Endothelial Cell Migration During Angiogenesis

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Circ Res. 2007;100:782-794
doi: 10.1161/01.RES.0000259593.07661.1e
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571

This Review is part of a thematic series on **Migration of Vascular Cells**, which includes the following articles:

Endothelial Cell Migration During Angiogenesis

Mechanisms of Vascular Smooth Muscle Cell Migration
Leukocyte Migration in the Vascular Wall
Molecular Mechanisms of Endothelial Cell Migration
Endothelial Migration in Vascular Development

Kathy K. Griendling, Editor

Endothelial Cell Migration During Angiogenesis

Laurent Lamalice,* Fabrice Le Boeuf,* Jacques Huot

Abstract—Endothelial cell migration is essential to angiogenesis. This motile process is directionally regulated by chemotactic, haptotactic, and mechanotactic stimuli and further involves degradation of the extracellular matrix to enable progression of the migrating cells. It requires the activation of several signaling pathways that converge on cytoskeletal remodeling. Then, it follows a series of events in which the endothelial cells extend, contract, and throw their rear toward the front and progress forward. The aim of this review is to give an integrative view of the signaling mechanisms that govern endothelial cell migration in the context of angiogenesis. **(*Circ Res*. 2007;100:782-794.)**

Key Words: endothelial cells ■ chemotaxis ■ haptotaxis ■ mechanotaxis ■ migration ■ angiogenesis ■ VEGF ■ integrins ■ actin ■ remodeling

Endothelial cells derive from the successive differentiation of mesodermal cells into hemangioblasts, which leads to the formation of the first vascular structures that are called primitive blood islands. The hemangioblasts from the center of the islands give rise to the hematopoietic stem cells, whereas the peripheral hemangioblasts differentiate into angioblasts, the precursors of mature endothelial cells. Under the influence of vascular endothelial growth factor (VEGF), the angioblasts and newly formed endothelial cells migrate on a matrix constituted mainly of collagen and hyaluronan, allowing the fusion of the blood islands, their remodeling into tubular structures, and the formation of the first primitive vascular plexus. These tubules remodel through vasculogenesis into larger vessels, leading to vascularization of the embryo.[1] In contrast to vasculogenesis, angiogenesis refers to the formation of new blood vessels from preexisting ones (Figure 1). It is required in many physiological and pathological conditions, including embryonic development, wound healing, tissue regeneration, and tumor growth. The process is regulated by a tight balance between pro- and antiangiogenic agents and involves a cascade of events of which migration of capillary endothelial cells is an essential component.[2] The basic concepts of the cellular and molecular machinery underlying endothelial cell migration have been obtained mostly from observations of cell culture systems (see the online data supplement, available at http://circres.ahajournals.org). However, the 3D environment within a whole organism is far more complex, and the cells must move between and among other cells while they interpret attractive and repulsive cues to choose their path. They must integrate and coordinate their adhesion with their surroundings and sense when to start and stop moving. Genetic dissection using in particular zebrafish[3,4] and mouse embryo[5] now offers the possibility to understand endothelial cell migration in a physiological context. The aim of this review is to make an integrative update on the signaling and physiological aspects of endothelial cell migration in angiogenesis.

Original received October 18, 2006; revision received January 11, 2007; accepted January 24, 2007.
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Circulation Research **is available at http://circres.ahajournals.org** **DOI: 10.1161/01.RES.0000259593.07661.1e**

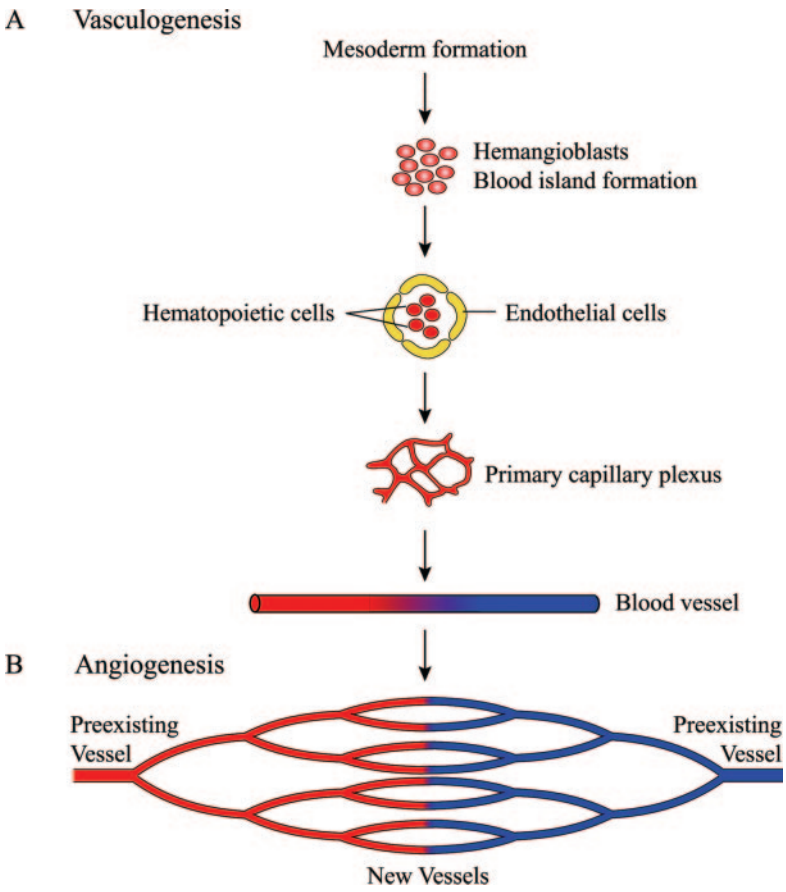

Figure 1. Genesis of the vascular system. A, During development, mesodermal cells differentiate into hemangioblasts leading to the formation of primitive blood islands. Then, the peripheral hemangioblasts differentiate into angioblasts, the precursors of endothelial cells. Following chemotactic and haptotactic activation, endothelial cells migrate allowing the fusion of the blood islands and their remodeling into tubular structures, giving rise to the first primitive vascular plexus. These vascular plexuses remodel into larger vessels, through the process of vasculogenesis, which leads to vascularization of the embryo. B, In contrast to vasculogenesis, angiogenesis is a neovascularization process by which new blood vessels form from preexisting ones. Adapted from Karkkainen et al.[125]

Actin Remodeling at the Heart of Endothelial Cell Migration: An Overview

Actin is a major cytoskeletal component of endothelial cells. It is composed of 43-kDa monomeric globular subunits (G-actin) that polymerize into helical filaments (F-actin). The assembly of F-actin is tightly associated with the hydrolysis of ATP by its intrinsic ATPase activity. Mg^{2+}/ATP-bound monomeric G-actin is incorporated into growing filaments at the barbed end. ATP-actin is then converted into ADP-actin, as actin monomers are shifted along the filaments toward the pointed end.[6] The constant remodeling of the actin cytoskeleton into filopodia, lamellipodia, and stress fibers (Figure 2) is essential for cell migration, as briefly overviewed below and discussed in detail by K. Mizumo and colleagues in a forthcoming article within this thematic review series.

Filopodia are membrane projections that contain long parallel actin filaments arranged in tight bundles. These particular structures act as sensors of motile stimuli. Classically, the formation of filopodia is regulated by activation of the small GTPase Cdc42 that associates with Wiskott–Aldrich syndrome proteins (WASPs). Lamellipodia are cytoplasmic protrusions that form at the leading edge of spreading or migrating cells.[7] These protrusions are approximately 1 to 5 μm wide and approximately 2 μm thick. The formation of lamellipodia is associated with important actin polymerization involving Rac and Arp2/3 complex. Stress fibers are actin filaments of inverted polarity linked by α-actinin and myosin and distributed along contractile fibers.[8] All 3 structures are essential to drive the several steps of actin-based endothelial cell motility as shown in (Figure 3A): (1) sensing of the motogenic signal by filopodia; (2) formation and protrusion of lamellipodia and pseudopodia-like forward extension; (3) attachment of the protrusions to the extracellular matrix (ECM); (4) stress fiber-mediated contraction of the cell body to allow forward progress; (5) rear release; and (6) recycling of adhesive and signaling materials.

Endothelial Cell Migration During Angiogenesis

Endothelial cell migration involves 3 major mechanisms, namely chemotaxis, the directional migration toward a gradient of soluble chemoattractants; haptotaxis, the directional migration toward a gradient of immobilized ligands; and mechanotaxis, the directional migration generated by mechanical forces.[9] Endothelial cell migration during angiogenesis is the integrated resultant of these 3 mechanisms. Typically, chemotaxis of endothelial cells is driven by growth factors such as VEGF and basic fibroblast growth factor (bFGF), whereas haptotaxis is associated with increased endothelial cell migration activated in response to integrins binding to ECM component.[5,10] Because of their location at the inner face of blood vessels, endothelial cells are constantly in contact with shear stress, which contributes to activate migratory pathways. In fact, there is now accumulating evidence that fluid shear stress initiates mechanotaxis and modulates the various steps of migration including extension at the leading edge, adhesion to the matrix, and

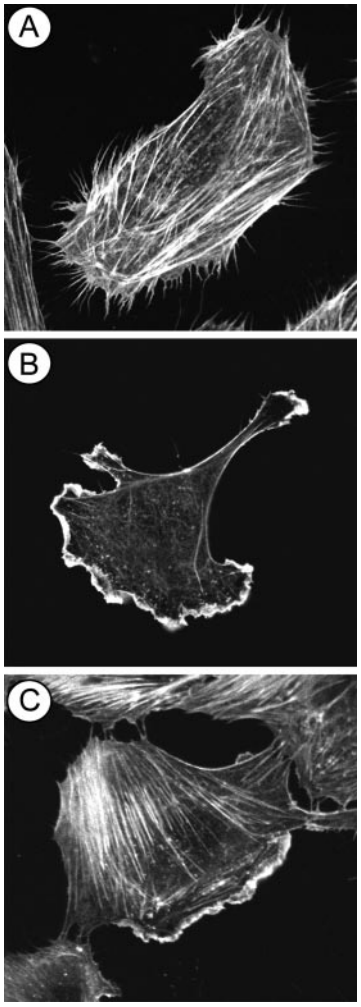

Figure 2. Actin structures involved in endothelial cell migration. HUVECs treated with VEGF form actin structures involved in endothelial cell migration (Houle François and J.H., unpublished observations, 2006). A, The filopodia are filamentous membrane projections that contain long parallel actin filaments arrange in tight bundles. These structures act as sensors of motile stimuli. B, The lamellipodia are cytoplasmic protrusions that contain a thick cortical network of actin filaments. They are found at the leading edge of migrating cells allowing their swimming-like motility. C, Stress fibers are bundles of actin filaments associated with myosin II and accessory proteins such as tropomyosins. These structures are anchored at focal adhesions and are required for the traction of the rear of the cells toward the leading edge during migration.

release of adhesions at the rear.[9] Hence, endothelial cell migration is a mechanically integrated molecular process that involves dynamic, coordinate changes in cell adhesion, signal transduction, and cytoskeletal dynamics and organization.

