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Endothelial Cell Dysfunction and the Pathobiology of Atherosclerosis

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Abstract

Dysfunction of the endothelial lining of lesion-prone areas of the arterial vasculature is an important contributor to the pathobiology of atherosclerotic cardiovascular disease. Endothelial cell dysfunction (ECD), in its broadest sense, encompasses a constellation of various non-adaptive alterations in functional phenotype, which have important implications for the regulation of hemostasis and thrombosis, local vascular tone and redox balance, and the orchestration of acute and chronic inflammatory reactions within the arterial wall. In this review, we trace the evolution of the concept of endothelial cell dysfunction, focusing on recent insights into the cellular and molecular mechanisms that underlie its pivotal roles in atherosclerotic lesion initiation and progression; explore its relationship to classic, as well as more recently defined, clinical risk factors for atherosclerotic cardiovascular disease; consider current approaches to the clinical assessment of endothelial cell dysfunction; and outline some promising new directions for its early detection and treatment.

Keywords

Atherosclerosis; Endothelial Cell Dysfunction; Vascular Homeostasis

Subject Terms

Vascular Disease; Atherosclerosis; Endothelium/Vascular Type/Nitric Oide; Pathophysiology

Introduction and Overview

Homeostasis in the cardiovascular system is maintained by a hierarchy of interacting genetic and epigenetic programs, the disequilibrium of which contributes to the pathogenesis of complex disease processes such as atherosclerosis and its life-threatening complications, myocardial infarction and stroke. Vascular endothelium, the continuous cellular lining of the cardiovascular system, "Nature's container for blood"[1], is an important locus of critical regulatory nodes in this homeostatic network. In health, this distributed organ supports the

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normal functioning of the various tissues and organs of the body; in disease, its dysfunction within the walls of large arteries is an important contributor to the local and systemic manifestations of atherosclerotic cardiovascular disease (ACVD)[2].

Endothelial cell dysfunction (ECD) [Dialogue Box], manifested in lesion-prone areas of the arterial vasculature, results in the earliest detectable changes in the life history of an atherosclerotic lesion[3, 4] — the focal permeation, trapping and physicochemical modification of circulating lipoprotein particles in the sub-endothelial space[5]. This sets into motion a complex pathogenic sequence[4–11], initially involving the selective recruitment of circulating monocytes from the blood into the intima, where they differentiate into macrophages and internalize modified lipoproteins to become foam cells (the hallmark of early fatty streak lesions); multiple chemokines and growth factors elaborated by activated endothelium and macrophages then act on neighboring smooth muscle cells (or their precursors[12]) to induce their proliferation and synthesis of extracellular matrix components within the intimal compartment, thus generating a fibromuscular plaque. Progressive structural remodeling of developing lesions results in the formation of a fibrous cap, overlying a lipid-rich, necrotic core consisting of oxidized lipoproteins, cholesterol crystals and cellular debris, and is accompanied by varying degrees of matrix remodeling and calcification. The lateral edges of these complicated plaques contain a rich population of inflammatory cells (activated macrophages and T-cells, natural killer T-cells, dendritic cells), which further modulate the endothelial proinflammatory phenotype, and contribute to structural instability of the plaque through the proteolytic modification of its extracellular matrix components[13–15]. In unstable or vulnerable plaques, this may result in a catastrophic transition in the life history of an atherosclerotic lesion—frank plaque rupture, with luminal release of the highly thrombogenic contents of the necrotic core, triggering an atherothrombotic occlusion[16–19]. Alternatively, significant clinical sequelae also can result from superficial intimal erosions, without evidence of plaque rupture[19]. The latter critical transition appears to be a consequence of endothelial cell apoptosis, with localized endothelial denudation and the triggering of thrombus formation[20]. These superficial erosions typically occur on the surface of lesions containing abundant smooth muscle cells and proteoglycans, but few macrophages, and characteristically are associated with regions of disturbed blood flow. Other stable lesions, characterized by a thick fibrous cap, and less lipid and inflammatory cell content, can progressively encroach on the vessel lumen causing ischemic symptoms, but typically do not precipitate atherothrombotic events. In a given individual, multiple atherosclerotic plaques can co-exist—each at its own stage of pathobiologic evolution[17, 19].

Given its multiple, mechanistically important roles throughout this complex series of events, endothelial cell dysfunction thus appears to constitute a pathogenic *sine qua non* for atherosclerotic cardiovascular disease. In this review, we will trace the evolution of the concept of endothelial cell dysfunction, focusing on recent insights into the cellular and molecular mechanisms that underlie its pivotal roles in atherosclerotic lesion formation and progression; explore its relationship to classic, as well as more recently defined, clinical risk factors for ACVD; consider approaches to the detection of ECD; and outline some promising new directions for its treatment.

Understanding Endothelial Cell Dysfunction

The evolution of our understanding of the vital functions of the vascular endothelium and the various manifestations of its dysfunction in the context of ACVD resembles the parable of "The Blind Men and the Elephant"[21]. Probed with the tools of classic cell biology and physiology, the endothelium initially was characterized as a vast, selectively permeable interface[22], separating the vascular and interstitial compartments of the body and serving as a gateman, regulating the transport of fluid and macromolecules via an elaborate system of transcellular vesicles and intercellular junctional complexes[23–26]. Localized loss of this selective barrier function (manifested as edema), coupled with the emigration of white blood cells (pus formation), have been recognized since antiquity as cardinal signs of inflammation-- the body's primal reaction to tissue injury or infection[27]. Examined from the perspective of classic biochemistry and pharmacology[28], and aided by the development of reliable methods for the *in vitro* culture of endothelial cells[29], a diverse repertoire of metabolic functions of the endothelium became apparent--- including the biosynthesis and degradation of vasoactive mediators, the enzymatic buffering of reactive oxygen species, the transport and metabolism of lipoproteins, the secretion and enzymatic remodeling of extracellular matrix components, the elaboration of various growth factors, cytokines and hormone-like substances, and the biosynthesis of prostaglandins (in particular prostacyclin) and other potent autocoids[30]. From the perspective of hemostasis and thrombosis, the intact, normally functioning endothelial lining provides an ideal container for blood; its luminal surface does not activate the intrinsic coagulation cascade or promote platelet adhesion, and actually exhibits anticoagulant and fibrinolytic properties[31]. Examined from a bioengineering perspective, the individual endothelial cells comprising the lining of various parts of the cardiovascular system are seen to function as local biomechanical transducers, sensing and transducing the diverse forces imparted by the pulsatile flow of blood into biological responses[32, 33]. Finally, when challenged with certain pro-inflammatory cytokines or bacterial products (e.g., endotoxins), endothelial cells undergo a coordinated program of gene activation, which (reversibly) alters many of these vital functional properties, presumably serving as an adaptive response to potentially noxious stimuli[30]. Thus, when viewed from these different perspectives, the vascular endothelium can be variously characterized as a distributed organ, a dynamically adaptable interface, and, at the individual cell level, an integrator of the local pathophysiological milieu. And, dysfunction of the endothelium, in the broadest sense, would encompass various non-adaptive alterations in its normal functional phenotype, with important implications for the regulation of hemostasis and thrombosis[31], local vascular tone[30] and redox balance[34], and the orchestration of acute and chronic inflammation[30].

While the term "endothelial dysfunction" has clearly found its place in the lexicon of modern Cardiovascular Medicine[35–37], the evolution of this working concept has a rich history dating from the early practice of Anatomical Pathology, in the 1850's, and continuing through the development of the modern field of Vascular Cell Biology, in the latter half of the 20th century. This conceptual evolution has contributed in important ways to our present day understanding of the cellular and molecular mechanisms of atherosclerotic cardiovascular disease. Its origins can be traced to the writings of Rudolph Virchow[38], who called attention to the localized accumulation of circulating lipids and other

macromolecular components of plasma at sites of early lesion formation (lipid insudation), which was detectable at autopsy and presumably reflected a localized change in endothelial permeability in life. Subsequently, the focal, non-random distribution of this permeability change was graphically illustrated by the mapping of blue and white areas, *en face*, in the aortas of experimental animals following injection of the plasma protein-binding azo dye Evans Blue[39, 40]. Blue areas were also associated with an increased rate of endothelial cell turn-over in normal animals [40], suggesting that they represented areas of chronic intimal injury, which, in animals made hypercholesterolemic by dietary alterations, were correlated with developing atherosclerotic lesions[41]. When examined ultrastructurally at the pre-lesional stage, these lesion-prone areas exhibited alterations in endothelial morphology, striking changes in the amount and composition of extracellular matrix, and the accumulation of extracellular liposomes, derived from plasma lipoproteins which had become trapped in the subendothelial space and undergone complex biochemical and physicochemical alterations[5, 42]. While these detailed morphologic descriptions of early intimal changes strongly supported the notion of localized dysfunction of the endothelial lining, the pathophysiological stimuli, underlying mechanisms and consequences for atherogenesis remained to be established.

Early characterizations of endothelial cell dysfunction, in the context of atherogenesis, focused on a loss of anatomical integrity of the intima, as exemplified by the seminal "Response-to-Injury Hypothesis" of Ross and Glomset[7], which stimulated much fruitful activity in the field. As originally formulated, the Response-to-Injury Hypothesis postulated that the initiating event in the atherogenic process was some form of overt injury to the intimal endothelial lining, induced by noxious substances (e.g., oxidized cholesterol, constituents of cigarette smoke, hyperhomocystemia, hyperglycemia, etc.) or altered hemodynamic forces (e.g., blood flow disturbances generated by hypertension). In particular, focal endothelial desquamation was envisioned as an inciting stimulus for platelet adhesion and the localized release of platelet-derived growth factors, which then would elicit the migration, proliferation and phenotypic modulation of medial smooth muscle cells, thus generating a fibromuscular plaque[6]. The experimental demonstration that this sequence of events could be induced simply by balloon catheter denudation of the intima lent support to this hypothesis[43], and catalyzed an abundance of studies of the potential roles of growth factors and injury-repair mechanisms in the atherogenic process. However, the direct relevance of this form of endothelial injury to the development of natural atherosclerosis was unclear, given that careful morphologic examination of the earliest fatty streak lesions in diet-induced animal models failed to demonstrate overt intimal injury or platelet adhesion[44, 45]. With the expanded awareness of the repertoire of vital functions of endothelial cells and their capacity for dynamic phenotypic modulation, the term "endothelial (cell) dysfunction" was introduced into the mainstream of atherosclerosis research[46, 47]. Various pathophysiologic stimuli of endothelial cell dysfunction (TABLE 1) soon became the focus of fruitful investigation by multiple groups, and the molecular manifestations of ECD began to be characterized in detail[11, 15, 19, 30, 35].

Nitric Oxide and Endothelial Cell Dysfunction

A major conceptual advance in the field of Vascular Cell Biology was the demonstration that the endothelial lining is a source of various autocoid factors that can influence the behavior of other types of cells within the vessel wall (pericytes, smooth muscle) and in the circulating blood (platelets and leukocytes). Using cultured vascular cells, as well as bioassays with isolated organs and vascular strips, several endothelial-derived autocrine and paracrine mediators were identified[48–53]. In particular, endothelial-derived prostacyclin and platelet-derived thromboxane A2 were characterized as mutually antagonistic components of a dynamic "hemostatic/thrombotic balance" at the vessel-blood interface[30, 31], and thus as potential targets for therapeutic modulation. In fact, pharmacologic manipulation of this aspect of arachidonic acid metabolism remains an important clinical strategy in the treatment and prevention of ACVD[52]. Collectively, these studies documented an active role of the vascular endothelium in the maintenance of blood fluidity and the regulation of vascular tone, and provided the experimental tools and conceptual framework to further define the roles of endothelium in cardiovascular physiology and pathophysiology.

In 1980, Furchgott and Zawadzki demonstrated that vasodilation induced by acetylcholine was dependent on the presence of an intact endothelium and appeared to be mediated by a potent humoral factor[54], later known as endothelium-derived relaxing factor (EDRF). Several studies documented that EDRF was a labile substance with a half-life of only seconds in oxygenated physiological media[55]. Release of EDRF from blood vessels was observed under basal conditions, as well as after stimulation with acetylcholine[56]. The biological effects of EDRF were shown to be inhibited by hemoglobin[57], and to be mediated by stimulation of a soluble guanylate cyclase with the consequent elevation of intracellular cyclic GMP levels in vascular smooth muscle cells[58]. Based on the similarities in the pharmacological properties of EDRF and NO, EDRF subsequently was identified as NO or a labile nitroso species[59, 60]. A detailed comparison of the biological actions of EDRF and NO on vascular smooth muscle strips[61] and platelets[62] also showed that EDRF and NO were indistinguishable. In 1988, L-Arginine was shown to be the precursor for the synthesis of NO by vascular endothelial cells[63].

Endothelial cells metabolize L-arginine via the endothelial isoform of nitric oxide synthase (eNOS)[64, 65] to form NO, with L-citrulline as a byproduct [Figure 1]. This overall process is subject to both transcriptional and complex post-translational regulation[66, 67]. A basal level production of NO by endothelial cells contributes to regulating vasomotor tone and preserving the non-thrombogenic behavior of the vascular lining. The synthesis of NO can be stimulated by receptor-dependent agonists (acetylcholine, bradykinin), non-receptor-dependent agonists (calcium ionophores), and also fluctuations in blood flow[66, 68]. Additionally, transcription of the *eNOS* gene is differentially regulated by fluid mechanical forces, such that endothelial cells in arterial geometries exposed to undisturbed laminar flow exhibit enhanced NO-forming capacity[2, 33]. Once NO is produced by eNOS, it can rapidly diffuse across cell membranes to act as a potent paracrine mediator, but it can also react with superoxide to form peroxynitrite anion, which leads to its inactivation. Particularly relevant for vascular homeostasis are the actions of NO on adjacent smooth muscle cells and circulating blood platelets and leukocytes. Many of these physiological actions are mediated

through activation of soluble guanylate cyclase (sGC), which catalyzes the conversion of guanosine triphosphate to cyclic guanosine monophosphate (cGMP). Other actions of NO are mediated by S-nitrosylation, the addition of an NO group to a Cys thiol to form an S-nitrosoprotein[69]. S-nitrosylation has been shown to modify the function of proteins in a reversible manner, which is analogous to phosphorylation[70]. In the cardiovascular system, S-nitrosylation of various target proteins has been demonstrated to modulate important cellular physiological processes including cell proliferation[60], apoptosis[71, 72], exocytosis[73], and ion channel activity[74], as well as blood flow and systemic oxygen delivery[75, 76].

The importance of endothelial-derived NO in the regulation of systemic blood pressure was first demonstrated in animal models, and then in humans, by using L-arginine-based competitive inhibitors. These inhibitors increase vascular resistance and blood pressure, and decrease blood flow[77]. Subsequent studies using genetically modified mice documented that *eNOS*-deficient mice are hypertensive, and that aortic rings isolated from these mice respond to acetylcholine with paradoxical vasoconstriction, but do relax in response to sodium nitroprusside or papaverine[78, 79]. Furthermore, these *eNOS*-deficient mice were found to display increased endothelial-leukocyte interactions, platelet aggregation and thrombosis[80, 81], and, when crossed with *ApoE*-deficient (hypercholersterolemic) mice, developed accelerated atherosclerosis[82].

