minimally described.

Cytoskeletal Proteins and the Physics of Cell Motility

The promigratory agents in Table 1 activate signal transduction cascades that trigger remodeling of the cytoskeleton, change the adhesiveness of the cell to the matrix, and activate motor proteins (Figure 1). To migrate, VSM cells need to extend lamellipodia toward the stimulus via actin polymerization, detach the trailing edge by degrading focal contacts, and generate force by myosin II in the body of the cell to propel the cell forward. Actin polymerization is coordinated by a large number of actin-binding proteins illustrated in Figure 1A. These proteins are regulated by Ca2+, phosphatidylinositol 4,5-bisphosphate (PIP2), small G proteins, and signaling cascades described below. For example, activation of the β isoform of PDGF receptor (PDGFR-β) is coupled via phosphatidylinositol 3-kinase (PI3K) and phospholipase Cγ to changes in myoplasmic calcium, hydrolysis of PIP2, and activation of mitogen-activated protein kinases (MAPK).44 Nucleation of new filaments is initiated by the actin-related protein 2/3 (ARP2/3) complex binding to the minus end and removal at the plus end of capping proteins that are sensitive to PIP2. Extension of new actin filaments is enhanced by
focal contacts at the trailing edge is necessary for the cell to
substrate and provide traction for propulsion of the cell over
leading edge provide adhesion of the lamellipodia to the
lipodia extension (Figure 1B). Nascent focal contacts at the
beyond the initial stages of actin polymerization and lamel-
extracellular matrix are necessary for migration to proceed
toward the stimulus.45,46

produce sufficient force to extend the leading edge of the cell
which serves to limit the length of filaments and to cause
depolymerization is promoted at the minus end by cofilin,
by gelsolin is promoted by Ca²⁺
to increase nucleation and branching. Actin-filament severing
each activate proteins of the actin-related protein 2/3 complex
protein (WASP), respectively. WAVE and WASP proteins
protein (WAVE) complex and Wiskott–Aldrich syndrome
protein (WAVE) to increase nucleotide exchange on G-actin monomers and enhances actin
from membrane phospholipid binding sites increases nucle-
concert with profilin. The formin mDia1 is activated by
formins (mDia1 and mDia2), which act on the plus end in
concert with profilin. The formin mDia1 is activated by
RhoA, and mDia2 is activated by Cdc42. Profilin liberated
from membrane phospholipid binding sites increases nucle-
otide exchange on G-actin monomers and enhances actin
polymerization. Filament branching is promoted by Rac and
Cdc42 activation of WASP family verprolin–homologous
protein (WAVE) complex and Wiskott–Aldrich syndrome
protein (WASP), respectively. WAVE and WASP proteins
each activate proteins of the actin-related protein 2/3 complex
to increase nucleation and branching. Actin-filament severing
by gelsolin is promoted by Ca²⁺, and nucleation is favored by
PIP₂ releasing gelsolin from plus ends of F-actin. Actin
depolymerization is promoted at the minus end by cofillin,
which serves to limit the length of filaments and to cause
turnover of existing filaments. These processes are thought to
produce sufficient force to extend the leading edge of the cell
toward the stimulus.45,46

Transient focal contacts between the cell membrane and
extracellular matrix are necessary for migration to proceed
beyond the initial stages of actin polymerization and lamel-
lipodia extension (Figure 1B). Nascent focal contacts at the
leading edge provide adhesion of the lamellipodia to the
substrate and provide traction for propulsion of the cell over
or through the extracellular matrix. Degradation of mature
focal contacts at the trailing edge is necessary for the cell to
release from the matrix and move forward. The composition
of focal contacts and mechanisms of initiation, maturation,
and degradation have been extensively studied in nonmuscle
cells.10,47,48 The major components of focal contacts have
been described in migrating VSM cells including focal
adhesion kinase (FAK),49 vinculin,50 paxillin,51,52 tensin,53
p130Cas,52 and Src.54 Focal adhesion kinase is a critical
regulator of cell migration that is upregulated following
intimal hyperplasia.55 Formation and degradation of focal
contacts is intimately associated with signal transduction
occurring during migration. Multiple signaling kinases asso-
ciate with focal contacts, including Src family members,
FAK, phosphoinositide-dependent kinase 1, MAPKs, and
PI3K. Phosphorylation of focal contact components including
FAK, paxillin, and tensin occurs during VSM migra-
tion,51,53,56 as does proteolysis of focal contact proteins by
metalloproteinases57,58 and calpain.59