Chemotactic Endothelial Cell Migration

Many different cytokines are involved in the regulation of chemotactic endothelial cell migration during angiogenesis. The 3 major promoters of this type of actin-based motility are VEGF, bFGF, and angiopoietins. Other contributing cytokines include: FGF-2, hepatocyte growth factor, platelet-derived growth factor, epidermal growth factor, transforming growth factor-β, interleukins, tumor necrosis factor-α, platelet-activating factor, ephrins, soluble adhesion molecules, endoglin, and angiogenin. For the sake of integration and understanding, we have concentrated our review on VEGF and angiopoietins.

Vascular Endothelial Growth Factors and Their Receptors

VEGFs encompass a family of structurally related proteins that include placental-derived growth factor, VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E.[11] Human VEGF-A monomers exist as 5 different isoforms, of which $VEGF_{165}$ is the most abundant and active form and is generally referred to as VEGF. VEGF plays major roles in regulating the functions of endothelial cells. It is a potent angiogenic agent that regulates all the key steps of the angiogenic process, including endothelial cell proliferation and migration.[11–13] Following activation by hypoxia, reactive oxygen species (ROS), and angiotensin II, VEGF is produced by several types of cells, notably cancer cells, endothelial cells, and vascular smooth muscle cells, and it modulates endothelial cell functions via auto and paracrine pathways.[14–16] Interestingly, the full-length $VEGF\text{-}A_{165}b$ isoform, present in conditioned medium, inhibits endothelial proliferation and migration in a dose-dependent manner, which suggests the existence of endogenous inhibitory forms of VEGF that may play a role in the transition of an antiangiogenic state to a proangiogenic phenotype.[17]

VEGF exerts its effects after binding to homologous membrane tyrosine kinase receptors, VEGFR-1 (Flt-1), VEGFR-2 (Flk1Kdr), and VEGFR-3 (Flt4), that are expressed mainly by blood vessel endothelial cells and lymphatic endothelial cells.[18] Although they have different biological activities, VEGFRs all play essential roles in VEGF-induced angiogenesis because knockout mice for VEGFR-1, VEGFR-2, and VEGFR-3 are all embryonic lethal as a result of vascular defects.[19–21] Intriguingly, the affinity of VEGFR-1 for VEGF is much higher than that of VEGFR-2, but the signaling induced by the latter is the major way by which VEGF regulates endothelial cell migration. Like other tyrosine kinase receptors, VEGFR-2 undergoes ligand-induced dimerization and oligomerization, which activates its intrinsic tyrosine kinase activity resulting into auto- and transphosphorylation on specific tyrosine residues in the cytoplasmic domain. These tyrosine residues, when phosphorylated, are involved as docking sites to recruit molecules containing SH2, or PTB domains, and to convey migratory signals to downstream pathways.[18] Major autophosphorylation sites on VEGFR-2 have been ascribed as Y1175 and Y1214.[22,23] Other putatively important phosphorylated sites include Y951 in the kinase insert domain and Y1054 and Y1059 in the tyrosine kinase catalytic domain.[24,25]

Vascular Endothelial Growth Factor Receptor-2 in Chemotactic Endothelial Cell Migration

Role of the Rho Small GTPases

The activation of the small GTPases of the Rho family is centrally involved in regulating endothelial cell migration in response to activation of VEGFR-2. In particular, Cdc42 is involved in the formation of filopodia; these structures that act as sensors, underlying the "guidance migratory mecha-

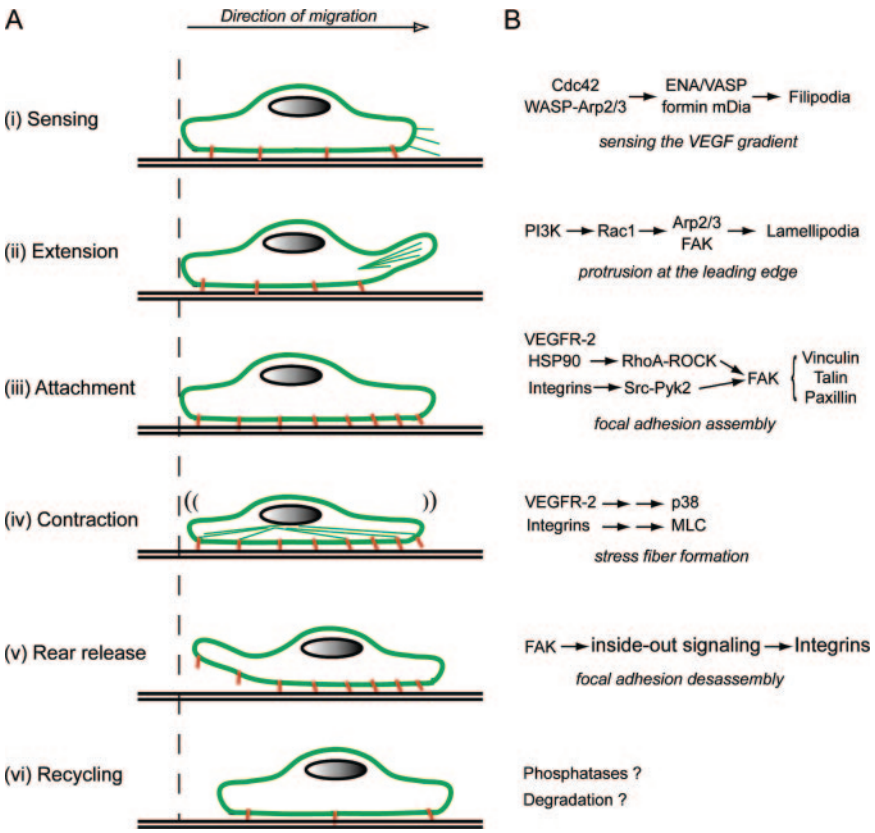

Figure 3. Major steps of endothelial cell migration. A, Endothelial cell migration can be divided in 6 sequential events: (i) Cdc42 dependent sensing of the motile stimuli by filopodia; (ii) cellular extension involving the Rac1-dependent formation of protruding lamellipodia; (iii) attachment of the protrusions to the extracellular matrix at focal adhesions; (iv) stress fiber–mediated contraction of the cell body allowing forward progression; (v) rear release by stress fiber–mediated traction forces; and (vi) recycling of the adhesive and signaling components. The major signaling events associated with each of these 6 steps are indicated in B.

nism" recently shown in early postnatal angiogenesis in the retina.[26] The concept of angiogenic guidance emerged by analogy with other guidance processes involving tip structures that contain sensor cells such as the growth cone in axonal guidance and tracheal tip cells during tracheal branching in *Drosophila*.[26–28] In both cases, the sensor cells use Cdc42-induced formation of dynamic filopodia to sense guidance cues and to migrate coordinately.[29,30] Interestingly, endothelial sprouts also extend multiple filopodia at their distal tips, indicating that growing vascular sprouts are endowed with specialized tip structures with potential functions in guidance and migration in response to a VEGF gradient.[26] The activation of Cdc42 downstream of VEGFR-2 is also involved in the formation of stress fibers by contributing to activate the p38 pathway.[23] On the other hand, VEGFR-2–mediated activation of Rac in concert with the activation of WAVE2 leads to the formation of lamellipodia assuring the swimming movement of the endothelial cells.[23,31]

RhoA, by contributing to phosphorylation of VEGFR-2 and activation of phosphatidylinositol 3-kinase (PI3K), is also an important determinant of endothelial cell migration.[32,33] The RhoA-mediated activation of PI3K regulates cell motility by generating phosphoinositides, like phosphoinositol$^{3-5}$ triphosphate, that influence various downstream motogenic events. In particular, the PI3K-derived inositol phosphates, in conjunction with calcium influx generated by phospholipase Cγ, regulate the function of a number of actin-regulating protein, such as profilin, cofilin and α-actinin.[12,34] PI3K also contributes to the activation of 3-phosphoinositide-dependent protein kinase-1 (PDK1), which leads to the activation of Akt/PKB (Akt/protein kinase B) and of a number of kinases of the AGC family potentially involved in cell migration.[35] Notably, as described below, eNOS is activated by Akt/PKB and, by producing NO, plays a role in endothelial cell migration and angiogenesis.[35] In addition to regulate endothelial cell migration via activation of the PI3K/NO pathway, the molecular link between VEGFR-2 and RhoA further involves activation of the Rho-associated kinase (ROCK) and focal adhesion kinase (FAK) phosphorylation on Ser732. This elicits an unfolding of FAK that unmasks Tyr407 making it accessible to Pyk2, also activated by VEGF downstream of $\alpha_v\beta_3$ integrins.[36,37] This aspect will be addressed in more details in the section on "haptotactic cell migration."

Role of NO

The role of NO as a major regulator of cell migration and angiogenesis is suggested by the observation that it is quickly produced by eNOS following its activation downstream of the VEGFR-2/PI3K/Akt-PKB axis in endothelial cells activated by VEGF.[35] It is further supported by the finding that knockout mice for eNOS show impaired angiogenesis in response to ischemia.[38,39] Moreover, inhibition of NO production blocks the chemotactic actions of VEGF, and NO transduces the increase of migration that results from VEGF exposure of bovine lung microvascular endothelial cells expressing an activated form of PKB.[40,41] Interestingly, eNOS is located in caveolae, a subset of lipid rafts that are prevalent on the plasma membrane of endothelial cells.[42] This particular localization is of utmost importance in modulating the

motogenic property of eNOS and NO. In particular, caveolin-1, a typical resident protein of caveolae, interacts with eNOS and negatively regulates its activity.[43] Given that caveolin-1 is also associated with VEGFR-2 and that the complex is dissociated by VEGF, an attractive possibility is that VEGF can contribute to activate eNOS and NO production by dissociating eNOS from caveolin-1.[44] Along these lines, eNOS cannot be properly activated in endothelial cells devoid of caveolae and the cells cannot migrate.[45,46] Together, these findings indicate that NO is an important messenger of VEGFR-2. It presumably modulates angiogenesis by inducing a vasodilatation-associated expansion of endothelial cell surface that enables a more proper response of the endothelium to angiogenic and promigratory agents.[12,40,47]

Role of Other Reactive Oxygen Species

The influence of ROS in regulating endothelial cell migration is not restricted to NO. In fact, ROS produced via activation of NADPH oxidase stimulate diverse redox signaling pathways leading to angiogenic responses including endothelial cell migration. In particular, VEGF stimulation increases ROS production via activation of Rac1-dependent NADPH oxidase and, thereafter, ROS are involved in VEGF-induced autophosphorylation of VEGFR-2.[48] The signaling properties of ROS are caused by the activation of several kinases including Src kinases and to the reversible oxidation of redox-sensitive protein tyrosine phosphatases (PTPs) and lipid phosphatase (PTEN).[49,50] In fact, several PTPs including SHP-1 and SHP-2 associate with VEGFR-2 in response to VEGF.[51,52] Their activation by ROS might contribute to stop the VEGF signals by dephosphorylating VEGFR-2. These specific aspects of VEGF signaling upstream of endothelial cell migration have recently been covered in an excellent review[48] and will not be further developed here.

