Mechanotransduction of shear stress occurs through changes in VE-cadherin and PECAM-1 tension: Implications for cell migration

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Recent work has shown that cadherins at cell-cell junctions bear tensile forces. Using novel FRET-based tension sensors, we showed first that in response to shear stress, endothelial cells rapidly reduce mechanical tension on vascular endothelial (VE)-cadherin. Second, we observed a simultaneous increase in tension on platelet endothelial cell adhesion molecule (PECAM)-1, induced by an interaction with vimentin. In this commentary, we discuss how our results fit with existing data on cadherins as important mediators of mechanotransduction, in particular, in cell migration where mechanical tension across cadherins may communicate the direction of movement. The ability of PECAM-1 to bear mechanical tension may also be important in other PECAM-1 functions, such as leukocyte transmigration through the endothelium. Additionally, our observation that vimentin expression was required for PECAM-1 tension and mechanotransduction of fluid flow suggests that intermediate filaments are capable of transmitting tension. Overall, our results argue against models where an external force is passively transferred across the cytoskeleton, and instead suggest that cells actively respond to extracellular forces by modulating tension across junctional proteins.

Introduction

Collective cell migration, the movement of cells as a group over long distances, is observed in developmental events from gastrulation to organogenesis. In particular, the migration of epithelial cells has been observed to consist of a sheet of cells moving, with minimal repositioning of individual cells within the sheet during migration. This strongly suggests that there exists a method of communication between cells at the leading edge and cells further back in order to coordinate the collective movement of the sheet of cells. Mechanical force has been proposed as a method of communication, that a “tug-of-war” of forces between cells drives and coordinates movement.1 The forces from the cell to the substrate are highest at the leading edge,1,2 and it was shown that the parallel component of the traction force (presumed to be junctional force) increases at cells further back from the leading edge.1 This polarization of forces requires the presence of cadherin-based junctional adhesions.2,3 Additionally, angiogenesis of endothelial cells, also a migration-dependent process, requires VE-cadherin.4 A recent study in drosophila showed a front-to-back gradient of E-cadherin tension that was required to communicate the direction of migration during embryo development.5 In light of these reports of cell junction and cadherin tension mediating migration, we will review our recent publication in which we examined the force changes on VE-cadherin and PECAM-1 in endothelial cells subjected to shear stress.6 In our study we reported that the stimulus of fluid flow triggers a rapid rearrangement of tension across these 2 proteins, indicating that both PECAM-1 and VE-cadherin forces are dynamic and actively regulated by the cell (Fig. 1). The forces across these 2 proteins are likely important drivers of signaling for a variety of cellular processes in the vascular system, including cell migration.

Keywords: cell-cell junctions, fluid shear stress, mechanotransduction, PECAM-1, VE-cadherin, vimentin intermediate filaments

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**Intercellular Junctions as Sites of Mechanotransduction**

Fluid shear stress, the frictional drag force exerted by blood flow on the endothelial cells that line blood vessels, is an important determinant of normal vessel morphogenesis. Furthermore, atherosclerosis arises at regions of arteries whose geometry leads to complex flow patterns with lower shear stress magnitude and changes of direction during the cardiac cycle. These flow patterns synergize with conventional risk factors such as high low-density lipoprotein (LDL) cholesterol to induce formation of atherosclerotic plaques. These effects of shear stress are mediated by specific mechanotransduction responses by the endothelium. Previous work identified a complex of proteins at cell-cell junctions, comprised of VE-cadherin, PECAM-1, and VEGFR2 (reviewed in) that mediates an important subset of the flow responses that contribute to both physiological remodeling of blood vessels and pathological remodeling in atherosclerosis. These data showed that force applied to PECAM-1 triggered many of the same responses as did flow, whereas no such effects were seen when force was applied to VE-cadherin. Thus, it was proposed that PECAM-1 was a direct mechanotransducer whereas VE-cadherin, though essential, played a distinct role. This model fit well with the proposal that force applied to the apical domain of the endothelial cells could be transmitted through the cytoskeleton to the cell-cell and cell-matrix adhesions, where other receptors could transduce these forces into biochemical signals.

To understand these issues in detail, we developed a FRET-based tension sensor that is capable of resolving tensile forces across specific proteins. We then developed VE-cadherin and PECAM-1 tension sensors to measure forces across these proteins at the onset of fluid shear stress. The VE-cadherin tension sensor revealed substantial myosin-dependent force across this molecule at cell-cell junctions in resting cells. Surprisingly, application of fluid shear stress reduced VE-cadherin tension, as well as the overall cell-cell junctional forces, and cell-matrix traction forces, all by approximately 25%. Thus, shear stress triggered a modest, global relaxation of cellular tension. By contrast, tension on PECAM-1 was initially undetectable but rapidly increased after flow. This pattern of responses is clearly inconsistent with the model of simple, passive force transfer and instead suggested a more complex, active response mechanism. Further work showed that the increase in tension across PECAM-1 was mediated by a rapid association of PECAM-1 with vimentin intermediate filaments. It was also dependent on myosin. Together, these data suggested that flow initially acts upon another mechanotransducer that triggers the association of PECAM-1 with vimentin filaments and transmission of force from myosin to PECAM-1. Force on PECAM-1 then triggers a second wave of signals. We speculated that this pathway may represent a mechanical analog of the amplification mechanisms frequently observed in biochemical pathways. In this case, the weak force from flow results in application of the much stronger force from myosin to PECAM-1.

