Role of Integrins in Endothelial Mechanosensing of Shear Stress

John Y.-J. Shyy, Shu Chien

Abstract—The focal pattern of atherosclerotic lesions in arterial vessels suggests that local blood flow patterns are important factors in atherosclerosis. Although disturbed flows in the branches and curved regions are proatherogenic, laminar flows in the straight parts are atheroprotective. Results from in vitro studies on cultured vascular endothelial cells with the use of flow channels suggest that integrins and the associated RhoA small GTPase play important roles in the mechanotransduction mechanism by which shear stress is converted to cascades of molecular signaling to modulate gene expression. By interacting dynamically with extracellular matrix proteins, the mechanosensitive integrins activate RhoA and many signaling molecules in the focal adhesions and cytoplasm. Through such mechanotransduction mechanisms, laminar shear stress upregulates genes involved in antiapoptosis, cell cycle arrest, morphological remodeling, and NO production, thus contributing to the atheroprotective effects. This review summarizes some of the recent findings relevant to these mechanotransduction mechanisms. These studies show that integrins play an important role in mechanosensing in addition to their involvement in cell attachment and migration. (Circ Res. 2002;91:769-775.)

Key Words: shear stress ■ mechanotransduction ■ endothelium ■ integrins ■ Rho

Located between the circulating blood and the vessel wall, vascular endothelial cells (ECs) are the primary cell type exposed to the shear stress resulting from blood flow. During the last 2 decades, the mechanotransduction mechanisms by which ECs convert shear stress stimulation to biochemical signals have been studied intensively with both in vivo and in vitro approaches. Experiments with the use of cultured ECs in flow channels, which allow the control of chemical and mechanical factors, facilitate the investigation of specific cellular responses to the applied mechanical forces. Knowledge emerging from interdisciplinary research involving vascular biology and bioengineering has demonstrated that mechanical sensing of shear stress can occur on both the abluminal and luminal sides of the EC membrane.

Integrins are membrane-associated glycoproteins composed of \( \alpha \) and \( \beta \) subunits. To date, 18 \( \alpha \) and 8 \( \beta \) subunits have been identified in mammalian cells. Each subunit has a large extracellular domain, a transmembrane spanning region, and a short cytoplasmic domain (see review\(^3\)). The extracellular domain binds directly to extracellular matrix (ECM) proteins, such as vitronectin, fibronectin, laminin, and collagen. The cytoplasmic domains of both the \( \alpha \) and \( \beta \) subunits interact with signaling molecules and cytoskeletal proteins to regulate cellular events, such as signal transduction, cytoskeletal organization, and cell motility via the modulation of integrin affinity and/or avidity. Affinity modulation involves changes in integrin heterodimer conformation that lead to an increased binding effectiveness for their ligands, whereas...
avidity modulation involves changes in lateral mobility and clustering of heterodimers to facilitate cell binding to multivalent matrices (see reviews2-3).

Integrin activation is directly associated with members of the Rho small GTPase family, including RhoA, Cdc42, and Rac (see reviews2-4). Rho family GTPases are converted from the inactive GDP-bound form to the active GTP-bound form in response to stimuli such as serum and lysophosphatidic acid to effect responses such as cell adhesion, stress fiber formation, and motility enhancement (see review8). GDP/GTP cycling is regulated by guanine nucleotide exchange factors, GTPase-activating proteins, and guanine nucleotide dissociation inhibitors (see review6). RhoA, Cdc42, and Rac have distinct functions in regulating the actin-based cytoskeletal structure. RhoA increases cell contractility, focal adhesions, and actin stress fiber formation; Cdc42 regulates filopodia formation; and Rac regulates membrane ruffling (see reviews6-7).