The exact nature of spatial and temporal regulation of
proteins in nascent focal contacts in VSM is mostly unde-
finied. It is also not entirely clear to what extent signaling
promotes assembly of proteins in parallel or in series. In
nonmuscle motile cells, integrin clustering recruits tensin and
many signaling components, including small G proteins,
PAK, extracellular signal-regulated kinase (ERK), and
FAK.60,61 Integrin ligation plus aggregation stimulates addi-
tion of structural proteins, including vinculin and α-actinin.
Tyrosine phosphorylation by FAK and Src family tyrosine
kinases is necessary for proper assembly, as is filamentous
actin. The assembly process is regulated and sequential with
protein components entering individually or in small pre-
formed complexes.51 A good example of sequential addition
of structural proteins is that a paxillin–green fluorescent
protein fusion protein has been observed to appear in focal
contacts before actinin–green fluorescent protein.62 A good
example of proteins entering as complex is that paxillin can
join the nascent focal contact along with GIT1 (G-protein
receptor kinase–interacting protein), PAK, extracellular signal-regulated kinase (ERK), and
PAK, interacting exchange factor (PIX).63 Formation of nascent
focal contacts containing adaptor proteins talin, vinculin,
paxillin, and α-actinin requires 4 events: integrin ligation,
integrin clustering, phosphorylation of several components by
tyrosine kinases, and an intact cortical F-actin meshwork.60
The critical features of focal contacts at the leading edge are
the ability to form rapidly, persist long enough to transmit
traction force to the matrix and then slide toward the center of
the cells and either undergo turnover or mature to stable focal
contacts. At the rear of the cell larger, more stable focal
contacts must disassemble for the cell to move forward.

Traction forces transmitted through focal contacts to the
matrix are generated by activation of myosin II motors via
Ca²⁺ activation of myosin light chain kinase (MLCK) and
phosphorylation of the 20-kDa regulatory myosin light
chains. Ca²⁺-independent activation of myosin II may also
occur through activation of RhoA and ROCK. Although the
basic elements of force generating mechanisms are known to
exist in migrating VSM cells, the details of how cells respond
to the physical nature of the matrix and how myosin motors
are regulated in migrating cells compared with intact muscle
remain to be defined. Recent work from Ingber and col-

