STRUCTURAL DYNAMICS IN LIVING ENDOTHELIAL CELLS INDUCED BY HEMODYNAMIC SHEAR STRESS

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The development of atherosclerosis *in vivo* occurs at arterial locations such as bifurcations and regions of high curvature that exhibit a complex fluid dynamic force profile [1]. Endothelial cells (ECs) at the interface between blood and tissue surrounding the blood vessel adapt to this hemodynamic environment both structurally and functionally, and endothelial dysfunction in these regions is associated with inflammatory processes responsible for atherogenesis [2]. However, the mechanisms by which ECs sense changes in hemodynamics and transduce mechanical stimuli into biochemical signaling are not well understood.

Biochemical signaling is rapidly initiated at multiple locations within ECs following alterations in the fluid shear stress profile acting at the cell surface [1]. For example, activation of heterotrimeric G proteins occurs within seconds after a change in magnitude or temporal gradient of shear stress, and the specificity of the activation depends whether the G proteins are located near cell edges or are distributed across the apical surface [3]. Increases in ion conductance across the apical membrane suggest that channel proteins can respond directly to shear forces [4]. In addition to these events at or near the apical cell surface, rapid phosphorylation of structural and signaling proteins also occurs at other sites within the cell. Chronically increased shear stress induces β-catenin transmigration from adherens junctions to the nucleus where it may interact with transcription factors to modulate gene expression [5]. Signaling molecules such as focal adhesion kinase [6] and paxillin [7] associated with focal contacts at the interface between ECs and the extracellular matrix are also rapidly phosphorylated after increased shear stress. Thus, hemodynamic forces also modulate integrin-mediated adhesion and migration [8].

Since changes in magnitude or gradient of shear stress initiate several signaling events on the same time scale (seconds), it is likely that cellular mechanotransduction results from the integration of messages from various sites in the cell. In order for this to occur, force acting at the surface must be transmitted to these intracellular locations instantaneously. The cytoskeleton plays a crucial role in such intracellular force transmission that initiates mechanochemical signaling [9]. Measurements of structural dynamics in cytoskeletal filaments and scaffold proteins associated with focal contacts and intercellular junctions are revealing new details of mechano-communication both within single cells and among adjacent cells, suggesting that ECs integrate multiple spatial and temporal events in response to local hemodynamic stimuli.

MATERIALS AND METHODS

DNA plasmids encoding structural proteins fused to either green fluorescent protein (GFP) or a red fluorescent variant (DsRed) were transfected into bovine aortic ECs (Lipofectin, Gibco, Gaithersburg, MD). ECs transiently expressed GFP-vimentin (a gift from R. D. Goldman, Northwestern Univ.), GFP-actin (Clontech, Palo Alto, CA), and/or paxillin-DsRed (a gift from A. F. Horwitz, Univ. of Virginia).

Red fluorescent microspheres (0.1 μm, Molecular Probes, Eugene. OR) were dried onto glass coverslips (Bioptechs, Butler, PA) and served as fiducial markers during time-lapse analysis. Cells expressing GFP fusion proteins were plated over the microspheres. Coverslips were assembled into a parallel plate flow chamber (Bioptechs) and maintained at 37 ºC.

Wide-field fluorescence optical sections were acquired using a DeltaVision microscopy system (Applied Precision, Issaquah, WA). Multiple-wavelength 3-D stacks of images were acquired at intervals ranging from 30 sec to 3 min. A step change in flow was imposed so that wall shear stress was 12 dyn/cm^2, and image acquisition continued for an additional 30-60 min. Image restoration was performed by 3-D constrained iterative deconvolution (softWoRx, Applied Precision) using an experimentally acquired point spread function. MatLab (Mathworks, Natick, MA) was used for additional image analysis.

