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PROSPECTS

Mechanotransduction in Endothelial Cell Migration

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Abstract The migration of endothelial cells (ECs) plays an important role in vascular remodeling and regeneration. EC migration can be regulated by different mechanisms such as chemotaxis, haptotaxis, and mechanotaxis. This review will focus on fluid shear stress-induced mechanotransduction during EC migration. EC migration and mechanotransduction can be modulated by cytoskeleton, cell surface receptors such as integrins and proteoglycans, the chemical and physical properties of extracellular matrix (ECM) and cell–cell adhesions. The shear stress applied on the luminal surface of ECs can be sensed by cell membrane and associated receptor and transmitted throughout the cell to cell–ECM adhesions and cell–cell adhesions. As a result, shear stress induces directional migration of ECs by promoting lamellipodial protrusion and the formation of focal adhesions (FAs) at the front in the flow direction and the disassembly of FAs at the rear. Persistent EC migration in the flow direction can be driven by polarized activation of signaling molecules and the positive feedback loops constituted by Rho GTPases, cytoskeleton, and FAs at the leading edge. Furthermore, shear stress-induced EC migration can overcome the haptotaxis of ECs. Given the hemodynamic environment of the vascular system, mechanotransduction during EC migration has a significant impact on vascular development, angiogenesis, and vascular wound healing. J. Cell. Biochem. 96: 1110–1126, 2005.

Key words: endothelial cells; mechanotransduction; cell migration; fluid shear stress; mechanotaxis

The migration of endothelial cells (ECs) plays an important role in vascular remodeling such as embryonic vasculogenesis, angiogenesis, and re-endothelialization in arteries after angioplasty and bypass procedures. Like other cell types, EC migration is a mechanically integrated molecular process that involves dynamic, coordinated changes in cell adhesions, cytoskeletal organization, and signal transduction. The migration process includes the protrusion of the leading edge, the formation of new adhesions at the front, the contraction of the cell, and the release of adhesions at the rear [Lauffenburger and Horwitz, 1996; Sheetz et al., 1998].

The chemical and physical factors in the vascular system regulate EC migration by different mechanisms such as chemotaxis (directional migration in response to a concentration gradient of chemoattractants), haptotaxis (directional migration in response to a gradient of immobilized ligands), and mechanotaxis (directional migration induced by mechanical forces). Since ECs make up the inner lining of blood vessels, they constantly experience fluid shear stress, the tangential component of hemodynamic stresses. While shear stress is applied on the luminal surface of ECs, the mechanical-chemical signaling can be transmitted throughout the cell and to cell–extracellular matrix (ECM) adhesions on the abluminal surface of ECs. There is accumulating evidence suggesting that fluid shear stress can modulate each step of the migration process, including the extension of the leading edge, adhesion to the matrix, and release of adhesions at the rear.

The importance of hemodynamic forces in EC migration did not receive much attention until the 1990s. In large vessels where convection is strong, mechanotaxis may have a more significant effect than chemotaxis. In the microcirculation, fluid shear stress may guide EC migration during angiogenesis, e.g., along the interstitial flow paths or tunnels during wound

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healing and lymphangiogenesis [Branemark, 1965; Moldovan et al., 2000; Boardman and Swartz, 2003].

Flow channels have been used to investigate the responses of cultured ECs to shear stress in vitro because the chemical and mechanical factors can be well controlled. Both in vivo and in vitro studies have shown that laminar shear stress can enhance EC migration in wound healing [Wu et al., 1995; Sprague et al., 1997; Tardy et al., 1997; Albuquerque et al., 2000; Hsu et al., 2001; Li et al., 2002]. Our time-lapse microscopy experiments have demonstrated that EC migration, rather than proliferation, is the major mechanism of wound healing, at least for the first 1–2 days. Laminar shear stress at the arterial level promotes lamellipodial protrusion and EC migration in the flow direction when the wound edge is perpendicular to the flow direction [Masuda and Fujiwara, 1993; van et al., 1994; Sprague et al., 1997; Hsu et al., 2001; Li et al., 2002], suggesting that the cytoskeleton and cell–ECM adhesions undergo polarized remodeling under flow. In addition, when the EC monolayer is wounded in parallel to the flow direction, the wound closure rate is also increased by shear stress [Albuquerque et al., 2000]. A possible explanation is that shear stress may promote the detachment at cell–cell adhesions, thus allowing EC to migrate into the wound [Hsu et al., 2001; Albuquerque and Flozak, 2002]. However, oscillatory disturbed flow induces large focal adhesions (FAs) and does not enhance EC migration [Hsu et al., 2001].

Here, we will review the effects of laminar shear stress on EC migration and the mechanisms of mechanotransduction, focusing on the roles of the cytoskeleton, cell surface receptors-integrins and proteoglycans, ECM, and cell–cell adhesions in the transduction and conversion of extracellular mechanical signals into intracellular signals and the coordination of EC migration in response to laminar shear stress.

ROLES OF THE CYTOSKELETON IN SHEAR STRESS-INDUCED EC MIGRATION

Cytoskeleton as Mechanotransducer

The cytoskeleton includes three types of protein filaments—actin filaments, microtubules, and intermediate filaments. The cytoskeletal filaments are interconnected, anchored at cell–ECM adhesions and cell–cell junctions, and provide mechanical support for the cell body. For example, actin filaments bind to cell–ECM adhesions through a protein complex that involves talin, vinculin, α-actinin, and filamin. Such a complex not only couples cell–ECM receptors such as integrins to the actin–myosin contractile apparatus, but also sequesters signaling molecules that participate in integrin signaling, e.g., focal adhesion kinase (FAK), Shc and Crk [Critchley, 2000]. Extracellular disturbances of the cytoskeletal networks may change the intracellular distribution of mechanical stress and strain, which may be sensed by the cells and result in signaling events. The cytoskeletal networks not only transduce mechanical signals throughout the cells, but may also modulate the activity of the signaling molecules associated with the cytoskeleton.