In a classic study, the clinical significance of EDRF for human atherosclerotic cardiovascular disease was first demonstrated by Ganz and colleagues, using coronary angiography[83]. The coronary arteries of individuals with advanced atherosclerotic disease showed a dose-dependent, paradoxical vasoconstriction in response to graded concentrations of acetylcholine, in contrast to the vasodilation observed in individuals with angiographically normal coronary arteries. Importantly, coronary vessels with minimal disease also constricted in response to acetylcholine, while coronary vessels from all three patient groups dilated in response to nitroglycerin. Hypercholesterolemia-dependent alterations in endothelium-dependent vasodilation were also documented in animal models of atherosclerosis[84–86]. Taken together, these observations suggested that a deficiency in endothelial NO production or its bioavailability, in both humans and animals, might actually precede the formation of clinically significant atherosclerotic lesions. Impairment of flow-mediated vasodilation, measured non-invasively in peripheral vessels such as the brachial artery, appears to correlate with direct measurements of altered vasoreactivity in the coronary circulation[87]. Peripheral manifestations of endothelial dysfunction have also been shown to be an independent predictor of ACVD events[36]. This has stimulated the development and application of various FDA-approved devices to measure flow-mediated arterial dilation as a clinically useful index in the assessment of cardiovascular disease. However, as considered in greater detail below, the pathogenesis of endothelial cell dysfunction and its pathophysiological consequences extend beyond endothelial nitric oxide metabolism.

Endothelial Proinflammatory Activation and Endothelial Cell Dysfunction in Atherogenesis

The involvement of the endothelial lining of small blood vessels (e.g., post-capillary venules) in acute inflammatory responses, induced by injury or infection, has long been

appreciated[25, 26]. Indeed, the cardinal signs of inflammation (rubor, calor, tumor, dolor) elicited by the action of histamine and other phlogistic agents can be mechanistically related to their effects on microvascular tone, permeability and leukocyte diapedesis. These acute responses, which have been referred to as “endothelial stimulation”, or “Type I Activation”[88], are rapid in onset, self-limited in nature, and typically do not result in sustained morphological or functional changes. In contrast, endothelial cells can undergo a dramatic modulation in their functional phenotype in response to certain bacterial products, such as Gram-negative endotoxins, and other pathogen-associated molecular patterns (PAMPs), modified lipoproteins, and other damage-associated molecular patterns (DAMPs), or cytokines, such as Interleukin-1 (IL-1), Tumor Necrosis Factor (TNF) and Interferon-gamma[89]. The hallmark of this form of endothelial response, termed “Type II Activation” [88–90], is the activation of pleiotropic transcription factors, such as Nuclear factor-kappa-B (NFκB), resulting in the expression of various effector proteins with important pathophysiologic implications. Originally described as “endothelial activation antigens”[91], detectable on the surface of cultured human endothelial cells *in vitro* following their incubation with cytokines or endotoxin, these effector proteins include Class-II major histocompatibility antigens, involved in antigen presentation; inducible endothelial-leukocyte adhesion molecules, such as ELAM-1 (E-selectin) and VCAM-1; procoagulant molecules, such as Tissue Factor, and secreted chemokines, such as Interleukin-8 (IL-8) and Monocyte Chemotractant Protein-1 (MCP-1)[92–95]. Together this activation program confers a localized, temporally coordinated, “proinflammatory endothelial phenotype” [Figure 2], which is detectable *in vivo*, at sites of inflammation, in the microvasculature of humans and experimental animals[96, 97], and has become a keystone in our understanding of the active role that endothelial cells play in acute and chronic inflammatory responses, and related disease processes[30].

A critical link between the activation program manifested by microvascular endothelium during inflammatory reactions and arterial endothelial cell dysfunction in atherogenesis came with the discovery of the Athero-ELAM (VCAM-1)[98]. Initially characterized as a cytokine-inducible activation antigen in cultured endothelial cells which exhibited a sustained pattern of expression (in contrast to “acute ELAMs” such as E-selectin), VCAM-1 was found to have selective adhesivity for mononuclear leukocytes and lymphocytes, via their expression of its counterreceptor VLA-4[99–101]. Importantly, its expression *in vivo*, in human coronary atherosclerotic plaques and in both dietary and genetic animal models of hypercholesterolemia is localized to the intact endothelium overlying atherosclerotic lesions, and actually precedes the earliest recruitment of mononuclear leukocytes to nascent lesions[102–105]. Further studies implicated components of oxidized lipoproteins, such as lysophosphatidylcholine, as potent stimuli for its expression, thus mechanistically linking VCAM-1 induction to the atherogenic process[106]. Additionally, its spatial pattern of expression *in vivo* in various animal models of atherosclerosis is localized to those regions of the arterial vasculature that are most susceptible to lesion formation[103, 104], and the detection of its soluble isoform in circulating plasma is correlated clinically with disease severity (lesion burden) in patients with atherosclerosis[107]. Finally, its genetic deficiency in mouse models of atherosclerosis results in a reduction in lesion formation[108]. These findings thus highlighted the pathogenic significance of VCAM-1 in the context of atherogenesis,

and also implicated it as a molecular marker of endothelial cell dysfunction in the clinical setting[35].

A second important link between atherogenesis and proinflammatory endothelial activation is the intrinsic capacity of activated vascular cells (endothelium, smooth muscle) to secrete chemokines, such as IL-1, MCP-1, granulocyte-monocyte stimulating factor (GM-CSF) and IL-8, thus generating localized, intercellular autocrine and paracrine signaling loops within the vessel wall[53, 90]. Coupled with the influx of various classes of T-lymphocytes and monocytes/macrophages[109], each contributing their particular repertoire of cytokines (in part triggered by the recognition of antigens on native and oxidized lipoproteins), a complex immuno-regulatory network is thus established within the developing atherosclerotic lesion, which has both local and systemic consequencies[15, 110–112]. Locally, the balance of pro- and anti-inflammatory mediators (e.g., IL-1; IL-11), as well as agents that promote the resolution of inflammation (e.g., Resolvins)[113] can contribute to lesion progression or regression, while systemic manifestations, such as the induction of acute-phase reactants (e.g., C-reactive protein) by endothelial-derived Interleukin-6 (IL-6), can serve as useful diagnostic and prognostic indices[114].

It is noteworthy that NFκB appears to play a central role in the proinflammatory activation of endothelium in atherogenesis. Various of the pathophysiologic stimuli of ECD have been implicated in NFκB signaling in arterial endothelium[115]; expression of many of the effector molecules associated with ECD in atherogenesis (e.g., VCAM-1, MCP-1) are under NFκB control; and certain flow-sensitive endothelial genes (e.g., platelet-derived growth factor-B) have non-canonical NFκB binding sites (“shear-stress-response elements”) in their promoters[116]. Interestingly, in atherosclerosis-prone arterial geometries, endothelial NFκB appears to be primed for enhanced activation in response to systemic stimuli [Figure 2], such as bacterial endotoxin or hypercholesterolemia[117]. Recent studies suggest that NFκB activation may also result in fundamental changes in endothelial chromatin structure, through the formation of super-enhancer complexes, which can confer an epigenetic level of regulation to the proinflammatory endothelial phenotype in atherogenesis[118].

This expanded appreciation of the roles of inflammatory and immuno-regulatory mediators and cellular processes in atherogenesis has led to a reformulation of the original “Response-to-Injury Hypothesis” into what is now termed the “Inflammatory Hypothesis of Atherothrombosis”[11, 13–15]. In addition to providing a unifying view of the pathophysiologic mechanisms involved in lesion initiation, progression and critical transitions, which are linked to many of the known risk factors for ACVD, the basic premise that atherosclerosis is a chronic inflammatory disease has important and timely clinical implications. These include the recent revision of the traditional clinical risk factors[119] to include systemic indices of inflammatory status[120, 121], and, as considered below, the initiation of randomized placebo-controlled clinical trials of anti-inflammatory treatments for ACVD[122]. Importantly, the evolution of the concept of endothelial cell dysfunction has provided the conceptual framework for these paradigm-shifting, basic to translational advances.

Hemodynamics, Endothelial Cell Dysfunction and the Focal Nature of Atherosclerosis

An intriguing aspect of the pathobiology of atherosclerosis is the observation that the earliest lesions, in both humans and various experimental animal models, characteristically develop in a distinctive, non-random pattern, the geometry of which correlates with arterial branch points and other regions of altered hemodynamics[123–125]. Detailed studies of the blood flow in these lesion-prone regions has revealed complex, disturbed laminar flow patterns (flow separation, recirculation, reattachment), which create significant temporal and spatial gradients over relatively short distances, resulting in a high oscillatory index and a low time-averaged wall shear stress[32, 33]. Initial hypotheses suggested that these conditions might result in longer dwell times for lipoproteins, favoring their increased permeation into the vessel wall[126], or that disturbed flow might physically disrupt endothelial integrity, creating foci of injury/repair reminiscent of the Response-to-Injury Hypothesis of Atherosclerosis[7]. As discussed above, however, the latter explanation was challenged by detailed morphologic studies documenting the presence of an intact endothelial lining in atherosclerosis-susceptible regions during early lesion formation[44, 45, 127]. The subsequent development of experimental fluid mechanical devices that could reproduce defined laminar, turbulent and disturbed flows over the surface of cultured endothelial cell monolayers *in vitro*[33, 128–131] revealed that the fluid mechanical forces generated by arterial blood flow could also act directly on endothelial cells to alter their morphological and functional properties. For example, exposure of cultured endothelial cells to undisturbed laminar shear stresses induces changes in cell shape, alignment, surface glycocalyx and cytoskeletal organization that mimic the morphology of aortic endothelium *in vivo* in lesion-resistant geometries[132–137]. In contrast, exposure to disturbed flows induce endothelial cell turnover and senescence [138, 139], and increased oxidative stress[34], as well as alterations in cell shape and the organization of cytoskeletal and intercellular junctional proteins changes[140–143] similar to those seen in lesion-prone areas of the arterial vasculature *in vivo*[33]. These observations suggested that distinct hemodynamic forces might constitute a local risk factor for endothelial cell dysfunction in atherogenesis[144].

A fundamental link between hemodynamic forces and atherogenesis came with the demonstration that the expression of various endothelial genes important in hemostasis and thrombosis, growth regulation and proinflammatory activation were transcriptionally regulated by defined fluid mechanical stimuli[145–149]. Since several of these genes were directly involved in endothelial adhesion biology (e.g., ICAM-1), these observations suggested a novel paradigm linking biomechanical stimulation with endothelial activation[150]. Further mechanistic analyses revealed the existence of shear stress response elements (SSREs) in the promoters of pathophysiologically relevant genes such as the platelet-derived growth factor, eNOS and VCAM-1, that acted to up- or down-regulate gene transcription[116, 151, 152]. Interestingly, clusters of multiple genes appeared to be differentially responsive to the spatial and temporal properties of the applied shear stresses, thus suggesting a complex, dynamic system of biomechanical endothelial gene regulation. In early studies, Topper and co-workers utilized high-throughput molecular biological techniques to define the effects of undisturbed laminar shear stresses, similar to those present in lesion-resistant arterial geometries, on the expression of endothelial genes that might have special relevance for atherogenesis[153]. Notably, several pathophysiologically

important endothelial genes showed a sustained, differential upregulation with undisturbed laminar flow, compared with turbulent flow. These included COX-2 (the inducible isoform of cyclo-oxygenase), eNOS, and manganese-dependent superoxide dismutase--- enzymes that exert potent anti-thrombotic, anti-adhesive, anti-inflammatory and anti-oxidant effects, both within the vascular lining and in interacting cells, such as platelets, leukocytes and vascular smooth muscle. These findings, together with the observation that, in the face of potent systemic drivers of cardiovascular risk (e.g., hypercholesterolemia)[12, 111, 154], certain regions of the arterial vasculature remain relatively resistant to the development of atherosclerotic lesions, led to the formulation of an alternative hypothesis for the selective distribution of atherosclerotic lesions, the "Atheroprotective Gene Hypothesis" [155–157]. This hypothesis postulated that undisturbed laminar shear stresses upregulate the coordinated expression of a set of atheroprotective genes in endothelial cells, which then act locally in lesion-resistant areas to offset the effects of systemic risk factors in ACVD. The critical testing of this hypothesis entailed a four-step experimental approach—first, a detailed characterization of the actual near-wall shear stress profiles in atheroprone and atheroprotected arterial geometries; second, the recreation of these complex flows, with spatial and temporal fidelity, over cultured human endothelial monolayers; third, a genome-wide comparative analysis of the resultant transcriptomes and their correlation with known pathogenic mechanisms in atherosclerosis; and fourth, the validation of these genetic expression programs in endothelial cells in atherosclerosis-resistant and atherosclerosis-susceptible vascular geometries *in vivo*.

The combined efforts of several groups subsequently produced a robust body of experimental observations linking specific patterns of hemodynamic stimulation with the transcriptional regulation of functional phenotypes in endothelium *in vitro* and *in vivo*[2, 130, 158–163]. In *in vitro* model systems, individual genes with special pathophysiologic significance for atherogenesis (e.g., IL-8, VCAM-1) were found to show a differential expression pattern that correlated with atheroprone waveforms, while clusters of genes associated with vasoprotection were coordinately upregulated by atheroprotective waveforms[161] [Figure 3]. Characterization of mRNAs expressed *in vivo* in endothelial cells selectively isolated from lesion-prone geometries in experimental animals provided important validation of these *in vitro* results[163]. Taken together, these studies led to the recognition that a major cause of endothelial cell dysfunction in lesion-prone arterial geometries appeared to be the absence of undisturbed laminar shear stresses, which normally serve as a positive regulator of a vasoprotective endothelial phenotype in vascular homeostasis [Figure 4][2, 33, 164]. This realization motivated the comparative bioinformatic analysis of the endothelial transcriptomes elicited by atheroprotective versus atheroprone fluid mechanical stimulation, with special attention focused on transcriptional regulators[161, 165, 166]. Among the transcription factors most responsive to hemodynamic stimuli, the zinc finger transcription factor, Kruppel-like Factor 2 (KLF2) appeared to show the strongest differential upregulation by atheroprotective waveform stimulation[166]. Expression of KLF2 had been previously demonstrated in the endothelium of atheroresistant regions of human arteries specimens, using *in situ* hybridization[167]. When experimentally introduced into cultured human endothelial monolayers, via viral vectors, KLF2 coordinately upregulated a large number of the genes that where associated with

atheroprotective hemodynamic stimulation[166, 168]. Taken together these observations focused attention on KLF2 as a potential critical regulatory node in the endothelial homeostatic network[2]. Several studies documented that expression of KLF2 in endothelial cells promotes an anti-inflammatory, anti-thrombotic endothelial phenotype, which is explained, at least in part, by its antagonism of the NFκB pathway[169]. In addition, the expression of KLF2 was found to regulate other endothelial cell functions important for atherogenesis, including endothelial barrier function[170], metabolism[171], and the release of microRNAs via the shedding of endothelial microvesicles[172]. Endothelial KLF2 expression also stimulates the production of several autocoids, including NO and natriuretic-peptide C (CNP), which have been shown to be deficient in dysfunctional endothelium *in vivo*[173]. Furthermore, mice genetically deficient in KLF2 display enhanced atherosclerotic plaque formation when compared to wild type controls[174].

Importantly, the statins, a widely prescribed class of cardio-protective drugs, were found to upregulate KLF2 expression in cultured human endothelial cells at pharmacologically relevant doses[175, 176]. This class of HMG CoA reductase inhibitors was originally designed to reduce the biosynthesis of endogenous cholesterol, and thus treat hypercholesterolemia—a major risk factor in ACVD. Biochemically they block production of mevalonate, which forms two major downstream products: farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP). Statin-mediated upregulation of KLF2 in human endothelial cells is dependent on the depletion of GGPP[175–177], which is known to prenylate several members of the Rho superfamily. Notably, upregulation of KLF2 is critical for many of the statin-dependent transcriptional changes in endothelial cells, thus implicating KLF2 in the so-called pleiotropic, non-lipid-lowering, beneficial cardiovascular effects of this class of cardiovascular drugs[178, 179].

