Spatial Hemodynamics, the Endothelium, and Focal Atherogenesis
A Cell Cycle Link?

Peter F. Davies

The characteristics of arterial flow and the hemodynamic forces associated with them have important effects upon endothelial biology\(^1\) that have long been implicated in the nonrandom distribution of atherosclerotic lesions.\(^2\) At branches, curvatures, and bifurcations of large elastic and muscular distributing arteries, separations of the flow streamlines create regions of disturbance that correlate closely with the early appearance of atherosclerosis. Within the regions of flow disturbance, the cardiac cycle imposes complicated spatial patterns of flow that include nonuniform, multidirectional pulsatile forces at variable frequencies. Rapidly changing gradients of shear stress arising from flow reversals and secondary flows frequently result in lower average levels of shear stress.\(^3\) The endothelial cells at these locations are subject to shear stress forces that vary considerably over short distances of the monolayer such that cells separated by tens of microns consistently experience significantly different hemodynamic environments. This leads to regional heterogeneity of endothelial exposure to flow forces within the same vascular bed\(^4–6\) as well as cell-to-cell heterogeneity arising from subcellular differences in cell surface geometry.\(^7\) Such spatially defined hemodynamic patterns are postulated to underlie the focal origin of atherosclerotic lesions by inducing small groups of endothelial cells toward a proatherosclerotic phenotype through differential mechanosignaling, transcription, and protein expression.\(^8\)

Nearly 30 years ago, Wright\(^9\) conducted the first studies of endothelial cell proliferation in vivo that are now recognized as relevant to endothelial regional heterogeneity. She noted that although the fraction of mitotic endothelial cells is extremely low throughout the arterial tree, there are loci of proliferating cells associated with curvatures and near branch arteries, regions of predictable disturbed flow. The association of endothelial cell cycle activity with atherosclerotic susceptible regions in animal models has since been further defined,\(^10\) and in vitro models of disturbed flow support the in vivo data. For example, extreme flow disturbance (turbulence in vitro) stimulated large and widespread increases in cell cycle entry,\(^11\) and the creation of controlled shear stress gradients in vitro promoted highly localized regions of cell proliferation associated with the flow disturbance.\(^6,12\) The mechanisms by which shear stress may initiate endothelial proliferation has long been presumed to involve force-induced cell separation and loss of contact inhibition between confluent quiescent endothelial cells. However, more subtle signaling cascades may also be involved.

In this issue of Circulation Research, Akimoto and coworkers,\(^13\) in confirmation of early studies by Levesque et al,\(^14\) noted that shear stresses above \(\approx 1\) dyn/cm\(^2\) significantly suppressed \(G_0/G_1 \rightarrow S\) phase transition of confluent (but still proliferating) bovine aortic endothelial cells. They propose that shear stress is linked to endothelial cell \(S\) phase transition through the cyclin-dependent kinase (cdk)-retinoblastoma protein (pRb) regulatory pathway and speculate that such a mechanism may be relevant to events occurring at low shear stress regions in disturbed flow. \(S\) phase entry in eukaryotic cells is largely regulated by phosphorylation of pRb through the activities of cdk2 and cdk4. Decreases in pRb phosphorylation and cdk2/cdk4 activities in endothelial cells were noted after exposure to significant levels of shear stress, suggesting that upstream regulation of the cdk5s is mechanically sensitive. Specific cdk inhibitory proteins regulate cdk activities by protein-kinase binding. Members of the Cip/Kip (p21\(^{\text{Sdi1/Cip1/Waf1}}\); p27\(^{\text{Kip1}}\)) and Ink4 (p15\(^{\text{Kip1}}\); p16\(^{\text{Ink4a}}\)) families inhibit the activities of various cdk5s.\(^15\) In the present study, shear stress increased the levels of p21 mRNA within 15 minutes and doubled p21 protein levels within 2 hours. Some selectivity was present because, in contrast to p21, another member of the Kip family, p27\(^{\text{Kip1}}\) protein, was unaffected by shear stresses as high as \(30\) dyn/cm\(^2\). Removal of the flow forces reversed the inhibition of proliferation and reduced p21 mRNA levels to those of control cells in static culture within 6 hours. Akimoto et al\(^13\) suggest that low shear stress such as may occur in preatherosclerotic disturbed flow regions in vivo favors \(G_0/G_1 \rightarrow S\) transition and hence cell proliferation through release of p21 suppression of cdk activity (see Figure).

