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Endothelial Dysfunction in Atherosclerotic Cardiovascular Diseases and Beyond: From Mechanism to Pharmacotherapies

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ABBREVIATIONS: AA, amino acid; ACE2, angiotensin-converting enzyme 2; ACEI, angiotensin-converting enzyme inhibitor; ADMA, asymmetric dimethylarginine; AGE, advanced glycation end product; AMPK, adenosine monophosphate activated protein kinase; ANG-2, angiopoietin-2; Ang-II, angiotensin II; ApoE, apolipoprotein E; ARB, angiotensin II receptor blocker; AT1R, Ang-II type 1 receptor; BH4, tetrahydrobiopterin; CCB, calcium channel blocker; COPD, chronic obstructive pulmonary disease; COX, cyclooxygenase; COXIB, COX-2 inhibitor; COVID-19, coronavirus disease 2019; CVD, cardiovascular disease; CXCL13, CXC chemokine ligand 13; DHFR, dihydrofolate reductase; DPP-4, dipeptidyl peptidase-4; EndoMT, endothelial-to-mesenchymal transition; eNOS, endothelial nitric oxide synthase; ER, endoplasmic reticulum; ERK, extracellular regulated protein kinase; ET-1, endothelin 1; FA, fatty acid; FAO, fatty acid oxidation; FGF, fibroblast growth factor; FMD, flow-mediated dilatation; FSP1, fibroblast-specific protein 1; GCH1, GTP cyclohydrolase 1; GLP-1RA, GLP-1 receptor agonist peptide-1; Hcy, homocysteine; HIF-1α, hypoxia-inducible factor 1α; H_2O_2, hydrogen peroxide; HOXA, homeobox A; HOXA-AS2, HOXA cluster antisense RNA 2; H_2S, hydrogen sulfide; HULC, hepatocellular carcinoma upregulated long noncoding RNA; HUVEC, human umbilical vein endothelial cell; ICAM-1, intercellular adhesion molecule-1; IKK, IκB kinase; IL, interleukin; IL-1β, interleukin-1β; KLF2, Kruppel-like factor 2; KLF4, Kruppel-like factor 4; LDL, low-density lipoprotein; LDLR, LDL receptor; lncRNA, long noncoding RNA; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; MDM2, murine double minute-2; MEG3, maternally expressed 3; MGO, methylglyoxal; miRNA, micro RNA; MMP, matrix metalloprotein; NAC, *N*-acetylcysteine; NET, neutrophil extracellular trap; NF-kB, nuclear factor kappa B; NLRP3, NOD like receptor family pyrin domain containing 3; NO, nitric oxide; NOX, NADPH oxidase; Nrf2, nuclear factor-like 2; oxLDL, oxidized LDL; PAI-1, plasminogen activator inhibitor-1; PAR1, protease-activated receptor 1; PCSK9, proprotein convertase subtilisin/kexin type 9; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; PGI_2, prostacyclin; PI3K, phosphatidylinositol 3-kinase; PM2.5, particulate matter; RAGE, receptor for advanced glycation end products; Rho, Ras homologous; ROS, reactive oxygen species; RvD1, resolvin D1; scRNA-seq, single-cell RNA-sequencing; SGLT2i, sodium-glucose cotransporter 2 inhibitor; SIRT1, sirtuin 1; SIRT6, sirtuin 6; α-SMA, α-smooth muscle actin; SNHG, small nucleolar host gene; STAT3, signal transducer and activator of transcription 3; TAZ, transcriptional coactivator with PDZ-binding motif; TF, tissue factor; TFEB, transcription factor EB; TNF-α, tumor necrosis factor α; TxA_2, thromboxane A_2; T2DM, type 2 diabetes; YAP, yes-associated protein 1; VCAM-1, vascular adhesion molecule-1; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; VE-PTP, vascular endothelial protein tyrosine phosphatase; vWF, von Willebrand factor; ZO-1, zonula occludens protein 1.

***Abstract*——The endothelium, a cellular monolayer lining the blood vessel wall, plays a critical role in maintaining multiorgan health and homeostasis. Endothelial functions in health include dynamic maintenance of vascular tone, angiogenesis, hemostasis, and the provision of an antioxidant, anti-inflammatory, and antithrombotic interface. Dysfunction of the vascular endothelium presents with impaired endothelium-dependent vasodilation, heightened oxidative stress, chronic inflammation, leukocyte adhesion and hyperpermeability, and endothelial cell senescence. Recent studies have implicated altered endothelial cell metabolism and endothelial-to-mesenchymal transition as new features of endothelial dysfunction. Endothelial dysfunction is regarded as a hallmark of many diverse human panvascular diseases, including atherosclerosis, hypertension, and diabetes. Endothelial dysfunction has also been implicated in severe coronavirus disease 2019. Many clinically used pharmacotherapies, ranging from traditional lipid-lowering drugs, antihypertensive drugs, and antidiabetic drugs to proprotein convertase subtilisin/kexin type 9 inhibitors and interleukin 1β monoclonal antibodies, counter endothelial dysfunction as part of their clinical benefits. The regulation of endothelial dysfunction by noncoding RNAs has provided novel insights into these newly described regulators of endothelial dysfunction, thus yielding potential new therapeutic approaches. Altogether, a better understanding of the versatile (dys) functions of endothelial cells will not only deepen our comprehension of human diseases but also accelerate effective therapeutic drug discovery. In this review, we provide a timely overview of the multiple layers of endothelial function, describe the consequences and mechanisms of endothelial dysfunction, and identify pathways to effective targeted therapies.**

***Significance Statement*——The endothelium was initially considered to be a semipermeable biomechanical barrier and gatekeeper of vascular health. In recent decades, a deepened understanding of the biological functions of the endothelium has led to its recognition as a ubiquitous tissue regulating vascular tone, cell behavior, innate immunity, cell-cell interactions, and cell metabolism in the vessel wall. Endothelial dysfunction is the hallmark of cardiovascular, metabolic, and emerging infectious diseases. Pharmacotherapies targeting endothelial dysfunction have potential for treatment of cardiovascular and many other diseases.**

I. Introduction

The endothelium is a continuous cell monolayer lining the vascular arterial, venous, and lymphatic vessels. The endothelium is often referred to as a barrier, but this is much too static a description, as the endothelium actively integrates, coordinates, and compartmentalizes blood to enable its function of supplying energy to the surrounding tissues and organs (Gimbrone and García-Cardeña, 2016a). The endothelium is designated as a disseminated organ with a total weight similar to other large human organs and an area of several thousand square meters. The endothelium has its anatomic presence between the blood, tissues, and organs and also serves much broader functions in terms of vascular quiescence and protection from blood clotting, tissue injury, and other mostly ischemic pathologies (Gimbrone and García-Cardeña, 2016a). Critically, the luminal surface of the endothelium does not normally activate the intrinsic coagulation cascade or promote platelet adhesion. A multitude of physical and biochemical insults can impact the function of the endothelium. In the mid-1980s, the phenomenon of endothelial dysfunction was described (Gimbrone, 1980) as reduced vasodilatory capacity occurring alongside

various vascular pathologies, with its importance derived from its potential relationship with the development of cardiovascular disease (CVD) (Gimbrone, 1980; Ross, 1986, 1999; Libby, 2002; Tabas et al., 2007).

The concept of compromised endothelial cell function has coevolved with the advent of modern vascular biology in the second half of the 20th century. The phenomenon of endothelial dysfunction has been known in the literature for at least 25 years (Anderson et al., 1995), and it may be narrowly defined as reduced vasodilatory capacity or, more broadly, as any change that impacts the vasoprotective homeostatic function of the endothelium. Importantly, endothelial dysfunction appears to be reversible in response to therapeutic interventions targeting the risk factors that correct the endothelial dysfunction (Heitzer et al., 1996a,b; Lesniewski et al., 2011; Hung et al., 2016; Jiang et al., 2017). Endothelial dysfunction is very widely used both as a research tool and as one of the risk factors for CVD. For example, hyperlipidemia precipitates endothelial dysfunction, and lipid-lowering therapies lead to the reversal of endothelial dysfunction (Chłopicki and Gryglewski, 2005).