The integrin-elicited signaling events are commonly investigated by allowing cells to attach to ECM proteins or treating cells with monoclonal antibodies (mAbs) against integrins.8 Shear stress is associated with an increase in the expression of p21, an inhibitor of cyclin-dependent kinases (cdk2 and cdk4).10,11 Physiological levels of shear stress upregulate gene products involved in cytoskeletal remodeling (eg, β-actin and myosin heavy chain), cell cycle arrest (eg, cyclin D1 and GADD45), EC survival (eg, Tie2 and Flk-1), and antioxidation (eg, heme oxygenase-1 and cytochrome P-450).16-18 The results from these high-throughput analyses allow us to generate new hypotheses to elucidate how blood flow regulates groups of proatherogenic or antiatherogenic genes in the arterial wall.

It has been shown that shear stress activates many Thr/Ser and Tyr kinases located in the cell membrane, focal adhesions, and cytoplasm. These kinases include focal adhesion kinase (FAK), c-Src, phoshatidylinositol 3-kinase (PI3K), myosin light chain kinase, Akt kinase, IκB kinases, and receptor tyrosine kinase Flk-1.19-25 Some of these kinase pathways have also been shown to be modulated by blood flow in animal models in vivo. The PI3K-Akt and protein kinase A pathways regulate synergistically the activation of endothelial NO synthase,23,26 leading to an increase in NO production. Wortmannin, a PI3K inhibitor, significantly reduces the NO-induced vasodilation in perfused rat mesenteric arterial beds.27 En face confocal microscopy has shown that treating LDL receptor knockout mice with lipopolysaccharide or feeding these animals with an atherogenic diet results in nuclear factor-κB activation and an upregulation of nuclear factor-κB-inducible genes, such as vascular cell adhesion molecule-1 and E-selectin, in ECs predominantly in proatherogenic regions.28 Many of the shear stress–activated kinases, eg, extracellular signal–regulated kinase (ERK) and FAK, are known to be regulated by integrins. Such a unique correlation is an important clue for the involvement of integrins in mechanotransduction.

Responses of ECs to Shear Stress
Recent studies have shown that laminar shear stress modulates many genes related to vascular biology, including cell fate (ie, cell cycle progression and apoptosis). Laminar shear stress suppresses the G1- to S-phase transition in ECs,10,11 and this is associated with an increase in the expression of p21, an inhibitor of cyclin-dependent kinases (cdks), at mRNA and protein levels. These changes are accompanied by decreases in the phosphorylation of retinoblastoma protein (Rb) and the activities of cdk2 and cdk4.10,11 Physiological levels of laminar shear stress prevent apoptosis of ECs in response to a variety of stimuli, including tumor necrosis factor-α, oxidized LDL, and angiotensin II.12,13 There is evidence that the laminar shear stress–induced antiapoptotic effect is mediated by the upregulation of superoxide dismutase and NO synthase.14 Analysis of EC apoptosis in human carotid atherosclerotic plaques has shown a preferential occurrence of apoptosis in the downstream side of the plaques, where shear stress is low and unsteady, compared with the upstream parts.15

Most of these studies on the effects of shear flow on gene expression were performed on a “single-gene” basis. Recently developed microarray technology enables the comprehensive screening of gene expression files. Such analysis has been used to compare the transcriptional profiles in ECs exposed to prolonged laminar shear stress or turbulent flow versus static controls. The results indicate that laminar shear stress upregulates gene products involved in cytoskeletal remodeling (eg, β-actin and myosin heavy chain), cell cycle arrest (eg, cyclin D1 and GADD45), EC survival (eg, Tie2 and Flk-1), and antioxidation (eg, heme oxygenase-1 and cytochrome P-450).16-18 The results from these high-throughput analyses allow us to generate new hypotheses to elucidate how blood flow regulates groups of proatherogenic or antiatherogenic genes in the arterial wall.
In complementary experiments, integrin activation due to cell attachment was shown to be sufficient for SREBP activation. Blocking integrins with RGD peptide abolishes the shear stress–induced secretion of basic fibroblast growth factor and the antiapoptotic effect of shear stress. In addition to modulating the avidity and affinity of integrins, shear stress also increases the mRNA and protein levels of the α5 and α2 integrins in ECs. Compared with static controls, ECs preexposed to shear stress revealed a significant increase in their reattachment to ECM, and this increased attachment was blocked by RGD peptide, anti-α5 antibody, or anti-α2 antibody.