Role of Nck

In response to VEGF, the increased actin polymerization required to trigger actin-based motility involves the recruitment of Nck to VEGFR-2. At least 2 converging signaling mechanisms were identified in endothelial cells. A first signal that emanates from VEGFR-2/Nck is associated with the recruitment of Nck to phospho-Tyr1214 within VEGFR-2 and it mediates actin polymerization and formation of stress fibers downstream from sequential activation of Fyn, Cdc42, MKK3 (mitogen-activated protein kinase kinase 3), SAPK2p38α (stress-activated protein kinase 2/p38α), and MAPKAP K2 (mitogen-activated protein kinase activated protein kinase 2) and phosphorylation of heat shock protein 27 (HSP27).[2,13,23,53] HSP27 is an actin-capping protein, and its phosphorylation has been proposed to release it from actin filaments, thus allowing addition of actin monomers and elongation of the filaments.[12,54,55] A second mechanism downstream of Nck recruitment to VEGFR-2 in endothelial cells involves the nucleation factor N-WASP (neuronal WASP), which is relocalized at the cell surface by Nck.[56,57] Then, N-WASP activates the Arp2/3 complex, a key regulator of actin nucleation and stress fibers in motile cells. Interestingly, Nck recruitment to VEGFR-2 triggers the assembly of focal adhesions via PAK activation, which may also contribute to enable the bundling of actin filaments into stress fibers.[58] On the other hand, the activity of LIM kinase also regulates actin remodeling and formation of stress fibers downstream of PI3K/ROCK and p38/MAPKAP K2 activation by VEGF. In turn, activated LIM kinase leads to phosphorylation of cofilin impairing actin depolymerization and thereby potentiating the VEGF-induced actin reorganization into stress fibers and endothelial cell migration[56,59] (Figure 4A).

Intriguingly, the residue Y1214 within VEGFR-2 is phosphorylated in all endothelial cell types, whereas phosphorylation of Y951 is restricted to certain endothelial cells that belong to immature vessels devoid of pericytes.[24] The adapter molecule TSAd/VRAP is recruited to phospho-Y951 within VEGFR-2, where it is tyrosine phosphorylated and forms a complex with Src to regulate stress fiber formation and endothelial cell migration. In turn, this contributes to increase endothelial cell migration during pathological angiogenesis and may explain why the recruitment of TSAd/VRAP is associated with cancer angiogenesis.[24]

Overall, these findings indicate that endothelial cell migration induced by VEGF results from several signaling pathways downstream of VEGFR-2 (Figures 3A and 3B and 4). Notably, the complementary role of signaling through p38 (actin polymerization) and FAK (focal adhesion turnover) in contributing to the formation of stress fibers emerges as an integrating concept given that these structures generate the contraction force required to pull the back of the cell and allow directed migration (Figure 4A and 4B).

Angiopoietins As Inducers of Endothelial Cell and Pericyte Migration and Their Roles in Vascular Stability

Angiopoietins (Ang) 1 to 4 are proangiogenic growth factors that specifically activate endothelial cells in a paracrine manner. Their action depends mainly on their binding to the tyrosine kinase receptor Tie-2, whereas the ligand for the related Tie-1 receptor remains to be identified. Ang1 is an activator of Tie-2 and Ang2 is known to antagonize the binding of Ang1 to Tie-2. Nonetheless, Ang2 also activates Tie-2 in a context-specific manner.[60] Similar phenotypes are observed in knockout mice for Ang1 or Tie-2. The primary vasculature is normal, but the mice die at embryonic day 12.5 from endocardial and myocardial defects.[61] A role for Ang3 and Ang4 is less defined, and knockout-mouse experiments have yet to be done.[62] However, it seems that Ang4, but not Ang3, is capable of binding and activating Tie-2. Ang2 is found at high levels at sites of vascular remodeling in adults. Ang3 expression is high in most tissues, whereas Ang4 expression seems to be restricted to the lung. These 2 members of the family are considered as interspecies orthologs (Ang3, mouse; Ang4, human).[62,63]

Angiopoietins have low mitogenic or proliferative activity for endothelial cells.[64] Nevertheless, Ang1 promotes in vivo angiogenesis in a Matrigel plug assay and both Ang1 and Ang2 increase endothelial cell migration and sprouting.[60,65,66] In particular, Ang1 induces polarized lamellipodia formation that is associated with the spatial redistribution of RhoA and Rac1, which may modulate their migratory functions.[67]

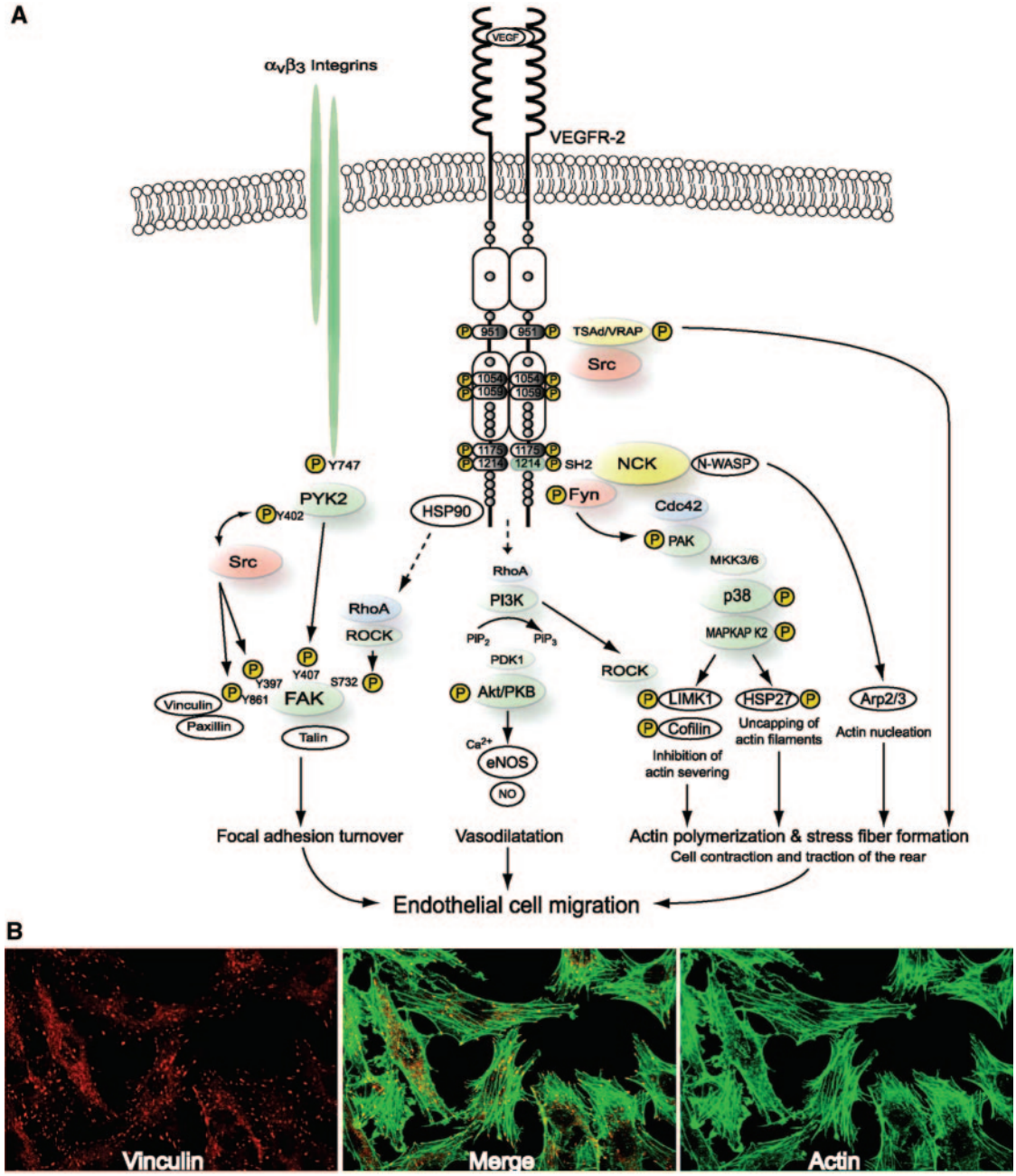

Figure 4. VEGFR-2 drives chemotactic endothelial cell migration by contributing to formation of stress fibers. A, The formation of stress fibers by binding of VEGF to VEGFR-2 requires a cooperative interaction between VEGFR-2 and integrins, especially integrin $\alpha_v\beta_3$. The activation of VEGFR-2 initiates its autophosphorylation on Tyr1214. Then, it follows the recruitment of Nck to VEGFR-2 and the sequential activation of Fyn and Cdc42, upstream of the p38 MAP kinase module. Activation of p38 leads to activation of MAPKAP K2 and LIM kinase, which contributes to increase the level of polymerized actin by phosphorylating HSP27 and cofilin, respectively. Of note, Nck can also trigger actin polymerization through activation of the WASP-Arp2/3 pathway. Concomitantly to the activation of p38, the binding of VEGF to VEGFR-2 triggers the recruitment of HSP90 to VEGFR-2, which initiates the activation of RhoA-ROCK and then the phosphorylation of FAK on Ser732. In turn, this changes the configuration of FAK, allowing the phosphorylation of Tyr407 by Pyk, downstream of integrin β_3. This favors the turnover of focal adhesions. Together, the p38-mediated actin polymerization and FAK-mediated turnover of focal adhesions contribute to the formation of stress fibers enabling cell migration by allowing endothelial cell contraction. The activation of PI3K downstream of VEGFR-2 activates Akt/PKB and eNOS. The subsequent production of NO is required for vasodilatation and endothelial cell migration. B, Actin stress fibers are anchored at focal adhesions, as shown in the colocalization of actin and vinculin, in porcine endothelial cells treated with VEGF.

Moreover, binding of Ang1 to Tie-2 initiates the recruitment of Dok-R to Tie-2, which elicits the activation of a signaling cascade that involves Nck and Crk, upstream of actin remodeling and cell migration.[68] Cell migration in response to Ang1 may also result from the activation of PI3K, downstream of Grb2 recruited to activated Tie-2.[69] In the presence of VEGF, Ang2 induces endothelial cell migration, proliferation, and sprouting, as well as an increase in the diameter of capillaries. In contrast, in the absence of VEGF, Ang2 does not promote cell migration; rather, it induces apoptosis of endothelial cells and regression of blood vessel.[70] Ang1 alone and Ang2 in the presence of VEGF are thus positive regulators of angiogen-

esis by a direct action on endothelial cell migration. Interestingly, hypoxia induces the expression of the angiopoietin-like family member ANGPTL4, which is followed by its storing in the surrounding ECM. In turn, immobilized ANGPTL4 limits the formation of focal adhesions by endothelial cells and thereby negatively regulates endothelial cell migration, angiogenesis as well as metastasis.[71,72] These new findings are important because they highlight other functions for angiopoietins in regulating endothelial cell migration and angiogenesis and because ANGPTL4 may reveal to be a useful new target to design antiangiogenic therapeutics in cancer.