### Table 2. Summary of Agents That Inhibit VSM Cell Migration

<table>
<thead>
<tr>
<th>Growth factors and cytokines</th>
<th>Hypoxia-inducible factor-1 (HIF1)³²⁰</th>
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<tbody>
<tr>
<td>Interleukin-1β (IL-1β)³²¹</td>
<td></td>
</tr>
<tr>
<td>Interleukin-10 (IL-10)²⁰²</td>
<td></td>
</tr>
<tr>
<td>Transforming growth factor-β₁ (TGFβ₁)²⁰⁰</td>
<td></td>
</tr>
<tr>
<td>Tumor necrosis factor-α (TNFα)²⁰¹</td>
<td></td>
</tr>
<tr>
<td>Extracellular matrix components</td>
<td>Heparin⁴⁰</td>
</tr>
<tr>
<td>Tissue inhibitors of metalloproteinases (TIMPs)⁴²,⁴³</td>
<td></td>
</tr>
<tr>
<td>Catecholamines</td>
<td>Epinephrine²⁰⁴</td>
</tr>
<tr>
<td>Dopamine²⁰⁵</td>
<td></td>
</tr>
<tr>
<td>Isoproterenol²⁰⁶</td>
<td></td>
</tr>
<tr>
<td>Cardiovascular Drugs and Other Molecules</td>
<td>cAMP²⁰⁷</td>
</tr>
<tr>
<td>Epoxidecosterienoic acids (ETs)²⁰⁸</td>
<td></td>
</tr>
<tr>
<td>Faudull⁸</td>
<td></td>
</tr>
<tr>
<td>NO donors²⁰⁹,²¹⁰</td>
<td></td>
</tr>
<tr>
<td>ω-3 fatty acids²¹¹</td>
<td></td>
</tr>
<tr>
<td>Paclitaxel⁶⁶</td>
<td></td>
</tr>
<tr>
<td>Peroxisome proliferator-activated receptor γ (PPAR-γ) activators³⁶¹</td>
<td></td>
</tr>
<tr>
<td>Rapamycin⁵⁶</td>
<td></td>
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<tr>
<td>Red wine polyphenols²¹²</td>
<td></td>
</tr>
<tr>
<td>Sphingosine-1 phosphate (S1P)³⁴</td>
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<td>Y-27632⁵⁷</td>
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leagues shows plating VSM cells on surfaces of differing densities of fibronectin and differing degrees of stiffness altered myosin light chain phosphorylation and traction forces. They also showed that inhibiting myosin ATPase with 2,3-butanediol 2-monoxime (BDM) reduced myosin light chain phosphorylation. These authors suggested that decreased adhesiveness or decreased stiffness of the matrix reduces prestress on the cytoskeleton, as does inhibition of myosin ATPase. Decreased stiffness in turn inhibited myosin phosphorylation by some undefined effect on biochemical signal transduction. These observations are consistent with a model in which myosin II in migrating cells generates traction forces on the matrix, and the matrix in turn regulates activation of myosin II depending on the stiffness of the matrix.

In addition to focal contact and actin filament remodeling, cell migration also requires remodeling of microtubules. The nucleus is reoriented during migration of fibroblasts, and the microtubule organizing center (MTOC) remains in the center of the cell (Figure 1). As cells migrate, the nucleus moves toward the rear of the cell. Nuclear movement is affected by Cdc42 regulation of myotonic dystrophy kinase-related Cdc42 binding kinase (MRCK). MRCK phosphorylates the 20-kDa myosin light chain, which activates actomyosin. Microtubules remodel actively during migration, and dynamic instability is required for VSM migration. Stabilization of microtubules with paclitaxel (Taxol) inhibits PDGF-stimulated VSM migration. Microtubules may also contribute to dissolution of stable focal contacts, thus allowing disengagement of the trailing edge of a migrating cell. Relatively few studies of VSM cell migration have linked specific signaling pathways to microtubule dynamics. Urokinase plasminogen activator has been proposed to promote migration, in part, by a pathway including PI3K, PIβ, and glycogen synthase kinase 3β (GSK3β). GSK3β might exert promigratory effects via the tumor suppressor APC (adenomatous polyposis coli), which can interact with microtubules and may ultimately affect actin polymerization via a Rac-dependent mechanism. However, direct evidence for APC interaction with microtubules and promotion of actin polymerization at the leading edge of migrating VSM cells remains to be seen.

**Signaling Cascades**

The actin cytoskeleton is regulated by multiple signaling pathways activated by both receptor tyrosine kinases (RTKs) and G protein–coupled receptors (GPCRs) (Figure 2). Key signaling components include trimeric G proteins, small G proteins, lipid kinases, Ca\(^{2+}\)-dependent protein kinases, ROCK, and MAPKs (Figure 2). Any stimulus that increases myoplasmic Ca\(^{2+}\) concentration will probably activate MLCK and myosin II. For example, Ca\(^{2+}\) oscillations were observed during PDGF-stimulated cell migration, and MLCK inhibitors block VSM cell migration. It is also
clear that many promigratory stimuli activate 1 or more pathways regulating actin polymerization, including RhoA and ROCK. Participation of other kinases will depend on the specifics of receptor signal transduction.

