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Choosing Sides in Polarized Endothelial Adaptation to Shear Stress

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Introduction

The endothelium is a primary integrator of biophysical and chemical cues that guide vascular wall physiology and pathology. Normally, arterial endothelial cells appear elongated longitudinally and rest on a basement membrane of collagen type IV and laminin. In atherosclerosis, lesions form primarily near arterial bifurcations and along the inner curvature of the aorta where complex spatiotemporal profiles of hemodynamic forces exist and where endothelial cells exhibit a non-polarized structure and upregulate expression of a provisional matrix enriched in fibronectin and fibrinogen. The regional heterogeneity in endothelial phenotype and matrix expression suggests that lesion progression requires transduction of mechanical cues associated with hemodynamic wall shear stress and artery wall stretch into biochemical signals for inflammation. Integrins have been proposed as candidate mechanotransducers capable of differentiating both physical cues and matrix composition, but an integrin-mediated mechanism that confers directionality in response to shear stress has remained elusive. In this issue of *Circulation Research*, Goldfinger et al.[1] report that shear stress activates protein kinase A (PKA) to phosphorylate α4 integrin locally at the downstream edge of endothelial cells, and phosphorylated α4 releases inhibition of the GTPase Rac1 to direct polarized reorganization of the cytoskeleton. The proposed mechanism is important not only because it improves understanding of intracellular spatial organization in mechanotransduction mechanisms but also because it suggests new avenues for engineering a healthy endothelium after bypass grafting or vascular stent procedures.

Spatial Organization during Endothelial Mechanotransduction

Endothelial cells associated with an atheroprotective phenotype exhibit planar polarity characteristics that include elongated shape, actin stress fibers oriented parallel to the shear stress direction, and microtubule organizing centers (MTOCs) located downstream of the nucleus. Goldfinger et al. propose that phosphorylated α4 integrin is localized preferentially near the downstream edge of the cell and serves as an early polarizing signal that is required for these adaptations to occur. What transmits the direction of shear stress to locations in the cell that drive these processes? One possibility involves the apical plasma membrane itself.

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The lateral mobility of lipids in the plasma membrane is increased in regions downstream of the nucleus after onset of shear stress,[2] perhaps enabling increased activation of G protein–coupled receptors.[3] It is tempting to propose that this mechanism would also enhance transport rates of α4 integrins to enable spatial concentration near the downstream edge, but this hypothesis would require the unlikely assumption that α4 mobility is independent of interactions with the cytoskeleton. A second possibility for transmitting directional cues involves intracellular "decentralization" of force by transmission through the cytoskeleton from the apical surface to locations where signaling is initiated.[4, 5] This idea is supported by measurements of strain focusing in the cytoskeleton near adhesions and junctions[6] and by intracellular stress tomography after onset of shear stress.[7] For example, shear stress onset induces coordinated displacement of stress fiber termini, adhesion sites, and extracellular matrix fibrils in the downstream direction,[8] reflecting a coordinated redistribution of intracellular tension. It is likely that redistribution of cytoskeletal tension in response to shear stress contributes to spatially polarized phosphorylation of ligated α4 integrins, as has been demonstrated for other integrins in nascent focal adhesions.

Following integrin activation in this manner, spatial polarization of downstream signaling is required for endothelial cell adaptation to unidirectional shear stress. Shear stress onset induces conformational activation and new ligation of αVβ3 integrins near the cell periphery, leading to transient downregulation of the GTPase RhoA, and adaptive alignment of endothelial cell shape and stress fibers does not occur if any of these events is inhibited.[9] Activation of Rac1 locally near the downstream edges of endothelial cells is also required for shear stress–induced alignment.[10] Polarized Rac activity promotes actin polymerization associated with leading edge lamellipodia, and endothelial cells in subconfluent layers or at wound edges migrate parallel to shear stress in a process termed mechanotaxis.[11] However, a plausible link that translates shear stress–induced integrin activation into spatially polarized signaling has not been proposed until now.

An Integrin Whose Function is Not Adhesion Strengthening?

