Mechanisms of Endothelial Cell Heterogeneity in Health and Disease

William C. Aird

The endothelium is an expansive spatially distributed organ. Endothelial cells participate in a large number of physiological processes including the control of vasomotor tone, the trafficking of cells and nutrients, the regulation of permeability, and the maintenance of blood fluidity. In addition, the endothelium mediates new blood vessel formation, contributes to the balance of pro- and antiinflammatory mediators, and may play a role in antigen presentation. In accomplishing these tasks, the endothelium exhibits a remarkable “division of labor”. For example, arteriolar endothelium is primarily responsible for mediating vasomotor tone; endothelium in postcapillary venules regulates leukocyte trafficking; capillary endothelial cells display organ-specific barrier properties (eg, blood brain barrier versus fenestrated, discontinuous endothelium in hepatic sinusoids); and endothelial cells from different vascular beds balance local hemostasis via the expression of site-specific patterns of anticoagulants and procoagulants. In recent years, in vivo phage display and direct proteome mapping of the intact vasculature have revealed a rich diversity in endothelial cell surface markers.

Mechanisms of Endothelial Cell Heterogeneity

Any consideration of the mechanisms underlying endothelial heterogeneity is best framed around the time-honored debate of nature versus nurture (which will be addressed here in reverse order) (Figure).

Nurture

Site-specific endothelial cell phenotypes may be initiated and maintained by signals residing in the extracellular environment. The endothelium is analogous to a barcode reader, constantly taking inventory of its surrounding extracellular environment on the luminal side (circulating blood and its constituents), the abluminal side, and at the endothelial junctions. Environmental cues may be classified into biomechanical or biochemical. Biochemical forces include shear stress and strain. Examples of biochemical signals include pH, oxygenation, growth factors, cytokines, chemokines, hormones, serine proteases, lipoproteins, sphingolipids, nucleosides, complement, and components of the extracellular matrix (ECM). Endothelial cells are capable of sensing these and many other extracellular cues. The net signal input is transduced by a series of nonlinear signaling pathways which typically begin at the cell surface and end with posttranscriptional modifications or changes in gene expression. Phenotypic responses include (but are certainly not limited to) changes in cell shape, gene and/or protein expression, migration, proliferation, apoptosis, barrier function, hemostatic balance, and leukocyte adhesion.

The nature of the input varies across the vascular tree. For example, endothelial cells residing in arteries are exposed to relatively high levels of shear stress (compared with veins and capillaries). Moreover, endothelial cells from distinct regions of the artery experience different flow patterns; whereas the majority of the arterial endothelium is exposed to undisturbed laminar flow (or pulsatile unidirectional shear stress), regions of complex geometry (eg, at curvatures or bifurcations) are subjected to disturbed laminar, and occasionally turbulent, flow. Endothelial cells residing in different arteries across the body are exposed to a similar composition of circulating mediators and blood vessel wall-derived signals (exceptions include the pulmonary artery which receives mixed-venous blood; and certain vascular beds that have two arterial beds separated by capillaries, eg, kidney glomerulus). In contrast to arteries, the composition of the blood bathing venous endothelial cells is predicted to vary across the vasculature depending on the net exchange of substances that occurs in the pre-venular capillaries. The capillary endothelium is exposed to the most striking variation in microenvironment because these cells are separated from underlying organ parenchyma by only a thin layer of ECM and occasional pericytes.

The nature of the input also varies over time. Examples of temporal changes include hemodynamic fluctuations associated with the cardiac cycle, exercise, or disease (eg, hypertension), and biochemical shifts associated with diurnal rhythms, postprandial state, transient bacteremia, aging, or disease (eg, hyperlipidemia and diabetes).

Because net signal input varies in space and time, and because endothelial cells have evolved to sense and respond to the extracellular environment, the endothelium displays phenotypic diversity. The importance of the microenvironment in maintaining endothelial heterogeneity is evidenced by the remarkable degree of phenotypic drift that occurs when endothelial cells are removed from their native tissue environment and cultured in vitro. For example, in one study, 41% of luminal endothelial cell membrane proteins isolated from rat lung were no longer detected when the microvascular endothelial cells were grown in culture. As another example, DNA microarrays of postcapillary high endothelial...
venule (HEV) endothelial cells derived from human tonsils (freshly isolated or grown in culture for 2 days) and human umbilical vein endothelial cells revealed that a significant number of HEV-specific transcripts were downregulated or lost during the first two days of culture.6

**Nature**
The second mechanism underlying endothelial cell heterogeneity is site-specific epigenetic modification. In multicellular organisms, the epigenetic code is responsible for translating the hardwired blueprint-like genome into cell type- and tissue-specific expression patterns and phenotypes. Epigenetic properties are transmitted through S phase from parental cells to their progeny without the need for ongoing signals that initiated these changes. Epigenetically-encoded information is created by DNA methylation, histone modification, and chromatin remodeling. An important feature of these modifications is that they are reversible and highly dynamic. They may change with aging and/or disease. Importantly, they may be amenable to therapeutic manipulation.