The atheroprotective flow-mediated expression of KLF2 in endothelial cells is dependent of the activation of the MEKK3/MEK5/ERK5/MEF2 signaling pathway, and several characterized modifiers including AMPK, SIRT1, PKCζ, SUMO specific protease 2 and histone deacetylase 5 [2, 180]. Activation of MEK5 and/or ERK5 *per se* leads to endothelial vasoprotection in both a KLF2-dependent and KLF2-independent manner, suggesting that additional targets of these kinases may be involved in mediating the beneficial effects of flow[181, 182]. One such target is the transcription factor Kruppel-like factor 4 (KLF4). KLF4 also is upregulated by atheroprotective flow in cultured endothelial cells via MEK5/ERK5/MEF2 signaling[181, 183]. KLF4 expression promotes vasoprotective gene expression in cultured endothelial cells, via several downstream transcriptional targets that are also activated by KLF2[181, 183, 184]. Using endothelial-selective gain-of-function and loss-of-function approaches, an important role for KLF4 in atheroprotection has been documented in a mouse model of atherosclerosis[184].

Another noteworthy flow-mediated transcriptional regulator of atheroprotection is NF-E2-related factor-2 (Nrf2). Nrf2 is activated by atheroprotective flow in cultured endothelial cells, via the phosphoinositol 3-kinase/Akt and Erk5 pathways and controls a series of downstream target genes that play a role in the regulation of intracellular redox balance, as well as resistance to extracellular oxidant stresses[185–187]. *In vivo*, Nrf2 is differentially expressed in those regions of the vasculature that are relatively atherosclerosis-resistant, thus

further suggesting its pathophysiological relevance[185, 188]. Recent studies have documented that KLF2 and Nrf2 act independently in the activation of flow-mediated gene expression, but suggest a requirement for KLF2 expression for full activity of Nrf2 in mediating antioxidant vasoprotection[189, 190]. Together, these two transcription factors account for the activation of ~70% of the atheroprotective flow-induced endothelial transcriptome[190], thus pointing to their critical role as master regulators of the vasoprotective endothelial phenotype [Figures 3 and 4].

Beyond their major effects at a transcriptional level, atheroprotective and atheroprone flow stimulation appear to influence endothelial gene expression via two additional mechanisms: microRNAs (miRNAs) and epigenetic modifications[191, 192][Figure 4]. Initial *in vivo* miRNA profiling studies, comparing arterial regions in normal adult swine, identified high expression of miR10a in atheroprotective regions[193]. In cultured endothelial cells, a major action of miR10a is to down-regulate the NFκB pro-inflammatory pathway. Atheroprotective flow also has been shown to upregulate the expression of miR19-a[194], miR-23b[195], and miR101[196] in cultured endothelial cells, leading to the suppression of endothelial cell proliferation. In contrast, expression of miR92a[197], miR663[198], miR712[199] and miR34a[200] is downregulated by atheroprotective flow and upregulated by atheroprone flow in cultured endothelial cells. The atheroprotective flow-dependent suppression of miR92a expression results in the upregulation of KLF2 and KLF4 and certain of their downstream transcriptional targets *in vitro* and *in vivo*[197, 201]. Inhibition of miRNA-92a in endothelial cells modulates endothelial activation in response to shear stress and oxidized LDL, and limits the development of atherosclerosis in LDL receptor-deficient mice at least in part by increasing the expression of KLF2 and KLF4, thus suppressing endothelial activation[202]. Inhibition of the expression of miR663 suppresses atheroprone flow-mediated endothelial activation[198]. miR712 downregulates the expression of tissue inhibitor of metalloproteinase 3 (TIMP3) leading to the stimulation of endothelial inflammation, permeability and atherosclerotic lesion formation in mice[199]. Similarly, the downregulation of miR34a contributes to the atheroprotective flow-mediated suppression of endothelial inflammation by downregulating NFκB signaling[200]. Finally, atheroprotective flow also induces the secretion of the miR143–145 cluster via a KLF2-dependent pathway. These secreted miRNAs can act on neighboring vascular smooth muscle cells to regulate their turnover and phenotype, and reduce atherosclerotic lesion size in ApoE-deficient mice[172]. The broad reaching implications of miRNAs for the diagnosis and treatment of ACVD are the subject of another review in this Compendium on Atherosclerosis series.

Recently, it has become apparent that hemodynamic forces can modulate endothelial gene expression at an epigenetic level. For example, two independent studies have shown that atheroprone flow significantly modulates endothelial DNA methylation patterns via alterations in DNA methyltransferase (DNMT) activity, in particular DNMT1[203, 204]. In addition, at the single gene level, disturbed flow was shown to increase methylation of the proximal promoter of *KLF4* in endothelial cells leading to the suppression of its expression by blocking a MEF2 binding site, and by regulating DNMT3A. This MEF2 site was also hypermethylated in swine aortic endothelium isolated from atherosclerosis susceptible regions of the aorta where *KLF4* expression is low[205]. In light of the recent awareness of the

global role of epigenetic changes in pro-inflammatory endothelial cell activation[118], this new area of flow-mediated gene regulation may offer interesting opportunities for targeted pharmacological interventions.

In addition to the basic role that the anatomical configuration of large arteries plays in defining distinct hemodynamic environments, physiological factors that alter systemic and/or local blood flow also can significantly influence endothelial functional phenotype. For example, aerobic exercise appears to exert some of its well described beneficial cardiovascular effects by modifying flow patterns in the vicinity of arterial branch points, thus altering the wall shear stresses experienced by the endothelial lining[206]. A transition to more undisturbed laminar flow in lesion-prone geometries should favor the increased expression of certain atheroprotective genes (e.g., eNOS), the inhibition of arterial stiffness, and the restoration of a vasoprotective endothelial phenotype[207, 208].

Multiple atherosclerotic lesions in a given human subject can progress, regress, or remain stable, each behaving in an autonomous fashion, thus suggesting that local factors are important determinants of their pathobiology [209]. Utilizing a combination of intravascular ultrasound, biplane angiography and blood flow measurements, Stone and coworkers undertook a detailed assessment of endothelial wall shear stresses and local arterial wall morphology in the coronary arteries of patients with acute coronary syndromes-- at the time of intravascular intervention (stenting) and 6 months later[210]. In addition to providing fresh insights into the natural history of coronary atherosclerotic disease, these and other vascular profiling studies suggested that endothelial shear stresses were a potentially useful predictor of subsequent pathobiological behavior of a given lesion[210–212]. These observations were then extended in the Prediction of Progression of Coronary Artery Disease and Clinical Outcome Using Vascular Profiling of Shear Stress and Wall Morphology (PREDICTION) Study, which demonstrated a strong correlation between localized, low (disturbed) endothelial shear stresses and subsequent lesion progression to a clinical event[213]. Taken together, these studies suggest that vascular profiling, combined with biological imaging techniques, potentially could be used to guide more targeted interventional therapies in acute coronary syndromes[214–216].

CLINICAL ASSESSMENT OF ENDOTHELIAL CELL DYSFUNCTION AND ITS RELATIONSHIP TO CARDIOVASCULAR RISK

Given its involvement at all stages of ACVD progression, and its predictive significance for cardiovascular events[36], there has been considerable interest in the detection and monitoring of ECD in the clinical setting. While direct measurement of impaired endothelial-dependent dilation during coronary angiography remains the historical “gold-standard”[83], various indirect approaches, such as the measurement of brachial artery diameter via non-invasive ultrasound imaging in response to flow-mediated reactive hyperemia, have found their way into general practice[36]. The concordance of these measurements of peripheral arterial vasoreactivity with coronary artery endothelial-dependent vasodilation has been established in controlled comparative studies[87], lending further support to their use for cardiovascular risk evaluation in a given patient. Since endothelial cell dysfunction also has been implicated in the pathophysiology of various disease states in addition to ACVD, e.g.,

hypertension, diabetes, chronic heart and renal failure[217], these indirect indices of ECD may have broader clinical significance.

In light of the expanded appreciation of the multiple roles that endothelium plays in cardiovascular homeostasis, the search for clinically useful measures of ECD, which more directly reflect the pathobiology of ACVD at the cellular and molecular level, has burgeoned[218]. These efforts encompass a broad spectrum of activity—including the validation of circulating plasma biomarkers that reflect pathogenic events occurring within developing lesions *per se* (e.g., the secretion of inflammation-associated cytokines, such as IL-1 and IL-6, or the shedding of induced cell surface adhesion receptors, such as sVCAM, sE-selectin), as well as the measurement of systemic indices of the inflammatory state, such as C-reactive protein. Efforts in biomarker discovery now extend well beyond the validation of individual candidate molecules of interest, and are employing the power of high-throughput genomics, proteomics and metabolomics, coupled with bioinformatic analyses. Complementary to these blood-based analytical approaches has been the emergence of novel imaging strategies to assess the clinical significance of individual atherosclerotic lesions, at a given moment, in a patient at risk[219, 220]. By employing state-of-the-art molecular imaging technologies to localize pathogenic events in arterial endothelium (e.g., expression of VCAM-1), this approach ideally would enable the assessment of the natural history of lesions, in real-time, thus potentially allowing the identification of a unstable plaque before its transition into a clinical event, or the evaluation of novel therapeutic strategies designed to target ECD *per se*[221]. If implemented in a cost-effective fashion, these approaches could represent a significant advance over the current use of traditional imaging techniques, which assess segmental arterial stenosis or vascular calcification, to guide interventional therapies. A separate review in this Compendium on Atherosclerosis series is devoted to this topic.

At the present time, considerable attention is focused on the application of high-sensitivity C-reactive protein (hsCRP) as a robust, clinical useful biomarker in the assessment of ACVD in individual patients and populations at risk[222]. C-reactive protein is one of a class of acute phase reactants elaborated by the liver in response to systemic inflammatory stimuli such as IL-6. In the context of atherogenesis, the proinflammatory activation of endothelial cells would be an important source of IL-6 generation. Significantly, in prospective epidemiological studies of initially healthy men and women, hsCRP has been shown to predict future risk for heart attack and stroke, independent of plasma LDL cholesterol associated risk[223, 224]. Multiple studies also have shown that statins reduce hsCRP in a manner largely independent of their action in reducing blood LDL cholesterol levels[225], thus suggesting that these widely prescribed agents are exerting their potent cardiovascular protective effects via both lipid-lowering and anti-inflammatory actions[226]. The demonstration that statins can act directly on endothelial cells to activate KLF2, resulting in a vasoprotective endothelial phenotype [Figure 4], provides an important mechanistic insight into the interrelationships of statin therapy, hsCRP measurements and ACVD risk. The ability of arterial endothelial cells, in a lesion-susceptible geometry, to resist multifactorial pathophysiologic stimuli of ECD [Table 1], through the coordinated expression of multiple atheroprotective genes, in effect reflects their reduced proinflammatory set-point. Thus, hsCRP might be viewed as a systemic index of this local pathophysiologic state. This

awareness has led to the development and validation of improved algorithms for the assessment of cardiovascular risk--The Reynolds Risk Scores for Women and Men[120, 121], which incorporate indices of systemic proinflammatory reactivity.

Taken together, these considerations provide cogent epidemiological support for the hypothesis that atherosclerosis is indeed a chronic inflammatory process. The critical testing of this hypothesis is the goal of two ongoing prospective clinical trials assessing different anti-inflammatory approaches to ACVD prevention—CIRT (Cardiovascular Inflammation Reduction Trial), which is evaluating low-dose methotrexate, and CANTOS (Canakinumab Anti-inflammatory Thrombosis Outcomes Study), which is evaluating IL-1β inhibition[122].

OPPORTUNITIES AND FUTURE DIRECTIONS

What does the future hold for our understanding of the role(s) of endothelial cell dysfunction in the pathogenesis of ACVD, and, importantly, its more effective detection, treatment and prevention in the clinical setting? At present, three areas of opportunity warrant consideration: first, understanding the various roles of endothelial metabolism in health and disease; second, characterizing the clinical consequences of genetic variations in atheroprotective genes; and third, targeting endothelial cell dysfunction via novel therapeutic strategies.

Endothelium is both the largest distributed organ in the human body (with an aggregate mass comparable to other vital organs such as the kidney), and an integral part, anatomically and functionally, of each of the body's composite tissues and organs[227]. As we have considered in detail in this review, endothelial cell dysfunction in response to specific pathophysiological stimuli (e.g., hypercholesterolemia and other dyslipidemias, diabetes, obesity, hypertension, aging) has important local manifestations within the walls of arteries in lesion-susceptible regions, but also can be detected in non-lesion regions of the vasculature[36]. A systemic context for ECD becomes especially relevant when considering the interrelationships of type 2 diabetes, insulin resistance, the Metabolic Syndrome and ACVD risk[228, 229]. One important mechanistic link appears to be the capacity of lipid-laden, dysfunctional adipocytes to generate a variety of proinflammatory "adipokines" (e.g., TNF), which can act locally within adipose tissue to activate macrophages and microvascular endothelium, and also systemically to stimulate NFκB-dependent pathways in other target tissues, including the NFκB-primed arterial endothelial cells in lesion-susceptible regions[14, 230, 231]. Within the endothelium, *per se*, the nuclear receptor PPARγ, an important transcriptional regulator of lipid metabolism, appears to play an central role in controlling metabolic and vascular responses to a high fat diet, as well as the pharmacologic effects of certain anti-diabetic agents[232]. Further, there is increasing evidence that a broad spectrum of basic metabolic pathways in endothelium are modulated by specific pathophysiologic stimuli associated with ECD[171, 233]. In contrast, relatively little effort has been given to applying state-of-the-art metabolomic approaches to characterize endothelial metabolism in the context of ACVD. Such studies offer much potential for enriching our understanding of the role endothelial metabolism in maintaining vascular homeostasis, and pointing the way to biomarkers useful for the diagnosis of early ECD in individuals at risk[234].

Currently, there is a growing awareness that Cardiovascular Medicine is poised to become more “personalized” and “precise” through the translation of genome-based discoveries into clinical practice[235]. Indeed, several of the reviews in this Compendium on Atherosclerosis series are related to this theme. These efforts are deeply rooted in the fundamental insights into ACVD pathogenesis that the study of familial hypercholesterolemias and other dyslipidemias have provided. Recent translational activities centered on the association of mutations in the gene encoding proprotein convertase subtilisin/kexin type 9 (PCSK9) with alterations in LDL receptor intracellular trafficking and hypercholesterolemia provide a vivid example of continued progress in this realm[221]. Given the state of our current understanding of the endothelial expression of atheroprotective genes and their critical roles in the generation of a vasoprotective phenotype [Figure 4], detailed analyses of the genetics of ECD in ACVD would appear to be a timely undertaking. The ACVD-association of specific alterations in the genes encoding key transcriptional regulators, such as KLF2, or major downstream effectors, of the vasoprotective endothelial phenotype, would not only further substantiate the Atheroprotective Gene Hypothesis, but would enable better definition of cardiovascular risk in a given patient or population.

Endothelial cell dysfunction appears to be a reversible process. However, to date, treatment of ECD has been focused largely on ameliorating known risk factors for ACVD rather than specifically targeting endothelial-based mechanisms[36]. Looking to the future, the development of pharmacomimetics of the natural, flow-mediated vasoprotective endothelial phenotype would appear to be a potentially fruitful strategy. One can envision such drugs acting on endothelial cells in atheroprone regions of the arterial vasculature to re-program their expression of a vasoprotective phenotype, thus offsetting the effects of systemic risk factors, such as hypercholesterolemia, and slowing the progression of atherosclerotic lesion development. Novel biomarkers of ECD, directly related to intrinsic, vessel wall processes, could serve as a guide in the application of these endothelial-targeted agents. As detailed above, the statins may indeed be exerting some of their well-recognized pleiotropic (i.e., non-lipid lower) beneficial effects via endothelial KLF2 activation[175, 176]. However, members of this class of HMG-CoA reductase inhibitors were not optimized around this endothelial-targeted action, thus highlighting the opportunity for the future development of selective therapies for endothelial cell dysfunction in atherogenesis [Figure 4].