In addition to their roles in regulating endothelial cell migration, another major contribution of angiopoietins to angiogenesis relies on their essential role in vessel stabilization and remodeling in vivo. The vessel stabilization induced by Ang1 may result directly from an inhibition of endothelial permeability or indirectly through stimulation of endothelial cell-dependent release of attractants like TGF-β and platelet-derived growth factor-B, which leads to an increased migration of pericytes and their recruitment to the nascent vessel.[73] The angiopoietin-mediated recruitment of pericytes to the growing vessels is further regulated by FGF-4–induced expression of VEGF, which contributes to increase the levels of matrix metalloproteinase-1 (MMP-1) and inhibits tissue inhibitor of metalloproteinase-1 (TIMP-1), thus facilitating pericyte invasion via ECM degradation.[74,75] Following their recruitment to the nascent vessels, pericytes play a major role in vascular stability by inducing the deposit of a matrix and initiating signals that allows endothelial cell differentiation and quiescence.[76]

In sum, angiopoietins are major regulators of angiogenesis either by a direct modulation of endothelial functions such as actin remodeling and migration or by an indirect effect that is mediated through cytokines released by activated endothelial cells, which in turn activates pericytes via a paracrine pathway.

Haptotactic Endothelial Cell Migration

Gradients of immobilized ECM components such as collagen drive endothelial cell migration independently of chemotactic cytokines.[77] This type of migration is called haptotaxis, and it may be defined as directed cell crawling toward ECM proteins. The adhesive interactions that trigger haptotaxis of endothelial cells are governed mainly by components of ECM and integrins.

Role of ECM in Haptotactic Endothelial Cell Migration

The vascular endothelium is supported by an ECM that is assembled by endothelial cells, pericytes, and supporting smooth muscle cells. This ECM is critical for endothelial cell migration. In fact, depending on its nature and the cellular context, it may promote or inhibit endothelial cell migration. In the absence of angiogenic stimulus, the ECM contributes to maintain the endothelial cells in a quiescent state. However, during the early steps of angiogenesis, the ECM is broken down by metalloproteases, which initiates motogenic signals generated either by the proteolytic fragments or the release of embedded angiogenic stimuli (VEGF, bFGF).[78] We review here the role of ECM in initiating and sustaining endothelial cell migration.

Vascular endothelial cells should adhere to ECM to migrate either dependently or independently of chemoattractants. In that regard, many of the interstitial and provisional ECM components encountered during angiogenesis, such as fibrin and collagen I, are capable of supporting chemotactic migration.[79] On the other hand, several studies have shown that gradients of ECM components such as fibronectin can, by themselves, guide and regulate the speed of haptotactic cell migration.[80,81] Although the significance of haptotaxis in vivo has been proven for several types of cells including T cells, its role is still debated in the case of endothelial cells.[77,82] Nevertheless, given that interstitial collagen has marked haptotactic properties in vitro, it is plausible that the high concentrations of interstitial collagen encountered by endothelial cells during sprouting may drive haptotactic cell migration in vivo.[83] Haptotaxis may be especially important in driving endothelial cell migration during repair of large vessel.[84]

The individual importance of specific ECM proteins in supporting haptotactic and chemotactic endothelial cell migration is still unclear because of signaling and functional overlap. For example, cytokine-dependent chemotactic cell migration may be supported by functional converging signaling initiated by the attachment to 2 different ECM. Moreover, a given integrin can bind to similar sites (eg, RGD peptide) on 2 different ECM to promote similar haptotactic signals. Furthermore, a variety of ECM components provide sufficient support for endothelial cell migration, although not with equal potency. In addition, there is evidence indicating that components of ECM function cooperatively.[85] For example, laminins 8 and 10 have complementary effects on human skin dermal microvascular endothelial cells migration and tube formation.[86] Interestingly, as we reported above for ANGPTL4, ECM proteins or their breakdown products may act as scaffolds that sequester cytokines that may either initiate or inhibit endothelial cell migration. These ECM/cytokine scaffolds may provide determinant cues to guide endothelial cell migration and sprout formation. In particular, collagenous matrices may function as release carriers of bFGF and VEGF. This feature is clinically promising and offers the possibility to generate a scaffold able to control angiogenesis and tissue regeneration.[87,88]

Endothelial cells are connected to ECM at focal adhesions. These latter are sites of tight adhesion between the membrane and the ECM on one hand, and the membrane and the cytoskeleton on the other. They are assembled following the recruitment of signaling molecules such as FAK and paxillin and of structural and membrane actin-anchoring proteins such as talin, vinculin, tensin, and α-actinin, which links the microfilament network to the adhesive molecules integrins at their sites of clustering.[89,90] These proteins provide a structural link allowing the anchorage of stress fibers to the membrane and to integrins. In migrating endothelial cells, focal adhesions and actin stress fibers are aligned in the direction of migration, supporting their participation in the process of actin-based motility (Figure 4B).[91] Moreover, in migrating endothelial cells, the forward movement is tightly

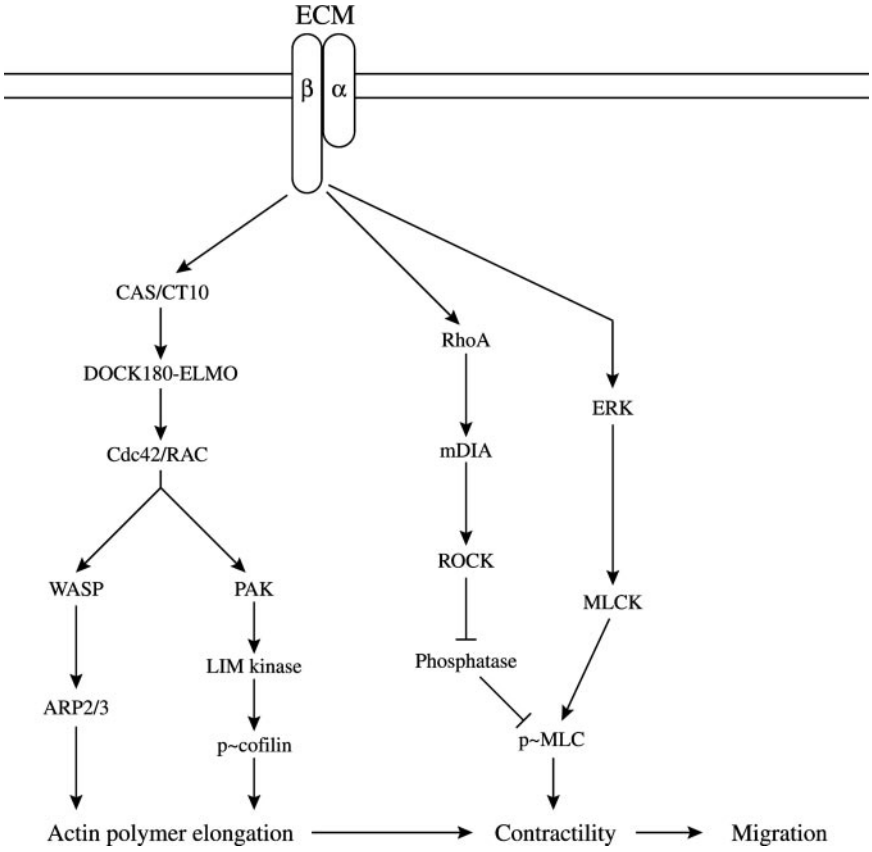

Figure 5. Integrin-driven haptotactic endothelial cell migration. Several integrins, namely $\alpha_v\beta_3$ and $\alpha_v\beta_5$, are involved in driven haptotactic cell migration. Following their binding to proteins of the extracellular matrix, integrins cluster and become activated. Several pathways are then activated that converge on actin polymerization, cellular contractility and cell migration. A putative integrating model involves the activation of integrins, which triggers the recruitment of the docking scaffold module Cas/Crk upstream of the GTPase-activating proteins such as DOCK1 and ELMO. Then follows the activation of the small GTPases Rac1 and Cdc42, which leads to actin polymerization via the activation of the WASP–Arp2/3 and PAK–LIM kinase–cofilin pathways. Concomitantly, the activation of integrins activates the RhoA and ERK pathways leading to increase endothelial contractility by contributing to phosphorylation of myosin light chain II via ROCK and MLC kinase, respectively. Together, integrin-mediated actin polymerization and contractility trigger haptotactic endothelial cell migration.

associated with the rapid assembly/disassembly of the focal adhesions, which allows the adhesion/de-adhesion processes inherent to migration.

Integrins in Haptotactic Cell Migration

Integrins are a family of heterodimeric transmembrane adhesion receptors that contains 16 α and 8 β subunits that associate to form 24 different receptors that bind to ECM with distinct yet often overlapping specificity.[92] Several studies pointed to $\alpha_v\beta_3$ integrin, a receptor for both fibronectin and vitronectin, and $\alpha_v\beta_5$ integrin, a vitronectin receptor, as major players in blood vessel formation.[93] Indeed, the blockade of both $\alpha_v\beta_3$ and or $\alpha_v\beta_5$ integrins disrupts tumor and experimental angiogenesis. For example, patients with melanoma benefit from Vitaxin, a β_3 integrin antagonist.[94,95] On the other hand, genetic experiments showed that successful vasculogenesis and angiogenesis depend on fibronectin and $\alpha_5\beta_1$ integrin rather than on α_v integrins.[93] Accordingly, antagonists of integrin $\alpha_5\beta_1$ block tumor angiogenesis and have entered clinical trials.[96] The apparent discrepancy between these results is difficult to explain but likely results from cellular context specificity. Nevertheless, they all point to the crucial role played by integrins in angiogenesis.