Endothelium is unique as a distributed organ because it is directly exposed to pressure and flow, with uniform and disturbed patterns that change in association with the cardiac cycle (Ramanathan and Skinner, 2005). Much of the development of atherosclerosis occurs in the subendothelial space (neointima), which is under at least partial control and hormonal regulation by the endothelium (Little et al., 2008a,b, 2010). A notable feature of the location and distribution of atherosclerotic plaques is that they do not occur randomly and are not evenly distributed, instead occurring at focal points of arterial branching and other such points of altered hemodynamic forces (Glagov et al., 1988). Studies of fluid dynamics in experimental vascular systems show that mechanical forces generated by arterial blood flow

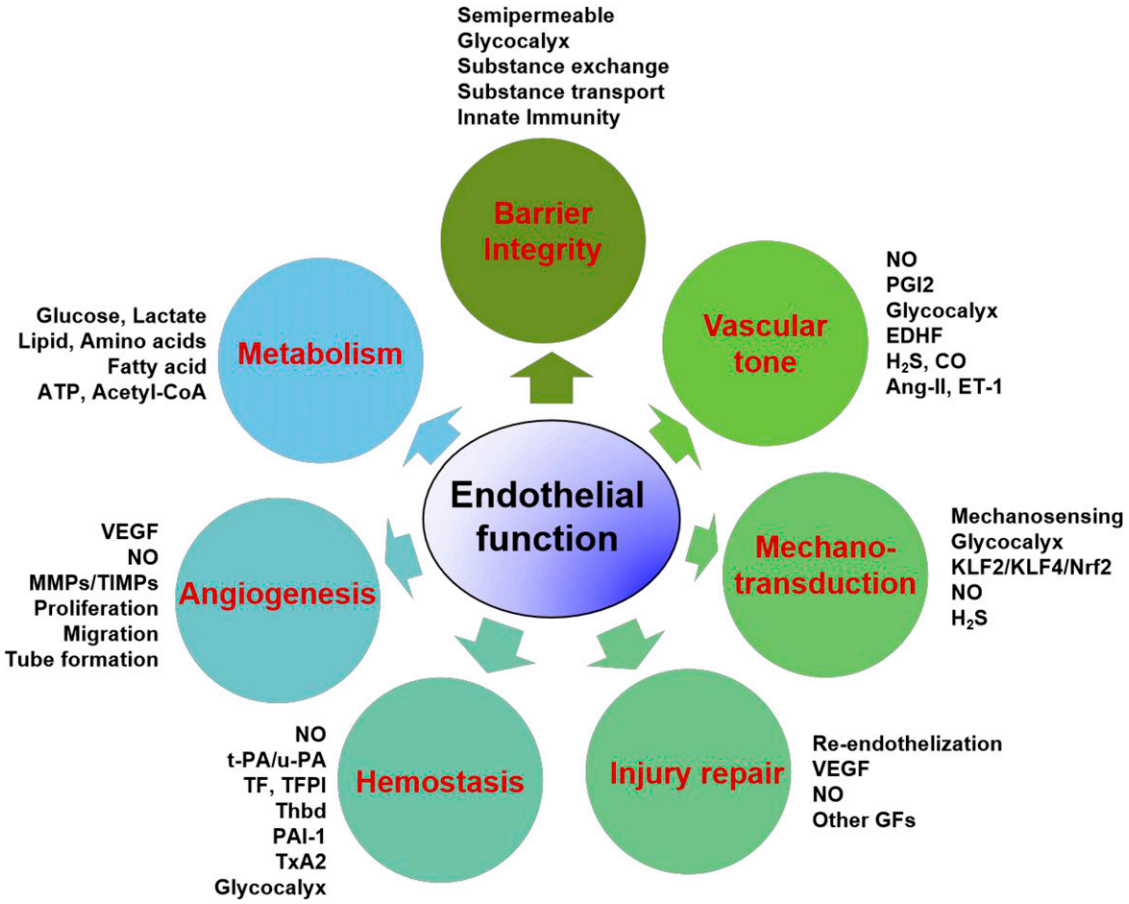

Fig. 1. Endothelial function. The healthy endothelium serves diverse biologic functions, including 1) serving as a semipermeable barrier and regulating substance exchange/transport and innate immunity; 2) regulation of vascular tone by balancing the production of vasodilators [such as NO, PGI_2, H_2S, and endothelium-derived hyperpolarizing factor (EDHF)] and vasoconstrictors (such as ET-1 and Ang-II); 3) mediating mechanotransduction via mechanosensors/mechanosensitive complexes and gene expression via transcription factors KLF2/KLF4/Nrf2, leading to increased NO and H_2S production; 4) repairing vascular injury by accelerating re-endothelialization and with the assistance of VEGF, NO, and other growth factors (GFs); 5) regulating hemostasis by secreting antiplatelet and anticoagulant molecules such as NO, tissue plasminogen activator (t-PA), urokinase-type plasminogen activator (u-PA), tissue factor pathway inhibitor (TFPI), and thrombomodulin (Thbd) while reducing TF and PAI-1; 6) regulating angiogenesis via producing factors, including, VEGF, NO, MMPs/tissue inhibitor metalloproteinase (TIMPs), and triggering endothelial proliferation, migration, and tube formation; and 7) serving as an important cell type for the metabolism of glucose, lipids, amino acids, fatty acids, ATP, lactate, and acetyl-CoA and maintaining oxygen and nutrient supply to all tissues in the body. The glycocalyx microstructure is involved in regulating multiple aspects of endothelial function as depicted.

could alter morphologic and functional properties of endothelial cells. Further studies showed that hemodynamic stimuli could alter the gene expression patterns of endothelial cells, and this led to the atheroprotective gene hypothesis (Traub and Berk, 1998; Topper and Gimbrone, 1999), in which local blood flow conditions rendered the straight regions of blood vessels with unidirectional blood flow resistant to systemic risk factors for atherosclerosis. Thus, factors impacted by blood flow need to be considered in the context of rendering specific vessels susceptible to the focal development of atherosclerotic plaques.

In preparing this review, we have determined that there are two aspects of endothelial dysfunction that warrant discussion: the narrow application related to vasodilatation and the broader context of oxidative stress, inflammation, infection, and beyond. Indeed, the endothelium forms a gateway for bidirectional cellular traffic in cardiovascular health and disease. We suggest that discussion of both the narrow and broad definitions of endothelial (dys)function are needed to adequately characterize the role of endothelial dysfunction in panvascular disease (Ge and Wang, 2018) and the impact of therapeutic interventions. It follows that the major interest in endothelial dysfunction is the extent to which it serves as a biomarker or effector of the development and progression of atherosclerosis (Goncharov et al., 2017). This review examines in detail the various factors and novel regulators that impact on endothelial dysfunction in CVD. We also examine the role of therapeutic agents in preventing or reversing endothelial dysfunction, with the goal of describing the current state of knowledge and identifying new areas for investigation.

II. Endothelial Function

A. Regulation of Vascular Tone

Regulation of vascular tone is clearly an important function of the endothelium (Fig. 1). The discovery that endothelium-derived relaxing factor was nitric oxide (NO) was a substantial breakthrough in the area that awarded Furchgott, Ignarro, and Murad the Nobel Prize in Physiology or Medicine in 1998 (Ignarro et al., 1987; Furchgott, 1996; Murad, 2006). NO is one of the primary vasodilatory molecules generated by the endothelium. In healthy endothelial cells, NO is produced from L-arginine via endothelial nitric oxide synthase (eNOS), with help from tetrahydrobiopterin (BH4) as a cofactor (Förstermann and Münzel, 2006). Then, NO diffuses into subjacent vascular smooth muscle cells, eliciting cGMP-dependent vasodilatation by the activation of guanylate cyclase.

Endothelium, the most interior layer of blood vessels, is constantly exposed to shear stress generated by flowing blood. The biomechanical force generated by fluid shear stress is sensed by mechanosensors/mechanosensitive complexes on the surface of endothelial cells and is transduced into biochemical signals that regulate vascular tone and homeostasis (Chatterjee, 2018). Fluid shear stress controls vascular homeostasis mainly by promoting eNOS-derived NO production via multiple mechanisms. In this regard, the discovery of novel mechanosensor/mechanosensitive complexes as well as "blood flow mimetic" compounds holds therapeutic potential for maintaining/restoring vascular homeostasis and thereby preventing CVD (Xu, 2020).

In addition to NO, various molecules stimulate vasodilation by eliciting endothelium-derived hyperpolarization. These molecules include hydrogen sulfide (H_2S), carbon monoxide, arachidonic acid metabolites, and H_2O_2 (Shimokawa, 2014). Once released from the endothelium, vasodilator prostacyclin (PGI_2) stimulates prostacyclin receptor and activates adenylyl cyclase in smooth muscle cells, subsequently activating the cAMP/protein kinas A (PKA) signaling pathway to reduce calcium-mediated vascular tone. Regulation of vascular tone serves as an important function of the endothelium in maintaining vascular homeostasis (Fig. 1).