RESULTS

Strain Focusing in the Intermediate Filament Cytoskeleton

Time-lapse analysis of the GFP-vimentin–labeled intermediate filament (IF) cytoskeleton demonstrated that onset of an arterial magnitude of unidirectional laminar shear stress induced increased filament displacement that was superimposed on constitutive movement [10]. Filament displacement increased with height in the

cell and in cytoplasmic regions downstream from the nucleus [10]. In order to detect filament network deformation, the Lagrangian strain tensor was computed from selected optical sections extracted from 4-D images of GFP-vimentin [11]. Onset of shear stress induced an increase in IF strain that was highly localized within the cell. Spatial concentration of strain magnitude occurred at discrete sites throughout the cytoplasm, including near the coverslip. These locations of *strain focusing* often corresponded to closely interconnected network elements in the IF cytoskeleton. Strain focusing also occurred near the nuclear boundary or near cell-cell boundaries. In addition, the orientation of principal strain along the upstream edge of the cell was often rotated after onset of shear stress from parallel to perpendicular to the cell edge. The axis of principal strain at these locations was oriented parallel to IFs along the cell edge during no-flow intervals, and onset of shear stress caused the principal strain axis to be reoriented perpendicular to these edge filaments.

Structural Dynamics in the Actin Cytoskeleton

In order to examine whether structural dynamics in the actin cytoskeleton were altered in similar fashion by onset of shear stress, ECs expressing GFP-actin were analyzed. Under no-flow conditions, small displacements of GFP-actin–labeled microfilaments were detected. Onset of shear stress induced displacement in the microfilament network in a manner similar to that measured in the IF cytoskeleton. For example, microfilament connections near the coverslip were displaced and deformed, and microfilaments often bent or bowed within minutes after onset of shear stress.

Onset of shear stress also induced a change in the dynamics of actin-containing edge ruffles. Under no-flow conditions, cells extended lamellipodia for small distances in apparently random directions. Within a minute after onset of unidirectional shear stress, new lamellipodia containing GFP-actin were extended, and prominent filopodia often began to retract. The direction and degree of ruffling was dependent on the degree of confluence and locations of physical cell-cell contact. In subconfluent monolayers, ruffles were extended along cell edge locations where no physical contact with an adjacent cell could be detected. In contrast, cells within a confluent monolayer in some experiments appeared to extend ruffles in the upstream direction (against the flow). The degree of actin-mediated ruffling induced by onset of shear stress was less pronounced in confluent monolayers than in subconfluent layers. In all cases, however, this rapid actin response was transient, subsiding after ~10–20 min.

Flow-Induced Focal Adhesion Dynamics

Since IF strain focusing and microfilament displacement near the coverslip was consistent with locations of focal adhesion sites, ECs were transfected with paxillin-DsRed, and movement of focal contacts was observed. Under no-flow conditions, focal contacts in central regions of the cell were stationary. After onset of shear stress, most focal contacts remained stationary. However, some paxillin-containing structures were rapidly displaced within minutes. The rapid displacement of these focal contacts correlated with microfilament displacement, as observed in ECs expressing both GFP-actin and paxillin-DsRed. Paxillin was not detected in most edge ruffles induced by shear stress.

DISCUSSION

These measurements demonstrate that hemodynamic shear stress acting at the cell surface is transmitted to remote locations within the cell, including near the coverslip. IF deformation and microfilament displacement on this short time scale suggests that cytoskeletal tension is rapidly redistributed within the cytoplasm following onset of shear stress. Furthermore, displacement of focal contacts correlates with microfilament displacement near the basal cell surface. This observation, coupled with strain focusing at discrete locations near the coverslip, suggests that hemodynamic force is concentrated by the cytoskeleton near sites rich in signaling molecules that initiate mechanotransduction.

Rapid edge ruffling constitutes a novel acute response to hemodynamic shear stress. Flow-induced ruffling and migration have been reported in single ECs [8], but the measurements presented here represent the first observations of directional ruffling in confluent monolayers. Since the ruffling is transient, occurs within minutes, and does not correspond to focal adhesion formation, this actin-mediated ruffling may represent a novel sensing mechanism for mechanical forces that include shear stress and cell-cell interactions. These observations are consistent with the hypothesis that cells sense micro-scale stimuli on their surface and transmit forces *via* the cytoskeleton to initiate an integrated cellular mechanotransduction response [9].

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