

# What Underlies Endothelial Shear Sensing? The Matrix, of Course

Lance S. Terada

A number of chronic vascular diseases preferentially arise in distinct zones of arterial conduits such as intracranial aneurysms, which occur inside regions of curvature or at bifurcations, and pulmonary plexiform lesions, which occur just beyond dichotomous branch points. The geometry of these shapes strongly suggests that fluid dynamics play a key role in the pathogenesis of these lesions. Early atheromas have long been known to develop in similar locations, which are predicted to experience severe dynamic perturbations in shear rates such as flow reversal and separation bubbles. One might expect that progression of intimal and subintimal lesions would further distort shear distribution, leading to propagation of the disease. Thus, atherosclerosis is perhaps the most common human disease strongly influenced by mechanical signals, one of perhaps many diseases of mechanosensation. What pathways are activated by shear, and how is this sensed?

The majority of arterial surfaces experience laminar flow, which is relatively continuous across time and space. Endothelium exposed to such laminar flow is relatively quiescent, and the transcriptome of continuously sheared endothelium demonstrates expression of genes promoting survival and suppression of genes associated with proliferation and inflammation.<sup>1</sup> Conversely, disturbed flow activates a panel of inflammatory, apoptotic, and procoagulant genes, supporting the atherogenic nature of abnormal mechanical signals.<sup>2</sup> Downstream of the actual mechanosensors, a number of kinase pathways appear important in activating such broad cellular responses, such as extracellular signal-regulated kinase (ERK)5 and AMP-activated protein kinase.<sup>3,4</sup>

Because cells anchor themselves to the underlying matrix to resist shear forces, integrins or associated focal contact proteins would seem to be logical candidates as proximal mechanosensors. Within seconds of cell shearing, both platelet endothelial cell adhesion molecule (PECAM)-1 and vascular endothelial growth factor receptor-2 become tyrosine-phosphorylated, and direct traction on PECAM-1 using magnetic beads also initiates PECAM-1 phosphorylation and ERK signaling.<sup>5,6</sup> More recently, these 2 proteins were shown to be coupled through VE-cadherin, acting as an adaptor, in a

shear-sensitive complex activating phosphatidylinositol 3-kinase upstream of  $\alpha_v\beta_3$  integrin.<sup>7</sup> Other mechanosensory complexes likely contribute to shear stress detection. Mechanical stretching of the focal contact protein p130Cas causes extension of its substrate domain, rendering it susceptible to phosphorylation by c-Src.<sup>8</sup> This phosphorylation allows recruitment of C3G and, thus, activation of the small GTPase Rap1. Thus integrin complexes themselves may sense shear.

In this issue of *Circulation Research*, Orr et al<sup>9</sup> examine another layer of complexity to integrin mechanosensation, which is the effect of different matrix proteins on shear signaling. Because different integrins are used to discriminate between various extracellular matrix proteins, one might generalize this observation to postulate that specific integrins may also be used to differentially report other environmental cues, such as shear forces. Here, Orr et al study this possibility by studying shear-dependent NF- $\kappa$ B activation on different matrices. This article is the latest in an elegant series of studies by this group on matrix specificity. In prior work, these authors established that initiation of laminar flow activates NF- $\kappa$ B only in endothelial cells plated on fibronectin or fibrinogen but not collagen.<sup>10</sup> By itself, this simple observation provided convincing evidence that integrins are, in fact, critical mechanotransducers of shear stress. The investigators further demonstrated that even in nonatherogenic wild-type mice, arterial regions susceptible to disturbed shear had focal increases in fibronectin and the NF- $\kappa$ B targets intercellular adhesion molecule-1 and vascular cell adhesion molecule-1.<sup>10</sup> In ApoE-null mice, such changes were more prominent; interestingly, these focal matrix alterations preceded fatty streak formation and leukocyte infiltration, suggesting a very proximal and perhaps primary role for matrix-dependent shear signaling in atherogenesis.

In a subsequent study by this group, irregular shear was found to activate p21-activated kinase (PAK), a Ste20 family kinase, again in a matrix-specific fashion on fibronectin but not collagen.<sup>11</sup> The matrix dependence of PAK activation by disturbed shear holds relevance for several reasons. First, PAK is known to interact functionally with integrins by being activated at focal contacts and mediating directional sensing and migration.<sup>12</sup> Second, activation of the PAK agonist Rac1 is also matrix-specific,<sup>13</sup> and finally, PAK presents a potential link to another shear-dependent signaling process, the production of reactive oxidants.