The endothelium also produces several vasoconstrictor molecules, such as endothelin 1 (ET-1), angiotensin II (Ang-II), thromboxane A_2 (TxA_2), thrombin (Miller, 2006; Sharma et al., 2018), superoxide anion, and other endothelium-derived contracting factors (Fig. 1). These molecules are released to regulate platelet activity, the coagulation cascade, and the fibrinolytic system under physiologic conditions. However, in most cases, excess release of vasoconstrictor molecules is the primary event in hypertension leading to endothelial dysfunction and the progression of CVD. Thus, the balance of vasodilators and vasoconstrictors is finely tuned to regulate arterial structure and remodeling. A decreased release of vasodilators and the augmented production of vasoconstrictors are hallmarks of endothelial dysfunction and make up a leading cause of the development of many cardiovascular comorbidities.

B. Regulation of Endothelium Integrity

The vascular endothelium acts as a semipermeable barrier between the vascular smooth muscle cells and vascular lumen and provides a nonthrombotic lining for the cardiovascular system (Abdelsalam et al., 2019). Macromolecules can cross the endothelial barrier via various mechanisms, for example, through the endothelial cell-to-cell junctions, endothelial cells themselves, lateral diffusion around the cell, vesicular transport, or via endothelial gaps. Recent studies demonstrate that low-density lipoprotein (LDL) can be transcytosed across the endothelial monolayer via either LDL receptor (LDLR) (Kraehling et al., 2016) or the scavenger receptor B1 (SR-B1) (Armstrong et al., 2015; Kraehling et al., 2016; Fung et al., 2017; Huang et al., 2019).

Several microstructures in endothelial cells are also essential for endothelial cell integrity. These microstructures include surface glycoproteins, proteoglycan core proteins, sialoglycoproteins, glycosaminoglycans, and other components, which together are defined as the endothelial glycocalyx (Harding et al., 2019) (Fig. 1). The glycocalyx is implicated in regulating endothelial function via mechanotransduction (Tarbell and Ebong, 2008). In particular, the glycocalyx is implicated in the maintenance of endothelial phenotype by responding to hemodynamic forces to elicit flow-dependent NO production (Ebong et al., 2014; Harding et al., 2018) and to regulate transendothelial permeability (Singh et al., 2007), leukocyte adhesion, and transmigration across the endothelium (Constantinescu et al., 2003). For example, increased eNOS expression in response to flow exposure at physiologic levels of shear stress is regulated by the following components of the glycocalyx: the major glycosaminoglycan heparan sulfate and the proteoglycan core protein glypican-1 that heparan sulfate covalently binds to (Ebong et al., 2014; Harding et al., 2018). Under uniform flow conditions of high physiologic shear stress with an intact glycocalyx, low endothelial permeability is observed; however, permeability is increased in disturbed flow conditions with low physiologic shear stress, in which glycocalyx components heparan sulfate and sialic acid have been shown to be reduced (Mitra et al., 2017, 2018; Mensah et al., 2020). Several studies have identified a reduction in the amount of glycocalyx in diseases such as atherosclerosis (Cancel et al., 2016), diabetes (Nieuwdorp et al., 2006), and COVID-19 (Fraser et al., 2020), which may contribute to vascular dysfunction associated with these complications.

Sphingosine-1-phosphate, a biologically active molecule associated with high-density lipoprotein (Wilkerson et al., 2012), promotes endothelial barrier function and vascular stability (Garcia et al., 2001; Xiong and Hla, 2014) by attenuating the degradation of the glycocalyx (Mensah et al., 2017; Zhang et al., 2016a, 2017b). These observations point to the importance of considering the development of therapeutics targeting the glycocalyx to combat these and other conditions (Fig. 1), as will be further discussed later in this review.

The loss of endothelial barrier integrity leads to vascular hyperpermeability and vascular swelling/edema during various diseases, including sepsis, ischemia, and trauma (Fig. 1). Various physiologic and pathophysiological stimuli can provoke acute and chronic changes in endothelium permeability. For instance, vascular endothelial growth factor (VEGF), histamine, thrombin, and other acute inflammatory regulators [interleukin 1β (IL-1β) and tumor necrosis factor α (TNF-α)] can act on endothelial cells, thereby compromising cell-cell connections at the level of tight junctions and adhesion complexes (Lum and Malik, 1996). The bacterial endotoxin lipopolysaccharide (LPS) results in excessive endothelium permeability by stimulating Ras homologous (Rho) A, Rho kinase, and small GTPases (Joshi et al., 2014). Excessive endothelial permeability may also result from a high level of oxidative stress and inflammation, leading to a reduction in NO availability (Yang et al., 2013b) and disruption of adherens junction proteins (such as VE-cadherin), tight junction proteins (zonula occludens 1 and occludins), and gap junction proteins (connexins) (Komarova et al., 2017). It is well known that Ang-II can cause hyperpermeability of the endothelium through oxidative stress and inflammation, leading to abnormal metabolism of NO (Husain et al., 2015). The receptor for advanced glycation end products (RAGE) also modulates Ang-II–induced endothelial permeability. Ang-II increases the permeability of the endothelial barrier, which can be ameliorated by soluble RAGE via disrupting the high-mobility group protein 1–mediated Ang-II type 1 receptor (AT1R)/RAGE crosstalk (Jeong et al., 2019).

Flavivirus nonstructural protein 1 is a secreted glycoprotein that induces high endothelial permeability in vitro and in vivo by destroying endothelial glycocalyx components and causing tissue-specific vascular leakage in mice (Puerta-Guardo et al., 2019; Biering et al., 2021). A recent study has reported that diminished endothelial glycocalyx triggered by a high-fat diet inhibits NO production; thus, impairing vasodilation and nonstructural protein 1 protein may also regulate vascular tone by degrading glycocalyx components (Kang et al., 2020). The mechanosensing ion channel Piezo1, located on endothelial cell membranes, is activated by high pressure and other mechanical stimuli and may impair the endothelial barrier. Piezo1 promotes the internalization and degradation of the endothelial adhesion junction protein VE-cadherin to induce high pulmonary vascular permeability. The loss of Piezo1 in endothelial cells prevents the degradation of adhesion junction protein and vascular hyperpermeability. Therefore, piezo1 mediates pressure-induced hyperpermeability of pulmonary vessels by initiating the degradation of VE-cadherin (Friedrich et al., 2019). NOD like receptor (NLR) family pyrin domain containing 3 (NLRP3) inflammasome activation also mediates endothelial barrier dysfunction in type 2 diabetes (T2DM), leading to hyperpermeability of the endothelium. Li et al. (2019e) reported that acarbose can inhibit NLRP3 inflammasome activation in vascular endothelial cells of T2DM rats and has inhibitory effects on endothelial hyperpermeability. In addition, acarbose blocks the production of NADPH oxidase (NOX) 4–dependent superoxide anion generation, thereby inhibiting the NLRP3 inflammasome and enhancing the expression of ZO-1 and VE-cadherin, in turn diminishing hyperpermeability of blood vessels.

The preservation of endothelial barrier integrity is critical for maintaining vascular homeostasis. Recent studies have reported some emerging new therapeutic

targets based on the inhibition of endothelial hyperpermeability. For example, overexpression of dynamin-related protein-1 prevents LPS-induced endothelial hyperpermeability in rats; conversely, its inhibition increases LPS-induced hyperpermeability (Luo et al., 2020). Puerarin, one of several known plant-derived isoflavones, also reduced LPS-induced hyperpermeability in vascular endothelial cells through inhibiting downregulation of VE-cadherin (Deng et al., 2019). CXC chemokine ligand 13 (CXCL13) is a chemokine family member that plays an important role in LPS-induced endothelium hyperpermeability. Knockdown of CXCL13 in human umbilical vein endothelial cells (HUVECs) inhibits LPS-induced endothelial hyperpermeability through regulating the $p38^{MAPK}$ signaling pathway (Chen et al., 2020b). This demonstrates that dynamin-related protein-1 and CXCL13 have the potential to be developed as therapeutic targets to inhibit endothelium hyperpermeability.