There is evidence that the extracellular mechanical force can be sensed by either cytoskeleton-linked receptors or the cytoskeleton structure itself. Direct application of force on integrin adhesion receptors increases the stiffness of the cytoskeleton, while force applied on non-adhesion receptors does not [Wang et al., 1993]. On the other hand, disruption of actin filaments inhibits shear stress activation of FAK [Li et al., 1997], and the knockdown of vimentin-type intermediate filaments decreases the assembly of FAs induced by shear stress [Tsuruta and Jones, 2003]. These results suggest that actin and intermediate filaments are involved in the mechanotransduction to cell–ECM adhesions. Consistently, mechanical strain mapping has shown that shear stress induces mechanical stretching at cell boundaries [Helmke and Davies, 2002], indicating that shear stress applied on the luminal surface of ECs can be transmitted to the abluminal surface of the cells. Besides cell–ECM adhesions, the cytoskeleton may also transduce mechanical signals to cell–cell junctions. Disruption of actin filaments increases the solubility of α-catenin and gap formation and inhibits cell alignment induced by shear stress [Schnittler et al., 2001]. The mechanical coupling of the cytoskeleton with cell–ECM adhesions and cell–cell junctions provides an efficient way to transduce mechanical signals throughout the cells, mechanotransduction plays an important role in coordinating EC migration in response to shear stress.

Structural models have been developed to describe the cytoskeletal networks. For example,

in the tensegrity model, the cytoskeleton is a pre-stressed tensegrity structure composed of molecular struts (microtubules) and cables (actin and intermediate filaments) [Ingber, 2003]; in the percolation model, the cytoskeleton forms randomly organized and interconnected networks [Forgacs, 1995]. Although these two models differ in the description of cytoskeletal organization, large deformations, and cytoskeletal remodeling, theoretically they both allow the propagation of mechanical and chemical signals through the networks.

Cytoskeleton as Mechano-Effector

The actin cytoskeleton plays a central role in coordinating cell migration. The dynamic reorganization of the actin cytoskeleton during cell migration leads to protrusion at the leading edge and retraction at the rear [Lauffenburger and Horwitz, 1996; Cooper and Schafer, 2000]. Actin assembly and disassembly is modulated by binding proteins such as Arp 2/3 complex, cofilin, gelsolin, profilin, and filamin, as well as by signaling molecules. The Rho family small GTPases Rho, Rac, and Cdc42 are major regulators of the actin cytoskeleton and cell migration [Van Aelst and D'Souza-Schorey, 1997; Ridley, 2001]. Rho induces the formation of actin stress fibers and FAs and stimulates cell contraction through the downstream effectors p160ROCK and mDia [Ridley et al., 1992; Nobes and Hall, 1995; Watanabe et al., 1999]. At the leading edge of the cell, Rac can promote lamellipodia formation by regulating the Wiskott–Aldrich syndrome protein (WASP)-family proteins (e.g., SCAR1, WAVE), p65PAK, LIM-kinase, and gelsolin [Nobes et al., 1995; Arber et al., 1998; Arcaro, 1998; Azuma et al., 1998; Bear et al., 1998; Yang et al., 1998; Miki et al., 1998b; Edwards et al., 1999; Machesky et al., 1999; Yamazaki et al., 2003]. Cdc42 can regulate N-WASP, p65PAK, LIM-kinase for filopodia formation [Ramesh et al., 1997; Suetsugu et al., 1998; Miki et al., 1998a; Edwards et al., 1999].

The remodeling of cytoskeleton can be regulated by mechanotransduction. Fluid shear stress induces EC alignment and the increase of stress fibers in EC monolayers [Dewey, 1984; Franke et al., 1984; Levesque and Nerem, 1985; Ives et al., 1986; Galbraith et al., 1998]. Cell alignment and actin stress fibers require signal transduction through intracellular calcium release, tyrosine kinases, microtubule integrity, Rho/p160ROCK, Rac, Cdc42, p38 mitogen-activated protein kinase (p38MAPK), and p65PAK [Girard and Nerem, 1993; Malek and Izumo, 1996; Li et al., 1999; Azuma et al., 2001; Tzima et al., 2001; Birukov et al., 2002; Wojciak-Stothard and Ridley, 2003], suggesting that shear stress activates multiple signaling pathways to regulate actin re-organization and changes in cell morphology. Furthermore, shear stress induces remodeling of microtubules and intermediate filaments [Galbraith et al., 1998; Helmke et al., 2000]. The microtubule organization center reorients to the downstream side of the nucleus relative to the flow direction and is regulated by Cdc42 [Tzima et al., 2003].

In subconfluent ECs, within seconds to minutes, shear stress induces lamellipodia formation around the cell periphery without significant remodeling of actin fibers, as demonstrated by time-lapse microscopy of green fluorescence protein (GFP)-actin [Li et al., 2002]. This early lamellipodia formation may result from shear stress-induced membrane deformation and/or signaling events (e.g., Rac activation). Stable lamellipodial protrusion and actin polymerization in the flow direction can be observed thereafter [Li et al., 2002], which is likely mediated by Rho GTPases as shown in recent studies. By using affinity-precipitation and fluorescence energy transfer assay, Tzima et al. [2002, 2003] have shown that shear stress-induced Cdc42 and Rac activity is transient and polarizes in the flow direction. A recent study shows that Rac, but not Cdc42 and PI-3 kinase, is required for the directional migration of ECs, although Rac, Cdc42, and PI-3 kinase all regulate the migration speed [Wojciak-Stothard and Ridley, 2003]. Over-expression of Rac in either constitutively activated mutant or negative mutant form disrupts the polarized lamellipodia formation, and inhibits cell migration [Hu et al., 2002], suggesting that spatially polarized Rac activity rather than the overall Rac activity is important for directional migration of ECs. In addition, inhibition of Rho blocks directional migration under flow, but enhances migration speed, possibly by decreasing cell–ECM adhesions [Wojciak-Stothard and Ridley, 2003]. Since complete inhibition of Rho or p160ROCK disrupts actin filaments and inhibits EC migration [Hsu et al., 2001; Shiu et al., 2004], a medium level of Rho activity may be needed for the most efficient EC migration. Rho activity is also required for the generation of

traction force through actin fibers and cell–ECM adhesions in response to shear stress [Shiu et al., 2003] (see the section on "Roles of Integrins and Proteoglycans in Shear Stress-Induced EC Migration").