**Receptor Crosstalk: Sphingosine-1 Phosphate and PDGF**

Most in vitro studies of VSM cell migration use a single agent to stimulate migration. VSM cells migrating in vivo are probably exposed to multiple promigratory and antimigratory molecules simultaneously. Therefore receptor crosstalk is likely to occur and have significant effects on migration. A good example is the interaction of sphingosine-1 phosphate (S1P) and PDGF during cell migration. As noted above, PDGFR-β knock out causes defects in vascular development attributable to failure of VSM migration. The S1P1 receptor knockout mouse undergoes a similar failure of vascular development caused by inhibition of VSM migration. This is consistent with a promigratory effect of S1P on VSM cell migration in vitro but is inconsistent with several reports of antimigratory effects of S1P. Activation of S1P1 receptors promotes migration, whereas activation of S1P5 receptors generally antagonizes cell migration in nonmuscle cells. S1P and PDGF might enhance migration in vivo during development or during atherogenesis in 2 ways: (1) by enhancing synthesis of the other agent causing sequential activation of receptors; and (2) by interactions of the S1P1 receptor with the PDGFβ receptor. There are several potential mechanisms of receptor interaction, including transactivation of S1P receptors by PDGF and S1P receptors serving as adaptors for PDGF receptor activation of focal adhesion signaling. Another somewhat different mechanism has been observed in airway smooth muscle cells. PDGFR receptor activation causes release of sphingosine kinase from the smooth muscle cell, which is hypothesized to produce S1P from sphingosine secreted from other cell types. S1P then activates a S1P1/PDGFR-β complex that enhances migration. It is possible that VSM cell migration in vivo is mediated by sequential receptor activation after mediator synthesis, receptor transactivation, and/or receptor–receptor complex formation. The extent to which these mechanisms occur during VSM migration is not well defined, nor is it clear how common receptor crosstalk is between other RTKs and GPCRs in migrating VSM cells.

**Small G Proteins and ROCK**

The small G proteins (Ras, Rho, Rac, Cdc42) are very early elements in signaling pathways that promote cell migration. Both RTKs and GPCRs activate several small G proteins via regulation of guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins. Although there are a few studies of GEFs in VSM cell migration, little is known of the details of either GEFs or GTPase-activating proteins in small G-protein function in smooth muscle.

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 ROCK1 and ROCK2 in VSM, and Rho kinase inhibition by C3 exoenzyme, fasudil, or Y-27632 blocks VSM cell migration. ROCK phosphorylates the myosin binding subunit of myosin light chain phosphatase, thus reducing phosphatase activity. This is a prominent mechanism of agonist-induced calcium sensitization of smooth muscle contraction that contributes significantly to cell migration as well. ROCK can also directly phosphorylate myosin light chains in vitro at Ser19, but this mechanism is debatable in differentiated vascular smooth muscle. In cultured 3T3 fibroblasts, ROCK directly phosphorylates myosin II located at the core of the cell but not in the periphery. It may be that migrating cells regulate actomyosin differently from differentiated cells and that ROCK can substitute for MLCK or act in concert with MLCK to phosphorylate myosin II during migration. In addition to effects on myosin II phosphorylation, ROCK promotes actin polymerization by activation of LIM kinase. Activation of LIM kinase will inhibit cofilin-mediated actin depolymerization favoring increased F-actin during migration. Although there are reports of dynamic changes in cofilin and LIM kinase expression in vascular smooth muscle, it is not known whether this mechanism is necessary for cell migration.

Activation of Rac1 has been observed during VSM migration stimulated by PDGF. Rac2 was recently reported to be inducible by inflammatory cytokines in VSM and to promote migration by interacting with a cytokine-inducible scaffold protein, allograft inflammatory factor-1. Rac1 may be under negative control by the Slit axon guidance proteins, which suggests a balance of positive and negative migratory factors may determine smooth muscle cell location during development in a fashion similar to the developing nervous system. Rac1 and Rac2 both activate PAKs, which are also positively modulated by reactive oxygen species, Ca²⁺, and protein kinase C.