C. Endothelial Cell Metabolism

Endothelial cells are metabolically active per se and also function as a critical influence on nutrient and oxygen supply to all tissues of the body. The major energy source for the endothelium is glycolysis. During glycolysis, 85% of ATP is produced via converting glucose to lactate with the help of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) enzyme (De Bock et al., 2013). Hexokinase 2 regulates endothelial cell glycolysis via phosphorylation of glucose into glucose-6-phosphate (Yu et al., 2017). Endothelial cells use fatty acid oxidation (FAO) as their secondary source of energy and produce ATP via the adenosine monophosphate activated protein kinase (AMPK) signaling pathway (Dagher et al., 2001). Carnitine palmitoyltransferase 1A is the main regulator of FAO, which transfers fatty acids (FAs) from the cytoplasm to the mitochondria via the carnitine palmitoyl shuttle, where FAO occurs (Currie et al., 2013). Endothelial cells also use amino acid (AA) metabolism to maintain their proliferative and vasodilator functions (Huang et al., 2017a). Glutamine is one of the AAs differentially used by endothelial cells (Huang et al., 2017a). Endothelial glutaminase activity catalyzes the metabolism of glutamine into α-ketoglutarate; pharmacological inhibition of glutaminase induces endothelial senescence and decreases endothelial cell proliferation (Huang et al., 2017a; Kim et al., 2017a). Arginine is another notable AA that is metabolized by endothelial cells. Arginine, the substrate of eNOS, is required for the production of vasoprotective NO (Morris, 2009). The specific functions of other AA metabolism in endothelial cells need further investigation.

Alteration in endothelial cell metabolism contributes to cardiovascular disorders through endothelial dysfunction. For example, disturbed flow alters endothelial cell metabolism via activating hypoxia-inducible factor 1α (HIF-1α), which induces endothelial cell proliferation and inflammation by activating glycolytic enzymes, conditions supporting the initiation of atherosclerosis (Feng et al., 2017). Altered endothelial cell metabolism contributes to pressure overload–induced heart failure. Ablation of endothelial sirtuin 3 alters glycolysis via disruption of endothelial glucose transport to cardiomyocytes and leads to pressure overload–induced heart failure (Zeng et al., 2020a). Inhibition of glycolysis using 2-deoxy-D-glucose induces endothelial cell cytotoxicity (Merchan et al., 2010). Furthermore, carnitine palmitoyltransferase 1A inhibition (the rate-limiting enzyme in FA oxidation), either by pharmacological or genetic manipulation, can lead to defects in vascular sprouting, increased hyperpermeability, decreased endothelial cell proliferation, and reduced FA oxidation (Schoors et al., 2015). The multiligand receptor CD36, expressed in endothelial and parenchymal cells, facilitates tissue uptake of FA, and its loss leads to metabolic effects in parenchymal cells (Son et al., 2018). Recently, it has been suggested that mitochondrial ATP drives endothelial FA uptake and transportation via acyl-CoA formation independent of total cellular ATP level. In this regard, niclosamide, a medication used to treat tapeworm infestations, suppressed endothelial FA uptake and transport via mitochondrial uncoupling, whereas fatty acid transport protein 4 promoted FA uptake (Ibrahim et al., 2020).

The evidence that links aberrant endothelial cell metabolism to vascular dysfunction and metabolic diseases is strengthened by a recent study (Sun et al., 2020) showing that deficiency of endothelial transcription factor EB (TFEB) leads to impaired glucose tolerance via reduced Akt signaling and reduced insulin receptor substrate 1 and 2 expression. This study uncovered a novel role of TFEB in endothelial cell metabolism and identified TFEB as a potential therapeutic target for treating various vascular and metabolic diseases. In comparison with other aspects of endothelial function, endothelial cell metabolism is a relatively new area of investigation (Fig. 1). Further exploration of endothelial cell metabolism in various physiologic and pathologic scenarios will provide us new perspectives and opportunities in cardiovascular research.

D. Platelet Activity and Interaction

Platelets and endothelial cells share several mechanisms of activation and therefore serve as attractive common targets for pharmacological intervention. Similar to endothelial cells, platelets contain specialized granules called Weibel-Palade bodies that secrete von Willebrand factor (vWF), P-selectin, and cytokines (Denorme et al., 2019; Holthenrich et al., 2019). Endothelial and platelet degranulation of Weibel-Palade bodies together regulate thrombosis and hemostasis, with vWF promoting platelet-to-platelet as well as platelet-to-endothelial adhesion through the glycoprotein1b receptor (Kroll et al., 1991). P-selectin promotes

platelet-to-endothelium as well as endothelium-to-leukocyte and platelet-to-leukocyte interactions (Muller, 2002). Platelets and endothelial cells both contain and are coregulated by NO synthase (Matsushita et al., 2003; Gkaliagkousi et al., 2007). Platelets contain serotonin-enriched dense granules that, like Weibel-Palade bodies, undergo membrane fusion and exocytosis when the platelet is activated by external stimuli (Matsushita et al., 2003; Ambrosio and Di Pietro, 2017). Platelet serotonin release impacts endothelium-dependent and non–endothelium-dependent changes in vascular tone and inflammation (Golino et al., 1989; Kataoka et al., 2016; Ntelis et al., 2019). To abrogate some of the adverse events of both endothelial and platelet activation (sometimes referred to as thromboinflammation), therapeutic agents targeting receptors common to both endothelial cells and to platelets is a rational therapeutic strategy. One such target is protease-activated receptor 1 (PAR1). PAR1 is a G protein–coupled receptor and, when activated by circulating thrombin, leads to degranulation of platelets and activation of endothelial cells. By utilizing PAR1 antagonists and PAR1 inhibitory strategies, the thromboinflammatory contributions to sepsis and inflammatory and fibrotic pulmonary diseases can be diminished (Howell et al., 2005; Jose et al., 2015; Sinha et al., 2018). Overall, much more work is needed to better understand bidirectional communication between endothelial cells and platelets in the etiology of various diseases including CVD. Advances in deciphering this bidirectional communication could potentially offer new therapeutic targets in treating CVD and its clinical sequelae.

E. Regulation of Hemostasis

The vascular endothelium provides a dynamic interface between tissue and blood, thereby regulating hemostasis and thrombosis (Fig. 1). Hemostasis is a complex biologic process that requires various interactions between the damaged vessel wall, blood cells, and coagulation-associated proteins [including protein C, thrombin, tissue factor (TF), tissue plasminogen activator, vWF]. Endothelial cells participate in all major hemostatic events after vascular injury and restrict clot formation to the specific areas that require hemostasis to restore vascular integrity (Verhamme and Hoylaerts, 2006; Yau et al., 2015). Vascular injury triggers endothelial cell activation and exposure of the subendothelial space, including adventitial fibroblasts and medial smooth muscle cells. The exposure of subendothelial collagen to blood allows platelet adhesion to the exposed surface of collagen via vWF binding. This platelet adhesion and later aggregation is responsible for preventing blood loss. Simultaneously with these events, smooth muscle cells and activated endothelial cells express TF and procoagulant factors, which interact with circulating clotting factor VII and promote the generation of thrombin. Circulating platelets adhering to an injury site upon activation by thrombin form platelet aggregates, with further activation of other procoagulant factors. This "carpet" of platelets physically hinders leukocyte adhesion and transmigration into sites of vascular injury [reviewed in Yau et al. (2015)] (Fig. 1). From this perspective, the intact and normal functioning of endothelium is pivotal, and it is a potential target for hemostasis control. Better understanding of endothelial cell regulation of hemostasis is needed to support development of drugs with minimal or no bleeding risk for the treatment of hemostatic disorders, e.g., thrombosis and atherosclerosis inter alia.

F. Endothelial Injury and Repair

Early characterization of atherosclerosis by Ross and Glomset postulated that atherosclerosis occurred as a response to "injury" to arterial endothelium, followed by endothelial peeling, platelet adherence, aggregation, and degranulation at the site of exposed endothelial surface. Hence, these events in response to endothelial injury lead to atherogenesis (Ross et al., 1977). According to this response-to-injury hypothesis, injury to the vascular endothelium is a common and early event in CVD. It has been proposed that healthy endothelial cells are maintained by a population of cells called endothelial progenitor cells, believed to be derived from bone marrow, which stimulate re-endothelialization and maintain vascular integrity with assistance from other supporting cells, such as pericytes. Key factors involved in endothelial injury are exposure to bacterial or viral infection, hypoxia, modified LDL, ROS, oscillatory shear stress, tobacco and waterpipe smoking, hyperglycemia, and advanced glycation end products (Abraham and Distler, 2007). These factors lead to endothelial dysfunction, triggering a series of biochemical and molecular reactions, including increases in leukocyte adhesion to endothelium, increased vascular permeability, and propagation of the inflammatory response. Particulate matter (PM2.5) increases levels of early biomarkers of CVD, such as high-sensitivity C-reactive protein, and leads to inflammation, atherothrombotic risk, and endothelial dysfunction (Al Rifai et al., 2017). High plasma glucose levels induce endothelial cell injury via downregulation of circular homeodomain interacting protein kinase 3 RNA (Cao et al., 2018). Oxidized LDL (oxLDL) induces endothelial cell injury via impairing cholesterol efflux, increased apoptosis signal-regulated kinase 1/NLRP3 inflammasome signaling, and endoplasmic reticulum stress (Hang et al., 2020). Methylglyoxal (MGO) is a reactive dicarbonyl metabolite of glucose that induces endothelial dysfunction by reducing eNOS activity and NO production. MGO quickly interacts with lipids, proteins, and nucleic acids to form advanced glycation end products (AGEs). Dhar et al. (2010) demonstrated that MGO mimics endothelial dysfunction induced by high glucose. Moreover, MGO scavengers have the potential to inhibit