Focused and regulated production of oxidants is known to mediate activation of a number of pathways, including the shear-responsive NF- $\kappa$ B and ERK5.<sup>14,15</sup> Initiation of shear and abrupt cessation of shear both initiate oxidant production, the latter through the NADPH oxidase Nox2.<sup>16</sup> Like PAK,

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oxidant production is also linked to integrin attachment. Cells initiate an oxidant burst upon matrix attachment, and the Nox2 adaptor p47<sup>phox</sup> translocates to tension-bearing focal complexes at the leading edge of endothelial cells migrating on fibronectin to initiate oxidant signaling.<sup>17</sup>

In the present study, Orr et al<sup>9</sup> use the matrix specificity of PAK activation to further dissect the role of oxidants in shear-dependent NF- $\kappa$ B activation. Linking their prior studies together, they found that PAK is activated and mediates NF- $\kappa$ B activation in endothelial cells plated on fibronectin but not collagen in vitro and at shear-sensitive sites in vivo. NF- $\kappa$ B is known to be oxidant-sensitive, and a logical link between oxidants and PAK is through Nox2. PAK activates Nox2 through p47<sup>phox</sup> phosphorylation in microglial cells stimulated with phorbol 12-myristate 13-acetate or *N*-formyl-methionyl-leucyl-phenylalanine<sup>18</sup> and in endothelial cells migrating or following stimulation with HIV-1 Tat, vascular endothelial growth factor receptor, or tumor necrosis factor.<sup>17,19</sup> Surprisingly, the study by Orr et al suggests that the response to oscillatory shear differs from these stimuli in that PAK does not activate NF- $\kappa$ B through a direct activation of an oxidase. Instead, this study shows that shear-dependent oxidant production, unlike PAK activation, is matrix-independent. Whereas oxidants were found to be necessary for NF- $\kappa$ B activation by oscillatory shear, exogenous H<sub>2</sub>O<sub>2</sub> activated NF- $\kappa$ B only in cells plated on fibronectin in a PAK-dependent fashion. Thus, although both oxidant production and PAK activation are necessary for NF- $\kappa$ B activation, Orr et al were able to separate the effects of oxidants and PAK through differential matrix sensitivity. The resultant model suggests that matrix-specific PAK activation may permit oxidant-dependent NF- $\kappa$ B-inducing kinase/NF- $\kappa$ B activation in response to disturbed shear.

This study raises a number of interesting questions. First, it is not clear how reactive oxidant and PAK signals are integrated. Oxidants frequently signal through focal initiation of common-use signaling devices such as tyrosine phosphorylation pathways and Ca<sup>2+</sup> transients.<sup>19</sup> Notably, fibronectin-dependent proliferative and motility signaling requires collaboration between PAK and tyrosine kinases such as FAK and Src. In addition, attachment to fibronectin activates PAK through the Ca<sup>2+</sup>-binding protein CIB1, offering another possible point of convergence between PAK and reactive oxidants.<sup>20</sup> Second, it is not clear how provisional matrix proteins like fibronectin end up at regions of disturbed shear, to permit PAK-dependent signaling. Paracellular permeability is increased at such regions to allow diffusion into the subendothelial zone, but this permeability appears itself to require provisional matrix attachment,<sup>11</sup> suggesting that permeability is a secondary process. Abnormal shear by itself may stimulate endothelial production of provisional matrix proteins, a scenario that may reflect a homeostatic attempt to remodel the vascular wall at points of low or irregular shear.

This latter possibility may underlie a teleologic explanation for the evolution of such shear-dependent pathways that can incidentally be subverted to cause atherosclerosis. If so, these pathways may recapitulate ontogenic programs enacted during vascular development. For instance, yolk sac vasculogenic remodeling requires shear stress in mice.<sup>21</sup> Similarly,

flow directly specifies artery versus vein determination.<sup>22</sup> Tension, albeit different from flow-induced shear, accelerates capillary formation during lung morphogenesis.<sup>23</sup> Such mechanical determinants of vascular remodeling find broad correlates in metazoan development, including the mechano-transduction of left-right axis determination, gastrulation, dorsal closure, and nephron development. The findings of Orr et al may, therefore, be expected to find relevance in a variety of diseases, and shed further insight into the means by which cells sense and respond to their physical environment, which includes both matrix and movement.

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## Disclosures

None.

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