Lessons from the endothelial junctional mechanosensory complex

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Abstract

Mechanotransduction plays a key role in both normal physiology and in diseases such as cancer, atherosclerosis and hypertension. Nowhere is this more evident than in the vascular system, where fluid shear stress from blood flow plays a critical role in shaping the blood vessels and in determining their function and dysfunction. Responses to flow are mediated in part by a complex of proteins comprised of PECAM-1, VE-cadherin and VEGFR2 at endothelial cell-cell junctions; all proteins that clearly have other, non-mechanical functions. We review recent progress toward understanding the functions and mechanisms of mechanotransduction by this complex and suggest some principles that may apply more broadly.

Introduction

Physical forces are key determinants of morphogenesis during embryonic development and during growth and remodeling in adults. Diseases such as cancer, atherosclerosis and hypertension involve pathological remodeling processes in which mechanical forces play important roles. A better understanding of these phenomena therefore offers hope for development of new therapeutic approaches. The basis for these effects appears to be mechanotransduction, in which cells convert forces to biochemical information. Indeed, subjecting cells to mechanical force activates numerous signaling pathways that mediate subsequent responses. Nowhere is this more evident than in the vascular system, where fluid shear stress from blood flow plays a critical role in shaping the blood vessels and in determining their function and dysfunction. Responses to flow are mediated in part by a complex of proteins comprising PECAM-1 (platelet endothelial cell adhesion molecule 1), VE-cadherin (vascular-endothelial cadherin) and VEGFR2 (vascular endothelial growth factor receptor 2) at endothelial cell-cell junctions. The importance of this system is illustrated by the fact that variants in genes encoding PECAM-1 are linked to early onset atherosclerosis and increased risk of cardiovascular disease, whereas loss of either VE-cadherin or VEGFR2 are embryonically lethal because of vascular endothelial abnormalities. This article briefly reviews recent progress toward understanding the functions and mechanisms of mechanotransduction by this complex. We also suggest some principles derived from these studies that may apply more broadly.

Mechanotransduction in the vascular system

Mechanical forces from blood flow exert strong regulatory influences on the physiology and pathology of the cardiovascular system [1]. The major forces of interest are fluid shear stress, which acts on the endothelium, and circumferential strain, which acts on the entire vessel wall. One reason for intense interest in this system is that atherosclerosis occurs selectively at curved or branched regions of arteries that experience low time-averaged shear stress, shear stress reversal, and spatial and temporal gradients in shear stress [2,3]. By contrast, risk factors such as high LDL (low-density lipoprotein) cholesterol, smoking, diabetes, etc are fairly uniform throughout the circulation. These data suggest the existence of synergy between mechanical forces and

circulating factors in lesion formation. Forces from blood flow are also essential for proper embryonic development of the heart and vasculature [4,5].

Proposed mechanotransducers for flow include cell-cell junctions [6], heterotrimeric G-proteins [7], primary cilia [8], caveolae [9], integrins [10], the glycocalyx [11], intermediate filaments [12], nucleus [13], ion channels [14], and the actin cytoskeleton [15]. The total force from physiological levels of fluid shear stress from blood flow is very low compared to traction forces or force from wall stretch, suggesting that mechanotransduction represents specific sensing of the environment rather than passive responses by the cell to force. In most cases, the responses to fluid shear stress are endothelial-specific [16,17], suggesting that the responses to shear stress involve endothelial-specific proteins. A complex of proteins at cell-cell junctions is the best studied mechanotransducer in this system, and thus, is discussed here in detail.

Junctional complex is responsible for transducing shear stress

A mechanosensory complex at cell-cell junctions, consisting of PECAM-1, VE-cadherin, and VEGFR2, is required for the activation of a number of shear-dependent signaling pathways [6]. These include the pathways that mediate both cell alignment and activation of many of the pro-atherosclerotic pathways.