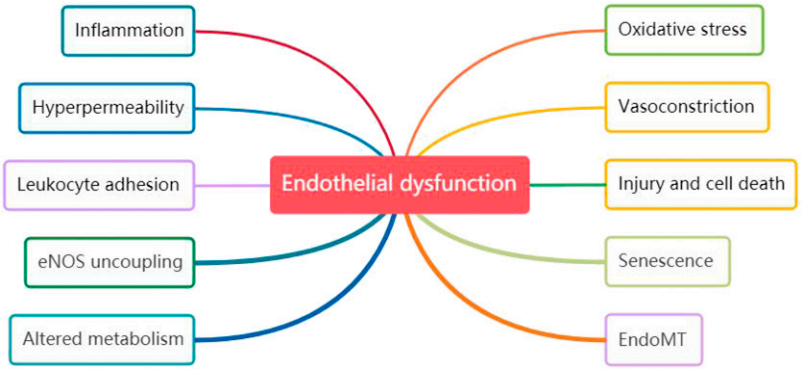

Fig. 2. Multiple layers of endothelial dysfunction. Endothelial dysfunction is characterized by a shift from executing physiologic functions of the vascular endothelium to inflammation, hyperpermeability, leukocyte adhesion, eNOS uncoupling, altered endothelial cell metabolism, oxidative stress, vasoconstriction, injury and cell death, senescence, and EndoMT. Endothelial dysfunction is more of a composite of these dysfunctional aspects, with impaired vasodilatation being one important layer of endothelial dysfunction.

MGO and high-glucose–induced endothelial dysfunction. More recently, MGO has been shown to induce endothelial cell dysfunction via the K_{ATP}/mitogen-activated protein kinase (MAPK) signaling pathway (Wang et al., 2019b). Targeting AGE and MGO in vascular endothelium may afford novel approaches to CVD-associated vascular injury by preventing endothelial cell loss, preserving the pool of healthy endothelial cells (Fig. 1).

III. Layers of Endothelial Dysfunction

A. Oxidative Stress

Oxidative stress occurs when the balance between pro-oxidant and antioxidant systems is disrupted. Oxidative stress in endothelial cells can be induced by exposure to factors such as oxLDL (Gradinaru et al., 2015), high plasma glucose, free fatty acids (Sun et al., 2019a), homocysteine (Xu et al., 2019c), aging (Haas et al., 2020), uric acid (Ko et al., 2019), Ang-II (Rao et al., 2020), airborne fine particulate matter (Riggs et al., 2020), arsenic (Guo et al., 2020), trimethylamine-*N*-oxide (a gut flora–dependent metabolite) (Piotrowska et al., 2018; Brunt et al., 2020), and other agents (Yan et al., 2017b; Mongiardi et al., 2019) (Figs. 2 and 3).

ROS in endothelial cells are mainly derived from xanthine oxidase, NADPH oxidases, uncoupled eNOS, and dysfunctional mitochondria (Schulz et al., 2014). Excessive ROS levels oxidize macromolecules such as nucleic acids, lipids, and proteins. Importantly, in vascular homeostasis, the antioxidant system, including superoxide

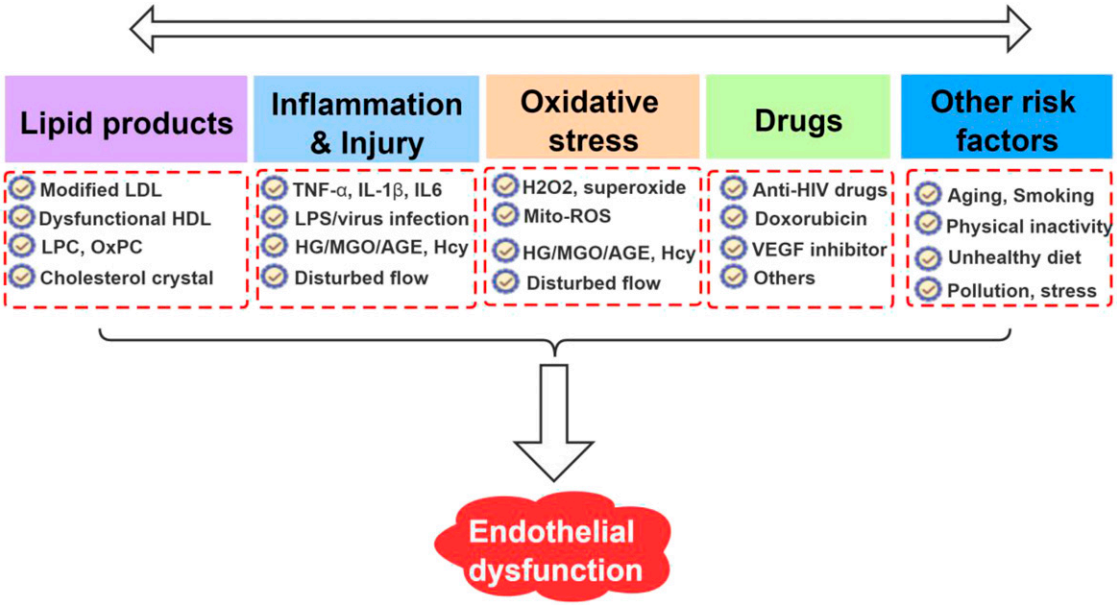

Fig. 3. Triggers of endothelial dysfunction. There are different factors that can trigger endothelial dysfunction: 1) lipid [via modified LDL, dysfunctional high-density lipoprotein (HDL), lysophosphatidylcholine (LPC), oxidized phosphatidylcholine (OxPC), and cholesterol crystal]; 2) inflammation [by proinflammatory cytokines IL-1β, IL-6, TNF-α, and high-sensitivity c-reactive protein, LPS or virus infection, high glucose (HG)/methylglyoxal (MGO)/AGE, Hcy, and disturbed flow]; 3) oxidative stress including H_2O_2, superoxide, mitochondrial ROS, hyperglycemia-associated stimuli, and disturbed flow; 4) different drugs such as anti–human immunodeficiency virus (HIV) drugs, anticancer agent doxorubicin, and vascular endothelial growth factor (VEGF) inhibitors may also trigger endothelial dysfunction; 5) other risk factors associated with endothelial dysfunction include aging, smoking, physical inactivity, unhealthy diet, irradiation, air pollution (i.e., PM2.5), and psychologic stress etc.

dismutase, catalase, glutathione peroxidase, thioredoxin, and peroxiredoxin, dominates over the pro-oxidant pathways (Liu et al., 2017; Lovatt et al., 2020; Poznyak et al., 2020). Recent studies have shown that clusterin (Ren et al., 2019), heat shock protein 22 (Yu et al., 2019), and nicotinamide nucleotide transhydrogenase (Rao et al., 2020) inhibit mitochondrial ROS formation and suppress diabetes/Ang-II–induced endothelial dysfunction, respectively. Sirtuin 3 inhibition, degradation of Nrf2, production of mitochondrial ROS, and deficiency of CR6 interacting factor 1 induce premature senescence in endothelial cells (Kim et al., 2020). As meta-regulators of gene expression, microRNAs also regulate endothelial function by influencing oxidative stress. microRNA-92a promotes oxidative stress and accelerates endothelial dysfunction (Shang et al., 2017), whereas miR-195-5p is reported to preserve endothelial function (Xu et al., 2020b). Kuosmanen et al. (2018) showed that miR-21-5p, miR-100-5p, and miR-126-3p might mediate downregulation of Nrf2 during endothelial cell senescence (Kuosmanen et al., 2018). In addition, a reduction in levels of *S*-adenosylhomocysteine, the precursor of homocysteine, by inhibition of *S*-adenosylhomocysteine hydrolase activity using short hairpin RNA (shRNA) induces endothelial dysfunction in mice through a mechanism mediated by p66shc upregulation (Xiao et al., 2019b).