**p21-Activated Protein Kinases**

PAKs phosphorylate several substrates that regulate cell migration. Phosphorylation of LIM kinase by PAK results in phosphorylation of cofilin, which promotes actin depolymerization. PAK phosphorylation of MLCK inhibits myosin phosphorylation. In contrast, PAK has also been reported to phosphorylate myosin light chains directly, which would activate actomyosin. PAK phosphorylates 2 actin-binding proteins, cortactin and caldesmon. PAK phosphorylation of h-caldesmon activates muscle actomyosin, thus increasing muscle contraction. However, l-caldesmon is the predominant caldesmon isoform expressed in cultured VSM. It is phosphorylated during PDGF-induced VSM migration, and l-caldesmon phosphorylation is necessary for stress fiber disassembly. PAK also phosphorylates cortactin, which reduces its binding to actin and may reduce actin branching. In addition to profound effects on the actin cytoskeleton and myosin motor protein, PAK may modify microtubule and intermediate filament structure. The activity of the microtubule-stabilizing protein Op18/stathmin is inhibited by Rac1 activation of PAK, resulting in stabilization of microtubules. PAK also phosphorylates desmin and vimentin to enhance intermediate filament turnover. Although expression of kinase-inactive PAK inhibits smooth muscle cell migration very effectively, it is not clear...
which of the positive or negative effects of PAK on cytoskeletal filaments, myosin, and focal adhesions predominate during VSM migration. Nor is it clear how the diverse effects of PAK enzymatic activity and chaperone function are orchestrated to promote coordinated cell movement. Spatial and temporal control of PAK function is mediated by several adaptor proteins in nonmuscle cells, including Nck, PAK-interacting exchange factor, and paxillin kinase linker (PKL). It is not known whether these proteins play similar roles in migrating VSM cells.

**Src Family Tyrosine Kinases**

Src and PI3Ks are proximal components of signaling cascades activated by both growth factor receptors (RTKs) and GPCRs (see Figure 2). Smooth muscle cells express Src family members, including Src, Lyn, Lyn, and Yes. PDGF activates Src during VSM cell migration, and inhibiting Src with drugs or anti-Src antibodies inhibits PDGF-induced VSM cell migration. Src activity is not necessary for cell attachment, but cell spreading and chemotaxis both depend on Src activity and PI3K activity. Likely downstream targets of Src include the ERK MAPK signaling cascade and focal contact proteins FAK, p130Cas, and paxillin. Defining the position of Src family kinases in signaling cascades and defining the substrates important for cell migration is a challenging task because of redundancy in Src signaling. The multiple Src family members expressed in smooth muscles have similar target phosphorylation sites and, therefore, may have overlapping substrate selectivity. Furthermore, chemical Src inhibitors do not discriminate well among Src family members. The fact that single and multiple Src family knockout mice are available, and that viral vectors can be used in rescue strategies, provides an avenue for some progress, but this has not been actively pursued.

**Phosphatidylinositol 3-Kinases**

PI3Ks are proximal components of many signaling pathways in VSM that control diverse cellular functions, including proliferation, cell survival, and cell motility. They phosphorylate phosphatidylinositol at position D3 of the inositol ring, resulting in activation of several signaling cascades implicated in VSM cell migration, including ERK and p38 MAPKs. PI3K has also been recently linked to remodeling of microtubules through Akt, GSK3β, and adenomatous polyposis coli. Evidence for the necessity of PI3K in VSM cell migration is mixed. In some cases, chemical inhibitors of p38 MAPKs, MLCK, focal adhesion kinase, and calpain. Indirect, longer-term effects on cell migration might be exerted by regulating transcription factors that control expression of promigratory growth factors, matrix proteins, and matrix metalloproteinases.