In a monograph entitled “Endothelium: Its Development, Morphology, Function, and Pathology”, published in 1954 and totaling 155 pages, Dr. Rudolf Altschul, Professor of Histology at the University of Saskatchewan began his state-of-the-art review with the statement-- “While working on problems of arteriosclerosis, I have realized not only how little I knew about endothelium, but also how much I ought to know for the proper understanding of arteriosclerosis.” [236]. Several decades later, this insightful comment continues to motivate and illuminate our understanding of ACVD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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NON-STANDARD ABBREVIATIONS AND ACRONYMS

ACVD	Atherosclerotic Cardiovascular Disease
ApoE	Apolipoprotein E
Athero-ELAM	Atherosclerosis-associated Endothelial-Leukocyte Adhesion Molecule
CNP	C-type Natriuretic Peptide
DAMP	Damage-Associated Molecular Patterns
DNMT1	DNA Methyl Transferase-1
ECD	Endothelial Cell Dysfunction
EDRF	Endothelial-Derived Relaxing Factor
eNOS	endothelial Nitric Oxide Synthase
ELAM	Endothelial-Leukocyte Adhesion Molecule
ERK5	Extracellular signal-Regulated protein Kinase 5
hs-CRP	high-sensitivity C-Reactive Protein
ICAM-1	Intercellular Cell Adhesion Molecule-1
KLF2	Kruppel-Like Factor-2
KLF4	Kruppel-Like Factor-4
MEF2	Myocyte-specific Enhancer Binding Factor-2
MEK5	Mitogen/Extracellular signal-regulated Kinase 5
MEKK3	Mitogen-activated protein kinase/Extracellular-regulated kinase Kinase Kinase-3
miRNA	microRNA
Nrf2	Nuclear Factor Erythroid 2-related factor-2
PAMP	Pathogen-Associated Molecular Patterns
sELAM	soluble Endothelial-Leukocyte Adhesion Molecule
SSRE	Shear Stress Response Element
sVCAM-1	soluble Vascular Cell Adhesion Molecule-1
TIMP3	Tissue Inhibitor of Metalloproteinase-3

TNF	Tumor Necrosis Factor
VCAM-1	Vascular Cell Adhesion Molecule-1
VLA-4	Very Late Antigen-4

REFERENCES

1. Gimbrone MA Jr. Vascular endothelium: nature's blood-compatible container. Ann N Y Acad Sci. 1987; 516:5–11. [PubMed: 3439744]
2. Gimbrone MA, García-Cardeña G. Vascular endothelium, hemodynamics, and the pathobiology of atherosclerosis. Cardiovasc Pathol. 2013; 22:9–15. [PubMed: 22818581]
3. Stary HC. Natural history and histological classification of atherosclerotic lesions: an update. Arterioscler Thromb Vasc Biol. 2000; 20:1177–1178. [PubMed: 10807728]
4. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. Arterioscler Thromb Vasc Biol. 2000; 20:1262–1275. [PubMed: 10807742]
5. Simionescu N, Vasile E, Lupu F, Popescu G, Simionescu M. Prelesional events in atherogenesis. Accumulation of extracellular cholesterol-rich liposomes in the arterial intima and cardiac valves of the hyperlipidemic rabbit. The American journal of pathology. 1986; 123:109–125. [PubMed: 3963146]
6. Ross R, Glomset JA. Atherosclerosis and the arterial smooth muscle cell: Proliferation of smooth muscle is a key event in the genesis of the lesions of atherosclerosis. Science. 1973; 180:1332–1339. [PubMed: 4350926]
7. Ross R, Glomset JA. The pathogenesis of atherosclerosis (first of two parts). N Engl J Med. 1976; 295:369–377. [PubMed: 819830]
8. Ross R. George Lyman Duff Memorial Lecture. Atherosclerosis: a problem of the biology of arterial wall cells and their interactions with blood components. Arteriosclerosis. 1981; 1:293–311. [PubMed: 6287982]
9. Ross R. The pathogenesis of atherosclerosis--an update. N Engl J Med. 1986; 314:488–500. [PubMed: 3511384]
10. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature. 1993; 362:801–809. [PubMed: 8479518]
11. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med. 1999; 340:115–126. [PubMed: 9887164]
12. Tabas I, Garcia-Cardena G, Owens GK. Recent insights into the cellular biology of atherosclerosis. J Cell Biol. 2015; 209:13–22. [PubMed: 25869663]
13. Libby P. Inflammation in atherosclerosis. Nature. 2002; 420:868–874. [PubMed: 12490960]
14. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med. 2005; 352:1685–1695. [PubMed: 15843671]
15. Hansson GK, Libby P. The immune response in atherosclerosis: a double-edged sword. Nature reviews Immunology. 2006; 6:508–519.
16. Davies MJ. Stability and instability: two faces of coronary atherosclerosis. The Paul Dudley White Lecture 1995. Circulation. 1996; 94:2013–2020. [PubMed: 8873680]
17. Schwartz SM, Galis ZS, Rosenfeld ME, Falk E. Plaque rupture in humans and mice. Arterioscler Thromb Vasc Biol. 2007; 27:705–713. [PubMed: 17332493]
18. Fuster V, Moreno PR, Fayad ZA, Corti R, Badimon JJ. Atherothrombosis and high-risk plaque: part I: evolving concepts. J Am Coll Cardiol. 2005; 46:937–954. [PubMed: 16168274]
19. Libby P. Mechanisms of acute coronary syndromes. N Engl J Med. 2013; 369:883–884. [PubMed: 23984747]

20. Quillard T, Araujo HA, Franck G, Shvartz E, Sukhova G, Libby P. TLR2 and neutrophils potentiate endothelial stress, apoptosis and detachment: implications for superficial erosion. Eur Heart J. 2015; 36:1394–1404. [PubMed: 25755115]
21. GM, S. The Udana: The solemn utterances of the Buddha: Forgotten Books. 1902.
22. Krogh, A. The anatomy and physiology of capillaries. Rev. and enl. ed ed: New Haven : Yale university press. London: H. Milford, Oxford university press; 1929.
23. Palade GE. Fine structure of blood capillaries. JAppl Physiol. 1953; 24
24. Karnovsky MJ. The ultrastructural basis of capillary permeability studied with peroxidase as a tracer. J Cell Biol. 1967; 35:213–236. [PubMed: 6061717]
25. Majno, G. Ultrastructure of the vascular membrane. Washington DC: American Physiological Society; 1965. Section 2, Circulation
26. Cotran, R. The fine structure of the microvasculature in relation to normal and altered permeability. Philadelphia: WB Saunders Co; 1967.
27. Majno, G. The Healing Hand. Man and wound in the ancient world. Cambridge: University Press; 1975.
28. Fishman AP. Endothelium: a distributed organ of diverse capabilities. Ann N Y Acad Sci. 1982; 401:1–8. [PubMed: 6963119]
29. Gimbrone MA Jr. Culture of vascular endothelium. Progress in hemostasis and thrombosis. 1976; 3:1–28. [PubMed: 181786]
30. Gimbrone MA Jr. Vascular Endothelium in Health & Disease. Haber (ed) Molecular Cardiovascular Medicine. ed. 1994:49–62.
31. Gimbrone, MA, Jr. Vascular Endothelium in Hemostasis & Thrombosis. Edinburgh: Churchill Livingstone; 1986.
32. Davies PF. Flow-mediated endothelial mechanotransduction. Physiological reviews. 1995; 75:519–560. [PubMed: 7624393]
33. Chiu JJ, Chien S. Effects of disturbed flow on vascular endothelium: pathophysiological basis and clinical perspectives. Physiological reviews. 2011; 91:327–387. [PubMed: 21248169]
34. Harrison D, Griendling KK, Landmesser U, Hornig B, Drexler H. Role of oxidative stress in atherosclerosis. Am J Cardiol. 2003; 91:7a–11a.
35. Gimbrone, MA. Atherogenesis: Current Concepts. In: Schoen, FG., Jr, editor. Cardiovascular Pathology Clincopathologic Correlations and Pathogenic Mechanisms. Baltimore: William & Wilkens; 1995. p. 1-11.MA
36. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation. 2004; 109:III27–III32. [PubMed: 15198963]
37. De Caterina, R.; Libby, P., editors. Endothelial dysfunctions and vascular disease. Oxford: Blackwell Futura; 2007.
38. Virchow R. Der Ateromatose Prozess der Arterien. Wien Med Wochenschr. 1856:825–827.
39. Caplan BA, Gerrity RG, Schwartz CJ. Endothelial cell morphology in focal areas of in vivo Evans blue uptake in the young pig aorta I. Quantitative light microscopic findings. Exp Mol Pathol. 1974; 21:102–117. [PubMed: 4138848]
40. Caplan BA, Schwartz CJ. Increased endothelial cell turnover in areas of in vivo Evans Blue uptake in the pig aorta. Atherosclerosis. 1973; 17:401–417. [PubMed: 4123526]
41. Gerrity RG, Naito HK, Richardson M, Schwartz CJ. Dietary induced atherogenesis in swine. Morphology of the intima in prelesion stages. The American journal of pathology. 1979; 95:775–792. [PubMed: 453335]
42. Simionescu N, Mora R, Vasile E, Lupu F, Filip DA, Simionescu M. Prelesional modifications of the vessel wall in hyperlipidemic atherogenesis. Extracellular accumulation of modified and reassembled lipoproteins. Ann N Y Acad Sci. 1990; 598:1–16. [PubMed: 2248429]
43. Spaet TH, Stemerman MB, Veith FJ, Lejnieks I. Intimal injury and regrowth in the rabbit aorta; medial smooth muscle cells as a source of neointima. Circulation research. 1975; 36:58–70. [PubMed: 1116230]
44. Davies PF, Bowyer DE. Scanning electron microscopy: arterial endothelial integrity after fixation at physiological pressure. Atherosclerosis. 1975; 21:463–49. [PubMed: 1148038]

45. Silkworth JB, McLean B, Stehbens WE. The effect of hypercholesterolemia on aortic endothelium studied en face. Atherosclerosis. 1975; 22:335–348. [PubMed: 1201140]
46. Gimbrone, MAJ. Endothelial Dysfunction and the Pathogenesis of Atherosclerosis, Proceedings of the Fifth International Symposium. Springer-Verlag New York: Springer US; 1980.
47. Gimbrone, MAJ. Vascular Endothelium and atherosclerosis. In: IMs, editor. Vascular Injury and Atherosclerosis. New York: Marcel Dekker; 1981. p. 25-52.
48. Gimbrone MA Jr, Alexander RW. Angiotensin II stimulation of prostaglandin production in cultured human vascular endothelium. Science. 1975; 189:219–220. [PubMed: 1138377]
49. Weksler BB, Marcus AJ, Jaffe EA. Synthesis of prostaglandin I2 (prostacyclin) by cultured human and bovine endothelial cells. Proc Natl Acad Sci U S A. 1977; 74:3922–3926. [PubMed: 333448]
50. Moncada S, Herman AG, Higgs EA, Vane JR. Differential formation of prostacyclin (PGX or PGI2) by layers of the arterial wall. An explanation for the anti-thrombotic properties of vascular endothelium. Thrombosis research. 1977; 11:323–344. [PubMed: 335560]
51. Vane JR. The Croonian Lecture, 1993. The endothelium: maestro of the blood circulation. Philosophical transactions of the Royal Society of London Series B, Biological sciences. 1994; 343:225–246. [PubMed: 8146236]
52. Weitz, J. Hemostasis, thrombosis, fibrinolysis and cardiovascular disease. In: Mann, Z.; Libby; Bonow, editors. Braunwald's Heart Disease. Philadelphia: Elsevier Saunders; 2015. p. 1809-1833.
53. Libby P, Ordovas JM, Auger KR, Robbins AH, Birinyi LK, Dinarello CA. Endotoxin and tumor necrosis factor induce interleukin-1 gene expression in adult human vascular endothelial cells. The American journal of pathology. 1986; 124:179–185. [PubMed: 3526909]
54. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature. 1980; 288:373–376. [PubMed: 6253831]
55. Griffith TM, Edwards DH, Lewis MJ, Newby AC, Henderson AH. The nature of endothelium-derived vascular relaxant factor. Nature. 1984; 308:645–647. [PubMed: 6424031]
56. Rubanyi GM, Lorenz RR, Vanhoutte PM. Bioassay of endothelium-derived relaxing factor(s): inactivation by catecholamines. The American journal of physiology. 1985; 249:H95–H101. [PubMed: 3874557]
57. Martin W, Villani GM, Jothianandan D, Furchgott RF. Blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation of rabbit aorta by certain ferrous hemoproteins. The Journal of pharmacology and experimental therapeutics. 1985; 233:679–685. [PubMed: 2861277]
58. Rapoport RM, Murad F. Agonist-induced endothelium-dependent relaxation in rat thoracic aorta may be mediated through cGMP. Circulation research. 1983; 52:352–357. [PubMed: 6297832]
59. Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci U S A. 1987; 84:9265–9269. [PubMed: 2827174]
60. Ignarro LJ, Buga GM, Wei LH, Bauer PM, Wu G, del Soldato P. Role of the arginine-nitric oxide pathway in the regulation of vascular smooth muscle cell proliferation. Proc Natl Acad Sci U S A. 2001; 98:4202–4208. [PubMed: 11259671]
61. Hutchinson PJ, Palmer RM, Moncada S. Comparative pharmacology of EDRF and nitric oxide on vascular strips. European journal of pharmacology. 1987; 141:445–451. [PubMed: 3499329]
62. Radomski MW, Palmer RM, Moncada S. Comparative pharmacology of endothelium-derived relaxing factor, nitric oxide and prostacyclin in platelets. British journal of pharmacology. 1987; 92:181–187. [PubMed: 3311265]
63. Palmer RM, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature. 1988; 333:664–666. [PubMed: 3131684]
64. Sessa WC, Harrison JK, Barber CM, Zeng D, Durieux ME, D'Angelo DD, Lynch KR, Peach MJ. Molecular cloning and expression of a cDNA encoding endothelial cell nitric oxide synthase. J Biol Chem. 1992; 267:15274–15276. [PubMed: 1379225]
65. Nishida K, Harrison DG, Navas JP, Fisher AA, Dockery SP, Uematsu M, Nerem RM, Alexander RW, Murphy TJ. Molecular cloning and characterization of the constitutive bovine aortic endothelial cell nitric oxide synthase. J Clin Invest. 1992; 90:2092–2096. [PubMed: 1385480]
66. Sessa WC. eNOS at a glance. Journal of cell science. 2004; 117:2427–2429. [PubMed: 15159447]