In light of the important role of oxidative stress in endothelial dysfunction, detecting novel markers (e.g., oxidatively modified lipids and proteins) of oxidative stress and applying antioxidants may represent a promising strategy for the prevention of endothelial dysfunction–associated diseases. Although many studies have been focused on the antioxidative effects of the established cardiovascular drugs, new compounds targeting oxidative stress have also been developed (Scioli et al., 2020). In the past two decades, several clinical trials and meta-analyses have been conducted to evaluate the beneficial effects of antioxidants such as vitamin C, vitamin E, xanthine oxidase inhibitors, α-lipoic acid, and the synthetic agent NXY-059 (Khaw et al., 2001; Ziegler et al., 2004; Shuaib et al., 2007; Bredemeier et al., 2018). However, the protective effects were not observed in large clinical trials. Recent years have witnessed the development of Nrf2 activators (Sharma et al., 2017; Cuadrado et al., 2018), NADPH oxidase inhibitors (Anvari et al., 2015; Gray et al., 2017b), and the ROS scavenger mitoQ (Gioscia-Ryan et al., 2018) as promising novel antioxidant approaches. In addition, the poly(ADP-ribose) polymerase inhibitor PJ-34 ameliorates cerebromicrovascular endothelial function and improves cognitive performance in aged mice by decreasing oxidative stress (Tarantini et al., 2019).

The role of oxidative stress in endothelial dysfunction and CVD has been well recognized. Further exploration of novel therapeutic targets in regulating exaggerated ROS generation and mitigating existing oxidative stress and the accompanying CVD is essential for designing novel antioxidant therapies. The optimal timing of the window of administration of antioxidants at different stages of CVD is another important consideration.

B. Inflammation

Vascular inflammation plays an important role in the initiation and progression of atherosclerosis and other forms of CVD (Haybar et al., 2019) (Figs. 2 and 3). In response to injury, endothelial cells become activated and produce interleukin (IL)-8, chemokines, colony-stimulating factors, interferons, monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1), P-selectin, E-selectin, vascular adhesion molecule-1 (VCAM-1), growth factors, and other inflammatory factors. These substances attract monocytes and neutrophils, which attach to the activated endothelium, penetrate the arterial wall, and initiate inflammation (Chistiakov et al., 2018). Proinflammatory mediators such as TNF-α and IL-1β stimulate endothelial cells to secrete other proinflammatory cytokines, including IL-6, that stimulate liver cells to produce and release diverse acute-phase reactants, including fibrinogen and c-reactive protein, that modulate both chronic inflammation and the acute-phase response (Libby, 2017). However, the anti-inflammatory cytokines IL-35 and IL-10 block endothelial activation via reducing mitochondrial ROS production (Li et al., 2020c).

The transcription factor NF-κB represents a master regulator of vascular inflammation by increasing expression of TNF-α, IL-6, MCP-1, and IL-1β in response to LPS stimulation in human peripheral blood mononuclear cells (Zhang et al., 2016b). LPS released into the blood may cause endothelial injury and promote inflammatory responses via enhancing interferon-induced protein with tetratricopeptide repeats 1 expression in HUVECs (Wang et al., 2020b). Angiopoietin-2 (ANG-2) is another endothelium glycoprotein that causes endothelial inflammation to initiate angiogenesis and atherosclerosis. In hyperinsulinemic insulin-resistant subjects, insulin increases Ang-II expression in endothelial cells through inhibiting NO bioavailability via the p38 MAPK-cFOS pathway and enhances inflammation in a paracrine manner. Hyperinsulinemic serum–induced endothelial inflammation can be prevented by inhibiting ANG-2 with a neutralizing antibody (Chandel et al., 2020). Furthermore, mitochondrial dysfunction promotes vascular inflammation and evokes endothelial dysfunction by producing excessive ROS production and plays a crucial role in atherosclerosis. Murine double minute-2 (MDM2) promotes oxLDL-induced inflammation by regulating mitochondrial damage in human aortic endothelial cells. Moreover, downregulation of MDM2 reduced oxLDL-induced secretion of IL-1β, IL-6, and TNF-α; conversely, MDM2 contributes to exacerbation of inflammation (Zeng et al., 2020b). In addition to NF-κB

and TLR4, yes-associated protein 1 (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) also mediate TNF-α and LPS-induced proinflammatory responses in the endothelium (Wang et al., 2016a,b; Xu et al., 2016a; Zhou et al., 2020c). Recent studies have also revealed that TFEB, a Kruppel-like factor 2 (KLF2)-dependent target formed under laminar flow, exerts anti-inflammatory effects in diabetic mice by suppressing IκB kinase (IKK) activity (Song et al., 2019).

Inhibition of endothelium inflammation has emerged as a novel therapeutic strategy to reverse endothelial dysfunction Activation of KLF2 and Kruppel-like factor 4 (KLF4) (master regulators of vascular homeostasis) inhibits inflammation in endothelial cells in response to proinflammatory cytokines (SenBanerjee et al., 2004; Hamik et al., 2007). More recently, activation of G coupled–protein receptor 81 has been reported to inhibit endothelial inflammation via reversing oscillatory shear stress–induced KLF2 downregulation and upregulating expression of MCP-1 and VCAM-1 (Sun et al., 2019b). Pharmacological activation of KLF2 by lipid-lowering statins modulates endothelial function (SenBanerjee et al., 2005) and prevents atherosclerosis by upregulating several of its downstream transcriptional targets (Parmar et al., 2005). KLF2 induction by sirtuin 1 (SIRT1) activator also suppresses vascular inflammation and confers a vasoprotective endothelial phenotype (Gracia-Sancho et al., 2010). The antimalarial drug halofuginone protects endothelial cells from inflammation via inhibiting LPS-induced attachment of monocytes to HUVECs by inhibiting the expression of adhesion molecules including VCAM-1 and E-selectin (Zhong et al., 2020). Moreover, *N*-methyl-2-pyrrolidone, a pharmaceutical solvent, has also been reported to inhibit inflammation via activation of KLF2 (Roche-Molina et al., 2020). In addition, butyrate (a short-chain fatty acid) inhibits endothelial inflammation via inhibiting the expression of MCP-1, VCAM-1, E-selectin, and IL-8 production and rescues the reduced KLF2 expression (Wang et al., 2020e). Another immunomodulatory agent, laquinimod, inhibits TNF-α–induced endothelial inflammation by increasing KLF2 expression via activating the extracellular regulated protein kinases (ERK) 5 pathway (Jiang et al., 2020a). In addition, Aloperine (an alkaloid) prevents endothelial inflammation, leukocyte adhesion to activated endothelial cells, and atherogenesis via the activation of atheroprotective transcriptional factor KLF2 through suppression of the phosphorylation of p53 protein (Li et al., 2020a).

Endothelial inflammation has long been intensively investigated in light of the crucial role of inflammation in the initiation and progression of CVD (Figs. 2 and 3). Therapeutic agents that inhibit NF-κB, YAP/TAZ, and TLR4, and those that activate KLF2/KLF4 and TFEB, hold great potential in treating endothelial dysfunction and CVD. The discovery of novel transcription factors that regulate endothelial inflammation is an active area of investigation. Further identification of novel drugs or repurposed drugs targeting inflammation-associated transcription factors is warranted.

C. Leukocyte Adhesion and Transmigration

The healthy vascular endothelium maintains a hemostatic equilibrium, thereby preventing atherothrombosis. One notable early cause of endothelial dysfunction is endothelial injury followed by increased leukocyte adhesion, rolling, and transmigration into the subendothelial space. Under pathologic conditions, certain proinflammatory mediators, TNF-α and IL-1β in particular, may exacerbate endothelial dysfunction by upregulating the expression of adhesion molecules such as VCAM-1, ICAM-1, E-selectin, and MCP-1, leading to increased adhesion of leukocytes to the endothelium and their migration across the endothelium (Steyers and Miller, 2014). The excessive transmigration of leukocytes is accentuated by cardiovascular risk factors such as hyperlipidemia, hyperglycemia, aging, and smoking and is often associated with chronic inflammation. The leukocyte adhesion cascade is usually divided into four different sequential steps: tethering, slow rolling, firm arrest, and transmigration (Ley et al., 2007). During tethering, leukocytes interact with P- or E-selectin on the endothelial cell surface. Shortly afterward, additional selectins and other adhesive molecules are engaged, leading to sustained or slow rolling. In the third phase, leukocyte integrins become activated, leading to firm cellular arrest on the surface of the endothelium (Anderson et al., 2019). Leukocyte adhesion to the endothelium represents the initiating event in the inflammatory phase of atherogenesis. Strategies to block leukocyte adhesion hold the promise of limiting endothelial dysfunction and slowing the progression of atherosclerosis (Fig. 2).