The p38 MAPK cascade has also been implicated in VSM cell migration with the use of both chemical inhibitors of p38 MAPK and gene transfer approaches. A signaling cascade comprising MAPK kinase 3 (MKK3), p38 MAPKs, and heat shock protein 27 (HSP27) is necessary for migration of both smooth muscle cells and endothelial cells. The p38 MAPK cascade is activated by numerous promigratory stimuli, including PDGF, angiotensin II, S1P, and thrombin. Thrombin stimulates this pathway via reactive oxygen species. Chemical inhibitors of p38 MAPK block vascular remodeling in a rabbit injury model, although, interestingly, blockade of p38 MAPK did not block migration of cultured cells in this case. Inhibition of p38 MAPK also inhibits reorientation of cultured VSM in response to stretch, suggesting p38 MAPK and its targets are early sensors of both the chemical and mechanical forces that promote cell migration.

**Mitogen-Activated Protein Kinases**

MAPK cascades are among the most common and important signaling cascades activated by both receptor and nonreceptor tyrosine kinases. Defining the role of ERK, p38 MAPK, and C-Jun N-terminal kinase (JNK) MAPK families in cell migration is facilitated by effective and relatively selective small molecule inhibitors. There are also good molecular tools used to express kinase-dead or constitutively active kinases. In early studies, PD98059 was used to inhibit MAPK/ERK1 (MEK1), the immediate upstream activator of ERKs, and to inhibit rat VSM cell migration. In the same study, antisense oligonucleotide against ERKs also partially inhibited PDGF-BB-induced migration. ERK activation is typically biphasic in response to growth factor stimulation, with early activation within minutes being required for cell migration and later ERK activity being associated with cell proliferation. Many of the promigratory stimuli listed in Table 1 activate ERK MAPKs in VSM. Although ERK activation has been described as a necessary event in VSM cell migration, there are also studies citing no effect of ERK inhibition, or even ERK-mediated inhibition of migration. This suggests that a canonical Ras/Raf/MEK/ERK signaling cascade can, and probably does, participate in VSM migration but that there is redundancy in the signaling networks that, in some experiments, compensates for ERK inhibition. Figure 2 shows examples of signaling redundancy in regulation of actin polymerization and myosin II phosphorylation. It is clear that, in the absence of ERK activity, there are still multiple pathways by which myosin II motors can be activated.

Despite an extensive use of chemical and molecular tools in correlative studies, it is not entirely clear how ERK activation promotes smooth muscle cell motility. Possible downstream substrates that might directly contribute to enhanced motility include the actin-binding protein l-caldesmon, MLCK, focal adhesion kinase, and calpain. Indirect, longer-term effects on cell migration might be exerted by regulating transcription factors that control expression of promigratory growth factors, matrix proteins, and matrix metalloproteinases.
and mechanical milieu of the vessel wall. The effect of p38 MAPK inhibitors in vascular remodeling is likely to be a combination of effects on cell migration via inhibiting phosphorylation of heat shock protein 27 and actin remodeling as well as effects on transcription and translation of proinflammatory mediators and proteins regulating the cell cycle.

Less is known about the role of JNK MAPKs in VSM migration compared with ERK and p38 MAPKs. The chemical inhibitors of JNK family members are less selective than MEK and p38 MAPK inhibitors and therefore somewhat less useful for definitive studies of kinase action in cells. However, adenoviral overexpression of kinase-dead constructs provides some insight into the contribution of JNKs to migration. Overexpression of a kinase-dead mutants of each MAPK family partially inhibits migration induced by PDGF-BB.135 Overexpressing JNK1 also inhibits angiotensin II–induced migration.85 Protein kinase C, proline-rich tyrosine kinase-2 (PYK2), and Rho/ROCK were found to be upstream of JNK activation and cell migration, but the downstream targets of JNK are less clear. C-Jun has been reported to be necessary for PDGF-induced VSM migration,136 but how this protein affects migration is unknown. JNK can phosphorylate myosin light chains directly.142 Direct evidence of a role in VSM migration is provided by overexpressing dominant negative ILK, which blocks angiotensin II–stimulated migration.143 In the same study ILK expression in mouse aorta was downregulated by statin treatment, suggesting ILK may be one of the anti-atherogenic targets effected by statins.