Integrins modulate angiogenic migration by enabling endothelial cell to adhere to ECM (Figure 5). Notably, the activation of integrins allows the functional connection between focal adhesions and actin cytoskeleton that is required to drive cell migration.[10,97] Moreover, integrins are active in initiating and regulating angiogenic signaling.[98] Signaling from integrins requires their oligomerization and engagement with their ligands and it starts from focal adhesions. Activation of integrins at focal adhesions may be triggered by a higher density of ECM, which in turn stimulates Rac and Cdc42 to induce actin remodeling, presumably through activation of the Arp2/3 complex and induction of actin filament via WASP. Alternatively, the Rho GTPases also activate PAK, which enhances the level of polymerized actin by activating LIM kinase. Interestingly, Rac and Cdc42, via an inside-out mechanism, may recruit more integrins at the cell protrusions thus forming a positive-feedback loop to amplify the haptotactic signal that originates from the concentration gradient of ECM.[99] Possible elements upstream of the GTPases, especially Rac, may involve tyrosine phosphorylation of the docking protein Cas (Crk-associated substrate) and the recruitment and binding of Crk (CT10 regulator of kinase) and the activation of the GTPase-activating proteins DOCK180 and ELMO.[100] The Cas/Crk scaffold module is present in endothelial cells and its activation is essential for integrin-directed cell migration.[100,101] In particular, the activation of this pathway is induced following the activation of integrin $\alpha_3\beta_1$ and Rac by laminin-10/11, which triggers the migration of endothelial cells.[102] On the other hand, integrin-induced activation of RhoA mediates the assembly and contraction of the actomyosin fibers, which in turn contributes to pulling the trailing edge forward during migration. The cascade downstream of RhoA involves a cooperative interaction between mDia and ROCK to induce the assembly of actomyosin fibers. In that regard, activation of ROCK inhibits myosin light chain (MLC) phosphatase, thus increasing the level of phospho-MLC and contraction of the acto-

myosin fibers.[98] Phosphorylation of MLC during collagen-mediated haptotaxis may also be induced via an ERK-dependent activation of MLC kinase.[10]

Crosstalk Between Integrins and Growth Factors

There is a wealth of evidence that supports that integrins and growth factor signaling pathways interact to co-coordinately integrate the message initiated by both types of receptors. In particular, VEGF and bFGF enhance the expression and the activation of several integrins, such as $\alpha_v\beta_3$, $\alpha_v\beta_5$ and $\alpha_5\beta_1$, that are involved in angiogenesis.[103] Conversely, several endogenous inhibitors of angiogenesis such as endostatin exert their functions by blocking integrins.[98] Other crosstalk mechanisms involve interaction at the receptors level. For example, $\alpha_5\beta_1$ integrin mediates fibronectin-induced epithelial cell proliferation through activation of the EGF receptors.[104] Similarly, $\alpha_v\beta_3$ integrin associates with platelet-derived growth factor-β and VEGFR-2 to potentiate their activity.[105–107] Little is known, however, about how the signals initiated by growth factor/integrin receptor complexes are integrated by the cells to activate the appropriate targets. Integrin-linked kinase integrates the insulin and fibronectin-dependent signals and FAK integrates the signal generated by integrins and the EGF and platelet-derived growth factor receptors.[108,109] FAK is a converging signaling point between VEGFR-2 and integrin $\alpha_v\beta_3$ and it controls the assembly/disassembly of focal adhesions that is necessary with regulation of actin polymerization for endothelial cell migration. More recently, as mentioned previously, we found that activation of VEGFR-2 initiates the phosphorylation of Ser732 within FAK, which triggers a conformational change within the FAK structure, thereby unmasking Tyr407 making it accessible to direct phosphorylation by Pyk2, downstream of integrin β_3.[37] Interestingly, FAK is also central to the regulation of endothelial cell migration by the VEGFR-2/integrin $\alpha_v\beta_5$ complex.[110,111] Some findings indicate that Src kinase can coordinate specific growth factor and ECM inputs by recruiting integrin $\alpha_v\beta_5$ into a FAK-containing signaling complex during growth factor-mediated biological responses.[112] Thus, it seems that integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ can induce endothelial cell migration in response to VEGF by cooperating with VEGFR-2. Nevertheless, if evidence exist that signals from these integrins transit through similar molecules such as Src and FAK, it is clear that both integrins have different implications in angiogenesis.[113] Notably, integrin $\alpha_v\beta_5$ but not $\alpha_v\beta_3$ requires insulin-like growth factor stimulation for integrin-mediated cell migration in vitro and metastasis in vivo.[113]

Several studies have shown that cell–cell adhesions inhibit cell migration and that they should be quickly broken to allow migration. In that regard, VEGF is very efficient in disrupting endothelial cell–cell contacts by dissociating the VE-cadherin/β-catenin complex at adherens junctions.[114,115] Interestingly, recent studies indicate that VEGFR-2–mediated angiogenic signaling is amplified by its association with VE-cadherin. Given that $\alpha_2\beta_1$ integrin–mediated adhesion on type I collagen decreases the localization of E-cadherin and β-catenin in cell–cell contacts, this finding raises the possibility that integrins may regulate VEGF signaling by modulating the VE-cadherin/VEGFR-2 interaction.[116,117] These recent discoveries are important because they highlight that the VEGF-productive signaling does not only require crosstalk with integrins but also with cadherins.

Overall, the crosstalk between integrins and growth factors receptors enhances the individual potency of both receptors in signaling to cell migration. Nevertheless, much remains to be done to fully understand the mechanisms involved and how signals from both receptors contribute to coordinately integrate chemotaxis and haptotaxis.

Mechanotactic Endothelial Cell Migration

Given that endothelial cells line the interior of the blood vessels, they are in constant contact with fluid shear stress, the tangential component of hemodynamic stress.[9] Shear stress is a friction exerted on the endothelium by the blood flow. If uniformly applied over the entire surface of the endothelium, the force exerted by shear stress is of 1 pN/μm^2, which is roughly 1000 to 5000 times weaker than the traction forces of focal adhesions.[118] However, shear stress regulates the migration of endothelial cells, presumably more so than does chemotaxis in large vessels where convection is stronger.[9] In the microcirculation, shear stress may guide endothelial cells along the interstitial tunnels during wound healing. In that case, it promotes the formation of lamellipodia and endothelial cell migration in the flow direction. This suggests that the cytoskeleton and the cell–ECM interaction are polarized under flow. Shear stress influences each step of cell migration, as described in Figure 6, and it influences both chemotactic and haptotactic cell migration. The endothelial cells response to shear stress involves dissociation of the cell–cell contacts and the activation of several signaling pathways that remodel the actin cytoskeleton to trigger endothelial cell migration in a polarized manner.[9]

There is evidence indicating that the extracellular mechanical force generated by shear stress is sensed by integrins or by the cytoskeleton itself. The integrins lie on the basal surface of the cells, whereas blood flow exerts its pressure on the apical surface of the endothelium. A layer of glycocalyx protects the endothelium and the embedded glycoproteins, such as syndecan-4, transmit the forces generated by shear stress to integrins and to cell–cell adhesion components, via signaling to the cytoskeleton.[118] Integrins are thus activated by shear stress, which in turn contributes to activate outside-in and inside-out motogenic signaling pathways involving particularly the Rho GTPases.

The small Rho-family GTPases are crucial regulators of actin dynamics in response to shear-stress. In fact, shear stress differentially regulates Rho GTPases at different location inside endothelial cells, presumably through regulating microtubules dynamics. In particular shear stress may induce microtubule elongation in the flow direction, which in turn activates Rac to promote actin polymerization and thus lamellipodia protrusion in the flow direction.[9] On the other hand, Rac promotes microtubule elongation through PAK, thus forming a positive feedback loop for microtubule elongation and Rac activation at the cell front. Meanwhile, Rac inhibits the activation of RhoA in the lamellipodia so that RhoA activity is restricted to the back of the cell, generating

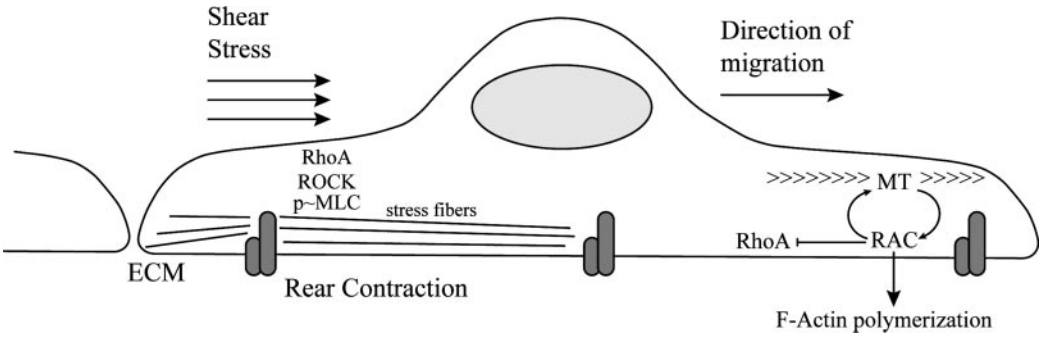

Figure 6. Shear stress and mechanotactic endothelial cell migration. Shear stress, the tangential component of hemodynamics stress, is sensed by the luminal membrane of endothelial cells and its associated receptors. The shear stress signal is then transmitted throughout the cells, where it activates intricate network of outside-in– and inside-out–directed signaling pathways that contribute to cell migration. In particular, shear stress regulates the Rho GTPases at different locations inside the cells in part by regulating microtubule dynamics. For example, shear stress contributes to microtubule elongation (MT), which in turn activates Rac and promotes actin polymerization and polarized protrusion of lamellipodia in the flow direction, favoring directed migration. This initiates a positive feedback loop because activated Rac may promote further elongation of the microtubule. On the other hand, the activation of Rac inhibits RhoA in the lamellipodia so that activated RhoA remains confined at the rear of the cells, where it would be involved in conferring contractility and rear detachment. Activation of RhoA and cell contraction may also result from increased membrane fluidity, leading to ROCK-induced activation of MLC. Hence, shear stress regulates in part endothelial cell migration by favoring the Rac-mediated formation of lamellipodia and RhoA-dependent increase of endothelial contractility. Adapted from Li.[9]

the contraction required for rear detachment and migration.[119] Alternatively, mechanotransduction through cell–ECM adhesions, cell–cell junctions, and endothelial cell plasma membranes may be involved in the shear stress activation of Rho GTPases. In particular, by increasing membrane fluidity, shear stress initiates signaling cascades from caveolae, which regulates the activity of specific Rho GTPases, especially RhoA, and eNOS.[9,33,120,121] In turn, this will increase the contractility of stress fibers at the rear of endothelial cells. In addition, shear stress, by regulating the Ca^{2+} exchange from the caveolae, may enhance the activity of ROCK increasing actin/myosin contraction and promoting detachment at the rear of migrating endothelial cells.

Hence, shear stress is a continuous stimulus for endothelial cells and, by mechanically influencing the response of endothelial cells to haptotactic and chemotactic signals, it importantly contributes to angiogenic cell migration. A very nice and complete review on the topic has recently been published by the Li et al.[9]

Concluding Remarks

Endothelial cell migration is an essential component of angiogenesis that requires a tight regulation of the contractile and noncontractile states of the cell. These processes require the integration of signals elicited by chemotactic, haptotactic, and mechanotactic stimuli. In turn, this is associated with the activation of intracellular pathways that converge on cytoskeleton remodeling. This includes the pathways involving activation of the small GTPases of the Rho family, PI3K and eNOS; SAPK2/p38; and phosphorylation of FAK. A critical question for future studies will be to determine how these pathways interact and how their timing is adjusted in the cell to generate appropriate cell migration. Future studies should also attempt to integrate linear data into a 4D model defining the spatiotemporal parameters that regulate the motile potential of a moving cell. Another major challenge for the future is to better understand tensegrity and especially how actin, microtubules, and intermediate filaments cooperate to coordinately regulate cell migration, as previously proposed.[122–124] This will yield major breakthroughs in understanding cell migration and angiogenesis and will hopefully identify new targets for chemotherapeutic interventions.

Acknowledgments

We thank François Houle for reading the manuscript, for help with preparing the figures, and for providing the photographic images presented in Figure 2.

Sources of Funding

The work was supported by a grant from The Canadian Institutes of Health and Research.

Disclosures

None.