D. Apoptosis and Cell Death

After endothelial injury, apoptosis may be initiated. Apoptosis is a key feature of vascular injury, leading to vascular leakage, inflammation, and coagulation. Many atherogenic factors, including high circulating concentrations of LDL, oxLDL, AGE, disturbed blood flow, tobacco smoking, elevated levels of Ang-II, and oxidative stress, can induce endothelial cell apoptosis (Mannarino and Pirro, 2008). Although the development of atherosclerosis is mainly associated with lipid accumulation in vessel walls, it is also coupled with the apoptosis of vascular cells, especially in the later stages characterized by plaque rupture. During apoptosis, endothelial cell–derived extracellular vesicles contribute to atherosclerosis progression (Paone et al., 2019).

Increased levels of MGO induce endothelial cell apoptosis via an increase of cytosolic phospholipase A2, which correlates with the progression of atherosclerosis.

Moreover, MGO-induced apoptosis was attenuated by inhibition of cytosolic phospholipase A2 in HUVECs (Yuan et al., 2017). According to previous reports, several microRNAs (miRNAs) are involved in endothelial cell apoptosis, including miR-210 (Li et al., 2017d). oxLDL treatment upregulated miR-210 and induced endothelial cell apoptosis in human endothelial cells and in a mouse model of atherosclerosis by targeting 3-phosphoinositide–dependent protein kinase-1 (Li et al., 2017d). A recent study has demonstrated that Ang-II induces vascular endothelial cell apoptosis and ROS production, which is accompanied by a decrease in cell viability. This process of apoptosis was protected by overexpression of sirtuin 6 (SIRT6) via the activation of the Nrf2-antioxidant response element signaling pathway (Yang et al., 2019). Furthermore, TNF-α–induced apoptosis during inflammation in mice is prevented by expression of physiologic levels of TGF-β–activated kinase-1 (Naito et al., 2019).

Other types of cell death such as pyroptosis (Jia et al., 2019) and ferroptosis (Wang and Tang, 2019; Xiao et al., 2019a, Bai et al., 2020) have recently been implicated in oxLDL-induced endothelial cell dysfunction and atherosclerosis, although the detailed mechanisms involved remain unclear. Further elucidation of the precise role of each of these novel modes of cell death may yield new therapeutic targets for promoting endothelial cell survival under diseased conditions and ameliorating various types of endothelial dysfunction–related diseases (Fig. 2).

E. Endothelial Nitric Oxide Synthase Uncoupling

eNOS is well known for its role in catalyzing NO production, with L-arginine being the substrate and BH4, heme, flavin mononucleotide, flavin adenine dinucleotide, and NADH being the cofactors. eNOS uncoupling, characterized by the transition of eNOS from catalyzing NO production to generating superoxide anion ($O2\bullet^{-}$) accompanied by the "electron leak" to molecular oxygen, is another major cause of endothelial dysfunction (Karbach et al., 2014). eNOS uncoupling not only reduces NO production but also further exacerbates oxidative stress. Moreover, the superoxide anion may react with NO to form peroxynitrite anion ($ONOO^{-}$), which exhausts NO, reduces NO bioavailability, and contributes to endothelial dysfunction (Xu et al., 2016b; Daiber and Chlopicki, 2020) (Fig. 2).

The risk factors for eNOS uncoupling include tobacco smoking, hyperlipidemia, hypertension, hyperglycemia, hyperhomocysteinemia, ischemia/reperfusion, and oxidative stress; thus, eNOS uncoupling is a hallmark of various CVDs and conditions such as atherosclerosis, diabetes, stroke, cardiac hypertrophy, abdominal aortic aneurysm, aging, and pulmonary hypertension (Daiber and Chlopicki, 2020; Wu et al., 2021). Oxidative stress induced by 1-palmitoyl-2-(5′-oxo-valeroyl)-sn-glycero-3-phosphocholine, an oxidation product of oxLDL, can cause eNOS uncoupling and impair vasodilation by decreasing NO production while increasing superoxide anion generation (Yan et al., 2017a). Several mechanisms contribute to eNOS uncoupling, including BH4 deficiency, L-arginine deficiency, increased arginase activity, asymmetric dimethylarginine (ADMA) formation, oxidative disruption of the zinc-sulfur complex ($ZnCys_4$) of the eNOS dimer, phosphorylation of eNOS at Thr495 and Tyr657, and *S*-glutathionylation of eNOS (Daiber et al., 2019; Wu et al., 2021). The most important mechanism of eNOS uncoupling, BH4 deficiency, has been widely studied in CVD. On the one hand, GTP cyclohydrolase 1 (GCH1) is the rate-limiting enzyme in BH4 biosynthesis, and its downregulation or inhibition leads to eNOS uncoupling (Wu et al., 2021). On the other hand, dihydrofolate reductase (DHFR) in the salvage pathway of BH4 is critically implicated in preventing eNOS uncoupling (Crabtree et al., 2009). The phenotype of DHFR knockout mice includes hypertension and abdominal aortic aneurysm through eNOS uncoupling and mitochondrial dysfunction (Li et al., 2019c). Interestingly, in mice rendered hypertensive by chronic Ang-II infusion, and in bovine aortic endothelial cells treated with H_2O_2, DHFR deficiency could be rescued by overexpression of the cell cycle regulatory transcription factor E2F1 (E2 promoter binding factor 1) (Li et al., 2019a). Another transcription factor, KLF2, was shown to improve BH4 levels and *S*-glutathionylation of eNOS and appreciably diminish eNOS uncoupling in HUVECs subjected to hypoxia and reoxygenation injury (Wu et al., 2019). In addition, caveolae have been shown to promote eNOS coupling in the arteries of spontaneous hypertensive mice and HUVECs. In senescent endothelial cells, NOX4 diminishes eNOS coupling via inducing dissociation of eNOS and HSP90 (Lee et al., 2017), whereas heme oxygenase-1, which generates carbon monoxide, was shown to be essential for maintaining eNOS coupling status (Luo et al., 2018).

Because of the critical involvement of eNOS uncoupling in the pathogenesis of CVD, some therapeutic strategies have aimed to reverse or prevent eNOS uncoupling. In newborn pigs with pulmonary hypertension, BH4 oral therapy alone or with L-citrulline ameliorated pulmonary hypertension by recoupling eNOS and improving NO signaling (Dikalova et al., 2016, 2020). In the hypertensive models, *Lactobacillus fermentum* (Toral et al., 2018), the natural compound icariin (Long et al., 2018), or folate (BH4 replacement therapy) (Gao et al., 2009) reduces hypertension through recoupling eNOS. In addition to these animal studies, several clinical trials have been performed with BH4 therapy in patients with pulmonary arterial hypertension (National Institutes of Health clinical trials number NCT00435331), systemic hypertension (National Institutes of Health clinical trial number NCT00325962) and hypercholesterolemia (Cosentino et al., 2008). Of interest, BH4 administration could improve the brachial artery flow–mediated dilation in chronic smokers (Taylor et al., 2016).

Since eNOS recoupling or prevention of eNOS uncoupling represents an additional mechanism to preserve eNOS activity and increase NO bioavailability, investigation of novel transcriptional, post-transcriptional, and epigenetic regulators of the expression of key enzymes involved in regulating eNOS coupling and uncoupling is warranted. It can also be envisaged that addition of eNOS uncoupling inhibitors to existing therapies may deliver additional therapeutic gains.

F. Endothelial-to-Mesenchymal Transition

Endothelial-to-mesenchymal transition (EndoMT) (Souilhol et al., 2018) is another root for endothelial dysfunction in which endothelial cells lose endothelial characteristics but acquire mesenchymal-like morphology and gene expression patterns (Chen and Simons, 2016), such as the expression of fibroblast-specific protein 1 (FSP1), and α-smooth muscle actin (α-SMA) (Cho et al., 2018) (Fig. 2). EndoMT resembles the process of epithelial-to-mesenchymal transition and fibroblast-to-myofibroblast differentiation, precipitating cellular phenotypic changes and tissue damage. During EndoMT, endothelial cells lose their polarity and remodel endothelial cell-cell junctions that cause endothelial dysfunction. EndoMT is driven by TGF-β/Smad signaling, hypoxia, chronic inflammation, oxidized lipids, hyperglycemia, and ROS production (Evrard et al., 2016). During inflammation, EndoMT leads to endothelial dysfunction, and exposure of endothelial cells to inflammatory stimuli, such as IL-1β, TGF-β, and TNF-α, alone or in combination, converts endothelial cells to mesenchymal-like cells via the EndoMT process (Good et al., 2015; Rieder et al., 2011). Previous studies have suggested that inflammation governs EndoMT by two signaling pathways: one is the TGF-β pathway, and the other is the non–TGF-β pathway (Dejana et al., 2017). TGF-β can increase the expression level of transcription factors such as zinc finger E-box homeobox 1, Smads, Snail, and Slug. Activation of these transcription factors promotes the expression of mesenchymal markers—for example, smooth muscle protein 22α, α-SMA, collagen 1A1, vimentin, fibronectin, matrix metalloproteinase (MMP)-2, MMP-9, and FSP1 (Gonzalez and Medici, 2014; Pérez et al., 2017).