How does shear stress differentially regulate Rho GTPases at different locations inside the cell? A possible mechanism is the activation of specific Rho GTPases by microtubule dynamics. Shear stress increases microtubule polymerization in the flow direction [Hu et al., 2002]. Microtubule elongation at the front of the cell may activate Rac to induce lamellipodia formation, while the inhibition of microtubule elongation decreases cell spreading, lamellipodial protrusion, and cell migration [Gotlieb et al., 1983; Domnina et al., 1985; Bershadsky et al., 1991; Mikhailov and Gundersen, 1998; Ren et al., 1999; Waterman-Storer et al., 1999; Wittmann et al., 2003]. Indeed, the stabilization of microtubules with taxol inhibits shear stress-activation of Rac and lamellipodial protrusion in the flow direction [Hu et al., 2002]. These results suggest that shear stress may induce microtubule elongation in the flow direction, which in turn activates Rac to promote actin polymerization and thus lamellipodial protrusion in the flow direction (Fig. 1). On the other hand, Rac can promote microtubule elongation through p65PAK [Daub et al., 2001], thus forming a positive feedback loop for microtubule elongation and Rac activation at the cell front. In addition, activated Rac may inhibit Rho activity locally [Sander et al., 1999], and result in polarized activation of Rac (at the front) and Rho (at the rear) during cell migration. At the rear of the cell, it is also possible that microtubule shortening may activate Rho to induce actin contraction and tail detachment [Danowski, 1989; Enomoto, 1996; Liu et al., 1998; Elbaum et al., 1999; Ren et al., 1999] (Fig. 1).

Alternatively, mechanotransduction through cell–ECM adhesions, cell–cell junctions, and EC plasma membranes may be involved in the shear stress activation of Rho GTPases. It has been shown that shear stress can increase membrane fluidity, activate G proteins, and initiate signaling cascades from caveolae [Gudi et al., 1996; Park et al., 1998; Rizzo et al., 1998; Haidekker et al., 2000; Butler et al., 2001]. Activation of specific G proteins may regulate the activity of specific Rho GTPases [Harhammer et al., 1996; Fromm et al., 1997; Gohla et al., 1998; Ma et al., 1998; Mao et al., 1998; Sah et al., 2000; Sugimoto et al., 2003], and the association of Rho GTPases with caveolae modulates the activity of Rho GTPases [Gingras et al., 1998; Michaely et al., 1999; Kawamura et al., 2003]. There is evidence that caveolae and G proteins can mediate mechanotransduction. Within hours, shear stress induces the translocation of caveolae and the Gαq subunit to the trailing edge of migrating ECs [Isshiki et al., 2002]. This activation of Gαq may lead to the activation of Rho [Sah et al., 2000] and thus the increase of contractility of stress fibers at the rear of ECs. In addition, a shear stress-induced Ca^{2+} wave can be initiated from the caveolae at the rear of ECs [Yoshikawa et al., 1997; Isshiki et al., 2002], which may enhance the function of p160ROCK in actin-myosin contraction and potentially activate calpain to degrade FAs locally. Both pathways may promote detachment at the rear of migrating ECs (Fig. 1). Consistently, blocking Ca^{2+} channels on the plasma membrane inhibits shear stress-induced EC migration [Yoshikawa et al., 1999].

ROLES OF INTEGRINS AND PROTEOGLYCANS IN SHEAR STRESS-INDUCED EC MIGRATION

Integrins and Proteoglycans as Mechanotransducers

Integrins, the major cell–ECM adhesion receptors, are a family of transmembrane heterodimers composed of α and β subunits. The extracellular domain of integrins binds to ECM proteins such as fibronectin, vitronectin, and collagen. The cytoplasmic domain interacts with cytoskeletal proteins (e.g., paxillin, talin) and signaling molecules in the FA sites, e.g., FAK and c-Src [Hynes, 1992; Sastry and Horwitz, 1993; Schwartz et al., 1995]. Integrins also regulate Rho family small GTPases to modulate the remodeling of cytoskeleton and FAs [Keely et al., 1998; Schwartz and Shattil, 2000]. Thus, integrins can function as both adhesion receptors and signaling transducers to regulate cell migration.

Fluid shear stress induces the remodeling cell–ECM adhesions in a cell density-dependent manner. In a confluent EC monolayer, shear stress increases the size and decreases the number of FAs [Davies et al., 1994, 2003]. The remodeling of FAs is driven by the polymerization of actin filaments anchored at FAs, and there is no preferential direction of polymerization either upstream and downstream of ECs