References

1. Risau W, Flamme I. Vasculogenesis. *Annu Rev Cell Dev Biol*. 1995;11:73–91.
2. Rousseau S, Houle F, Landry J, Huot J. p38 MAP kinase activation by vascular endothelial growth factor mediates actin reorganization and cell migration in human endothelial cells. *Oncogene*. 1997;15:2169–2177.
3. Yaniv K, Isogai S, Castranova D, Dye L, Hitomi J, Weinstein BM. Live imaging of lymphatic development in the zebrafish. *Nat Med*. 2006;12:711–716.
4. Jin SW, Beis D, Mitchell T, Chen JN, Stainier DY. Cellular and molecular analyses of vascular tube and lumen formation in zebrafish. *Development*. 2005;132:5199–5209.
5. Giroux S, Tremblay M, Bernard D, Cardin-Girard JF, Aubry S, Larouche L, Rousseau S, Huot J, Landry J, Jeannotte L, Charron J. Embryonic death of Mek1-deficient mice reveals a role for this kinase in angiogenesis in the labyrinthine region of the placenta. *Curr Biol*. 1999;9:369–372.
6. Disanza A, Steffen A, Hertzog M, Frittoli E, Rottner K, Scita G. Actin polymerization machinery: the finish line of signaling networks, the starting point of cellular movement. *Cell Mol Life Sci*. 2005;62:955–970.
7. Small JV, Stradal T, Vignal E, Rottner K. The lamellipodium: where motility begins. *Trends Cell Biol*. 2002;12:112–120.
8. Small JV, Resch GP. The comings and goings of actin: coupling protrusion and retraction in cell motility. *Curr Opin Cell Biol*. 2005;17:517–523.
9. Li S, Huang NF, Hsu S. Mechanotransduction in endothelial cell migration. *J Cell Biochem*. 2005;96:1110–1126.

10. 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.
11. Zachary AA, Montgomery RA, Leffell MS. Factors associated with and predictive of persistence of donor-specific antibody after treatment with plasmapheresis and intravenous immunoglobulin. *Hum Immunol*. 2005; 66:364–370.
12. Rousseau S, Houle F, Huot J. Integrating the VEGF signals leading to actin-based motility in vascular endothelial cells. *Trends Cardiovasc Med*. 2000;10:321–327.
13. Lamalice L, Houle F, Huot J. Phosphorylation of Tyr1214 within VEGFR-2 triggers the recruitment of Nck and activation of Fyn leading to SAPK2/p38 activation and endothelial cell migration in response to VEGF. *J Biol Chem*. 2006;281:34009–34020.
14. Barleon B, Siemeister G, Martiny-Baron G, Weindel K, Herzog C, Marme D. Vascular endothelial growth factor up-regulates its receptor fms-like tyrosine kinase 1 (FLT-1) and a soluble variant of FLT-1 in human vascular endothelial cells. *Cancer Res*. 1997;57:5421–5425.
15. Chua CC, Hamdy RC, Chua BH. Upregulation of vascular endothelial growth factor by H2O2 in rat heart endothelial cells. *Free Radic Biol Med*. 1998;25:891–897.
16. Brogi E, Schatteman G, Wu T, Kim EA, Varticovski L, Keyt B, Isner JM. Hypoxia-induced paracrine regulation of vascular endothelial growth factor receptor expression. *J Clin Invest*. 1996;97:469–476.
17. Bates DO, Cui TG, Doughty JM, Winkler M, Sugiono M, Shields JD, Peat D, Gillatt D, Harper SJ. VEGF165b, an inhibitory splice variant of vascular endothelial growth factor, is down-regulated in renal cell carcinoma. *Cancer Res*. 2002;62:4123–4131.
18. Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling - in control of vascular function. *Nat Rev Mol Cell Biol*. 2006;7:359–371.
19. Fong GH, Rossant J, Gertsenstein M, Breitman ML. Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. *Nature*. 1995;376:66–70.
20. Shalaby F, Rossant J, Yamaguchi TP, Gertsenstein M, Wu XF, Breitman ML, Schuh AC. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. *Nature*. 1995;376:62–66.
21. Goishi K, Klagsbrun M. Vascular endothelial growth factor and its receptors in embryonic zebrafish blood vessel development. *Curr Top Dev Biol*. 2004;62:127–152.
22. Takahashi T, Yamaguchi S, Chida K, Shibuya M. A single autophosphorylation site on KDR/Flk-1 is essential for VEGF-A-dependent activation of PLC-gamma and DNA synthesis in vascular endothelial cells. *EMBO J*. 2001;20:2768–2778.
23. Lamalice L, Houle F, Jourdan G, Huot J. Phosphorylation of tyrosine 1214 on VEGFR2 is required for VEGF-induced activation of Cdc42 upstream of SAPK2/p38. *Oncogene*. 2004;23:434–445.
24. Matsumoto T, Bohman S, Dixelius J, Berge T, Dimberg A, Magusson P, Wang L, Wikner C, Qi JH, Wernstedt C, Wu J, Bruheim S, Mugishima H, Mukhopadhyay D, Spurklnd A, Claesson-Welsh L. VEGF receptor-2 Y951 signaling and a role for the adapter molecule TSAd in tumor angiogenesis. *EMBO J*. 2005;24:2342–2353.
25. Matsumoto T, Claesson-Welsh L. VEGF receptor signal transduction. *Sci STKE.* 2001;2001:RE21.
26. Gerhardt H, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, Abramsson A, Jeltsch M, Mitchell C, Alitalo K, Shima D, Betsholtz C. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. *J Cell Biol*. 2003;161:1163–1177.
27. Huot J. Ephrin signaling in axon guidance. *Prog Neuropsychopharmacol Biol Psychiatry*. 2004;28:813–818.
28. Cabernard C, Affolter M. Distinct roles for two receptor tyrosine kinases in epithelial branching morphogenesis in Drosophila. *Dev Cell*. 2005;9: 831–842.
29. Kater SB, Rehder V. The sensory-motor role of growth cone filopodia. *Curr Opin Neurobiol*. 1995;5:68–74.
30. Ribeiro C, Ebner A, Affolter M. In vivo imaging reveals different cellular functions for FGF and Dpp signaling in tracheal branching morphogenesis. *Dev Cell*. 2002;2:677–683.
31. Yamazaki D, Suetsugu S, Miki H, Kataoka Y, Nishikawa S, Fujiwara T, Yoshida N, Takenawa T. WAVE2 is required for directed cell migration and cardiovascular development. *Nature*. 2003;424:452–456.
32. van Nieuw Amerongen GP, Koolwijk P, Versteilen A, van-Hinsbergh VW. Involvement of RhoA/Rho kinase signaling in VEGF-induced endothelial cell migration and angiogenesis in vitro. *Arterioscler Thromb Vasc Biol*. 2003;23:211–217.
33. Gingras D, Lamy S, Beliveau R. Tyrosine phosphorylation of the vascular endothelial-growth-factor receptor-2 (VEGFR-2) is modulated by Rho proteins. *Biochem J*. 2000;348:273–280.
34. Qi JH, Claesson-Welsh L. VEGF-induced activation of phosphoinositide 3-kinase is dependent on focal adhesion kinase. *Exp Cell Res*. 2001;263:173–182.
35. Williams MR, Arthur JS, Balendran A, van der Kaay J, Poli V, Cohen P, Alessi DR. The role of 3-phosphoinositide-dependent protein kinase 1 in activating AGC kinases defined in embryonic stem cells. *Curr Biol*. 2000;10:439–448.
36. Le Boeuf F, Houle F, Huot J. Regulation of vascular endothelial growth factor receptor 2-mediated phosphorylation of focal adhesion kinase by heat shock protein 90 and Src kinase activities. *J Biol Chem*. 2004;279: 39175–39185.
37. Le Boeuf F, Houle F, Sussman M, Huot J. Phosphorylation of focal adhesion kinase (FAK) on Ser732 is induced by rho-dependent kinase and is essential for proline-rich tyrosine kinase-2-mediated phosphorylation of FAK on Tyr407 in response to vascular endothelial growth factor. *Mol Biol Cell*. 2006;17:3508–3520.
38. Garcia-Cardena G, Fan R, Shah V, Sorrentino R, Cirino G, Papapetropoulos A, Sessa WC. Dynamic activation of endothelial nitric oxide synthase by Hsp90. *Nature*. 1998;392:821–824.
39. Murohara T, Witzenbichler B, Spyridopoulos I, Asahara T, Ding B, Sullivan A, Losordo DW, Isner JM. Role of endothelial nitric oxide synthase in endothelial cell migration. *Arterioscler Thromb Vasc Biol*. 1999;19:1156–1161.
40. Morales-Ruiz M, Fulton D, Sowa G, Languino LR, Fujio Y, Walsh K, Sessa WC. Vascular endothelial growth factor-stimulated actin reorganization and migration of endothelial cells is regulated via the serine/threonine kinase akt. *Circ Res*. 2000;86:892–896.
41. Morales-Ruiz M, Lee MJ, Zollner S, Gratton JP, Scotland R, Shiojima I, Walsh K, Hla T, Sessa WC. Sphingosine 1-phosphate activates Akt, nitric oxide production, and chemotaxis through a Gi protein/phosphoinositide 3-kinase pathway in endothelial cells. *J Biol Chem*. 2001;276: 19672–19677.
42. Mineo C, Shaul PW. Circulating cardiovascular disease risk factors and signaling in endothelial cell caveolae. *Cardiovasc Res*. 2006;70:31–41.
43. Maniatis NA, Brovkovych V, Allen SE, John TA, Shajahan AN, Tiruppathi C, Vogel SM, Skidgel RA, Malik AB, Minshall RD. Novel mechanism of endothelial nitric oxide synthase activation mediated by caveolae internalization in endothelial cells. *Circ Res*. 2006;99: 870–877.
44. Labrecque L, Royal I, Surprenant DS, Patterson C, Gingras D, Beliveau R. Regulation of vascular endothelial growth factor receptor-2 activity by caveolin-1 and plasma membrane cholesterol. *Mol Biol Cell*. 2003; 14:334–347.
45. Sonveaux P, Martinive P, DeWever J, Batova Z, Daneau G, Pelat M, Ghisdal P, Gregoire V, Dessy C, Balligand JL, Feron O. Caveolin-1 expression is critical for vascular endothelial growth factor-induced ischemic hindlimb collateralization and nitric oxide-mediated angiogenesis. *Circ Res*. 2004;95:154–161.
46. Navarro A, Anand-Apte B, Parat MO. A role for caveolae in cell migration. *FASEB J*. 2004;18:1801–1811.
47. Parenti A, Morbidelli L, Cui XL, Douglas JG, Hood JD, Granger HJ, Ledda F, Ziche M. Nitric oxide is an upstream signal of vascular endothelial growth factor-induced extracellular signal-regulated kinase1/2 activation in postcapillary endothelium. *J Biol Chem*. 1998; 273:4220–4226.
48. Ushio-Fukai M. Redox signaling in angiogenesis: role of NADPH oxidase. *Cardiovasc Res*. 2006;71:226–235.
49. Kwon J, Lee SR, Yang KS, Ahn Y, Kim YJ, Stadtman ER, Rhee SG. Reversible oxidation and inactivation of the tumor suppressor PTEN in cells stimulated with peptide growth factors. *Proc Natl Acad Sci U S A*. 2004;101:16419–16424.
50. Chiarugi P, Cirri P. Redox regulation of protein tyrosine phosphatases during receptor tyrosine kinase signal transduction. *Trends Biochem Sci*. 2003;28:509–514.
51. Kroll J, Waltenberger J. The vascular endothelial growth factor receptor KDR activates multiple signal transduction pathways in porcine aortic endothelial cells. *J Biol Chem*. 1997;272:32521–32527.
52. Guo DQ, Wu LW, Dunbar JD, Ozes ON, Mayo LD, Kessler KM, Gustin JA, Baerwald MR, Jaffe EA, Warren RS, Donner DB. Tumor necrosis factor employs a protein-tyrosine phosphatase to inhibit activation of KDR and vascular endothelial cell growth factor-induced endothelial cell proliferation. *J Biol Chem*. 2000;275:11216–11221.