67. Dudzinski DM, Michel T. Life history of eNOS: partners and pathways. Cardiovasc Res. 2007; 75:247–260. [PubMed: 17466957]
68. Kuchan MJ, Frangos JA. Role of calcium and calmodulin in flow-induced nitric oxide production in endothelial cells. The American journal of physiology. 1994; 266:C628–C636. [PubMed: 8166225]
69. Stamler JS, Simon DI, Osborne JA, Mullins ME, Jaraki O, Michel T, Singel DJ, Loscalzo J. S-nitrosylation of proteins with nitric oxide: synthesis and characterization of biologically active compounds. Proc Natl Acad Sci U S A. 1992; 89:444–448. [PubMed: 1346070]
70. Hess DT, Stamler JS. Regulation by S-nitrosylation of protein post-translational modification. J Biol Chem. 2012; 287:4411–4418. [PubMed: 22147701]
71. Dimmeler S, Haendeler J, Nehls M, Zeiher AM. Suppression of apoptosis by nitric oxide via inhibition of interleukin-1beta-converting enzyme (ICE)-like and cysteine protease protein (CPP)-32-like proteases. The Journal of experimental medicine. 1997; 185:601–607. [PubMed: 9034139]
72. Kang-Decker N, Cao S, Chatterjee S, Yao J, Egan LJ, Semela D, Mukhopadhyay D, Shah V. Nitric oxide promotes endothelial cell survival signaling through S-nitrosylation and activation of dynamin-2. Journal of cell science. 2007; 120:492–501. [PubMed: 17251380]
73. Matsushita K, Morrell CN, Cambien B, Yang SX, Yamakuchi M, Bao C, Hara MR, Quick RA, Cao W, O'Rourke B, Lowenstein JM, Pevsner J, Wagner DD, Lowenstein CJ. Nitric oxide regulates exocytosis by S-nitrosylation of N-ethylmaleimide-sensitive factor. Cell. 2003; 115:139–150. [PubMed: 14567912]
74. Xu L, Eu JP, Meissner G, Stamler JS. Activation of the cardiac calcium release channel (ryanodine receptor) by poly-S-nitrosylation. Science. 1998; 279:234–237. [PubMed: 9422697]
75. Stamler JS, Jia L, Eu JP, McMahon TJ, Demchenko IT, Bonaventura J, Gernert K, Piantadosi CA. Blood flow regulation by S-nitrosohemoglobin in the physiological oxygen gradient. Science. 1997; 276:2034–2037. [PubMed: 9197264]
76. Haldar SM, Stamler JS. S-nitrosylation: integrator of cardiovascular performance and oxygen delivery. J Clin Invest. 2013; 123:101–110. [PubMed: 23281416]
77. Hobbs AJ, Higgs A, Moncada S. Inhibition of nitric oxide synthase as a potential therapeutic target. Annual review of pharmacology and toxicology. 1999; 39:191–220.
78. Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, Fishman MC. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. Nature. 1995; 377:239–242. [PubMed: 7545787]
79. Shesely EG, Maeda N, Kim HS, Desai KM, Krege JH, Laubach VE, Sherman PA, Sessa WC, Smithies O. Elevated blood pressures in mice lacking endothelial nitric oxide synthase. Proc Natl Acad Sci U S A. 1996; 93:13176–13181. [PubMed: 8917564]
80. Kuhlencordt PJ, Rosel E, Gerszten RE, Morales-Ruiz M, Dombkowski D, Atkinson WJ, Han F, Preffer F, Rosenzweig A, Sessa WC, Gimbrone MA Jr, Ertl G, Huang PL. Role of endothelial nitric oxide synthase in endothelial activation: insights from eNOS knockout endothelial cells. American journal of physiology Cell physiology. 2004; 286:C1195–C1202. [PubMed: 15075219]
81. Huang PL. Lessons learned from nitric oxide synthase knockout animals. Seminars in perinatology. 2000; 24:87–90. [PubMed: 10709868]
82. Kuhlencordt PJ, Gyurko R, Han F, Scherrer-Crosbie M, Aretz TH, Hajjar R, Picard MH, Huang PL. Accelerated atherosclerosis, aortic aneurysm formation, and ischemic heart disease in apolipoprotein E/endothelial nitric oxide synthase double-knockout mice. Circulation. 2001; 104:448–454. [PubMed: 11468208]
83. Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, Ganz P. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med. 1986; 315:1046–1051. [PubMed: 3093861]
84. Freiman PC, Mitchell GG, Heistad DD, Armstrong ML, Harrison DG. Atherosclerosis impairs endothelium-dependent vascular relaxation to acetylcholine and thrombin in primates. Circulation research. 1986; 58:783–789. [PubMed: 3087655]
85. Bossaller C, Habib GB, Yamamoto H, Williams C, Wells S, Henry PD. Impaired muscarinic endothelium-dependent relaxation and cyclic guanosine 5'-monophosphate formation in

atherosclerotic human coronary artery and rabbit aorta. J Clin Invest. 1987; 79:170–174. [PubMed: 2432088]

86. d'Uscio LV, Smith LA, Katusic ZS. Hypercholesterolemia impairs endothelium-dependent relaxations in common carotid arteries of apolipoprotein e-deficient mice. Stroke. 2001; 32:2658–2664. [PubMed: 11692031]
87. Anderson TJ, Gerhard MD, Meredith IT, Charbonneau F, Delagrange D, Creager MA, Selwyn AP, Ganz P. Systemic nature of endothelial dysfunction in atherosclerosis. Am J Cardiol. 1995; 75:71B–74B. [PubMed: 7801869]
88. Pober JS, Cotran RS. The role of endothelial cells in inflammation. Transplantation. 1990; 50:537–544. [PubMed: 2219269]
89. Pober JS, Cotran RS. Cytokines and endothelial cell biology. Physiological reviews. 1990; 70:427–451. [PubMed: 1690903]
90. Pober JS, Sessa WC. Evolving functions of endothelial cells in inflammation. Nature reviews Immunology. 2007; 7:803–815.
91. Pober JS, Bevilacqua MP, Mendrick DL, Lapierre LA, Fiers W, Gimbrone MA Jr. Two distinct monokines, interleukin 1 and tumor necrosis factor, each independently induce biosynthesis and transient expression of the same antigen on the surface of cultured human vascular endothelial cells. Journal of immunology. 1986; 136:1680–1687.
92. Pober JS, Gimbrone MA Jr. Expression of Ia-like antigens by human vascular endothelial cells is inducible in vitro: demonstration by monoclonal antibody binding and immunoprecipitation. Proc Natl Acad Sci U S A. 1982; 79:6641–6645. [PubMed: 6815654]
93. Bevilacqua MP, Pober JS, Mendrick DL, Cotran RS, Gimbrone MA Jr. Identification of an inducible endothelial-leukocyte adhesion molecule. Proc Natl Acad Sci U S A. 1987; 84:9238–9242. [PubMed: 2827173]
94. Gimbrone MA Jr, Obin MS, Brock AF, Luis EA, Hass PE, Hebert CA, Yip YK, Leung DW, Lowe DG, Kohr WJ, et al. Endothelial interleukin-8: a novel inhibitor of leukocyte-endothelial interactions. Science. 1989; 246:1601–1603. [PubMed: 2688092]
95. Rollins BJ, Yoshimura T, Leonard EJ, Pober JS. Cytokine-activated human endothelial cells synthesize and secrete a monocyte chemoattractant, MCP-1/JE. The American journal of pathology. 1990; 136:1229–1233. [PubMed: 2113354]
96. Cotran RS, Gimbrone MA Jr, Bevilacqua MP, Mendrick DL, Pober JS. Induction and detection of a human endothelial activation antigen in vivo. The Journal of experimental medicine. 1986; 164:661–666. [PubMed: 3723080]
97. Munro JM, Pober JS, Cotran RS. Tumor necrosis factor and interferon-gamma induce distinct patterns of endothelial activation and associated leukocyte accumulation in skin of Papio anubis. The American journal of pathology. 1989; 135:121–133. [PubMed: 2505619]
98. Cybulsky MI, Gimbrone MA. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science. 1991; 251:788–791. [PubMed: 1990440]
99. Osborn L, Hession C, Tizard R, Vassallo C, Luhowskyj S, Chi-Rosso G, Lobb R. Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. Cell. 1989; 59:1203–1211. [PubMed: 2688898]
100. Rice GE, Munro JM, Bevilacqua MP. Inducible cell adhesion molecule 110 (INCAM-110) is an endothelial receptor for lymphocytes. A CD11/CD18-independent adhesion mechanism. The Journal of experimental medicine. 1990; 171:1369–1374. [PubMed: 1691264]
101. Elices MJ, Osborn L, Takada Y, Crouse C, Luhowskyj S, Hemler ME, Lobb RR. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. Cell. 1990; 60:577–584. [PubMed: 1689216]
102. O'Brien KD, Allen MD, McDonald TO, Chait A, Harlan JM, Fishbein D, McCarty J, Ferguson M, Hudkins K, Benjamin CD, et al. Vascular cell adhesion molecule-1 is expressed in human coronary atherosclerotic plaques. Implications for the mode of progression of advanced coronary atherosclerosis. J Clin Invest. 1993; 92:945–951. [PubMed: 7688768]
103. Iiyama K, Hajra L, Iiyama M, Li H, DiChiara M, Medoff BD, Cybulsky MI. Patterns of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 expression in rabbit and mouse

atherosclerotic lesions and at sites predisposed to lesion formation. Circulation research. 1999; 85:199–207. [PubMed: 10417402]

104. Nakashima Y, Raines EW, Plump AS, Breslow JL, Ross R. Upregulation of VCAM-1 and ICAM-1 at atherosclerosis-prone sites on the endothelium in the ApoE-deficient mouse. Arterioscler Thromb Vasc Biol. 1998; 18:842–851. [PubMed: 9598845]
105. Li H, Cybulsky MI, Gimbrone MA Jr, Libby P. An atherogenic diet rapidly induces VCAM-1, a cytokine-regulatable mononuclear leukocyte adhesion molecule, in rabbit aortic endothelium. Arteriosclerosis and thrombosis : a journal of vascular biology / American Heart Association. 1993; 13:197–204. [PubMed: 7678986]
106. Kume N, Cybulsky MI, Gimbrone MA Jr. Lysophosphatidylcholine, a component of atherogenic lipoproteins, induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells. J Clin Invest. 1992; 90:1138–1144. [PubMed: 1381720]
107. De Caterina R, Basta G, Lazzerini G, Dell'Omo G, Petrucci R, Morale M, Carmassi F, Pedrinelli R. Soluble vascular cell adhesion molecule-1 as a biohumoral correlate of atherosclerosis. Arterioscler Thromb Vasc Biol. 1997; 17:2646–2654. [PubMed: 9409238]
108. Cybulsky MI, Iiyama K, Li H, Zhu S, Chen M, Iiyama M, Davis V, Gutierrez-Ramos JC, Connelly PW, Milstone DS. A major role for VCAM-1, but not ICAM-1, in early atherosclerosis. J Clin Invest. 2001; 107:1255–1262. [PubMed: 11375415]
109. Hansson GK. Immune mechanisms in atherosclerosis. Arterioscler Thromb Vasc Biol. 2001; 21:1876–1890. [PubMed: 11742859]
110. Steinberg D. The LDL modification hypothesis of atherogenesis: an update. Journal of lipid research. 2009; 50(Suppl):S376–S381. [PubMed: 19011257]
111. Steinberg D, Witztum JL. Oxidized low-density lipoprotein and atherosclerosis. Arterioscler Thromb Vasc Biol. 2010; 30:2311–2316. [PubMed: 21084697]
112. Eid RE, Rao DA, Zhou J, Lo SF, Ranjbaran H, Gallo A, Sokol SI, Pfau S, Pober JS, Tellides G. Interleukin-17 and interferon-gamma are produced concomitantly by human coronary artery-infiltrating T cells and act synergistically on vascular smooth muscle cells. Circulation. 2009; 119:1424–1432. [PubMed: 19255340]
113. Hasturk H, Abdallah R, Kantarci A, Nguyen D, Giordano N, Hamilton J, Van Dyke TE. Resolvin E1 (RvE1) Attenuates Atherosclerotic Plaque Formation in Diet and Inflammation-Induced Atherogenesis. Arterioscler Thromb Vasc Biol. 2015; 35:1123–1133. [PubMed: 25792445]
114. Libby P, Ridker P. Inflammation and atherothrombosis: from population biology and bench research to clinical practice. J Am Coll Cardiol. 2006:A33–A46.
115. Collins T, Cybulsky MI. NF-kappaB: pivotal mediator or innocent bystander in atherogenesis? J Clin Invest. 2001; 107:255–264. [PubMed: 11160146]
116. Resnick N, Collins T, Atkinson W, Bonthron DT, Dewey CF Jr, Gimbrone MA Jr. Platelet-derived growth factor B chain promoter contains a cis-acting fluid shear-stress-responsive element. Proc Natl Acad Sci U S A. 1993; 90:4591–4595. [PubMed: 8506304]
117. Hajra L, Evans AI, Chen M, Hyduk SJ, Collins T, Cybulsky MI. The NF-kappa B signal transduction pathway in aortic endothelial cells is primed for activation in regions predisposed to atherosclerotic lesion formation. Proc Natl Acad Sci U S A. 2000; 97:9052–9057. [PubMed: 10922059]
118. Brown JD, Lin CY, Duan Q, Griffin G, Federation AJ, Paranal RM, Bair S, Newton G, Lichtman AH, Kung AL, Yang T, Wang H, Luscinskas FW, Croce KJ, Bradner JE, Plutzky J. NF-kappaB directs dynamic super enhancer formation in inflammation and atherogenesis. Molecular cell. 2014; 56:219–231. [PubMed: 25263595]
119. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation. 1998; 97:1837–1847. [PubMed: 9603539]
120. Ridker PM, Buring JE, Rifai N, Cook NR. Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds Risk Score. JAMA. 2007; 297:611–619. [PubMed: 17299196]

121. Ridker PM, Paynter NP, Rifai N, Gaziano JM, Cook NR. C-reactive protein and parental history improve global cardiovascular risk prediction: the Reynolds Risk Score for men. Circulation. 2008; 118:2243–2251. 4p following 2251. [PubMed: 18997194]
122. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature. 2011; 473:317–325. [PubMed: 21593864]
123. Cornhill JF, Roach MR. A quantitative study of the localization of atherosclerotic lesions in the rabbit aorta. Atherosclerosis. 1976; 23:489–501. [PubMed: 57782]
124. Glagov S, Zarins C, Giddens DP, Ku DN. Hemodynamics and atherosclerosis. Insights and perspectives gained from studies of human arteries. Archives of pathology & laboratory medicine. 1988; 112:1018–1031. [PubMed: 3052352]
125. Giddens DP, Zarins CK, Glagov S. The role of fluid mechanics in the localization and detection of atherosclerosis. Journal of biomechanical engineering. 1993; 115:588–594. [PubMed: 8302046]
126. Caro CG, Fitz-Gerald JM, Schroter RC. Atheroma arterial wall shear. Observation, correlation and proposal of a shear dependent mass transfer mechanism for atherogenesis. Proceedings of the Royal Society of London Series B, Biological sciences. 1971; 177:109–159.
127. Stehbens WE. The role of hemodynamics in the pathogenesis of atherosclerosis. Progress in cardiovascular diseases. 1975; 18:89–103. [PubMed: 1098111]
128. Bussolari SR, Dewey CF Jr, Gimbrone MA Jr. Apparatus for subjecting living cells to fluid shear stress. The Review of scientific instruments. 1982; 53:1851–1854. [PubMed: 7156852]
129. Dewey CF Jr, Bussolari SR, Gimbrone MA Jr, Davies PF. The dynamic response of vascular endothelial cells to fluid shear stress. Journal of biomechanical engineering. 1981; 103:177–185. [PubMed: 7278196]
130. Blackman BR, Garcia-Cardena G, Gimbrone MA Jr. A new in vitro model to evaluate differential responses of endothelial cells to simulated arterial shear stress waveforms. Journal of biomechanical engineering. 2002; 124:397–407. [PubMed: 12188206]
131. Frangos JA, Eskin SG, McIntire LV, Ives CL. Flow effects on prostacyclin production by cultured human endothelial cells. Science. 1985; 227:1477–1479. [PubMed: 3883488]
132. Remuzzi A, Dewey CF Jr, Davies PF, Gimbrone MA Jr. Orientation of endothelial cells in shear fields in vitro. Biorheology. 1984; 21:617–630. [PubMed: 6487771]
133. Levesque MJ, Nerem RM. The elongation and orientation of cultured endothelial cells in response to shear stress. Journal of biomechanical engineering. 1985; 107:341–347. [PubMed: 4079361]
134. Zand T, Nunnari JJ, Hoffman AH, Savilonis BJ, MacWilliams B, Majno G, Joris I. Endothelial adaptations in aortic stenosis. Correlation with flow parameters. The American journal of pathology. 1988; 133:407–418. [PubMed: 3189514]
135. Koo A, Dewey CF Jr, Garcia-Cardena G. Hemodynamic shear stress characteristic of atherosclerosis-resistant regions promotes glycocalyx formation in cultured endothelial cells. American journal of physiology Cell physiology. 2013; 304:C137–C146. [PubMed: 23114962]
136. Wong AJ, Pollard TD, Herman IM. Actin filament stress fibers in vascular endothelial cells in vivo. Science. 1983; 219:867–869. [PubMed: 6681677]
137. Weinbaum S, Tarbell JM, Damiano ER. The structure and function of the endothelial glycocalyx layer. Annual review of biomedical engineering. 2007; 9:121–167.
138. Warboys CM, de Luca A, Amini N, Luong L, Duckles H, Hsiao S, White A, Biswas S, Khamis R, Chong CK, Cheung WM, Sherwin SJ, Bennett MR, Gil J, Mason JC, Haskard DO, Evans PC. Disturbed flow promotes endothelial senescence via a p53-dependent pathway. Arterioscler Thromb Vasc Biol. 2014; 34:985–995. [PubMed: 24651677]
139. Davies PF, Remuzzi A, Gordon EJ, Dewey CF Jr, Gimbrone MA Jr. Turbulent fluid shear stress induces vascular endothelial cell turnover in vitro. Proc Natl Acad Sci U S A. 1986; 83:2114–2117. [PubMed: 3457378]
140. Gabriels JE, Paul DL. Connexin43 is highly localized to sites of disturbed flow in rat aortic endothelium but connexin37 and connexin40 are more uniformly distributed. Circulation research. 1998; 83:636–643. [PubMed: 9742059]