By identifying some of the regulatory molecules, the present study adds more detail to the earlier findings that laminar shear stress suppresses endothelial proliferation\(^14\) whereas turbulent shear stress stimulates the cell cycle.\(^3\) Missing, of course, are the initiating links between the mechanical force and p21 induction, the latter detectable minutes after exposure to flow. Many other upstream events are stimulated by shear stress. These include activation of members of the protein kinase C (PKC) family that are
implicated in the control of p21^{cip1}, p27^{kip1}, and other cyclin-related inhibitory proteins. Ashton et al.\textsuperscript{16} have recently reported a critical role for PKC-\(\delta\) in p27^{kip1}-mediated S phase arrest of serum-stimulated endothelial cells. Flow-induced activation of phospholipase C (PLC) by G protein subunits\textsuperscript{17} generates diacylglycerol that may regulate PKC, and the potential role of G protein–coupled receptors as mechanotransduction elements linked to cell proliferation has been previously discussed.\textsuperscript{1} Induction of endothelial nitric oxide synthase, the nitric oxide generating enzyme that is stimulated by agonists and shear stress,\textsuperscript{18} increases expression of both p21^{cip1} and tumor suppressor protein p53, causing inhibition of S phase transition and suppressed proliferation.\textsuperscript{19,20} Also upstream is the activation by shear stress of Ras-dependent phosphatidylinositol-3 (PI-3) kinase and mitogen-activated protein (MAP) kinase pathways, stimulating ERK1/2 phosphorylation and apparently mediated by PKC.\textsuperscript{21} Although there is clearly an overlap, it remains unclear where receptor-mediated and shear-mediated pathways converge and diverge in flow-regulated proliferation. Many potential regulatory molecules such as transcription factors and small GTPases may in turn be specifically sensitive to shear stress.

Akimoto et al.\textsuperscript{3} did not address the role that contact inhibition of endothelial growth may play in modulating some of their observations. Cells were studied at confluence but with a significant cell fraction still in the cell cycle; in contrast, proliferation in the monolayer in vivo is almost completely suppressed. The release of suppression by no/low shear stress in disturbed flows in vivo is therefore counterruitive, because contact inhibition would need to be overcome. Comparison of the regulation of cell cycle by mechanical and cell-cell contact mechanisms would be useful, even limited to the pathways already described.

The link between atherosclerosis and endothelial proliferation/turnover is most probably at the cell junctions that regulate the permeability and transmural passage of larger blood molecules. These include proatherosclerotic lipoproteins and procoagulant proteins. During the cell cycle, significantly increased protein permeability is associated with regions of endothelial turnover.\textsuperscript{22}

To extend the present study to situations in which flow disturbances generate the low/no shear stresses that appear necessary for release of endothelial growth inhibition, several well described in vitro models of flow separation could be used. DePaula’s model\textsuperscript{6} provides distinct regions of unidirectional laminar flow, flow separations, reversals, stagnation regions, and defined gradients of shear stress. Endothelial cell migration and proliferation are associated with the disturbed flow regions of the model.\textsuperscript{6} It may therefore be possible to perform spatial assays of cell cycle regulatory pathways within the same experiment, despite small amounts of materials. It is certainly possible to assess gene expression in small groups of cells and single cells in such a model,\textsuperscript{6} an approach that can be applied to the entire endothelium, including proliferation-relevant genes, via high throughput microarrays. If individual endothelial cells can be harvested from predicted disturbed flow locations in arteries (and they can), cell cycle–related gene expression can be profiled, and the in vitro findings of Akimoto et al.\textsuperscript{3} will be more meaningfully related to Wright’s\textsuperscript{9} original in vivo observations.

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References


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