53. Yu J, Bian D, Mahanivong C, Cheng RK, Zhou W, Huang S. p38 mitogen-activated protein kinase regulation of endothelial cell migration depends on urokinase plasminogen activator expression. *J Biol Chem*. 2004;279:50446–50454.
54. Lavoie JN, Lambert H, Hickey E, Weber LA, Landry J. Modulation of cellular thermoresistance and actin filament stability accompanies phosphorylation-induced changes in the oligomeric structure of heat shock protein 27. *Mol Cell Biol*. 1995;15:505–516.
55. Landry J, Huot J. Modulation of actin dynamics during stress and physiological stimulation by a signaling pathway involving p38 MAP kinase and heat- shock protein 27. *Biochem Cell Biol*. 1995;73:703–707.
56. Gong C, Stoletov KV, Terman BI. VEGF treatment induces signaling pathways that regulate both actin polymerization and depolymerization. *Angiogenesis*. 2004;7:313–321.
57. Li W, Fan J, Woodley DT. Nck/Dock: an adapter between cell surface receptors and the actin cytoskeleton. *Oncogene*. 2001;20:6403–6417.
58. Stoletov KV, Ratcliffe KE, Spring SC, Terman BI. NCK and PAK participate in the signaling pathway by which VEGF stimulates the assembly of focal adhesions. *J Biol Chem*. 2001;276:22748–22755.
59. Kobayashi M, Nishita M, Mishima T, Ohashi K, Mizuno K. MAPKAPK-2-mediated LIM-kinase activation is critical for VEGF-induced actin remodeling and cell migration. *EMBO J*. 2006;25:713–726.
60. Teichert-Kuliszewska K, Maisonpierre PC, Jones N, Campbell AI, Master Z, Bendeck MP, Alitalo K, Dumont DJ, Yancopouos GD, Stewart DJ. Biological action of angiopoietin-2 in a fibrin matrix model of angiogenesis is associated with activation of Tie2. *Cardiovasc Res*. 2001;49:659–670.
61. Suri C, Jones PF, Patan S, Bartunkova S, Maisonpierre PC, Davis S, Sato TN, Yancopoulos GD. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. *Cell*. 1996;87:1171–1180.
62. Distler JH, Hirth A, Kurowska-Stolarska M, Gay RE, Gay S, Distler O. Angiogenic and angiostatic factors in the molecular control of angiogenesis. *Q J Nucl Med*. 2003;47:149–161.
63. Valenzuela DM, Griffiths JA, Rojas J, Aldrich TH, Jones PF, Zhou H, McClain J, Copeland NG, Gilbert DJ, Jenkins NA, Huang T, Papadopoulos N, Maisonpierre PC, Davis S, Yancopoulos GD. Angiopoietins 3 and 4: diverging gene counterparts in mice and humans. *Proc Natl Acad Sci U S A*. 1999;96:1904–1909.
64. Davis S, Aldrich TH, Jones PF, Acheson A, Compton DL, Jain V, Ryan TE, Bruno J, Radziejewski C, Maisonpierre PC, Yancopoulos GD. Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. *Cell*. 1996;87:1161–1169.
65. Mochizuki Y, Nakamura T, Kanetake H, Kanda S. Angiopoietin 2 stimulates migration and tube-like structure formation of murine brain capillary endothelial cells through c-Fes and c-Fyn. *J Cell Sci*. 2002;115:175–183.
66. Witzenbichler B, Maisonpierre PC, Jones P, Yancopoulos GD, Isner JM. Chemotactic properties of angiopoietin-1 and -2, ligands for the endothelial-specific receptor tyrosine kinase Tie2. *J Biol Chem*. 1998;273:18514–18521.
67. Cascone I, Audero E, Giraudo E, Napione L, Maniero F, Philips MR, Collard JG, Serini G, Bussolino F. Tie-2-dependent activation of RhoA and Rac1 participates in endothelial cell motility triggered by angiopoietin-1. *Blood*. 2003;102:2482–2490.
68. Jones N, Dumont DJ. The Tek/Tie2 receptor signals through a novel Dok-related docking protein, Dok-R. *Oncogene*. 1998;17:1097–1108.
69. Loughna S, Sato TN. Angiopoietin and Tie signaling pathways in vascular development. *Matrix Biol*. 2001;20:319–325.
70. Lobov IB, Brooks PC, Lang RA. Angiopoietin-2 displays VEGF-dependent modulation of capillary structure and endothelial cell survival in vivo. *Proc Natl Acad Sci U S A*. 2002;99:11205–11210.
71. Galaup A, Cazes A, Le Jan S, Philippe J, Connault E, Le Coz E, Mekid H, Mir L, Opolon P, Corvol P, Monnot C, Germain S. Angiopoietin-like 4 prevents metastasis through inhibition of vascular permeability and tumor cell motility and invasiveness. *Proc Natl Acad Sci U S A*. 2006;103:18721–18726.
72. Cazes A, Galaup A, Chomel C, Bignon M, Brechot N, Le Jan S, Weber H, Corvol P, Muller L, Germain S, Monnot C. Extracellular matrix-bound angiopoietin-like 4 inhibits endothelial cell adhesion, migration, and sprouting and alters actin cytoskeleton. *Circ Res*. 2006;99:1207–1215.
73. von Tell D, Armulik A, Betsholtz C. Pericytes and vascular stability. *Exp Cell Res*. 2006;312:623–629.
74. Deroanne CF, Hajitou A, Calberg-Bacq CM, Nusgens BV, Lapiere CM. Angiogenesis by fibroblast growth factor 4 is mediated through an autocrine up-regulation of vascular endothelial growth factor expression. *Cancer Res*. 1997;57:5590–5597.
75. Chantrain CF, Henriet P, Jodele S, Emonard H, Feron O, Courtoy PJ, DeClerck YA, Marbaix E. Mechanisms of pericyte recruitment in tumour angiogenesis: a new role for metalloproteinases. *Eur J Cancer*. 2006;42:310–318.
76. Armulik A, Abramsson A, Betsholtz C. Endothelial/pericyte interactions. *Circ Res*. 2005;97:512–523.
77. Davis GE, Senger DR. Endothelial extracellular matrix: biosynthesis, remodeling, and functions during vascular morphogenesis and neovessel stabilization. *Circ Res*. 2005;97:1093–1107.
78. Wang D, Anderson JC, Gladson CL. The role of the extracellular matrix in angiogenesis in malignant glioma tumors. *Brain Pathol*. 2005;15:318–326.
79. Dejana E, Languino LR, Polentarutti N, Balconi G, Ryckewaert JJ, Larrieu MJ, Donti MB, Mantovani A, Marguerie G. Interaction between fibrinogen and cultured endothelial cells. Induction of migration and specific binding. *J Clin Invest*. 1985;75:11–18.
80. Smith JT, Elkin JT, Reichert WM. Directed cell migration on fibronectin gradients: effect of gradient slope. *Exp Cell Res*. 2006;312:2424–2432.
81. Smith JT, Tomfohr JK, Wells MC, Beebe TP Jr, Kepler TB, Reichert WM. Measurement of cell migration on surface-bound fibronectin gradients. *Langmuir*. 2004;20:8279–8286.
82. Hauzenberger D, Klominek J, Bergstrom SE, Sundqvist KG. T lymphocyte migration: the influence of interactions via adhesion molecules, the T cell receptor, and cytokines. *Crit Rev Immunol*. 1995;15:285–316.
83. Senger DR, Perruzzi CA, Streit M, Koteliansky VE, de Fougerolles AR, Detmar M. The alpha(1)beta(1) and alpha(2)beta(1) integrins provide critical support for vascular endothelial growth factor signaling, endothelial cell migration, and tumor angiogenesis. *Am J Pathol*. 2002;160:195–204.
84. Herbst TJ, McCarthy JB, Tsilibary EC, Furcht LT. Differential effects of laminin, intact type IV collagen, and specific domains of type IV collagen on endothelial cell adhesion and migration. *J Cell Biol*. 1988;106:1365–1373.
85. Perruzzi CA, de Fougerolles AR, Koteliansky VE, Whelan MC, Westlin WF, Senger DR. Functional overlap and cooperativity among alphav and beta1 integrin subfamilies during skin angiogenesis. *J Invest Dermatol*. 2003;120:1100–1109.
86. Li J, Zhang YP, Kirsner RS. Angiogenesis in wound repair: angiogenic growth factors and the extracellular matrix. *Microsc Res Tech*. 2003;60:107–114.
87. Kanematsu A, Yamamoto S, Ozeki M, Noguchi T, Kanataki I, Ogawa O, Tabata Y. Collagenous matrices as release carriers of exogenous growth factors. *Biomaterials*. 2004;25:4513–4520.
88. Kanematsu A, Marui A, Yamamoto S, Ozeki M, Hirano Y, Yamamoto M, Ogawa O, Komeda M, Tabata Y. Type I collagen can function as a reservoir of basic fibroblast growth factor. *J Control Release*. 2004;99:281–292.
89. Huot J, Houle F, Rousseau S, Deschesnes RG, Shah GM, Landry J. SAPK2/p38-dependent F-actin reorganization regulates early membrane blebbing during stress-induced apoptosis. *J Cell Biol*. 1998;143:1361–1373.
90. Romer LH, Birukov KG, Garcia JG. Focal adhesions: paradigm for a signaling nexus. *Circ Res*. 2006;98:606–616.
91. Romer LH, McLean N, Turner CE, Burridge K. Tyrosine kinase activity, cytoskeletal organization, and motility in human vascular endothelial cells. *Mol Biol Cell*. 1994;5:349–361.
92. Eliceiri BP. Integrin and growth factor receptor crosstalk. *Circ Res*. 2001;89:1104–1110.
93. Serini G, Valdembri D, Bussolino F. Integrins and angiogenesis: a sticky business. *Exp Cell Res*. 2006;312:651–658.
94. Eliceiri BP, Cheresh DA. Adhesion events in angiogenesis. *Curr Opin Cell Biol*. 2001;13:563–568.
95. Tucker GC. Integrins: molecular targets in cancer therapy. *Curr Oncol Rep*. 2006;8:96–103.
96. Kim S, Bell K, Mousa SA, Varner JA. Regulation of angiogenesis in vivo by ligation of integrin alpha5beta1 with the central cell-binding domain of fibronectin. *Am J Pathol*. 2000;156:1345–1362.
97. Giancotti FG. Complexity and specificity of integrin signalling. *Nat Cell Biol*. 2000;2:E13–E14.
98. Guo W, Giancotti FG. Integrin signalling during tumour progression. *Nat Rev Mol Cell Biol*. 2004;5:816–826.