141. DePaola N, Davies PF, Pritchard WF Jr, Florez L, Harbeck N, Polacek DC. Spatial and temporal regulation of gap junction connexin43 in vascular endothelial cells exposed to controlled disturbed flows in vitro. Proc Natl Acad Sci U S A. 1999; 96:3154–3159. [PubMed: 10077653]
142. DePaola N, Gimbrone MA Jr, Davies PF, Dewey CF Jr. Vascular endothelium responds to fluid shear stress gradients. Arteriosclerosis and thrombosis : a journal of vascular biology / American Heart Association. 1992; 12:1254–1257. [PubMed: 1420084]
143. Wang C, Baker BM, Chen CS, Schwartz MA. Endothelial cell sensing of flow direction. Arterioscler Thromb Vasc Biol. 2013; 33:2130–2136. [PubMed: 23814115]
144. Gimbrone MA Jr, Cybulsky MI, Kume N, Collins T, Resnick N. Vascular endothelium. An integrator of pathophysiological stimuli in atherogenesis. Ann N Y Acad Sci. 1995; 748:122–131. discussion 131-2. [PubMed: 7535025]
145. Diamond SL, Sharefkin JB, Dieffenbach C, Frasier-Scott K, McIntire LV, Eskin SG. Tissue plasminogen activator messenger RNA levels increase in cultured human endothelial cells exposed to laminar shear stress. Journal of cellular physiology. 1990; 143:364–371. [PubMed: 2110169]
146. Takada Y, Shinkai F, Kondo S, Yamamoto S, Tsuboi H, Korenaga R, Ando J. Fluid shear stress increases the expression of thrombomodulin by cultured human endothelial cells. Biochem Biophys Res Commun. 1994; 205:1345–1352. [PubMed: 7802668]
147. Shyy YJ, Hsieh HJ, Usami S, Chien S. Fluid shear stress induces a biphasic response of human monocyte chemotactic protein 1 gene expression in vascular endothelium. Proc Natl Acad Sci U S A. 1994; 91:4678–4682. [PubMed: 8197118]
148. Ohno M, Cooke JP, Dzau VJ, Gibbons GH. Fluid shear stress induces endothelial transforming growth factor beta-1 transcription and production. Modulation by potassium channel blockade. J Clin Invest. 1995; 95:1363–1369. [PubMed: 7883983]
149. Lin MC, Almus-Jacobs F, Chen HH, Parry GC, Mackman N, Shyy JY, Chien S. Shear stress induction of the tissue factor gene. J Clin Invest. 1997; 99:737–744. [PubMed: 9045878]
150. Gimbrone MA Jr, Nagel T, Topper JN. Biomechanical activation: an emerging paradigm in endothelial adhesion biology. J Clin Invest. 1997; 99:1809–1813. [PubMed: 9109423]
151. Davis ME, Grumbach IM, Fukai T, Cutchins A, Harrison DG. Shear stress regulates endothelial nitric-oxide synthase promoter activity through nuclear factor kappaB binding. J Biol Chem. 2004; 279:163–168. [PubMed: 14570928]
152. Korenaga R, Ando J, Kosaki K, Isshiki M, Takada Y, Kamiya A. Negative transcriptional regulation of the VCAM-1 gene by fluid shear stress in murine endothelial cells. The American journal of physiology. 1997; 273:C1506–C1515. [PubMed: 9374635]
153. Topper JN, Cai J, Falb D, Gimbrone MA Jr. Identification of vascular endothelial genes differentially responsive to fluid mechanical stimuli: cyclooxygenase-2, manganese superoxide dismutase, and endothelial cell nitric oxide synthase are selectively up-regulated by steady laminar shear stress. Proc Natl Acad Sci U S A. 1996; 93:10417–10422. [PubMed: 8816815]
154. Williams KJ, Tabas I. The response-to-retention hypothesis of early atherogenesis. Arterioscler Thromb Vasc Biol. 1995; 15:551–561. [PubMed: 7749869]
155. Topper JN, Gimbrone MA. Blood flow and vascular gene expression: fluid shear stress as a modulator of endothelial phenotype. Mol Med Today. 1999; 5:40–46. [PubMed: 10088131]
156. Traub O, Berk BC. Laminar shear stress: mechanisms by which endothelial cells transduce an atheroprotective force. Arterioscler Thromb Vasc Biol. 1998; 18:677–685. [PubMed: 9598824]
157. Malek AM, Alper SL, Izumo S. Hemodynamic shear stress and its role in atherosclerosis. Jama. 1999; 282:2035–2042. [PubMed: 10591386]
158. Garcia-Cardena G, Comander J, Anderson KR, Blackman BR, Gimbrone MA Jr. Biomechanical activation of vascular endothelium as a determinant of its functional phenotype. Proc Natl Acad Sci U S A. 2001; 98:4478–4485. [PubMed: 11296290]
159. McCormick SM, Eskin SG, McIntire LV, Teng CL, Lu CM, Russell CG, Chittur KK. DNA microarray reveals changes in gene expression of shear stressed human umbilical vein endothelial cells. Proc Natl Acad Sci U S A. 2001; 98:8955–8960. [PubMed: 11481467]

160. Chen BP, Li YS, Zhao Y, Chen KD, Li S, Lao J, Yuan S, Shyy JY, Chien S. DNA microarray analysis of gene expression in endothelial cells in response to 24-h shear stress. Physiol Genomics. 2001; 7:55–63. [PubMed: 11595792]

161. Dai G, Kaazempur-Mofrad MR, Natarajan S, Zhang Y, Vaughn S, Blackman BR, Kamm RD, Garcia-Cardena G, Gimbrone MA Jr. Distinct endothelial phenotypes evoked by arterial waveforms derived from atherosclerosis-susceptible and -resistant regions of human vasculature. Proc Natl Acad Sci U S A. 2004; 101:14871–14876. [PubMed: 15466704]

162. Passerini AG, Polacek DC, Shi C, Francesco NM, Manduchi E, Grant GR, Pritchard WF, Powell S, Chang GY, Stoeckert CJ Jr, Davies PF. Coexisting proinflammatory and antioxidative endothelial transcription profiles in a disturbed flow region of the adult porcine aorta. Proc Natl Acad Sci U S A. 2004; 101:2482–2487. [PubMed: 14983035]

163. Davies PF. Endothelial transcriptome profiles in vivo in complex arterial flow fields. Annals of biomedical engineering. 2008; 36:563–570. [PubMed: 17978875]

164. Davies PF, Civelek M, Fang Y, Fleming I. The atherosusceptible endothelium: endothelial phenotypes in complex haemodynamic shear stress regions in vivo. Cardiovasc Res. 2013; 99:315–327. [PubMed: 23619421]

165. Dekker RJ, van Soest S, Fontijn RD, Salamanca S, de Groot PG, VanBavel E, Pannekoek H, Horrevoets AJ. Prolonged fluid shear stress induces a distinct set of endothelial cell genes, most specifically lung Kruppel-like factor (KLF2). Blood. 2002; 100:1689–1698. [PubMed: 12176889]

166. Parmar KM, Larman HB, Dai G, Zhang Y, Wang ET, Moorthy SN, Kratz JR, Lin Z, Jain MK, Gimbrone MA, García-Cardeña G. Integration of flow-dependent endothelial phenotypes by Kruppel-like factor 2. J Clin Invest. 2006; 116:49–58. [PubMed: 16341264]

167. Dekker RJ, van Thienen JV, Rohlena J, de Jager SC, Elderkamp YW, Seppen J, de Vries CJ, Biessen EA, van Berkel TJ, Pannekoek H, Horrevoets AJ. Endothelial KLF2 links local arterial shear stress levels to the expression of vascular tone-regulating genes. The American journal of pathology. 2005; 167:609–618. [PubMed: 16049344]

168. Dekker RJ, Boon RA, Rondaij MG, Kragt A, Volger OL, Elderkamp YW, Meijers JC, Voorberg J, Pannekoek H, Horrevoets AJ. KLF2 provokes a gene expression pattern that establishes functional quiescent differentiation of the endothelium. Blood. 2006; 107:4354–4363. [PubMed: 16455954]

169. Atkins GB, Simon DI. Interplay between NF-kappaB and Kruppel-like factors in vascular inflammation and atherosclerosis: location, location, location. Journal of the American Heart Association. 2013; 2:e000290. [PubMed: 23757395]

170. Lin Z, Natesan V, Shi H, Dong F, Kawanami D, Mahabeleshwar GH, Atkins GB, Nayak L, Cui Y, Finigan JH, Jain MK. Kruppel-like factor 2 regulates endothelial barrier function. Arterioscler Thromb Vasc Biol. 2010; 30:1952–1959. [PubMed: 20651277]

171. Doddaballapur A, Michalik KM, Manavski Y, Lucas T, Houtkooper RH, You X, Chen W, Zeiher AM, Potente M, Dimmeler S, Boon RA. Laminar shear stress inhibits endothelial cell metabolism via KLF2-mediated repression of PFKFB3. Arterioscler Thromb Vasc Biol. 2015; 35:137–145. [PubMed: 25359860]

172. Hergenreider E, Heydt S, Treguer K, Boettger T, Horrevoets AJ, Zeiher AM, Scheffer MP, Frangakis AS, Yin X, Mayr M, Braun T, Urbich C, Boon RA, Dimmeler S. Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. Nature cell biology. 2012; 14:249–256. [PubMed: 22327366]

173. Moyes AJ, Khambata RS, Villar I, Bubb KJ, Baliga RS, Lumsden NG, Xiao F, Gane PJ, Rebstock AS, Worthington RJ, Simone MI, Mota F, Rivilla F, Vallejo S, Peiro C, Sanchez Ferrer CF, Djordjevic S, Caulfield MJ, MacAllister RJ, Selwood DL, Ahluwalia A, Hobbs AJ. Endothelial C-type natriuretic peptide maintains vascular homeostasis. J Clin Invest. 2014; 124:4039–4051. [PubMed: 25105365]

174. Atkins GB, Wang Y, Mahabeleshwar GH, Shi H, Gao H, Kawanami D, Natesan V, Lin Z, Simon DI, Jain MK. Hemizygous deficiency of Kruppel-like factor 2 augments experimental atherosclerosis. Circulation research. 2008; 103:690–693. [PubMed: 18757824]

175. Parmar KM, Nambudiri V, Dai G, Larman HB, Gimbrone MA Jr, Garcia-Cardena G. Statins exert endothelial atheroprotective effects via the KLF2 transcription factor. J Biol Chem. 2005; 280:26714–26719. [PubMed: 15878865]

176. Sen-Banerjee S, Mir S, Lin Z, Hamik A, Atkins GB, Das H, Banerjee P, Kumar A, Jain MK. Kruppel-like factor 2 as a novel mediator of statin effects in endothelial cells. Circulation. 2005; 112:720–726. [PubMed: 16043642]

177. Chu UB, Duellman T, Weaver SJ, Tao Y, Yang J. Endothelial protective genes induced by statin are mimicked by ERK5 activation as triggered by a drug combination of FTI-277 and GGTI-298. Biochimica et biophysica acta. 2015; 1850:1415–1425. [PubMed: 25829196]

178. Liao JK, Laufs U. Pleiotropic effects of statins. Annual review of pharmacology and toxicology. 2005; 45:89–118.

179. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med. 2008; 359:2195–2207. [PubMed: 18997196]

180. Abe J, Berk BC. Novel mechanisms of endothelial mechanotransduction. Arterioscler Thromb Vasc Biol. 2014; 34:2378–2386. [PubMed: 25301843]

181. Villarreal G Jr, Zhang Y, Larman HB, Gracia-Sancho J, Koo A, Garcia-Cardena G. Defining the regulation of KLF4 expression and its downstream transcriptional targets in vascular endothelial cells. Biochem Biophys Res Commun. 2010; 391:984–989. [PubMed: 19968965]

182. Le NT, Takei Y, Izawa-Ishizawa Y, Heo KS, Lee H, Smrcka AV, Miller BL, Ko KA, Ture S, Morrell C, Fujiwara K, Akaike M, Abe J. Identification of activators of ERK5 transcriptional activity by high-throughput screening and the role of endothelial ERK5 in vasoprotective effects induced by statins and antimalarial agents. Journal of immunology. 2014; 193:3803–3815.

183. Ohnesorge N, Viemann D, Schmidt N, Czymai T, Spiering D, Schmolke M, Ludwig S, Roth J, Goebeler M, Schmidt M. Erk5 activation elicits a vasoprotective endothelial phenotype via induction of Kruppel-like factor 4 (KLF4). J Biol Chem. 2010; 285:26199–26210. [PubMed: 20551324]

184. Zhou G, Hamik A, Nayak L, Tian H, Shi H, Lu Y, Sharma N, Liao X, Hale A, Boerboom L, Feaver RE, Gao H, Desai A, Schmaier A, Gerson SL, Wang Y, Atkins GB, Blackman BR, Simon DI, Jain MK. Endothelial Kruppel-like factor 4 protects against atherothrombosis in mice. J Clin Invest. 2012; 122:4727–4731. [PubMed: 23160196]

185. Dai G, Vaughn S, Zhang Y, Wang ET, Garcia-Cardena G, Gimbrone MA Jr. Biomechanical forces in atherosclerosis-resistant vascular regions regulate endothelial redox balance via phosphoinositol 3-kinase/Akt-dependent activation of Nrf2. Circulation research. 2007; 101:723–733. [PubMed: 17673673]

186. Hsieh CY, Hsiao HY, Wu WY, Liu CA, Tsai YC, Chao YJ, Wang DL, Hsieh HJ. Regulation of shear-induced nuclear translocation of the Nrf2 transcription factor in endothelial cells. Journal of biomedical science. 2009; 16:12. [PubMed: 19272177]

187. Blagovic K, Kim LY, Voldman J. Microfluidic perfusion for regulating diffusible signaling in stem cells. PloS one. 2011; 6:e22892. [PubMed: 21829665]

188. Zakkar M, Van der Heiden K, Luong le A, Chaudhury H, Cuhlmann S, Hamdulay SS, Krams R, Edirisinghe I, Rahman I, Carlsen H, Haskard DO, Mason JC, Evans PC. Activation of Nrf2 in endothelial cells protects arteries from exhibiting a proinflammatory state. Arterioscler Thromb Vasc Biol. 2009; 29:1851–1857. [PubMed: 19729611]

189. Fledderus JO, Boon RA, Volger OL, Hurttila H, Yla-Herttuala S, Pannekoek H, Levonen AL, Horrevoets AJ. KLF2 primes the antioxidant transcription factor Nrf2 for activation in endothelial cells. Arterioscler Thromb Vasc Biol. 2008; 28:1339–1346. [PubMed: 18467642]

190. Boon RA, Horrevoets AJ. Key transcriptional regulators of the vasoprotective effects of shear stress. Hamostaseologie. 2009; 29:39–40. 41-3. [PubMed: 19151844]