PTEN
PTEN (Phosphatase and Tensin Homolog Deleted on Chromosome Ten) is a multifunctional phosphatase that acts primarily as a 3’ lipid phosphatase to dephosphorylate PIP3 to PIP2. Because there are many downstream targets of PI3K and PIP3, PTEN participates in diverse processes including transcription, protein synthesis, cell cycle progression, and cell migration.144,145 Studies in nonmuscle cells suggest that one critical function of PTEN in cell migration is to allow focal adhesions at the trailing edge to disassemble as part of the process of dissociating the rear of the cell from the extracellular matrix. Focal contact disassembly may be attributable in part to dephosphorylation of PIP3, and inactivation of ILK as well as direct dephosphorylation of FAK.146,147 Cell migration might also be enhanced through activation of Rac GEFs.148 The necessity for PTEN in VSM migration is suggested by the fact that overexpressing PTEN reduces PDGF-mediated migration.149 In contrast, reduced PTEN activity in a PTEN−/− mouse model had no effect on the development of atheroma.150 Therefore, although PTEN activity is necessary for key signaling processes in smooth muscle proliferation and migration, it is not clear whether or not modulation of PTEN activity alone is sufficient to alter the development of atherosclerotic lesions.

Cardiovascular Drugs and VSM Cell Migration
Many studies of VSM cell migration conclude with suggestions that inhibiting cell migration might be useful for preventing or reducing angiogenesis or reducing the sequelae of vascular injury. This seems reasonable in principle and is probably a contributing factor in the beneficial effects of rapamycin and paclitaxel incorporated into vascular stents.151 Cell migration is sensitive to the proliferative state of VSM cells. Nonproliferating cells in culture are not migratory.152,153 Growth-arrested cells do not migrate in response to PDGF, in part, because of uncoupling of PDGF activation of Ca2+/calmodulin-dependent protein kinase II (CaMKII).154 In proliferating cultures, a very small fraction of smooth muscle cells migrate in response to PDGF. When cultured rat VSM cells were synchronized by serum withdrawal and then stimulated to migrate, most migrating cells were in the G1(b) phase of the cell cycle.155 This is consistent with observations that several drugs that block cell cycle progression also block cell migration. Rapamycin inhibits cell cycle progression at the G1-to-S transition by disrupting several critical processes including cdk2 activation and Rb phosphorylation. Rapamycin also inhibits VSM cell migration,156 in part, by upregulating expression of the cdk inhibitor p27(Kip1).157 Paclitaxel stabilizes microtubules and blocks cell cycle progression. It inhibits VSM migration158 and is effective in drug-eluting stents to prevent restenosis. The antimigratory effect of paclitaxel is probably caused by combined inhibition of cell cycle progression and inhibition of dynamic instability of microtubules. Dynamic instability is necessary for proper dissolution of focal contacts at the trailing edge of migrating cells.159 Apolipoprotein E is thought to reduce migration by inhibiting ERK MAPK activation and cyclin D1 expression, thus preventing transition from G0 to G1 of the cell cycle.158 Sabiporide, an Na/H exchanger inhibitor, also blocks the G1-to-G0 transition and inhibits human pulmonary artery VSM migration.159 Peroxisome proliferator-activated receptor γ (PPARγ) activators have beneficial cardiovascular...
effects attributable, in part, to reducing migration. Troglitazone inhibits VSM migration160,161 as well as ERK MAPK activation, degradation of p27(Kip1), retinoblastoma protein phosphorylation, and cell cycle progression from G1 to S.162 In principle, any treatment that reduces cell cycle progression and proliferation should reduce the number of cells available to migrate in response to growth factors and other chemotactic stimuli.