PECAM-1

PECAM-1 is an Ig family transmembrane protein with a short cytoplasmic domain. In endothelial cells, it localizes mainly to intercellular junctions, where it binds PECAM-1 on neighboring cells. The PECAM-1 cytoplasmic tail contains two tyrosines (Y663 and Y686) each in an ITIM (immunoreceptor tyrosine-based inhibitory) motif [18]. These residues can be phosphorylated by src family kinases to bind the protein tyrosine phosphatase SHP2. Several mechanical stimuli increase PECAM tyrosine phosphorylation, including cyclic stretching of cells on elastic substrata, cell swelling in hyposomotic medium, and tension exerted directly on PECAM-1 by magnetic beads coated with PECAM antibodies [19,20]. This pathway has been implicated in activation of Erk (extracellular signal-regulated kinase) [20], transactivation of VEGFR2 [21] and production of nitric oxide in response to flow [22].

PECAM-1 knockout mice, though viable and fertile [23,24], have vascular defects. They exhibit reduced nitric oxide-dependent dilation of arterioles in high shear stress [25], and reduced collateral artery growth in a model of flow-induced vessel remodeling [26]. In mouse models of atherosclerosis, loss of PECAM decreased lesion size in the lesser curvature of the aortic arch, a well characterized region of disturbed fluid shear stress [27,28,29]. This effect was attributed to endothelial PECAM-1 as transplantation of bone marrow into irradiated recipients showed that the effect was independent of hematopoietic cells [27,28]. In humans, PECAM single nucleotide polymorphisms are linked to early onset of atherosclerosis and increased risk of cardiovascular disease [30]. Interestingly, one of these polymorphisms is located in the cytoplasmic tail and affects endothelial PECAM tyrosine phosphorylation [31,32], and thus, appears to affect intracellular signaling.

In vivo results, however, are complex. The effect of PECAM on atherosclerotic lesions was not clear in some regions of the vasculature; moreover, different mouse models of atherosclerosis gave different results [27,28,29]. The data support the idea that endothelial PECAM-1 is an important pro-atherosclerotic mechanosensor for the aortic arch but, at other sites, it can drive both pro- and anti-atherogenic responses [27,28]. Another complicating factor is that endothelial nitric oxide synthase is deregulated in the absence of PECAM-1 so that PECAM$^{-/-}$ mice have constitutively high nitric oxide production, which tends to suppress inflammation [33]. Additionally, PECAM-1 is expressed in leukocytes and platelets, where it suppresses cell activation, so that in PECAM$^{-/-}$ mice these cells are hyperactive [34,35].

VE-cadherin

The second component of the mechanotransduction complex is vascular endothelial (VE) cadherin. This classic cadherin is specific to endothelium, where it is a critical component of endothelial adherens junctions and is essential for limiting vascular permeability both *in vitro* and *in vivo* [36]. Loss or mutation of VE-cadherin leads to early embryonic lethality with severe vascular abnormalities [37], indicating a key role in development.

There is evidence for mechanotransduction by some cadherins; application of force to E-cadherin stimulates cellular signaling and actin assembly [38,39], and reinforcement of adherens junctions, which is a form of mechanotransduction [40,41,42]. However, although VE-cadherin is essential for endothelial responses to fluid shear stress as a component of the junctional complex, there is no evidence for VE-cadherin acting as a direct mechanotransducer in this context. Homophilic binding of VE-cadherin is not required for responses to shear stress, and direct application of force to VE-cadherin does not stimulate the shear pathway as does application of force to PECAM-1 [43,6]. Available evidence suggests that VE-cadherin functions mainly as an adapter in this