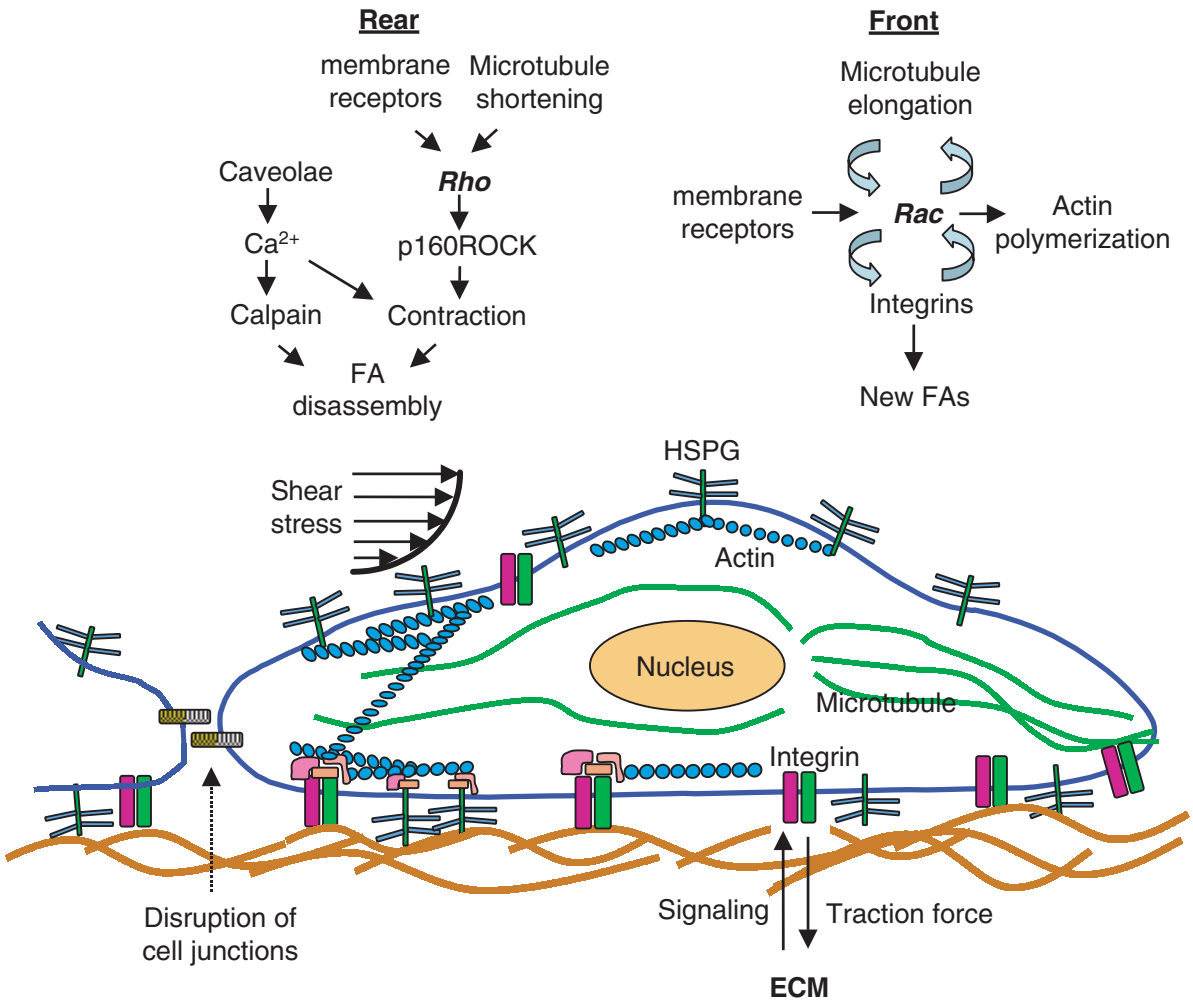

Fig. 1. A model of the polarized activation of signaling molecules by shear stress during endothelial cell (EC) migration. The shear stress applied on the luminal surface of ECs can be sensed by cell membrane and associated receptors, and transmitted throughout the cell to cell–extracellular matrix (ECM) adhesions and cell–cell adhesions. Shear stress induces directional migration of ECs by promoting lamellipodial protrusion and the formation of focal adhesions (FAs) in the flow direction and the disassembly of FAs at the rear. Persistent EC migration in the flow direction can be driven by polarized activation of signaling molecules and the positive feedback loops constituted by microtubules, Rac, and integrins at the front. Microtubule elongation at the front activates Rac to drive actin polymerization and lamellipodial protrusion and recruit/activate integrins for new FA formation. On the other hand, integrins activate Rac to form a positive feedback loop between Rac and integrins, and Rac promotes microtubule elongation form a positive feedback loop between Rac and microtubules. Other membrane receptors (e.g., G proteins, receptors in caveolae) may also induce polarized activation of Rac. At the rear of the cell, microtubule shortening or membrane receptors (e.g., G proteins) can activate the Rho-p160ROCK pathway and induce actin-myosin contraction to detach FAs. In addition, a shear stress-induced Ca^{2+} wave can be initiated from the caveolae at the rear of ECs to enhance the function of p160ROCK in actin-myosin contraction and potentially activate calpain to degrade FAs locally. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

[Noria et al., 2004]. Lamellipodia and filopodia are not observed during EC alignment in confluent monolayer [Noria et al., 2004], possibly due to the restriction of cell–cell adhesion, but transient gap formation and re-sealing at cell–cell junctions can be observed during EC morphological change under flow [Li, unpublished observation].

In subconfluent ECs, the remodeling of FAs is polarized and more dynamic. We have used time-lapse confocal microscopy to monitor the molecular dynamics of FAs labeled with GFP-FAK [Li et al., 2002]. The early response to shear stress is lamellipodial protrusion, followed by new FA formation under the lamellipodia, in the flow direction. The existing FAs under the main cell body either increase in size or merge under flow, which may be the immediate response to resist cell detachment. However, the clustering of FAs in subconfluent ECs is

transient, and the FAs disassemble shortly afterwards to allow for cell migration. New FAs form exclusively under new lamellipodia but not under the main cell body. During persistent EC migration under flow, the existing FAs usually remain stationary before disassembly, and serve as a "sliding track" for the cell body. Some of the FAs containing FAK are ripped off from the tail of migrating ECs. Whether this ripping off of FAs is due to the localized activation of calpain remains to be determined. These results suggest that shear stress enhances both the new FA formation at the front and the detachment at the rear of ECs.

Integrins can be activated by clustering or conformation (affinity) change. As implicated by the experiments described above, in response to shear stress, integrin clustering may be involved in integrin activation, especially in confluent ECs. Due to the dynamic nature of FA turnover, integrin affinity change may be important in integrin activation in subconfluent ECs during migration. By measuring integrin-Shc association, it has been shown that shear stress induces sustained activation of integrins [Chen et al., 1999b], which requires the dynamic binding of the matrix proteins with their specific integrin receptors such as $\alpha_5\beta_1$ and $\alpha_v\beta_3$ [Jalali et al., 2001; Shyy and Chien, 2002]. Furthermore, shear stress can increase integrin binding affinity for the ECM, as measured by using an antibody that recognizes activated $\alpha_v\beta_3$ [Tzima et al., 2001]; however, this integrin activation does not show polarized distribution, although the new FAs only form in the flow direction. In addition to post-translational activation of integrins, shear stress increases the expression of integrin $\alpha_5\beta_1$ to enhance cell migration [Urbich et al., 2002].

