Mechanosignal transduction coupling between endothelial and smooth muscle cells: role of hemodynamic forces

Tzung K. Hsiai

Department of Biomedical Engineering and Division of Cardiovascular Medicine, University of Southern California, Los Angeles, California

HEMODYNAMICS play an important role in the focal nature of atherosclerosis. Mounting evidence has demonstrated that fluid shear stress is intimately involved in the biological activities of vascular endothelial cells (ECs). However, the role of neighboring smooth muscle cells (SMCs) in modulating the endothelial phenotype in the presence of shear stress remains undefined. Emerging EC and SMC coculture systems have provided the basis to elucidate the mechanosignal transduction coupling between ECs and SMCs. Hasting et al. (9) as well as Chiu et al. (2–5) have demonstrated novel transcriptional regulation to support the notion that endothelial functional phenotypes are not only influenced by hemodynamic forces but also by neighboring SMCs.

Vascular ECs in resistant arteries are constantly exposed to the dynamic changes of blood flow, namely, hemodynamic forces. These homodynamic forces can be resolved into three components: 1) shear stress, the tangential frictional force acting at the EC surface; 2) hydrostatic pressure, the perpendicular force acting on the vascular wall; and 3) cyclic strain, the circumferential stretch of the vessel wall (6, 7, 11, 13, 17). Emerging lines of evidence have supported the role of shear stress in mechanosignal transduction between vascular ECs and SMCs (3, 9).

ECs are subject to fluid shear stress in the presence of neighboring SMCs (Fig. 1). The bidirectional communication between ECs and SMCs influences the homeostasis of the function and structure of the blood vessel wall. As an interface between blood and the vessel wall, ECs sense and respond to hemodynamic forces. EC and SMC coculture systems have elucidated novel transcriptional regulation between ECs and SMCs (4, 9), providing evidence that endothelial functional phenotypes are not only influenced by hemodynamic forces but also by neighboring SMCs (3, 9).

The coculture system represents a significant advance over homogeneous culture to assess the molecular mechanisms whereby shear stresses regulate EC function in the presence of SMCs (1, 14, 15). Using the perfused transcapillary coculture model, Remond et al. (15) reported that ECs protect against flow-induced SMC migration and flow-induced EC plasminogen activator inhibitor type 1. Using the parallel plate EC/SMC coculture system, Chiu et al. (4) reported that laminar shear stress significantly inhibits SMC-induced adhesion molecule gene expression. Furthermore, SMCs induce an upregulation of proinflammatory gene expression in ECs that are located in close proximity to SMCs. However, laminar shear stress acts as a negative regulator by regulating NF-κB binding sites in the promoters of these inflammatory genes expressed in the presence of SMCs (2). Chiu et al. (3) further elucidated the molecular mechanism whereby JNK and p38 in MAPK pathways are activated to induce E-selectin expression in cocultures. Gel shifting and chromatin immunoprecipitation assays showed that SMC coculture increased the NF-κB-promoter binding activity in ECs, whereas preshearing of ECs at 12 dyn/cm² inhibited coculture-induced EC signaling and E-selectin expression (3). Thus, mechanosignal transduction in the EC/SMC coculture system provides molecular insights into the atheroprotective role of unidirectional laminar shear stress (12 dyn/cm²) to downregulate proinflammatory gene expressions in ECs induced by coculture with SMCs.

The coculture in vitro model further allowed for testing the hypothesis that differential human-derived hemodynamic flow patterns applied to ECs influence SMC phenotypic modulation (1). Using a modified cone and plate flow device, Hasting et al. (9) developed a dynamic system to accommodate a 75-mm Transwell culture dish (polycarbonate, 10 μm thickness and 0.4 μm pore diameter, Corning) in which ECs were exposed to two distinct flow profiles, namely, pulsatile shear stress in the common carotid artery and oscillatory shear stress in the lateral wall or point of flow separation in the internal carotid artery (Fig. 2, A and C). Similar to the parallel plate coculture flow system (5), ECs and SMCs were separated by a porous membrane with only the EC side subjected to the flow condition (Fig. 2, B and D). The former enabled the testing of atherogenic hemodynamics in the presence of SMCs (1). The latter elucidated new insights into the molecular mechanisms of SMCs (2).
whereby laminar shear stress downregulated coculture induction of EC signaling and E-selectin expression (3). Both coculture systems demonstrated that cocultured SMCs tended to orient perpendicularly to the flow direction when ECs were exposed to a physiological range of shear stress (for example, 12 dyn/cm² for 24 h) (4, 9, 10).