Integrin-Associated Mechanotransduction in ECs
Integrins play a major role in the shear stress activation of signaling events in focal adhesions and the actin-based cytoskeleton. FAK and Shc are 2 molecules that have been shown to mediate the activation of downstream mitogen-activated protein kinases (MAPKs) by shear stress.

On activation, FAK is autophosphorylated at Tyr397 and associates with the Src homology 2 (SH2) domain of c-Src. As a result, c-Src phosphorylates paxillin and p130Cas, which serve as scaffolds for the recruitment of various adaptors and signaling intermediates (see reviews). Consisting mainly of the SH2 and Src homology 3 (SH3) domains, Crk is an adaptor protein that can bind to the phosphorylated paxillin and p130Cas and is involved in the integrin-mediated signaling. Among the several effector proteins bound to Crk, C3G is a guanine nucleotide exchange factor for Rap1, which is a member of Ras family G proteins (see review). The functions of Rap-1 may be different depending on cell types. Rap-1 antagonizes Ras in T cells but activates the Raf-ERK cascade, as does Ras, in PC12 cells and contributes to the integrin-mediated adhesion of T lymphocytes and embryonic fibroblasts. As shown in Figure 1, the activation of the integrin-dependent FAK/c-Src pathway can lead to ERK activation through paxillin, p130Cas, Crk, C3G, Rap-1, and Raf. Several lines of evidence indicate that shear stress activates this integrin-initiated signaling. First, tyrosine kinases in focal adhesions, eg, FAK and c-Src, are rapidly activated in ECs by shear stress. Second, shear stress causes tyrosine phosphorylation of p130Cas and its association with Crk in ECs and the consequent activation of ERK. Furthermore, the use of FAK(F397Y) and c-Src(K295R), the respective kinase-defective mutants of FAK and c-Src, blocks shear stress activation of ERK. Shear stress can enhance EC directional migration in flow channels. FAK signaling is also implicated in the shear stress–induced EC migration. Time-lapse confocal microscopy has revealed that FAK tagged with green fluorescence protein is recruited to new focal adhesions to support the protrusion of lamellipodia in...
the leading front and disappears at the rear of the cells. These findings suggest that dynamic remodeling of FAK facilitates the rear detachment while enhancing the formation of new focal adhesions at the leading edge. Sharing high homology with FAK, RAFTK/Pyk2 is another member of the FAK family. Tyrosine phosphorylation of RAFTK/Pyk2, as that of FAK, leads to the recruitment of c-Src, p130Cas, growth factor receptor binding protein 2 (Grb2), and the activation of ERK (see review52). Although there is yet no evidence that shear stress increases the phosphorylation of RAFTK/Pyk2, it is possible that this signaling molecule plays a role similar to that of FAK in mechanotransduction.

Although most integrins activate FAK, a subset, including αβv, αβ3, and αβ6, also activates Shc, which is an adaptor protein containing a C-terminal SH2 domain. Tyrosine-phosphorylated Shc becomes associated with the cognate receptor tyrosine kinases through SH2 binding and mediates the integrin-induced signal transduction. Shc is recruited to αβv, αβ3, and αβ6 in A431 cells after the conjugation of these integrins to their corresponding antibodies. As shown in Figure 1, caveolin-1 (Cav-1) and Fyn tyrosine kinase are 2 critical molecules for the Shc-dependent pathway. Cav-1, a major protein component of caveolae, is important for modulating multiple signaling molecules and cholesterol trafficking. With the use of various αv mutant constructs, Wary et al showed that integrin α subunit and Cav-1 interact predominantly within the lipid bilayer. In the same study, it was shown that Cav-1 is constitutively associated with the Src family member Fyn. On integrin ligation, Fyn is activated and binds to Shc, leading to the phosphorylation of Shc at Tyr317 and recruitment of the adaptor molecule Grb2. This sequence of events leads to the coupling of integrins to the Ras-ERK pathway, thus promoting cell cycle progression. Shear stress activation of αβv and β3 integrins is associated with an increased association of these integrins with Shc. It is likely that Cav-1 facilitates the linking of Shc, Fyn, and the αv-integrin subunit for mechanotransduction. This hypothesis is supported by the findings that shear stress activation of ERK is inhibited by a polyclonal anti-Cav-1 and that shear stress can activate Fyn (S. Jalali, S. Chien, unpublished data, 1998). The increased integrin association with Shc is functionally related to the shear stress activation of ERK and JNK and the consequent AP-1/TRE-mediated transactivation, inasmuch as a dominant-negative mutant of Shc inhibits these events.