system. Thus, VE-cadherin is essential for the physical association of PECAM-1 and VEGFR2, and for activation of VEGFR2 tyrosine kinase in response to flow or after application of force to PECAM-1 [6]. Interestingly, VE-cadherin associates with and modulates VEGFR2 function in response to VEGF as well [37]. However, the architecture of these complexes is poorly understood.
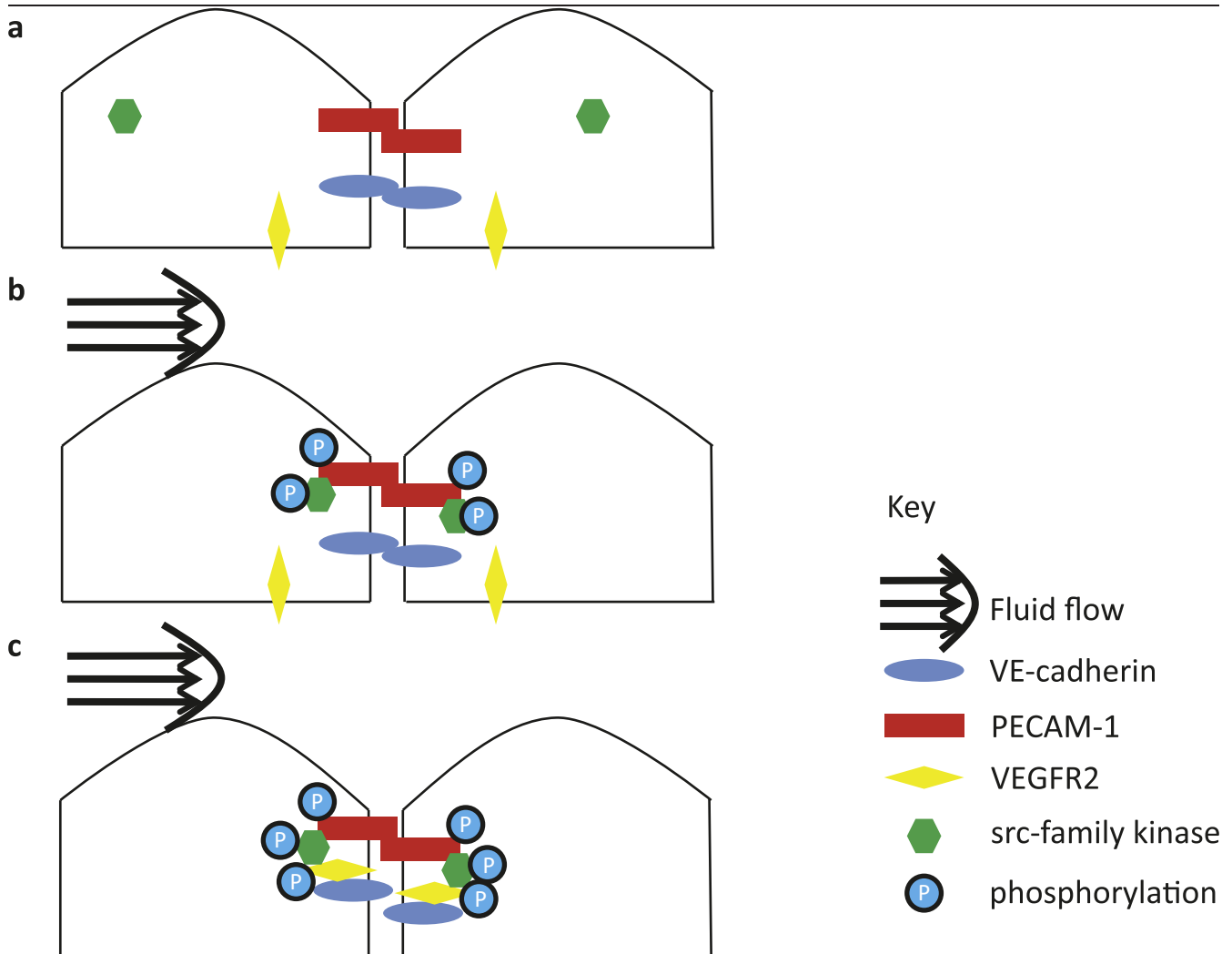
VEGFR2
The third member of the complex is VEGFR2 (called KDR in humans or FLK-1 in mice), the major receptor tyrosine kinase that mediates the angiogenic effects of VEGF.

VEGF binding triggers dimerization and activation of VEGFR2, followed by association with adapter and signaling proteins [44]. VEGFR2 is an essential protein since its deletion in mice leads to defective endothelial cell differentiation and embryonic death at E 8.5-9.5 due to defective endothelial cell differentiation [45].