As part of outside-in signaling, integrins can regulate the activity of Cdc42, Rac, and Rho [Hotchin and Hall, 1995; Clark et al., 1998; Price et al., 1998; Ren et al., 1999]. Fibroblasts plated on fibronectin exhibit an early activation of Cdc42 and Rac, and a delayed activation of Rho [Price et al., 1998; Ren et al., 1999], suggesting that Cdc42/Rac and Rho may be regulated through different mechanisms. Although integrin activation is required for the activation of Rac and Cdc42 and the deactivation of Rho by shear stress [Tzima et al., 2001, 2002, 2003], how non-polarized integrin activation induces polarized activation of Rho GTPases is not clear. One explanation is that other signaling pathways are required to collaborate with integrins to induce polarized activation of Rho GTPases, e.g., the polarized microtubule elongation and polarized caveolae-initiated signaling. For example, Rac activation due to microtubule elongation may recruit high affinity integrin $\alpha_v\beta_3$ to lamellipodia during endothelial migration [Kiosses et al., 2001], and integrins can further activate Rac [Clark et al., 1998; Price et al., 1998; del Pozo et al., 2004], thus forming a positive feedback loop between Rac activation and integrin activation in lamellipodia to drive persistent directional migration.

Besides Rho GTPases, integrin signaling through FAK and its associated pathways plays an important role in the regulation of cell migration [Schwartz et al., 1995; Cary et al., 1999; Schlaepfer et al., 1999]. FAK is a cytoplasmic tyrosine kinase that co-localizes with integrins in FAs. Integrin binding to ECM induces FAK activation and tyrosine phosphorylation in a variety of cell types [Schwartz et al., 1995]. Recent studies have provided direct evidence for the role of FAK in cell motility. Inhibition of FAK by a dominant negative FAK construct results in a decrease in EC motility [Gilmore and Romer, 1996]. A FAK homozygous knockout in mice is embryonically lethal, and cells cultured from these $FAK^{-/-}$ embryos display decreased motility in vitro [Ilic et al., 1995]. For monolayer ECs, shear stress activates FAK and the downstream signaling [Ishida et al., 1996; Li et al., 1997]. For individual ECs, shear stress recruits FAK to new FAs under lamellipodia in the flow direction [Li et al., 2002]. These results suggest that FAK plays a role in shear stress-induced EC migration. Upon cell adhesion, activation and autophosphorylation of FAK at Y397 allows FAK to associate with two other intracellular signaling molecules, Src and PI-3 kinase, via their SH2 domains [Cary et al., 1999]. FAK/Src association triggers downstream signaling events such as phosphorylation of p130CAS and extracellular-regulated kinase (ERK) kinase to mediate cell adhesion and migration [Burridge et al., 1992; Chen et al., 1994a; Schlaepfer et al., 1994; Vuori and Ruoslahti, 1995; Zhu and Assoian, 1995; Cary et al., 1998]. Consistently, inhibition of PI-3 kinase and ERK has been shown to decrease EC migration under flow [Urbich et al., 2002]. There is also evidence that FAK can crosstalk with Rho GTPases-mediated signaling. For example,

FAK phosphorylation leads to the increase of Rac activity [Hsia et al., 2003] and the decrease of Rho activity [Ren et al., 2000], which may contribute to polarized activation of Rho GTPases (Fig. 1). In parallel to FAK, adaptor protein Shc can also mediate integrin signaling [Wary et al., 1996]. Shear stress increases the association of integrin and Shc [Chen et al., 1999a; Jalali et al., 2001], and inhibition of Shc decreases shear stress-induced EC migration [Urbich et al., 2002].

Integrin-mediated cell adhesion and migration can be modified by cell surface proteoglycans such as syndecan-4 [Woods and Couchman, 1998; Couchman and Woods, 1999]. Syndecans are membrane glycoproteins that are usually substituted with heparan sulfate chains, and some also bear chondroitin sulfate. Syndecan-4 is localized at FAs [Woods and Couchman, 1994], and the clustering of syndecan-4 provides signals required for FA assembly [Longley et al., 1999; Saoncella et al., 1999]. It has been shown that syndecan-4 binds to the heparin-binding domain HepII of fibronectin [Woods et al., 2000]. There is also evidence that Rho is necessary for the formation of FAs induced by syndecan-4, and that protein kinase C and Rho coordinate FA formation [Defilippi et al., 1997; Saoncella et al., 1999].

Disruption of heparan sulfate proteoglycans (HSPGs) with heparinase decreased EC adhesion rate by 40% and adhesion strength by 33% [Moon et al., 2005]. HSPG disruption decreased stress fibers and the size of FAs, increased filopodia formation, and enhanced EC migration by promoting the detachment at the rear of cells. Under flow conditions, heparinase treatment increased EC migration speed, but inhibited shear stress-induced directionality of EC migration and the recruitment of phosphorylated FAK in the flow direction, suggesting that HSPGs are important for sensing the direction of shear stress [Moon et al., 2005]. In addition, decreasing cell adhesion by lowering fibronectin density enhanced EC migration under static and flow conditions, but did not affect the directional migration of ECs under flow. Based on our results, we propose that HSPGs play dual roles as mechanotransducers on the EC surface: (1) HSPG–matrix interaction on the abluminal surface regulates EC migration speed through an adhesion-dependent manner, and (2) HSPGs without binding to the matrix (e.g., on the luminal surface) are involved in sensing the direction of flow through an adhesion-independent manner.