The association of αβv and β3 with Shc has been used as a readout to test a working model for integrins in mechanotransduction. The central theme of this model is that a dynamic interaction between integrin and the cognate ECM protein is crucial for mechanotransduction (Figure 2A). Two approaches were used to block integrins from making new connections, thus inhibiting their dynamic interaction with ECM proteins. First, ECs were plated on flow channel coated with an anti-integrin mAb so that the ability of the integrin to make new connections was restricted by the strong mAb/integrin conjugation (Figure 2B, top). Second, the available integrin-binding sites of ECM proteins were blocked by their mAb to prevent any free integrin from making new ligations (Figure 2B, bottom). The shear stress–induced association of αβv and β3 with Shc was prevented by either of these 2 approaches, suggesting that a dynamic interaction between integrin and ECM ligands is essential for mechanotransduction. By analyzing the real-time images of the changes in area and topography of adhesion sites in living cells subjected to shear stress, Davies et al observed constant remodeling of focal adhesions in ECs under shear stress, with approximately equal gains and losses of focal adhesion areas as each site undergoes remodeling. The working model in Figure 2 shows that (1) the focal adhesions and the associated signaling molecules, eg, integrin and Shc, are dynamic in their interactions and that (2) mechanotransduction can occur at the abluminal side of ECs through the integrin-ECM interaction. These are in concert with the observations made by Davies et al.

**Role of RhoA in Mechanotransduction**

Chemical stimuli (eg, lysophosphatidic acid) cause a transient translocation of RhoA from cytosol to membrane or cytoskeleton on activation. Shear stress, such as produced by chemical stimuli, causes a transient increase in RhoA association with the membrane. Integrin activation in ECs by either attachment to ECM or mechanical shearing causes a 2-phase change in RhoA activity. EC attachment to ECM initially causes a decrease in RhoA activity, which has been linked to an activation of either FAK or Src, resulting in tyrosine phosphorylation and activation of p190 Rho GTPase–activating protein. This transient suppression is followed by a modest activation phase. Using Rho GTP loading assays, Tzima et al have shown that shear stress causes a similar 2-phase change in RhoA activity. The shear stress modulation of RhoA activity is attenuated if the new integrin-ECM interaction is inhibited by blocking the unoccupied ECM protein (fibronectin) sites with the blocking-type mAb. These results support the hypothesis that RhoA is also regulated by the dynamic integrin-ECM binding in ECs in response to shear stress (Figure 2).

The shear stress activation of Rho is linked functionally to EC migration, MAPK signaling, and the organization of the actin-based cytoskeleton. The inhibition of RhoA with C3 exoenzyme reduces EC migration under static and flow conditions. The attenuation of shear stress activation of JNK by RhoN19, a dominant-negative mutant of RhoA, suggests that RhoA regulates the shear stress induction of JNK-mediated AP-1/TRE activation. Shear stress–induced cell alignment and stress fiber formation were inhibited by RhoN19 and by a kinase-defective mutant of the Rho effector ROCK, which is a Rho-associated Ser/Thr kinase. RhoA regulates cofilin, an actin-depolymerizing protein, via ROCK and LIM kinase (LIMK), and this may provide one of the pathways that mediate the shear stress–induced actin reorganization. The RhoA-ROCK-LIMK-cofilin signal transduction pathway, which is known to modulate the spatial and temporal specificity of the actin assembly in response to various extracellular stimuli, has recently been shown to also regulate the shear stress activation of SREBP.