**Cadherins in Mechanotransduction**

There is also evidence that cadherins, including VE-cadherin, participate in mechanotransduction, specifically, the reorganization and strengthening of the junction in response to applied force. This occurs at least in part by recruiting additional cadherin molecules to the junction and also recruiting vinculin to strengthen the link between α-catenin and actin. We and others have observed that the tension across VE- and E-cadherin is dependent on actomyosin contractility, indeed, junctional assembly requires myosin activity. It may be relevant that the decrease in tension across VE-cadherin after application of flow required PECAM-1 expression and was blocked by an inhibitor of src family kinases (SFKs) (Conway, unpublished data). Potential SFK substrates include both cadherins and β-catenin, whose phosphorylation affects junctional stability. Tension applied to PECAM-1 triggers activation of SFKs. Interestingly, SFKs are also activated downstream of soluble factors such as VEGF and thrombin that regulate junctional stability, and through
Mechanical Force and Intermediate Filaments

Our finding that PECAM-1-dependent flow signaling involves intermediate filaments is consistent with impaired flow responses in vivo in vimentin knockout mice.27-29 Our data suggested that intermediate filaments transmit mechanical forces from actomyosin to PECAM-1. Recent studies have suggested that intermediate filaments are in fact extensible, reaching strains of up to 250% (3.5x the original length) before breaking.30 Electron microscopy of uniaxially stretched cells showed that strain induced the initially curly intermediate filaments to straighten, indicative of mechanical tension.30 The same study also observed buckling upon relaxation of strain.30 Intermediate filaments may therefore be better able to support tension than compression. Additionally, keratin intermediate filaments at epithelial desmosomes are typically observed to be linear/straight and in alignment with filaments on the neighboring cell; this alignment is lost further into the center of the cell, suggesting that there may exist an uneven distribution of force across the keratin network.31 Together, transmission of tension through vimentin intermediate filaments seems reasonable but much remains to be learned about the details of its mechanical functions.

Mechanical Forces on PECAM

In addition to fluid shear stress, exposure of ECs to cyclic stretch, swelling in hypoosmotic media, and pulling directly on PECAM with magnetic beads all stimulate tyrosine phosphorylation of the PECAM-1 cytoplasmic domain.10,35,36 These results led to the hypothesis that PECAM-1 is a mechanosensor in response to these diverse mechanical stimuli. In light of our recent results, however, these findings may be more consistent with a pathway in which different kinds of mechanical forces stimulate upstream pathways that result in force application to PECAM-1. In this regard, PECAM-1, similar to cadherins, has also been shown to stiffen in response to applied mechanical force,37 indicating force-dependent cytoskeletal assembly via PECAM-1. Thus, the PECAM-1 responses observed in response to flow may represent an instance of a more general behavior in which forces control cytoskeletal linkages to adhesion receptors, as observed with cadherins38 and integrins.39 This type of mechanism would allow cells to tune their cytoskeletal/adhesive structures in accordance with their mechanical environment.

In support of this notion, we found that ECs are able to adhere and spread on surfaces coated with a PECAM-1 antibody that binds to the extracellular domain of PECAM-1 and mimics ligation.6 Interestingly, ECs were also able to migrate on these surfaces (Conway, unpublished data). While migration via PECAM does not to our knowledge model any endothelial physiology, it may mimic transmigration across endothelial monolayers by leukocytes. PECAM-1 is involved in leukocyte transmigration in vitro40 and in vivo.41 During the process of para-cellular migration, PECAM-1 participates in homophilic adhesion between the leukocytes and endothelial cells. It was previously shown that during transmigration, neutrophils exert force on ECs as they transmigrate.52 Actin (but not microtubule) polymerization and depolymerization is required for neutrophil transmigration, and myosin contractility is required for complete transmigration of the neutrophil tail.43 In addition to actin, vimentin has been shown to actively reorganize in both the EC and leukocyte during transcellular migration, where the function of vimentin may be in anchoring and organizing the surface proteins required for transmigration.44 While mechanical force may be important in the opening of the EC junction, it has also been shown to be important for leukocytes to migrate through an already open junction.43 Mechanotransmission and transduction through PECAM-1 is thus a likely component of the tractive and regulatory events that govern leukocyte movement through EC junctions.

Conclusion and Perspectives

Recent experiments have shown that the mechanical force across cadherin may provide a directional cue for migrating cells.1-3,5 While it is tempting to assume the force changes on cadherin and other junction adhesion proteins arise from the extracellular force of leading cells pulling away, it equally possible that the changes in forces are also in part due to active regulation of cytoskeletal tension by the cell. Our results showed that application of shear stress to endothelial cells resulted in opposite force changes on PECAM-1 and VE-cadherin,6 which suggests an active and not passive response by the cell. The force imparted by shear stress is
approximately 2 orders of magnitude less than the overall force exerted by the cell on the matrix, which suggests that there is a signal amplification mechanism by the cell. Similar signal amplification mechanisms may exist in the context of cell migration to amplify the pulling force of the leading cell. Additionally, in light of our finding that vimentin filaments are involved in the mechanotransduction of shear stress, the contribution of intermediate filaments and mechanical force across their associated junction structures (e.g., PECAM-1 or desmosomes) to other mechanosensitive processes, such as collective cell migration, merits further investigation.

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