In addition to a physiological tissue analog to study EC function, ECs and SMCs in an EC/SMC coculture model can be exposed to atherogenic or atheroprone hemodynamics to assess SMC hyperplasia and migration into the collagen layer (16). Williams et al. (18) constructed porous poly(glycolic acid) tubular scaffolds that were seeded with ECs and SMCs under pulsatile shear stress in a bioreactor for 25 days. DiI-Ac-LDL uptake by ECs was compared with that in an EC/SMC coculture and an EC monoculture in the presence of laminar shear flow at 13.3 dyn/cm². It was found that the uptake of LDL by the EC-SMC coculture was much greater than that by the EC monoculture (12). A 10% cyclic strain was incorporated to alter the characteristics of a SMC-seeded collagen gel in the EC/SMC coculture system (10). This form of strain preconditioning resulted in a rearrangement of the vessel wall that yielded circumferentially oriented cells and collagen fibrils (10).

SMCs induce a paracrine effect by releasing agents that act on ECs that are mediated to upregulate proinflammatory genes (8). Chiu et al. (3) demonstrated that the cytokines IL-1β and IL-6 produced by ECs/SMCs can exert paracrine effects on ECs to induce E-selectin expression via receptor-interacting molecules IL-1 receptor-associated kinase and glycoprotein-130 (gp130) as well as intracellular signaling cascades JNK and p38 MAPK (Fig. 3). Hastings et al. (9) demonstrated a “mechanotranscriptional coupling” phenomenon between shear force-exposed endothelium and SMCs. Atheroprone flow (bidirectional oscillating flow at the lateral wall of the internal carotid artery) decreased genes associated with differentiated ECs (endothelial nitric oxide synthase, Tie2, and Kruppel-like factor 2) (KLF2) (Fig. 4). Atherogenic and atheroprotective hemodynamics regulate EC and SMC phenotypes. Using quantitative RT-PCR and chromatin immunoprecipitation analyses, Hastings et al. (9) compared the relative expression of inflammatory, quiescent contractile, and synthetic phenotypes in the EC/SMC coculture system in response to atheroprotective and atherogenic hemodynamics. eNOS, endothelial nitric oxide synthase; KLF, Kruppel-like factor; MCP-1, monocyte chemoattractant protein-1; SMα-Actin, smooth muscle α-actin; SMMHC, smooth muscle myosin heavy chain.
factor-2) and SMCs (smooth muscle α-actin and myocardin) and induced an inflammatory phenotype in ECs and SMCs (VCAM-1, IL-8, and monocyte chemoattractant protein-1) (Fig. 4). Furthermore, atheroprotective flow-induced SMC differentiation markers were regulated at the chromatin level, as indicated by decreased serum response factor binding to the smooth muscle α-actin–CArG promoter regions and deacetylation of histone H4 (smooth muscle α-actin, smooth muscle myosin heavy chain, and myocardin for the quiescent contractile phenotype), whereas serum response factor and histone H4 acetylation were enriched at the c-fos promoter in SMCs (Kruppel-like factor-4 and VCAM-1 for the synthetic phenotype) (9). Hemodynamic forces induced vascular EC and SMC priming toward a proatherogenic response, thus corroborating the coculture system as a physiologically relevant biomimetic vascular model.

In summary, interactions between vascular ECs and SMCs are important in regulatory processes to maintain vessel wall homeostasis. The in vitro coculture model using ECs and SMCs provides an entry point to elucidate the molecular mechanisms whereby atheroprotective or atheroprotective hemodynamics modulate EC and SMC phenotypes (12, 13, 16). Mounting evidence has supported the effect of shear stress on endothelial biology; however, the influence of SMCs remains largely unexplored. The recent study by Hastings et al. (9) and others (2, 10, 12, 14) demonstrate that the EC/SMC coculture system is a novel tool to investigate cell-cell and cell-extracellular matrix interactions and mechanotransduction coupling between ECs and SMCs.

REFERENCES