The EC surface is covered by a layer of glycocalyx, a network of glycoproteins and proteoglycans about 0.05–0.5 μm thick [Luft, 1966; Vink and Duling, 1996; Squire et al., 2001], which may function as a mechanotransducer for shear stress applied on the EC surface. The extensive polysaccharide structure of HSPGs sticking out on the luminal surface of ECs may function as sensors and transducers of shear stress, and transmits the extracellular stimulation via the interaction of HSPGs with plasma membranes and the underlying actin structure. This notion is supported by a recent theoretical analysis predicting that the glycocalyx can deform in response to shear stress and transmit the bending and torque into cells [Weinbaum et al., 2003].

ECM in Mechanotransduction

ECM provides mechanical support and chemical cues for cell adhesion, migration, proliferation, and differentiation. The chemical components, density and distribution of ECM play important roles in mechanotransduction during EC migration. The major ECM proteins that interact with vascular ECs include collagen, laminin, fibronectin, vitronectin, and fibrinogen. Various ECM proteins bind to different integrins, and may activate different signaling molecules. For example, EC migration on collagen type I matrix is mediated by $\alpha_2\beta_1$ and $\alpha_1\beta_1$ integrins [Senger et al., 1997; Davis et al., 2000, 2002]. Laminin, a major ECM protein in the basement membrane, preferentially binds to $\alpha_6\beta_1$ integrin in ECs [Sonnenberg et al., 1988]. Fibronectin mainly binds to $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins. Vitronectin and fibrinogen bind to $\alpha_v\beta_3$ integrin. The chemical components of ECM and the various integrins may differentially regulate intracellular signaling and cell migration. For example, each type of integrin regulates FAK and SHC activation differently [Wary et al., 1996], and integrin β_1 and β_3 differentially regulate the activity of Rac and Rho respectively [Miao et al., 2002]. In addition, different roles of integrins are also apparent in cell migration [Clyman et al., 1992; Chon et al., 1998; Jacques et al., 1998]. The relative contribution of different integrins to mechanotransduction during EC migration remains to be determined.

Many efforts have been made to identify the different cell binding domains (CBDs) in ECM,

e.g., the Arg-Gly-Asp (RGD) peptide in fibronectin and other ECM proteins. Fibronectin has multiple CBDs for integrin and non-integrin receptors on the cell surface [Ruoslahti and Pierschbacher, 1987; Mohri, 1997; Sharma et al., 1999]. Fibronectin primarily interacts with integrin β_1 or $\alpha_v\beta_3$ through RGD tripeptide in III_{10} region, and with other integrins like $\alpha_4\beta_1$ through CBDs such as Arg-Glu-Asp-Val (REDV) and Leu-Asp-Val (LDV) domains in the type III connecting strand region. Recently, Pro-His-Ser-Arg-Asn (PHSRN) sequence in the III_9 region of fibronectin has been identified as a synergy site that cooperates with the RGD sequence in mediating cell adhesion and migration [Obara et al., 1988; Aota et al., 1994]. Aside from interacting with integrins, fibronectin also binds to transmembrane HSPG (e.g., syndecan-4) at the cell surface via motifs in repeats 12–14 such as Pro-Arg-Ala-Arg-Ile (PRARI), which act in concert with fibronectin-integrin binding to stimulate FA formation [Beyth and Culp, 1984; Woods et al., 1986; Woods et al., 1993]. With increasing trends toward the fabrication of novel biomaterials (e.g., vascular grafts and stents) for vascular repair, the peptides derived from CBDs such as RGD have been immobilized on the engineered surface to enhance EC attachment and spreading [Lin et al., 1994, 2001; Chung et al., 2003]. By conjugating RGD peptides on non-fouling surfaces, we have demonstrated the increase of EC adhesion and spreading with the increase of RGD density, but no significant effects are detected for EC proliferation [Patel and Li, unpublished observation], suggesting that the minimal RGD density to support EC proliferation has a very low threshold.

The density of ECM proteins controls the level of integrin–ECM adhesive interaction and plays an important role in regulating cell migration. At low adhesiveness, the cell cannot form new adhesions at the front efficiently; at high adhesiveness, the cell cannot break the cell–ECM adhesions at the rear. Therefore, cell migration shows a maximum speed at the intermediate level of adhesiveness [Duband et al., 1991; DiMilla et al., 1993; Keely et al., 1995]. We have shown that EC migration on fibronectin (0.5–40 $\mu g/cm^2$) has a biphasic dependency on the density, with highest migration speed at $\sim 5\ \mu g/cm^2$ [Shiu et al., 2004]. Shear stress increases the migration speed at both high and low densities, suggesting that shear stress may enhance the adhesion at the front on the low fibronectin density and the detachment at the rear on the high fibronectin density.

The distribution of ECM proteins is also critical in controlling EC functions. It has been shown that the restriction of EC spreading on micropatterned fibronectin surfaces decreases proliferation and increases apoptosis in ECs [Chen et al., 1997; Dike et al., 1999]. Micropatterned matrix strips (10–15 μm wide) promote EC differentiation and directional migration [Dike et al., 1999; Li et al., 2001]. On ECM proteins with a density gradient, cell migration is driven by haptotaxis, e.g., from less adherent area to more adherent area [Carter, 1967]. Haptotaxis may be involved in EC migration during angiogenesis and large vessel repair [Herbst et al., 1988], and can be a useful tool to enhance EC migration and angiogenesis by controlling the spatial distribution of bioactive molecules (e.g., ECM and its derived peptides) in vascular grafts, stents, and scaffolds.