Flow stimulates ligand-independent activation of VEGFR2 and activation of downstream pathways such as Akt and MAP kinases [46,47,48]. Other flow pathways that are dependent on VEGFR2 include production of nitric oxide and activation of the adaptor protein

Figure 1. Potential mechanism for assembly of the mecanosensory complex

a) VE-cadherin (vascular-endothelial cadherin) and PECAM-1 (platelet endothelial cell adhesion molecule-1) engage in homophilic interactions at intracellular junctions in cells cultured under static conditions. b) When cells are exposed to fluid flow, cells are exposed to a shear stress force. At the onset of shear stress, a src-family kinase (evidently fyn) is activated by phosphorylation and is recruited to and phosphorylates PECAM-1 c) Once PECAM-1 is phosphorylated, VE-cadherin recruits VEGFR2 (vascular endothelial growth factor receptor 2) to the complex. VEGFR2 is then phosphorylated by the src kinase and activates downstream pathways such as Akt and MAP kinases. The specific details of how the complex is assembled (including other binding partners) is currently unknown. Abbreviations: PECAM, platelet endothelial cell adhesion molecule; VE, vascular-endothelial; VEGFR, vascular endothelial growth factor receptor.

Shc [43]. Tyrosines Y801 and Y1175 in the VEGFR2 cytoplasmic tail are essential for shear stress responses, possibly because upon phosphorylation they mediate association with phosphoinositide-3-kinase [6]. Chemical inhibition of VEGFR2 also blocked a transient increase in actin polymerization and cell stiffness in response to shear stress [49].

A model for the mechanosensory complex
A simple conceptual model for function of the mechanosensory complex is that force applied to PECAM-1 results in a conformational change that activates or recruits a src family kinase, recently shown to be fyn [19]. In the presence of VE-cadherin, VEGFR2 is brought into the complex, which facilitates its transactivation by fyn. This idea is consistent with the many studies in which activation of src family kinases mediate ligand-independent transactivation of receptor tyrosine kinases downstream of G-protein coupled receptors or integrins [50]. Activated VEGFR2 then mediates subsequent steps including activation of phosphoinositide-3-kinase and endothelial nitric oxide synthase [6] (see figure 1).

Lessons for other systems
One major conclusion from the analysis of mechanotransduction in blood vessels is that there are no dedicated mechanotransducers. Proteins implicated in mechanotransduction clearly have other, non-mechanical functions. It seems likely that, during evolution, proteins involved in a specific linkage or adhesion function were modified to allow cells to respond to forces as well. This conclusion is underscored by the cooperation between VE-cadherin and PECAM-1 in cell-cell junction stability as well as in flow-induced effects [51] and of VE-cadherin and VEGFR2 in responses to VEGF [37]. Indeed, there are interesting parallels between the ligand-independent and ligand-dependent functions of VE-cadherin and VEGFR2.

A second conclusion is that a limited number of proteins or protein domains function as physiologically important mechanotransducers. Physics indicates that every protein that bears force will undergo changes in conformation as tension or compression alters the stability of protein domains under force [52]. Thus, in principle, mechanotransduction could be highly distributed, with very large numbers of proteins participating. However, the biological evidence argues otherwise, since deletion of specific proteins such as PECAM-1 has drastic effects on endothelial mechanotransduction [6]. Genetic studies in model organisms lead to similar conclusions [37,45]. Protein domains that are designed to change conformation in ways that promote biologically meaningful signaling are likely to be relatively rare. Identifying those domains and understanding in detail the effects of force is the current challenge.

Abbreviations
Erk, extracellular signal-regulated kinase; ITIM, immunoreceptor tyrosine-based inhibitory; LDL, low-density lipoprotein; PECAM, platelet endothelial cell adhesion molecule; VE, vascular-endothelial; VEGFR, vascular endothelial growth factor receptor.

Competing interests
The authors declare that they have no competing interests.

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