191. Schober A, Nazari-Jahantigh M, Weber C. MicroRNA-mediated mechanisms of the cellular stress response in atherosclerosis. Nature reviews Cardiology. 2015; 12:361–374. [PubMed: 25855604]

192. Yan MS, Marsden PA. Epigenetics in the Vascular Endothelium: Looking From a Different Perspective in the Epigenomics Era. Arterioscler Thromb Vasc Biol. 2015; 35:2297–2306. [PubMed: 26404488]

193. Fang Y, Shi C, Manduchi E, Civelek M, Davies PF. MicroRNA-10a regulation of proinflammatory phenotype in athero-susceptible endothelium in vivo and in vitro. Proc Natl Acad Sci U S A. 2010; 107:13450–13455. [PubMed: 20624982]

194. Qin X, Wang X, Wang Y, Tang Z, Cui Q, Xi J, Li YS, Chien S, Wang N. MicroRNA-19a mediates the suppressive effect of laminar flow on cyclin D1 expression in human umbilical vein endothelial cells. Proc Natl Acad Sci U S A. 2010; 107:3240–3244. [PubMed: 20133739]

195. Wang KC, Nguyen P, Weiss A, Yeh YT, Chien HS, Lee A, Teng D, Subramaniam S, Li YS, Chien S. MicroRNA-23b regulates cyclin-dependent kinase-activating kinase complex through cyclin H repression to modulate endothelial transcription and growth under flow. Arterioscler Thromb Vasc Biol. 2014; 34:1437–1445. [PubMed: 24855060]

196. Chen K, Fan W, Wang X, Ke X, Wu G, Hu C. MicroRNA-101 mediates the suppressive effect of laminar shear stress on mTOR expression in vascular endothelial cells. Biochem Biophys Res Commun. 2012; 427:138–142. [PubMed: 22989749]

197. Wu W, Xiao H, Laguna-Fernandez A, Villarreal G Jr, Wang KC, Geary GG, Zhang Y, Wang WC, Huang HD, Zhou J, Li YS, Chien S, Garcia-Cardena G, Shyy JY. Flow-Dependent Regulation of Kruppel-Like Factor 2 Is Mediated by MicroRNA-92a. Circulation. 2011; 124:633–641. [PubMed: 21768538]

198. Ni CW, Qiu H, Jo H. MicroRNA-663 upregulated by oscillatory shear stress plays a role in inflammatory response of endothelial cells. American journal of physiology Heart and circulatory physiology. 2011; 300:H1762–H1769. [PubMed: 21378144]

199. Son DJ, Kumar S, Takabe W, Kim CW, Ni CW, Alberts-Grill N, Jang IH, Kim S, Kim W, Won Kang S, Baker AH, Woong Seo J, Ferrara KW, Jo H. The atypical mechanosensitive microRNA-712 derived from pre-ribosomal RNA induces endothelial inflammation and atherosclerosis. Nature communications. 2013; 4:3000.

200. Fan W, Fang R, Wu X, Liu J, Feng M, Dai G, Chen G, Wu G. Shear-sensitive microRNA-34a modulates flow-dependent regulation of endothelial inflammation. Journal of cell science. 2015; 128:70–80. [PubMed: 25395581]

201. Fang Y, Davies PF. Site-specific microRNA-92a regulation of Kruppel-like factors 4 and 2 in atherosusceptible endothelium. Arterioscler Thromb Vasc Biol. 2012; 32:979–987. [PubMed: 22267480]

202. Loyer X, Potteaux S, Vion AC, Guerin CL, Boulkroun S, Rautou PE, Ramkhelawon B, Esposito B, Dalloz M, Paul JL, Julia P, Maccario J, Boulanger CM, Mallat Z, Tedgui A. Inhibition of microRNA-92a prevents endothelial dysfunction and atherosclerosis in mice. Circulation research. 2014; 114:434–443. [PubMed: 24255059]

203. Dunn J, Qiu H, Kim S, Jjingo D, Hoffman R, Kim CW, Jang I, Son DJ, Kim D, Pan C, Fan Y, Jordan IK, Jo H. Flow-dependent epigenetic DNA methylation regulates endothelial gene expression and atherosclerosis. J Clin Invest. 2014; 124:3187–3199. [PubMed: 24865430]

204. Zhou J, Li YS, Wang KC, Chien S. Epigenetic Mechanism in Regulation of Endothelial Function by Disturbed Flow: Induction of DNA Hypermethylation by DNMT1. Cellular and molecular bioengineering. 2014; 7:218–224. [PubMed: 24883126]

205. Jiang YZ, Jimenez JM, Ou K, McCormick ME, Zhang LD, Davies PF. Hemodynamic disturbed flow induces differential DNA methylation of endothelial Kruppel-Like Factor 4 promoter in vitro and in vivo. Circulation research. 2014; 115:32–43. [PubMed: 24755985]

206. Taylor CA, Hughes TJ, Zarins CK. Effect of exercise on hemodynamic conditions in the abdominal aorta. Journal of vascular surgery. 1999; 29:1077–1089. [PubMed: 10359942]

207. Sessa WC, Pritchard K, Seyedi N, Wang J, Hintze TH. Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial cell nitric oxide synthase gene expression. Circulation research. 1994; 74:349–353. [PubMed: 7507417]

208. Seals DR, Desouza CA, Donato AJ, Tanaka H. Habitual exercise and arterial aging. J Appl Physiol (1985). 2008; 105:1323–1332. [PubMed: 18583377]

209. Chatzizisis YS, Coskun AU, Jonas M, Edelman ER, Feldman CL, Stone PH. Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: molecular, cellular, and vascular behavior. J Am Coll Cardiol. 2007; 49:2379–2393. [PubMed: 17599600]

210. Stone PH, Coskun AU, Kinlay S, Clark ME, Sonka M, Wahle A, Ilegbusi OJ, Yeghiazarians Y, Popma JJ, Orav J, Kuntz RE, Feldman CL. Effect of endothelial shear stress on the progression of coronary artery disease, vascular remodeling, and in-stent restenosis in humans: in vivo 6-month follow-up study. Circulation. 2003; 108:438–444. [PubMed: 12860915]

211. Stone PH, Coskun AU, Kinlay S, Popma JJ, Sonka M, Wahle A, Yeghiazarians Y, Maynard C, Kuntz RE, Feldman CL. Regions of low endothelial shear stress are the sites where coronary plaque progresses and vascular remodelling occurs in humans: an in vivo serial study. Eur Heart J. 2007; 28:705–710. [PubMed: 17347172]

212. Samady H, Eshtehardi P, McDaniel MC, Suo J, Dhawan SS, Maynard C, Timmins LH, Quyyumi AA, Giddens DP. Coronary artery wall shear stress is associated with progression and transformation of atherosclerotic plaque and arterial remodeling in patients with coronary artery disease. Circulation. 2011; 124:779–788. [PubMed: 21788584]

213. Stone PH, Saito S, Takahashi S, Makita Y, Nakamura S, Kawasaki T, Takahashi A, Katsuki T, Nakamura S, Namiki A, Hirohata A, Matsumura T, Yamazaki S, Yokoi H, Tanaka S, Otsuji S, Yoshimachi F, Honye J, Harwood D, Reitman M, Coskun AU, Papafaklis MI, Feldman CL. Prediction of progression of coronary artery disease and clinical outcomes using vascular profiling of endothelial shear stress and arterial plaque characteristics: the PREDICTION Study. Circulation. 2012; 126:172–181. [PubMed: 22723305]

214. Wentzel JJ, Chatzizisis YS, Gijsen FJ, Giannoglou GD, Feldman CL, Stone PH. Endothelial shear stress in the evolution of coronary atherosclerotic plaque and vascular remodelling: current understanding and remaining questions. Cardiovasc Res. 2012; 96:234–243. [PubMed: 22752349]

215. Papafaklis MI, Takahashi S, Antoniadis AP, Coskun AU, Tsuda M, Mizuno S, Andreou I, Nakamura S, Makita Y, Hirohata A, Saito S, Feldman CL, Stone PH. Effect of the local hemodynamic environment on the de novo development and progression of eccentric coronary atherosclerosis in humans: insights from PREDICTION. Atherosclerosis. 2015; 240:205–211. [PubMed: 25801012]

216. Corban MT, Eshtehardi P, Suo J, McDaniel MC, Timmins LH, Rassoul-Arzrumly E, Maynard C, Mekonnen G, King S 3rd, Quyyumi AA, Giddens DP, Samady H. Combination of plaque burden, wall shear stress, and plaque phenotype has incremental value for prediction of coronary atherosclerotic plaque progression and vulnerability. Atherosclerosis. 2014; 232:271–276. [PubMed: 24468138]

217. Hadi HA, Carr CS, Al Suwaidi J. Endothelial dysfunction: cardiovascular risk factors, therapy, and outcome. Vascular health and risk management. 2005; 1:183–198. [PubMed: 17319104]

218. Libby, PREG.; Ridker, P. Biomarkers, Proteomics, Metabolomics, and Personalized Medicine Braunwald's Heart Disease. 10th. Saunders, Philadelphia: Elsevier; 2015. p. 84-93.

219. Libby P, DiCarli M, Weissleder R. The vascular biology of atherosclerosis and imaging targets. J Nuclear Medicine. 2010:33S–37S.

220. Fernandez-Friera L, Ibanez B, Fuster V. Imaging subclinical atherosclerosis: is it ready for prime time? A review. Journal of cardiovascular translational research. 2014; 7:623–634. [PubMed: 25119855]

221. Rader DJ, Daugherty A. Translating molecular discoveries into new therapies for atherosclerosis. Nature. 2008; 451:904–913. [PubMed: 18288179]

222. Ridker PM, Kastelein JJ, Genest J, Koenig W. C-reactive protein and cholesterol are equally strong predictors of cardiovascular risk and both are important for quality clinical care. Eur Heart J. 2013; 34:1258–12561. [PubMed: 23376447]

223. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med. 1997; 336:973–979. [PubMed: 9077376]

224. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med. 2000; 342:836–843. [PubMed: 10733371]

225. Albert MA, Danielson E, Rifai N, Ridker PM, Investigators P. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. JAMA. 2001; 286:64–70. [PubMed: 11434828]

226. Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe CH, Pfeffer MA, Braunwald E. Pravastatin or Atorvastatin E and Infection Therapy-Thrombolysis in Myocardial Infarction I. C-reactive protein levels and outcomes after statin therapy. N Engl J Med. 2005; 352:20–28. [PubMed: 15635109]

227. Endothelial Biomedicine. New York: Cambridge University Press; 2007.

228. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nature reviews Immunology. 2011; 11:85–97.

229. Ridker, P.; Libby, P.; Buring, J. Braunwald's Heart Disease. 10th. Saunders, Philadelphia: Elsevier; 2015. Risk Markers and the Primary Prevention of Cardiovascular Disease.

230. Kloting N, Bluher M. Adipocyte dysfunction, inflammation and metabolic syndrome. Reviews in endocrine & metabolic disorders. 2014; 15:277–287. [PubMed: 25344447]

231. Baker RG, Hayden MS, Ghosh S. NF-kappaB, inflammation, and metabolic disease. Cell metabolism. 2011; 13:11–22. [PubMed: 21195345]

232. Kanda T, Brown JD, Orasanu G, Vogel S, Gonzalez FJ, Sartoretto J, Michel T, Plutzky J. PPARgamma in the endothelium regulates metabolic responses to high-fat diet in mice. J Clin Invest. 2009; 119:110–124. [PubMed: 19065047]

233. Eelen G, de Zeeuw P, Simons M, Carmeliet P. Endothelial cell metabolism in normal and diseased vasculature. Circulation research. 2015; 116:1231–1244. [PubMed: 25814684]

234. Goveia J, Stapor P, Carmeliet P. Principles of targeting endothelial cell metabolism to treat angiogenesis and endothelial cell dysfunction in disease. EMBO molecular medicine. 2014; 6:1105–1120. [PubMed: 25063693]

235. Ginsburg, G. Braunwald's Heart Disease. 10th. Philadelphia: Elsevier, Saunders; 2015. Personalized and Precision Cardiovascular Medicine; p. 57-63.

236. Altschul R and Co. NYTM. Endothelium: Its Development. Morphology, Function and Pathology. 1954

Dialogue Box: Endothelial Cell Dysfunction (ECD)

In the lexicon of modern Cardiovascular Medicine, the term “endothelial dysfunction” typically is used to refer to abnormalities in the production or bioavailability of endothelial-derived nitric oxide and resultant deleterious changes in vascular reactivity. For the purpose of this *Compendium on Atherosclerosis*, we use the term “endothelial cell dysfunction (ECD)” to encompass all of the maladaptive changes (multifactorial, localized and systemic, acute and chronic) in the functional phenotype of endothelial cells, which are associated with atherosclerotic cardiovascular disease.

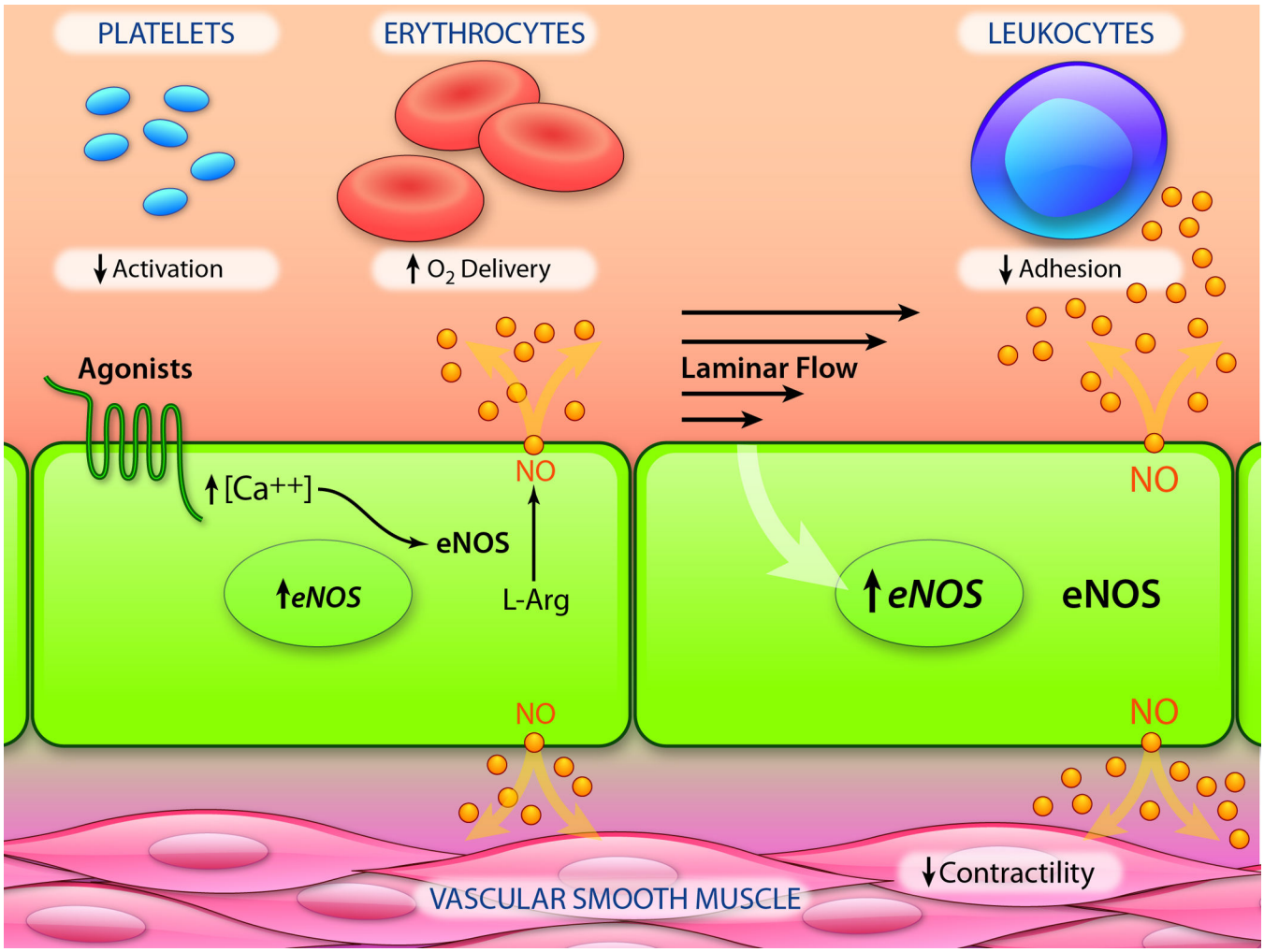

Figure 1. Endothelial-derived Nitric Oxide: Production and Biological Actions

Endothelial cells rapidly produce nitric oxide (NO) via a unique isoform of nitric oxide synthase (eNOS) in response to agonists (e.g., acetylcholine, bradykinin) and fluctuations in blood flow. Once generated, NO rapidly diffuses through the endothelial plasma membrane to activate guanylate cyclase in several cell types present in the blood (platelets, leukocytes) and also within the vessel wall (smooth muscle). Activation of guanylate cyclase in platelets results in inhibition of activation, adhesion and aggregation; in leukocytes, decreased adhesivity; in smooth muscle cells, dephosphorylation of myosin light chain and vasorelaxation. NO also reacts with hemoglobin in erythrocytes, enhancing oxygen delivery to tissues. Chronic exposure of endothelial cells to laminar flow results in transcriptional upregulation of eNOS, thus increasing their NO-forming capacity. (Illustration Credit: Ben Smith)