Statins are 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitors indicated for treatment of hyperlipidemias. Although inhibiting cholesterol synthesis is often the primary therapeutic goal, statins also have beneficial side effects as inhibitors of VSM proliferation, matrix protein synthesis, and cell migration.163,164 The many cardiovascular benefits of statins have led to the use of these drugs to help prevent atherosclerotic disease. An important mechanism underlying the pleiotropic effects of statins is inhibition of mevalonate synthesis and the isoprenylation reactions important in signal transduction by small G proteins.165 As seen in Figures 1 and 2, all of the small G proteins participate in cytoskeletal and focal contact remodeling that occurs during cell migration. It is therefore not surprising that cell migration is inhibited in vitro. Reduced cell migration and proliferation in vivo probably both contribute to achieving the therapeutic goal of inhibiting vascular wall remodeling.

Future Directions and Summary

Migration in Three Dimensions

There are interesting similarities between the response of VSM cells to injury or inflammation and the phenotype of metastatic cancer cells. In both cases, cells respond to environmental clues by proliferating, secreting signaling molecules that are chemotactic, degrading the basement membrane by secreting matrix metalloproteinases, and invading the matrix.166–168 Most studies cited in this review are of cells migrating in 2 dimensions on very stiff substrates, such as glass or porous polycarbonate membranes. Conditions for cell migration in vivo are very different. Cells interact with the matrix on all surfaces, not just on the basal surface. The shape of the cell and the types and kinetics of focal contacts may be fundamentally different. For example, fibroblasts embedded in 3D matrices form focal contacts using a more limited set of integrins than cells on 2D matrices.169 This might result from differences in matrix stiffness that alter mechanical signaling from the matrix.22,64,170 The distribution of key signaling molecules may be quite different, and the types and rates of biochemical reactions controlling the cytoskeleton may be quite different in flat cells on a 2D matrix versus more rounded spindle-shaped VSM cells seen in 3 dimensions. This raises some interesting questions about integrin activation in migrating VSM. Do the same sets of receptor (RTKs and GPCRs), integrins, and focal contact components mediate migration during development compared with an injury response? This question might not be answered accurately in 2D cell cultures. Advances in imaging technology now allow measurement of cell shape, migration velocity, and traction forces at high spatial and temporal resolution.171 Specific candidate proteins can be visualized by expressing fluorescent fusion proteins that often cause minimal or no disruption of protein function. Visualization of dynamic changes in actin filament structure by speckle microscopy is an instructive example.172 One goal for future studies might be to incorporate fluorescent markers into key proteins and track redistribution within the cell in 3 dimensions during migration. For example, trafficking of critical signal transduction proteins could be followed by covalently pulse labeling epitope tags with fluorescent substrates.173 These approaches are substantially more complex than imaging cells in 2D space, but they may provide information highly relevant to behavior of cells in vivo.174,175

Summary

Several thousand studies of VSM cell migration have been published since pioneering work of the early 1980s showing PDGF is a potent stimulant of VSM cell migration. This extensive literature includes an impressive number of physiologically relevant extracellular signaling molecules and drugs that either stimulate or inhibit migration. VSM cell migration may occur in vivo under a number of conditions: during growth of new vessels, during atherogenesis, and following vascular injury. In all 3 conditions, signaling molecules are produced that favor migration. Chemical and molecular interventions have been described that block migration and disrupt vessel remodeling. Some of the drugs that block migration are among the mainstays of cardiovascular medicine, such as the statins. Major signaling pathways common to most mammalian cells are activated to control cytoskeletal remodeling and focal contact remodeling. Chief among these are small G proteins, Rho kinase, actin-binding proteins, and myosin II motors. The biophysical events that cause cells to migrate are not studied in as much detail in VSM cells as in other cells, such as fibroblasts, neutrophils, and the amoeboid stage of Dictyostelium discoideum. However, motile cells all share the major features of cell polarization, dynamic cytoskeletal, and focal contact remodeling at the leading and trailing edge, generation of propulsive and traction forces by myosin motors—all of which result in coordinated movement along concentration gradients of soluble chemicals or matrix components. Advances in imaging technology and vascular tissue bioengineering offer an opportunity to conduct future studies on these interesting processes in 3D cultures. These approaches may provide some additional insights highly relevant to motile behavior of VSM cells in vivo.

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None.

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