### Mechanisms of EC heterogeneity

<table>
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<th><strong>Epigenetics</strong></th>
<th><strong>Microenvironment</strong></th>
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<td><strong>Artery</strong></td>
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<td>Major role in mediating phenotypic heterogeneity between arteries and veins</td>
<td>Major role in mediating phenotypic heterogeneity between arteries and veins/capillaries</td>
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<tr>
<td>Possible role in mediating phenotypic heterogeneity between different arteries</td>
<td>Major role in mediating phenotypic heterogeneity between areas of disturbed and undisturbed flow within a given artery</td>
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| **Vein** | +++  | ++  | ++  |
|-----------------|----------------------|
| Major role in mediating phenotypic heterogeneity between veins and arteries | Major role for low (and/or lack of high) flow rates in mediating phenotypic heterogeneity between veins and arteries/capillaries | Possible role in mediating phenotypic heterogeneity between veins and arteries/capillaries, owing to differences in oxygen content, pH, CO₂ (luminal) and composition of blood vessel wall (aboluminal) |
| Possible role in mediating phenotypic heterogeneity between different veins | Disturbed flow increases propensity for atherosclerosis | Likely role in mediating phenotypic heterogeneity between venous ECs since content of circulating blood depends on net exchange of substances that has occurred in preceding capillaries |

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| **Capillary** | +  | +  | ++++++++ |
|-----------------|----------------------|
| Likely major role in mediating phenotypic heterogeneity between nascent/naïve capillaries and large vessels | Major role for very low (and/or lack of high) flow rates in mediating phenotypic heterogeneity between capillaries and arteries/veins | Major role in mediating phenotypic heterogeneity between macrovessels and microvessels, since capillary endothelium is uniquely exposed to underlying tissue parenchyma |
| Possible role in mediating phenotypic heterogeneity between different capillary beds | May increase propensity for atherosclerosis | Major role in mediating phenotypic heterogeneity between different capillary beds since the microenvironment varies significantly between organs |

Mechanisms of endothelial cell heterogeneity. Relative importance of epigenetics and microenvironment in mediating site-specific phenotypes is indicated by +. The table is designed to provide a conceptual framework; the scores are largely speculative and will require ongoing experimental validation.
With regards to the endothelium, epigenetic changes are known to play key roles in mediating lineage determination and site-specific properties during embryonic development. For example, fate mapping studies in birds and Xenopus have revealed that distinct populations of precursors give rise to different subsets of endothelial cells. Moreover, studies in zebrafish embryos have demonstrated that site-specific markers of artery and veins are determined even before the onset of blood flow.9

From an operational standpoint, epigenetically determined properties may be defined as gene expression programs that are mitotically heritable and retained during multiple passages in culture. Viewed from this perspective, the most compelling evidence for the existence of epigenetically fixed differences in endothelial phenotypes is found in a study by Chi and colleagues, who demonstrated site-specific transcriptional programs in cultured endothelial cells from different segments of the vasculature, with most notable differences occurring between macrovascular and microvascular endothelial cells, and between arterial and venous endothelial cells.10 Unfortunately, few of these molecular differences were validated in situ. However, the shear number and reproducibility of site-specific transcripts in cultured cells implies biological relevance.

**Nature and Nurture**

In the final analysis, there is strong evidence to support a role for both mechanisms—environment and epigenetics—in mediating endothelial cell heterogeneity, thus creating a balance between stability and plasticity in gene expression and phenotype. For example, the study of human tonsillar endothelial cells referred to above demonstrated that some, but not all, site-specific transcripts were altered after 2 days of culture. In other words, whereas certain genes were sensitive to changes in the microenvironment, others were epigenetically programmed. It may be argued that arteries and veins display stereotypic functions in relatively fixed environments, and that the seeming propensity for heritable gene programs in these conduit vessels (eg, arterial-venous identity) reflects their need for stability over plasticity. In contrast, capillary endothelium mediates exchange of nutrients and gases between blood and underlying tissue, and is tightly coupled to the needs of the local environment (Figure). Given the remarkable spatial and temporal dynamics of the tissue microenvironment, plasticity of the microvascular endothelial cells may optimize their ability to “march to the tune” of the local environment.

**Endothelial Heterogeneity and Atherosclerosis**

Atherosclerosis is primarily a disease of large conduit arteries. An important question is how systemic changes in the circulation associated with atherosclerosis, eg, tobacco toxins, elevated cholesterol and/or glucose, result in characteristic lesions within such a defined segment of the vasculature. A common explanation is that systemic signals are channeled into local lesions by regional differences in flow patterns. This conclusion is supported by the clinical observation that saphenous veins, when grafted into the coronary circulation, tend to acquire artery-like properties including an increased propensity for atherosclerosis. A number of elegant in vitro and in vivo studies have demonstrated that endothelial cells exposed to disturbed flow versus nondisturbed flow display unique expression profiles.11,12 Although the detailed findings vary between studies, the data are consistent in supporting the notion that endothelial cells in regions of disturbed flow are primed to express a proinflammatory phenotype in response to systemic perturbations (such as those associated with atherosclerotic risk factors).