**Summary and Discussion**

Investigations on the effects of shear stress have demonstrated that integrins play significant roles in the shear-
elicited signaling in ECs. These studies demonstrate that integrins are involved not only in cell adhesion and migration but also in mechanotransduction. However, additional studies are needed to address the molecular basis of mechanosensing by integrins and its relation to vascular biology.

Although the apical membrane of ECs is directly exposed to flow, WOW-1 immunostaining at the basal side of the sheared ECs suggests the activation of integrins on the abluminal membrane. In line with this observation, shear stress also elicits the dynamic remodeling of focal adhesions on the basal side of ECs. These results raise the question of whether the inactive integrins on the apical membrane translocate to the basal membrane after their activation by shear stress or whether only the integrins interacting with ECM on the basal side are activated by shear stress. In either case, dynamic changes in the membrane would be required to either translocate the activated integrins or transduce the mechanical stimuli from the apical to the basal membrane. One possible mechanism by which shear stress activates integrins through the cell membrane is the shear-induced increase in membrane fluidity, which may lead to an augmented avidity of integrins. By increasing their lateral mobility in the plane of the membrane, which is followed by a conformational change, the integrins in the basal membrane can enhance their dynamic interaction with the cognate ECM proteins. The resulting increases in integrin avidity and affinity may thus activate some of the integrin-associated signaling molecules.

Many shear stress–activated signaling events depend on the actin-based cytoskeleton. Apparently, RhoA regulates the assembly of stress fiber in response to shear stress. However, the detailed molecule interplay among integrins, RhoA, and actin in mechanotransduction remains elusive. Within the RhoA signaling cascade, p21-activated kinase (PAK)-1, like ROCK, regulates cytoskeletal reorganization, actin polymerization, and focal adhesion formation. Both ROCK and PAK-1 can phosphorylate LIMK, and the activated LIMK (phosphorylated form) can then phosphorylate cofilin, which is an actin-regulatory protein. Dominant-negative mutants of PAK-1 block the shear stress–induced cytoskeletal remodeling, suggesting that other RhoA downstream effector proteins may also contribute to the mechanotransduction. An intact actin cytoskeleton is necessary for many, if not all, mechanotransduction processes. One possible explanation is that the cytoskeletal network facilitates the translocation of various signaling molecules from the focal adhesion site to the cytoplasm. In contrast, the tensegrity model suggests that the F-actin can be used for transmitting forces from integrins to intracellular organelles. How cytoskeleton coordinates with membrane fluidity and the dynamic integrin-ECM in-
teraction in regulating intracellular signaling needs further investigation.

The majority of the work cited in the present review are studies using laminar flow channels. A comparison between laminar versus disturbed flow in their modulation of integrins and the associated RhoA would be important in understanding their physiological and pathophysiological roles in vascular biology, because the preferential location of atherosclerotic lesion in branch points and curved regions may be related to the local disturbed flow patterns. Finally, like any molecular mechanisms elucidated from in vitro experiments, the mechanisms of mechanotransduction deduced from flow channels need to be verified by studies on the responses of vascular wall to various flow patterns. Davies et al have recently demonstrated the feasibility of such an experimental approach. By profiling the gene expression in single ECs isolated from pig common carotid artery and comparing these results from those from flow channel experiments, these authors suggested more expression heterogeneity in the disturbed than in the undisturbed flow area. Similarly, profiling the integrin-mediated transcription in the arterial tree would help us understand better the role of integrin-elicated mechanotransduction in health and disease.

Acknowledgments

This review was supported in part by NIH grants HL-19454, HL-62747, and HL-64382 (Dr Chien) and grants HL-56707 and HL-60789 (Dr Shyy) from the National Heart, Lung, and Blood Institute. Dr Shyy is an Established Investigator of the American Heart Association.

References


Role of Integrins in Endothelial Mechanosensing of Shear Stress
John Y.-J. Shyy and Shu Chien

Circ Res. 2002;91:769-775
doi: 10.1161/01.RES.0000038487.19924.18
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/91/9/769