Previous studies on haptotaxis used Boyden chambers with filters coated with matrix proteins on one side, but the matrix gradient and cell migration cannot be directly measured and characterized using this method. We have used microfabrication techniques to deposit matrix proteins onto substrates to create step changes of matrix density, and studied the crosstalk between haptotaxis and mechanotaxis induced by shear stress during EC migration on collagen. Analysis of time-lapse phase contrast microscopy videos revealed that shear stress at 2 dyn/cm^2 did not affect haptotaxis when compared to static control. However, shear stress at 3 dyn/cm^2 or higher was sufficient to drive EC migration in the flow direction against the collagen gradient (Hsu and Li, unpublished observation). Our findings suggest that fluid shear stress beyond a certain threshold can predominately regulate EC migration. Since the average level of fluid shear stress in the arteries is 10–20 dyn/cm^2, shear stress may be a major determinant of the direction of EC migration, especially for the individual cells at the wound edge without much cell–cell interaction. Although both ECM gradients and shear stress promote FA formation, the mechanism may be different. Higher ECM density may directly increase the rate of the binding between cell adhesion receptors and the underlying matrix, while shear stress may induce mechanotransduction through the cytoskeleton by

promoting directional microtubule elongation and by activating integrins and signaling molecules (e.g., Rac), thus promoting and stabilizing new FA formation at the front [Tzima et al., 2001, 2002; Hu et al., 2002; Li et al., 2002; Wojciak-Stothard and Ridley, 2003].

Inside-Out Mechanotransduction

Cell–ECM adhesions not only transduce the chemical and mechanical signals outside-in, i.e., from extracellular space to cytoplasm, but also transduce the signals inside-out through adhesion receptors to ECM. The feedback loop for outside-in and inside-out signaling plays an important role in coordinating cell migration. Cell migration is driven by asymmetric traction forces generated by the actin cytoskeleton and transduced through cell adhesion receptors. A migrating cell generates a pulling force in the front and a contraction force at the rear. The counter forces applied by the ECM to the cells, if not balanced, will be converted into the momentum for cell migration.

Inside-out signaling can be realized by the cytoskeleton and adhesion receptors. The major regulators of the cytoskeleton are Rho GTPases. Among Rho GTPases, Rho plays a major role in actin assembly and generating the force on the ECM, and Rac but not Cdc42 also contributes to force exerted on ECM [Zhong et al., 1998; Ridley, 2001; Li et al., 2003]. While Rac modulates the actin polymerization and force generation in the front, Rho may be involved in the contraction at the rear. In addition to the cytoskeleton, small GTPases also regulate integrin activation. Rac recruits high affinity integrin $\alpha_v\beta_3$ to lamellipodia during endothelial migration [Kiosses et al., 2001], while Ras suppresses integrin activation [Hughes et al., 1997]. The cytoplasmic domain of β integrins appears to mediate the integrin–ECM binding affinity and the force exerted on the ECM [Ylanne et al., 1993; Chen et al., 1994b; O'Toole et al., 1994]. Shear stress activates both Rac and integrins [Tzima et al., 2001, 2003; Hu et al., 2002; Wojciak-Stothard and Ridley, 2003], but which is activated first remains to be determined.

The increase of cytoskeletal contractility and the binding affinity of cell adhesion receptors will lead to the increase of the traction forces exerted on ECM. Over the past two decades, various techniques have been developed to study and measure the traction forces generated by cells during migration. Usually the traction forces are deduced from the deformation of a flexible substrate, e.g., the wrinkling of elastic membrane [Harris et al., 1980], the displacement of fluorescent beads in elastic substrate [Pelham and Wang, 1999; Balaban et al., 2001], and the array of cantilevers/posts [Galbraith and Sheetz, 1997; Tan et al., 2003]. By using the beads-in-substrate approach, the effect of shear stress on traction force generation has been studied. Within minutes, shear stress increases traction forces across the cell, with strongest traction forces at the periphery [Shiu et al., 2004], which may be correlated with the early increase of FA sizes and EC adhesions induced by shear stress [Li et al., 2002]. This increase of traction forces is dependent on shear stress-induced Rho activity because the inhibition of p160ROCK blocks the generation of traction forces [Shiu et al., 2004].

CELL–CELL ADHESIONS IN SHEAR STRESS-INDUCED EC MIGRATION

When ECs migrate from an existing monolayer or microvessels during wound healing or angiogenesis, cell–cell adhesions can significantly modulate the cell motility. Cell–cell adhesions not only directly couple ECs together, but also communicate with cell–ECM adhesions through the cytoskeleton and signaling molecules. Three major types of cell–cell adhesions are present in ECs. Adherens junctions mechanically couple ECs through the cytoskeleton linkage. Gap junctions allow the propagation of electrical and chemical signals between the cytoplasm of neighboring cells. Tight junctions control the permeability of the cells.

Vascular endothelial (VE)–cadherin is the major adhesion molecule at adherens junctions in ECs. The extracellular domain of VE–cadherin mediates the homotypic binding with the VE–cadherin in neighboring cells. The cytoplasmic domain of VE–cadherin interacts with β-catenin, plakoglobin, and p120 catenin. β-catenin and plakoglobin bind to α-catenin, which mediates the linkage of the cadherin–catenin complex to the actin cytoskeleton [Schnittler, 1998; Dejana et al., 1999]. There is evidence that plakoglobin is required to maintain adherens junctions under flow conditions [Schnittler et al., 1997]. Under static conditions, VE–cadherin and β-catenin appear at adherens junctions earlier than plakoglobin. The depletion of plakoglobin does not affect cell–cell

adhesions mediated by VE–cadherin and platelet–endothelial cell adhesion molecule 1 (PECAM-1), but results in junctional dissociation of ECs subjected to shear stress [Schnittler et al., 1997]. In an EC monolayer, shear stress disrupts adherens junctions and decreases the expression of VE–cadherin, α-catenin, and plakoglobin within hours, followed by the re-formation of adherens plaques anchored with stress fibers and an up-regulation of VE–cadherin, β-catenin, and α-catenin [Noria et al., 1999]. This is consistent with the remodeling of the cytoskeleton induced by shear stress, i.e., disruption of peripheral actin filaments and the re-formation of central stress fibers, suggesting a coordination of remodeling in the cytoskeleton and cell–cell adhesions. For a wounded monolayer, in contrast, instead of the re-formation of adherens junctions, ECs migrate away from the monolayer following the disruption of adherens junctions. Shear stress promotes the disruption of adherens junctions, with less or no adherens junctions at the wound edge to enhance EC migration [Hsu et al., 2001]. Chemical disruption of adherens junction further enhances the wound closure under flow [Albuquerque and Flozak, 2002]. These results implicate that cell–cell adhesions have inhibitory effects on EC migration, and this inhibition can be released by shear stress.