In this issue of *Circulation Research*, Deng et al describe differences in basal and inducible gene expression in cultured arterial versus venous endothelial cells, thus calling attention to the potential role for “nature” in mediating atherosclerosis. The authors used high density microarrays to examine gene expression profiles in human coronary artery endothelial cells (CAECs) and human saphenous vein endothelial cells (SVECs) under basal conditions and in response to one or another agonist, including oxidized LDL, tumor necrosis factor (TNF)-α, and interleukin (IL)-1. They identified 285 genes that were more highly expressed in SVECs, and 111 genes whose transcripts were more abundant in CAECs. The vein endothelial cell–selective transcripts were enriched for genes involved in inhibiting reactive oxygen species, attenuating the inflammatory response, and promoting proliferation. The artery-selective genes included inhibitors of cell proliferation and lipid metabolism. In response to ox-LDL, many more genes were upregulated and downregulated in venous compared with arterial cells, whereas TNF-α and IL-1 induced similar gene expression responses in both cell types. In venous endothelial cells, ox-LDL–activated genes involved protective pathways, whereas arterial endothelial cells demonstrated upregulation of cell adhesion molecules and genes involved in proliferation and apoptosis. The differential effect of ox-LDL on CAEC versus SVEC proliferation was validated in functional assays.

These findings support the conclusions of Chi et al, who also demonstrated cell subtype-specific differences in expression profiles in endothelial cell from many different sources, including coronary artery and saphenous vein.10 In addition, the present study extends the previous observations by: (1) offering a more focused annotation of genes related to atherosclerosis; (2) demonstrating functional relevance of differential gene expression at the level of cell proliferation; (3) reporting cell subtype- and mediator-specific differences in response to atherosclerotic stimuli; and (4) validating a small number of genes in situ.

Thus, existing data support a model of site-specific atherosclerosis in which both epigenetic and microenvironmental determinants play a role. According to this model, mitotically heritable differences in phenotype between venous and arterial endothelial cells may provide an epigenetic substrate for atherosclerosis (although it is possible that such differences actually provide a net protective effect against the disease). Regions of disturbed flow within arteries provide a microenvironmental niche that is primed for proinflammatory responses (this is now well established). The combined effect of epigenetics and microenvironment on arterial phenotype may result in “hot spots” which, when exposed to systemic...
changes associated with cardiovascular risk factors, undergo atherogenic change.

Questions and Future Studies

The current study, together with previous investigations, raises several interesting and important questions:

1. To what extent are the differences observed in cultured arterial versus venous endothelial cells reflective of differences in vivo?

It is formally possible that arterial and venous endothelial cells undergo variable degrees of phenotypic drift in vitro, and that site-specific signatures capture these variations. In data not shown, the authors of the present study claim that their in vitro DNA microarray results correlate with DNA microarray analyses of intact vessels. However, such a conclusion will require confirmatory assays, such as in situ hybridization or immunohistochemistry.

2. For those arterial-venous differences that hold true in vivo, which of their functional correlates are biologically relevant in determining susceptibility to atherosclerosis?

Although it is tempting (and perhaps useful) to speculate on the functional relevance of differential gene expression in arterial and venous endothelial cells, such predictions must ultimately be validated with functional assays, in vitro and more importantly in vivo. The authors have taken an initial step toward this goal by assaying for differences in cell proliferation under culture conditions. However, further studies are required to determine the functional relevance of site-specific differences in proliferative capacity (and other endothelial functions) in determining atherosusceptibility.

3. What effect does a mediator such as ox-LDL have on arterial and venous endothelial cells in the context of the native tissue environment of the cells?

Endothelial cell response to a particular agonist (eg, ox-LDL) is highly dependent on the net signal input (for an example, see reference 14). Owing to the complexity of the in vivo microenvironment (eg, the presence or absence of certain growth factors, cytokines, or chemokines; and/or differences in flow patterns), the site-specific patterns of ox-LDL response observed in culture may or may not hold true in the intact organism.

4. What is the clinical importance of delineating the relative role of epigenetics and microenvironment in mediating atherosclerosis?

Understanding the interplay between microenvironmental and epigenetic determinants in mediating atherosusceptibility has important therapeutic implications. Certain aspects of the microenvironment are fixed, whereas others are reversible. For example, heterogeneity in flow patterns—including disturbed flow—is a requisite feature of the cardiovascular system. However, an accentuation of these differences (eg, as occurs in hypertension) may be minimized with treatment. Therapeutic interventions such as lipid-lowering agents, smoking cessation, and diabetes control are all aimed toward modulating the microenvironment. From the standpoint of epigenetics, several open questions remain: (1) if any of the mitotically heritable arterial-specific markers render these vessel types susceptible to atherosclerosis; (2) to what extent is gender, aging, and/or comorbidity associated with changes in the endothelial epigenome; and (3) can these epigenetic determinants be pharmacologically manipulated?

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References


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