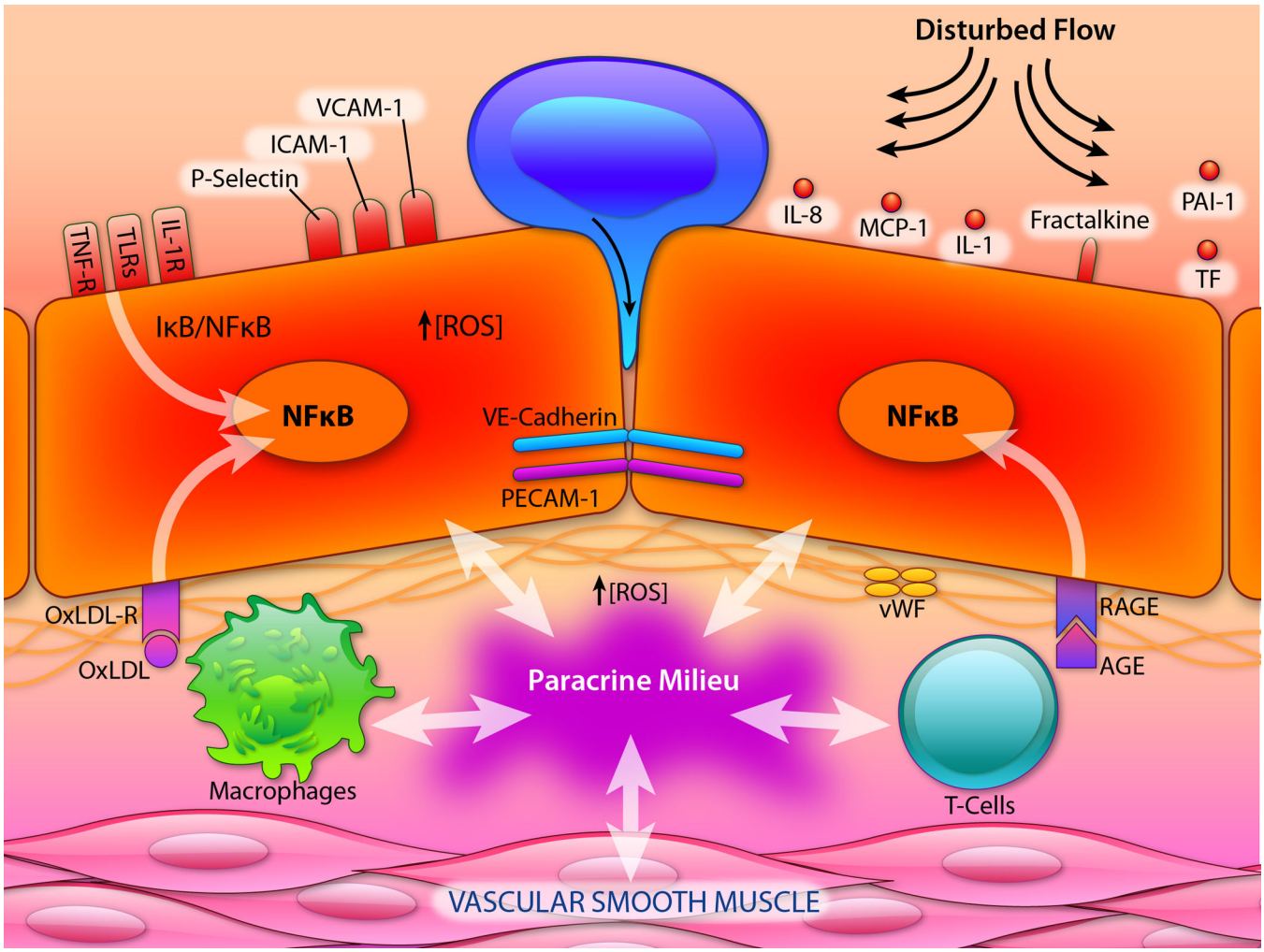

Figure 2. Endothelial Pro-Inflammatory Activation

In lesion-prone regions of the arterial vasculature, the actions of pro-inflammatory agonists (e.g., IL-1, TNF, endotoxin), oxidized lipoproteins (oxLDL) and advanced glycation end products (AGE), as well as biomechanical stimulation by disturbed blood flow, leads to endothelial activation. These biochemical and biomechanical stimuli signal predominantly via the pleiotropic transcription factor NFκB, resulting in a coordinated program of genetic regulation within the endothelial cell. This includes the cell surface expression of adhesion molecules (e.g., VCAM-1), secreted and membrane-associated chemokines (e.g., MCP-1, Fractalkine), and pro-thrombotic mediators (e.g., TF, vWF, PAI-1). These events foster the selective recruitment of monocytes and various types of T-lymphocytes, which become resident in the subendothelial space. The concerted actions of activated endothelial cells, smooth muscle cells, monocyte/macrophages and lymphocytes result in the production of a complex paracrine milieu of cytokines, growth factors and reactive oxygen species (ROS) within the vessel wall, which perpetuates a chronic pro-inflammatory state and fosters atherosclerotic lesion progression. (vWF, vonWillebrand Factor; PAI-1, Plasminogen Activator Inhibitor-1; RAGE, Receptor for AGE; OxLDL-R, Receptor for Oxidized LDL;

IL-R, TNF-R, Receptor(s) for IL-1, TNF; and TLRs, Toll-like Receptors). (Illustration Credit: Ben Smith).

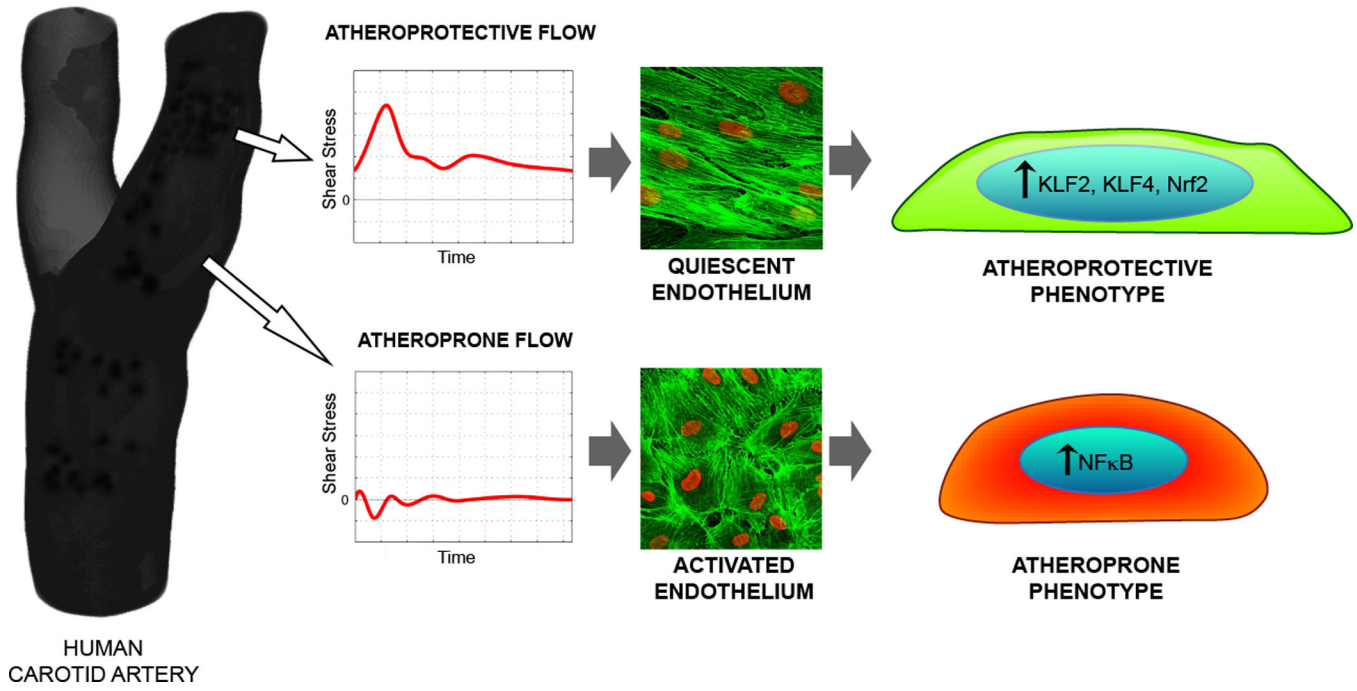

Figure 3. Hemodynamics and Endothelial Phenotypes

Computational analyses of *in vivo* blood flow patterns in the carotid artery bifurcation, in normal human subjects, yielded representative near-wall shear stress waveforms from two hemodynamically distinct, clinically relevant locations: the distal internal carotid (an atherosclerosis-resistant region) and the carotid sinus (an atherosclerosis-susceptible region). Exposure of cultured human endothelial monolayers to these two distinct biomechanical stimuli, resulted in markedly different cell morphologies (visualized here by cytoskeletal actin staining) and functional phenotypes. Pulsatile (unidirectional) laminar flow induced upregulation of key transcription factors (KLF2, KLF4, Nrf2), which orchestrated a multifunctional atheroprotective phenotype; in contrast, disturbed (oscillatory) flow resulted in enhanced expression of the pleiotropic transcription factor NFκB, resulting in a proinflammatory, atheroprone phenotype. (See Ref. 161 for details.)

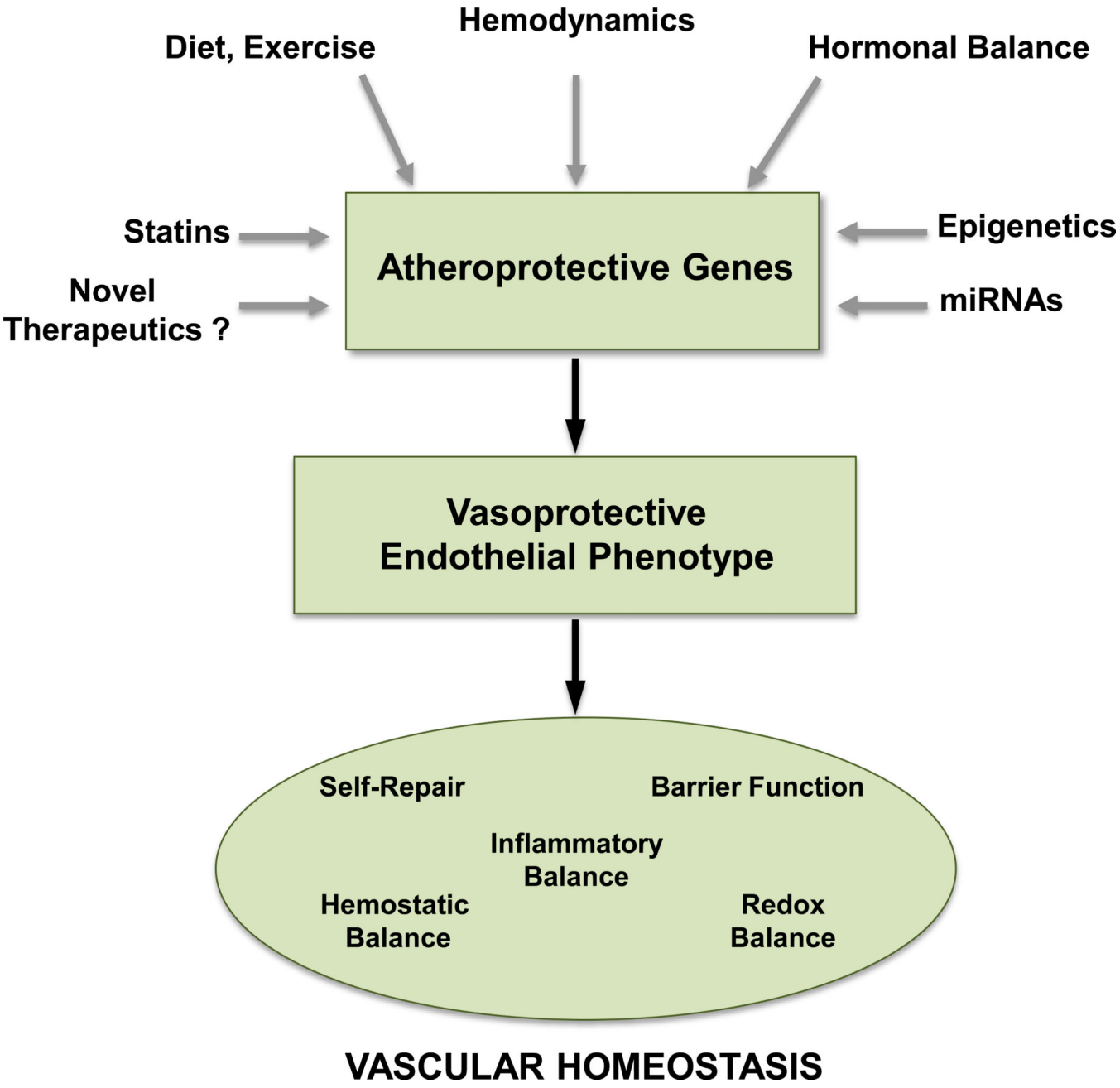

Figure 4. Endothelial Atheroprotective Genes and Vascular Homeostasis

The expression of atheroprotective genes in vascular endothelium is regulated by key transcriptional factors (e.g., KLF2, KLF4, Nrf2) in response to hemodynamic, hormonal and environmental stimuli. The coordination of this genetic program is further influenced by miRNAs, epigenetic modifications, and pharmacologic agents. The resultant vasoprotective endothelial phenotype supports a spectrum of functions critical to the maintenance of vascular homeostasis.

TABLE 1

Pathophysiological Stimuli of Endothelial Cell Dysfunction in Atherogenesis

- Hypercholesterolemia (e.g., oxidatively modified lipoproteins)
- Diabetes, Metabolic Syndrome (e.g., advanced glycation end products, reactive oxygen species, adipokines)
- Hypertension (e.g., angiotensin-II, reactive oxygen species)
- Sex Hormonal Imbalance (e.g., estrogen deficiency, menopause)
- Aging (e.g., advanced glycation end products, cell senescence)
- Oxidative Stress (multi-factorial)
- Proinflammatory Cytokines (e.g., Interleukin-1, Tumor Necrosis Factor)
- Infectious Agents (e.g., bacterial endotoxins, viruses)
- Environmental Toxins (e.g., cigarette smoke, air pollutants)
- Hemodynamic Forces (e.g., disturbed blood flow)