The early disruption of adherens junctions by shear stress is likely due to the post-translational regulation of the junction proteins. The tyrosine phosphorylation of catenins and VE–cadherin in ECs are associated with a decrease in cell–cell adhesion [Lampugnani et al., 1997]. Indeed, shear stress increases the phosphorylation of β-catenin in adherens junctions [Ukropec et al., 2002], which may account for the shear stress-induced junctional dissociation. Src may mediate the tyrosine phosphorylation of catenins and cadherins to destabilize adherens junctions [Behrens et al., 1993; Hamaguchi et al., 1993]. Since integrins $\alpha_5\beta_1$ and $\alpha_2\beta_1$ are located at cell borders and maintain cell–cell adhesions [Lampugnani et al., 1991], it is possible that shear stress-activation of integrins may activate Src to phosphorylate catenins and VE–cadherin. It is controversial whether Rho GTPases regulate adherens junctions in ECs. There is evidence that Cdc42 promotes but Rac inhibits the formation of adherens junctions [van Wetering et al., 2002; Kouklis et al., 2004], while there is evidence that VE–cadherin localization is not sensitive to Rho and Rac activity [Braga et al., 1999].

Besides the structural role of adherens junctions in EC wound healing, the complexes of cadherin–catenins can regulate intracellular signaling. Over-expression of VE–cadherin activates Rac and Cdc42 but de-activates Rho [Lampugnani et al., 2002; Kouklis et al., 2003]. Cytoplasmic p120 catenin, when not associated with cadherins, can form a complex with a guanine nuclear exchange factor Vav2 or RhoA-GDP, resulting in an increase of Cdc42/Rac activity and a decrease of RhoA activity, respectively [Anastasiadis and Reynolds, 2000; Noren et al., 2000], thus modulating the cytoskeleton and cell migration. Whether shear stress regulates Rho GTPases through VE–cadherin remains to be determined.

There is accumulating evidence that other receptors at cell–cell adhesions can mediate mechanotransduction. In response to shear stress, a complex containing VE–cadherin, β-catenin, and vascular endothelial growth factor receptor is formed, which is required for the shear stress-activation of Akt and p38MAPK [Shay-Salit et al., 2002]. PECAM-1 at cell–cell adhesions can also serve as a mechanotransducer. For example, PECAM-1 is required to activate downstream signaling such as ERK phosphorylation [Osawa et al., 2002], and shear stress induced dissociation of PECAM-1 and endothelial nitro oxide synthase (eNOS) at cell–cell adhesions can activate eNOS [Dusserre et al., 2004].

In addition to the adherens junctions, gap junctions and tight junctions in ECs are also regulated by shear stress. In gap junctions, laminar shear stress transiently increases the expression of connexin 43, while disturbed flow sustains the induction of connexin 43 but disorganizes the cell–cell communication [Cowan et al., 1998; Gabriels and Paul, 1998; DePaola et al., 1999]. In tight junctions, shear stress increases occludin phosphorylation and decreases occludin expression, which may be responsible for the increase of hydraulic conductivity of an EC monolayer [DeMaio et al., 2001]. The roles of gap junctions and tight junctions in mechanotransduction during EC migration await further investigation.

SUMMARIES AND PERSPECTIVE

In summary, the fluid shear stress-induced EC migration can be divided into four distinct

steps: (1) lamellipodia formation at the cell periphery without directional preference; (2) directional lamellipodial protrusion and new FA formation in the flow direction; (3) remodeling of pre-existing FAs; and (4) disassembly of FAs at the rear. Shear stress applied at the luminal surface of ECs can be sensed by cell membrane and associated receptors, and transduced immediately throughout cells (e.g., to cell–ECM adhesions and cell–cell adhesions) through the cytoskeleton. The first step could be due to the pure mechanical effect on cell membrane. The second step requires polarized mechano-chemical signaling (e.g., Rho GTPases), which may be initiated by polarized microtubule elongation and membrane associated receptors. The third step may involve the increase of cell adhesion to resist shear stress and the translocation/merging of FAs. The fourth step may involve active contraction and protease activity for FA disassembly. If cell–cell adhesions exist, the cells need to dissociate or re-organize cell–cell adhesions for cell migration.

The persistent EC migration in the flow direction can be driven by positive feedback loops constituted by Rho GTPases, the cytoskeleton, and FAs at the leading edge (Fig. 1). The microtubule-Rac and Rac-integrin loops can provide persistent signals to drive actin polymerization and FA formation at the front. Two critical issues need further studies: (1) how the positive feedback loops are initiated by shear stress, and (2) the mechanism of polarized activation of Rho GTPases by shear stress.

The interactions between cell adhesion receptors and ECM proteins mediate outside-in and inside-out mechano-chemical signaling. Different cell adhesion receptors and ECM proteins may have differential roles in EC migration. The density and distribution of ECM can modulate EC migration and mechanotransduction. The questions to be answered include: (1) the relative contribution of different integrins and cell surface proteoglycans to mechanotransduction and EC migration; (2) the roles of different ECM proteins and CBDs in mechanotransduction for EC migration; (3) the long-term dynamics of the traction forces induced by shear stress on ECM; and (4) the dependency of EC migration on ECM rigidity. The understanding of cell–ECM adhesions in mechanotransduction will help us establish the rational basis of engineering ECM for vascular tissue engineering.

The effects of shear stress on cell–cell adhesions have been demonstrated in previous studies. The future studies should address: (1) the mechanotransduction to and from cell–cell adhesions; and (2) the crosstalk between cell–cell adhesions and other mechanotransducers (e.g., cell–ECM adhesions).

Finally, the effect of shear stress on EC migration in three-dimensional ECM may result in mechanotaxis of ECs for angiogenesis. The shear stress regulation of matrix metalloproteinases and the contribution of matrix metalloproteinases to EC migration in three-dimensional ECM under flow conditions need to be investigated.

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