99. Hsu S, Thakar R, Liepmann D, Li S. Effects of shear stress on endothelial cell haptotaxis on micropatterned surfaces. *Biochem Biophys Res Commun*. 2005;337:401–409.
100. Chodniewicz D, Klemke RL. Regulation of integrin-mediated cellular responses through assembly of a CAS/Crk scaffold. *Biochim Biophys Acta*. 2004;1692:63–76.
101. Ohmori T, Yatomi Y, Okamoto H, Miura Y, Rile G, Satoh K, Ozaki Y. G(i)-mediated Cas tyrosine phosphorylation in vascular endothelial cells stimulated with sphingosine 1-phosphate: possible involvement in cell motility enhancement in cooperation with Rho-mediated pathways. *J Biol Chem.* 2001;276:5274–5280.
102. Gu J, Sumida Y, Sanzen N, Sekiguchi K. Laminin-10/11 and fibronectin differentially regulate integrin-dependent Rho and Rac activation via p130(Cas)-CrkII-DOCK180 pathway. *J Biol Chem*. 2001;276:27090–27097.
103. Byzova TV, Goldman CK, Pampori N, Thomas KA, Bett A, Shattil SJ, Plow EF. A mechanism for modulation of cellular responses to VEGF: activation of the integrins. *Mol Cell*. 2000;6:851–860.
104. Kuwada SK, Li X. Integrin alpha5/beta1 mediates fibronectin-dependent epithelial cell proliferation through epidermal growth factor receptor activation. *Mol Biol Cell*. 2000;11:2485–2496.
105. Borges E, Jan Y, Ruoslahti E. PDGF-receptor-(beta) and EGF-receptor-2 bind to the (beta)3 integrin through its extracellular domain. *J Biol Chem*. 2000;275:39867–39873.
106. Soldi R, Mitola S, Strasly M, Defilippi P, Tarone G, Bussolino F. Role of alphavbeta3 integrin in the activation of vascular endothelial growth factor receptor-2. *EMBO J*. 1999;18:882–892.
107. Masson-Gadais B, Houle F, Laferriere J, Huot J. Integrin alphavbeta3, requirement for VEGFR2-mediated activation of SAPK2/p38 and for Hsp90-dependent phosphorylation of focal adhesion kinase in endothelial cells activated by VEGF. *Cell Stress Chaperones*. 2003;8:37–52.
108. Dedhar S, Williams B, Hannigan G. Integrin-linked kinase (ILK): a regulator of integrin and growth-factor signalling. *Trends Cell Biol*. 1999;9:319–323.
109. Sieg DJ, Hauck CR, Ilic D, Klingbeil CK, Schaefer E, Damsky CH, Schlaepfer DD. FAK integrates growth-factor and integrin signals to promote cell migration. *Nat Cell Biol*. 2000;2:249–256.
110. Avraham HK, Lee TH, Koh Y, Kim TA, Jiang S, Sussman M, Samarel AM, Avraham S. Vascular endothelial growth factor regulates focal adhesion assembly in human brain microvascular endothelial cells through activation of the focal adhesion kinase and related adhesion focal tyrosine kinase. *J Biol Chem*. 2003;278:36661–36668.
111. Eliceiri B. Mechanisms of signal transduction in vascular permeability: potential targets. *Pathophysiol Haemost Thromb*. 2003;33(suppl 1):5–6.
112. Eliceiri BP, Puente XS, Hood JD, Stupack DG, Schlaepfer DD, Huang XZ, Sheppard D, Cheresh DA. Src-mediated coupling of focal adhesion kinase to integrin alpha(v)beta5 in vascular endothelial growth factor signaling. *J Cell Biol*. 2002;157:149–160.
113. Brooks PC, Klemke RL, Schon S, Lewis JM, Schwartz MA, Cheresh DA. Insulin-like growth factor receptor cooperates with integrin alpha v beta 5 to promote tumor cell dissemination in vivo. *J Clin Invest*. 1997;99:1390–1398.
114. Weis S, Shintani S, Weber A, Kirchmair R, Wood M, Cravens A, McSharry H, Iwakura A, Yoon YS, Himes N, Burstein D, Doukas J, Soll R, Losordo D, Cheresh D. Src blockade stabilizes a Flk/cadherin complex, reducing edema and tissue injury following myocardial infarction. *J Clin Invest*. 2004;113:885–894.
115. Weis S, Cui J, Barnes L, Cheresh D. Endothelial barrier disruption by VEGF-mediated Src activity potentiates tumor cell extravasation and metastasis. *J Cell Biol*. 2004;16:223–229.
116. Wallez Y, Vilgrain I, Huber P. Angiogenesis: the VE-cadherin switch. *Trends Cardiovasc Med*. 2006;16:55–59.
117. Grzesiak JJ, Smith KC, Chalberg C, Burton DW, Deftos LJ, Bouvet M. Type I collagen and divalent cation shifts disrupt cell-cell adhesion, increase migration, and decrease PTHrP, IL-6, and IL-8 expression in pancreatic cancer cells. *Int J Gastrointest Cancer*. 2005;36:131–146.
118. Katsumi A, Orr AW, Tzima E, Schwartz MA. Integrins in mechanotransduction. *J Biol Chem*. 2004;279:12001–12004.
119. Sander EE, ten Klooster JP, van Delft S, van der kammen RA, Collard JG. Rac downregulates Rho activity: reciprocal balance between both GTPases determines cellular morphology and migratory behavior. *J Cell Biol*. 1999;147:1009–1022.
120. Gingras D, Gauthier F, Lamy S, Desrosiers RR, Beliveau R. Localization of RhoA GTPase to endothelial caveolae-enriched membrane domains. *Biochem Biophys Res Commun*. 1998;247:888–893.
121. Gratton JP, Bernatchez P, Sessa WC. Caveolae and caveolins in the cardiovascular system. *Circ Res*. 2004;94:1408–1417.
122. Ingber DE. Integrins, tensegrity, and mechanotransduction. *Gravit Space Biol Bull*. 1997;10:49–55.
123. Ingber DE. Tensegrity. I. Cell structure and hierarchical systems biology. *J Cell Sci*. 2003;116:1157–1173.
124. Ingber DE. Tensegrity. II. How structural networks influence cellular information processing networks. *J Cell Sci*. 2003;116:1397–1408.
125. Karkkainen MJ, Makinen T, Alitalo K. Lymphatic endothelium: a new frontier of metastasis research. *Nat Cell Biol*. 2002;4:E2–E5.

Experimental models to study endothelial cell migration

A number of *in vitro* and in vivo assays have been developed for studying angiogenesis and endothelial cell migration.

Wound healing assay

In this assay, endothelial cells are cultivated as a confluent monolayer and the layer is scraped with a rubber policeman or the pointed end of a sterile tip to create a cell-free zone. Thereafter, cells are washed with medium and stimulated with pro-angiogenic agents. Over time, the cells migrate to close the wound and the process is monitored under phase contrast microscopy. Endothelial cell migration is quantified by measuring the width of the cell-free zone (distance between the edges of the injured monolayer) before and after different exposures after stimulation at 5 distinct positions (every 5 mm). [1]

Modified Boyden chambers assay

The Boyden chamber consists of an upper chamber that is separated from a lower chamber by a porous membrane coated with an appropriate matrix. The endothelial cells are seeded on the top of the membrane and the lower chamber is filled with medium containing chemotactic pro-angiogenic agent. The cells sense and migrate toward the source of the chemo-attractant. After four hours, the cells that have crossed the membrane are stained and counted under the microscope. This approach is useful for the statistical quantification of the chemotactic migration process *in vitro*.[2-4]

Tube formation in Matrigel

This assay is somehow at the frontier between *in vitro* and *in vivo* models. Matrigel is liquid as 4°C but becomes a solid gel at 37°C. Endothelial cells are embedded in the gel and cell migration is evaluated by determining the formation of tubular structures. This type of assay provides the advantage of studying endothelial cell migration and tubule formation in the presence of surrounding non-endothelial cells, thereby mimicking the *in vivo* situation. Alternatively, a Matrigel plug containing cells or an angiogenic factor can be implanted subcutaneously in mice to test the extent of CD31-positive vessels formed in the plug. [5, 6].

Chick chorioallatoic membrane

The chick chorioallantoic membrane (CAM) allows performing relatively inexpensive large scale *in vivo* studies. A test substance is prepared on a slow-release pellet or disc and implanted on the CAM through a window in the eggshell. The number of blood vessels can be counted using a stereomicroscope.[7] This type of assay is widely used and allows for the study of pro- or anti-angiogenic molecules.

Zebrafish

The zebrafish was brought to the scientific scene in the lasts years as a model of choice for studying vasculature, vessel functions, blood flow and developmental angiogenesis. The zebrafish embryos are transparent and therefore allow the observation of the vasculature with a great optical clarity. [7] The zebrafish model is used as a primary screening approach to assess compounds that affect angiogenesis.

References

1. Chai J, Jones MK, Tarnawski AS. Serum response factor is a critical requirement for VEGF signaling in endothelial cells and VEGF-induced angiogenesis. *Faseb J.* 2004;18:1264-1266.

2. Matsumoto T, Bohman S, Dixelius J, Berge T, Dimberg A, Magnusson P, Wang L, Wikner C, Qi JH, Wernstedt C, Wu J, Bruheim S, Mugishima H, Mukhopadhyay D, Spurkland A, Claesson-Welsh L. VEGF receptor-2 Y951 signaling and a role for the adapter molecule TSAd in tumor angiogenesis. *Embo J.* 2005; 24:2342-2353.

3. Rousseau S, Houle F, Landry J, Huot J. p38 MAP kinase activation by vascular endothelial growth factor mediates actin reorganization and cell migration in human endothelial cells. *Oncogene.* 1997; 15:2169-2177.

4. Lamalice L, Houle F, Huot J. Phosphorylation of Tyr1214 within VEGFR-2 triggers the recruitment of Nck and activation of Fyn leading to SAPK2/p38 activation and endothelial cell migration in response to VEGF. *J Biol Chem.* 2006; 281:34009-34020.

5. Takeda Y, Kazarov AR, Butterfield CE, Hopkins BD, Benjamin LE, Kaipainen A, Hemler ME. Deletion of tetraspanin CD151 results in decreased pathological angiogenesis in vivo and in vitro. *Blood.* Oct 5 2006.[Epub ahead of print]

6. Tong Q, Zheng L, Li B, Wang D, Huang C, Matuschak GM, Li D. Hypoxia-induced mitogenic factor enhances angiogenesis by promoting proliferation and migration of endothelial cells. *Exp Cell Res.* 2006; 312:3559-3569.

7. Staton CA, Stribbling SM, Tazzyman S, Tazzyman S, Hughes R, Brown NJ, Lewis CE. Current methods for assaying angiogenesis in vitro and in vivo. *Int J Exp Pathol.* 2004; 85:233-248.