Most work in integrin mechanosignaling has focused on explaining adhesion strengthening and cytoskeletal reinforcement or stiffening under an external applied stress.[12-14] In these models, α5β1 or αVβ3 integrins interact with "synergy" and "cell-binding" domains in type III repeats 9 and 10, respectively, of matrix fibronectin. Although adhesion strengthening occurs locally where forces are applied with micrometer scale probes, evidence for spatial polarity in response to a force gradient at the cell length scale (as might be the case for shear stress) is lacking. The CS-1 domain of fibronectin is a variably spliced segment containing the LDV (leucine-aspartate-valine) consensus sequence of amino acids that serves as a ligand for α4β1 and α4β7 integrins. Goldfinger et al. adhered endothelial cells on CS-1 fragment to limit ligated integrin to α4 only. This strategy revealed a role for α4 in sensing shear stress direction that may be distinct from the functions of α5 and αV in modulating mechanotransmission and cytoskeletal reinforcement.

How does α4 transmit the direction of shear stress? Previous work on cell migration suggests a mechanism.[15] α4 is phosphorylated on Serine-988 by PKA, preventing binding of paxillin. Along the sides and trailing edges of migrating cells where α4 is not phosphorylated, paxillin binds and recruits a GTPase-activating protein (GAP) for ADP-ribosylation factor (Arf). The Arf-GAP, known as GIT1, decreases Arf activity, causing local inhibition of Rac1 activity. The resulting spatial polarization of activated Rac leads to stabilization of a directional lamellipodium. Goldfinger et al. now suggest a similar role for PKA-mediated α4 phosphorylation in shear stress–induced directional Rac activation, lamellipodium stabilization, and cell migration. PKA was responsible for phosphorylating α4, since PKA inhibitors blocked α4 phosphorylation at the leading edge, Rac1 activation near the leading

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edge, and adaptive elongation and alignment of the cells. Thus, a primary role for α4 integrin in establishing planar polarity in response to unidirectional shear stress has been established.

Harnessing Mechano-Polarization Mechanisms

Several major questions remain to be answered in order to clear the path for engineering endothelial wound healing after bypass grafting or stent placement. For example, why is α4 phosphorylated by PKA only at the leading edge? In neutrophils, exposure to a spatial gradient of PKA inhibitor is sufficient to stimulate directional migration,[16] but it remains unknown whether PKA activation in endothelial cells is spatially localized near the leading edge after shear stress onset. On an in vivo matrix, local activation of PKA may depend on crosstalk with other newly ligated integrins. Shear stress induces activation of PKA and suppression of αVβ3 conformational activation in endothelial cells plated on collagen, whereas PKC is activated and α2β1 is suppressed in cells plated on fibronectin.[17] Thus, elucidating the relative roles of interacting integrin and matrix signals remains a hurdle to solving directional mechanosensing.

Alternate α4 phosphorylation sites may also play an important role in directional sensing. For example, overexpression in Chinese hamster ovary (CHO) cells of α4 with Tyrosine-991 mutated to alanine prevents paxillin binding to α4 and promotes leading edge spreading in response to shear stress,[18] suggesting that shear stress–induced tyrosine phosphorylation of α4 may counteract directional sensing independently of paxillin binding. Interestingly, wild type α4 expressed in CHO cells was phosphorylated on Ser-988 both at the leading and trailing edges of cells migrating in response to shear stress, and mutation of Ser-988 inhibited both leading edge extension and trailing edge retraction. Thus, Ser-988 phosphorylation may serve a dual role to enhance directional sensing in some cases.

Spatial polarization of α4-paxillin-GIT1 is not the only mechanism proposed to regulate spatial activation of Rac. Rac is activated in waves propagating from newly formed adhesions in cells on micropatterned fibronectin substrates,[19] suggesting that an alternative mechanism for establishing Rac polarity exists that depends on new ligation of αVβ3 and/or α5β1. However, a role for α4 ligation cannot be ruled out because micropatterns were generated with full-length fibronectin, so a “leading edge” would be determined by the geometry of the micropatterns.

Biomedical engineers seek the ability to harness mechanotransduction mechanisms to design substrates that enhance endothelial wound healing for development of artificial vascular grafts. On substrates coated with CS-1 fragment and exposed to arterial levels of shear stress, retention of some endothelial cell types but not others was improved, probably due to variability in expression levels of α4.[20] Even when α4 is exogenously overexpressed, the correlation between α4-paxillin–mediated signaling and adhesion strength is weak.[18] However, the ability to control directional migration to enhance wound healing or re-endothelialization may represent the real opportunity for improving therapies in patients with advanced atherosclerosis. The mechanism elucidated by Goldfinger et al. represents a major step in the right direction.

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