EndoMT serves as the link between atherosclerosis initiating factors such as tissue remodeling, inflammation, and disturbed blood flow and plaque formation (Chen and Simons, 2016). The initial step is the loss of protective endothelial fibroblast growth factor (FGF) signaling, which enables activation of the endothelial TGF-β signaling pathway and the induction of EndoMT (Chen and Simons, 2016). Indeed, there are a number of tissue remodeling processes that are influenced by the balance in the mutually antagonistic effects of FGF and TGF-β growth factor families (Schuliga et al., 2013). Once induced, EndoMT stimulates plaque growth and drives atherosclerosis progression (Chen et al., 2015). Moreover, EndoMT-derived fibroblasts are associated with an unstable plaque phenotype in atherosclerosis. Rupture of unstable atherosclerotic plaques and the ensuing thrombosis are the major clinical sequelae pathologic causes of CVD (Evrard et al., 2016; Li et al., 2017a). Conditioned media from endothelial cells undergoing EndoMT modulate macrophage phenotype in atherosclerosis, protect against oxLDL-induced macrophage inflammation (including TNF-α production and decreased expression of antigen-presenting cell molecules), and foster scavenger receptor–mediated oxLDL uptake (Helmke et al., 2019). This finding adds a new element to the complexity of multicellular interactions in the atherogenic context.

Since EndoMT plays a fundamental role in the development of many types of CVD, inhibiting EndoMT-dependent signaling pathways or targeting transcription factors responsible for EndoMT may hold therapeutic promise for treating these diseases. In light of the heterogeneous nature of endothelial cells, the technologies of lineage tracing and single-cell RNA-sequencing will facilitate the discovery of further novel roles of EndoMT in CVD and assist in identification of the heterogeneous role of endothelial cells in regulating tissue damage. Also, it is of interest to determine whether vasodilator agents such as NO and PGI_2 can improve endothelial function via modulating EndoMT.

G. Endothelial Cell Senescence

Aging represents a major risk factor for endothelial dysfunction and CVD. Senescence in endothelial cells is an initial step in a series of events that will culminate with the development and progression of CVD and other endothelial dysfunction–associated diseases (Fig. 2) (Minamino et al., 2002; van der Feen et al., 2020). Endothelial cell senescence is a biologic event that can damage the vascular endothelium and is triggered by atherogenic stimuli such as doxorubicin (Hwang et al., 2020), H_2O_2 (Liu et al., 2014), TNF-α (Khan et al., 2017), progerin (Bidault et al., 2020), high glucose (Shosha et al., 2018), native LDL (Oh et al., 2017), oxLDL (Jiang et al., 2020b), and some anti–human immunodeficiency virus drugs (lopinavir/ritonavir) (Auclair et al., 2014). The heightened status of oxidative stress and chronic inflammation within the endothelium accelerates the process of endothelial senescence, impacting the biologic functions of endothelial cells and favoring the development of CVD. Morphologically, when endothelial cells become senescent, they typically acquire a flattened and enlarged morphology. At the phenotypic level, senescent endothelial cells show prominent decline in DNA replication [evidenced by decreased markers of bromodeoxyuridine (BrdU), Ki-67, proliferating cell nuclear antigen (PCNA)], telomere shortening, upregulation of senescence-associated β-galactosidase activity, and cell cycle arrest (cyclin D1, p16, p21, p53). Senescent endothelial cells exhibit increased secretion of growth factors

(VEGF), proinflammatory mediators (IL-6, IL-8, TNF-α), C-C motif chemokine ligands (CCLs), and MMPs (termed as senescence-associated secretory phenotype) (Schafer et al., 2020). A number of abnormal molecules or molecular pathways are associated with these underlying pathophysiological changes, including SIRT1 (Guo et al., 2016), SIRT6 (Liu et al., 2014), Klotho (Romero et al., 2019), FGF21 (Yan et al., 2017c), and heme oxygenase-1 (Luo et al., 2018). Since the "inflamm-aging" of endothelial cells contributes to endothelial dysfunction and CVD, therapies such as resveratrol (Du et al., 2019), rapamycin (Sasaki et al., 2020), chlorogenic acid (Hada et al., 2020), and senolytic therapies [B-cell lymphoma 2 inhibitor ABT263 (Zhu et al., 2016), quercetin/dasatinib cocktail (Roos et al., 2016)] improve endothelium-dependent vasodilation and endothelial dysfunction in hypercholesterolemic conditions.

The importance of maintaining healthy and young endothelial cells is articulated in the famous saying of Dr. Thomas Sydenham: "Man is as old as his arteries." Being one of the most important cell types in human blood vessels, endothelial cells are the first-line gatekeepers of vascular health. Future discovery of endothelial enriched longevity genes or endothelial cell senescence–targeted therapeutic drugs or senolytics is promising to preserve endothelial health and maintain vascular functions.

To provide a snapshot of the triggers and features of endothelial dysfunction, we summarized currently known major contributors to endothelial dysfunction in Figs. 2 and 3.

IV. Clinical Assessment of Endothelial Function and Dysfunction

In some domains, endothelial dysfunction has drifted from the research environment into the clinical lexicon (Davignon and Ganz, 2004). Functional assessment of the endothelium includes analysis of the responsiveness of endothelial cells to stimulate vasodilation or vasoconstriction. The methods include flow-mediated dilation (FMD) in brachial artery, venous occlusion plethysmography, and pulse wave velocity (Sandoo et al., 2010). Evaluation of endothelial function has been proposed as a method with potential clinical applicability as a risk factor in the identification of cardiovascular complications, even in asymptomatic patients. Furthermore, measurement of FMD can stratify individuals at low, medium, or high risk of potential future cardiometabolic events.

Other tests for assessing endothelial dysfunction include the measurement of circulating biomarkers that reflect the occurrence of pathogenic events in the

Fig. 4. Endothelial dysfunction in CVD and beyond. Endothelial dysfunction is generally associated with many disease conditions due to imbalanced production of vasodilators and vasoconstrictors, or many other factors. Major disease conditions associated with endothelial dysfunction include atherosclerosis, hypertension and stroke, diabetes, peripheral arterial disease, metabolic syndrome (obesity, insulin resistance), chronic kidney disease, pre-eclampsia, recently reported COVID-19, and many others (not depicted here).

cardiovascular system, such as inflammation-related cytokine secretion (IL-1β and IL-6) or the secretion of cell surface induced adhesion molecules (E-selectin and VCAM-1), together with measurement of systemic indicators of inflammation (high-sensitivity c-reactive protein). These biomarkers have been found to be elevated in both coronary and peripheral atherosclerotic lesions, as well as in other CVD-related inflammatory conditions. These blood-based analyses of biomarkers are complemented by the emergence of new imaging strategies to evaluate the clinical significance of lesions in patients with atherosclerosis at a given time (Fernández-Friera et al., 2014; Libby et al., 2010). The use of state-of-the-art imaging technology to locate disease-causing events in the arteries (expression of VCAM-1) can allow for the identification of unstable plaques before they transition to clinical events (Rader and Daugherty, 2008).

Taken together, the gold standard for clinical evaluation of endothelial function is FMD in response to acetylcholine (Goligorsky, 2006; Flammer et al., 2012). There is a Food and Drug Administration–approved device to measure FMD in human subjects, but as yet, endothelial dysfunction in most settings has not emerged as a bone fide diagnosis or a treatment target in its own right. Nevertheless, it is possible that this might occur in the future if a stronger and more direct mechanistic association with atherosclerosis can be established (Ellins and Halcox, 2011). Furthermore, new noninvasive methods for extracting information about endothelial function from experimental studies to human patients are required.

V. Endothelial Dysfunction in Cardiovascular Disease and Beyond

Dysfunctional status of endothelial cells causes endothelial dysfunction, which is the cause of many types of CVD and related complications (Fig. 4). In the next section, we will provide an overview of diseases associated with endothelial dysfunction, with an aim of identifying novel therapeutic targets and providing a guide to future targeted drug discovery.

A. Atherosclerosis

Atherosclerosis is characterized by chronic inflammation and lipid accumulation within the arteries, ultimately restricting blood flow to the artery and leading to heart attack and ischemic stroke (Libby et al., 2019).

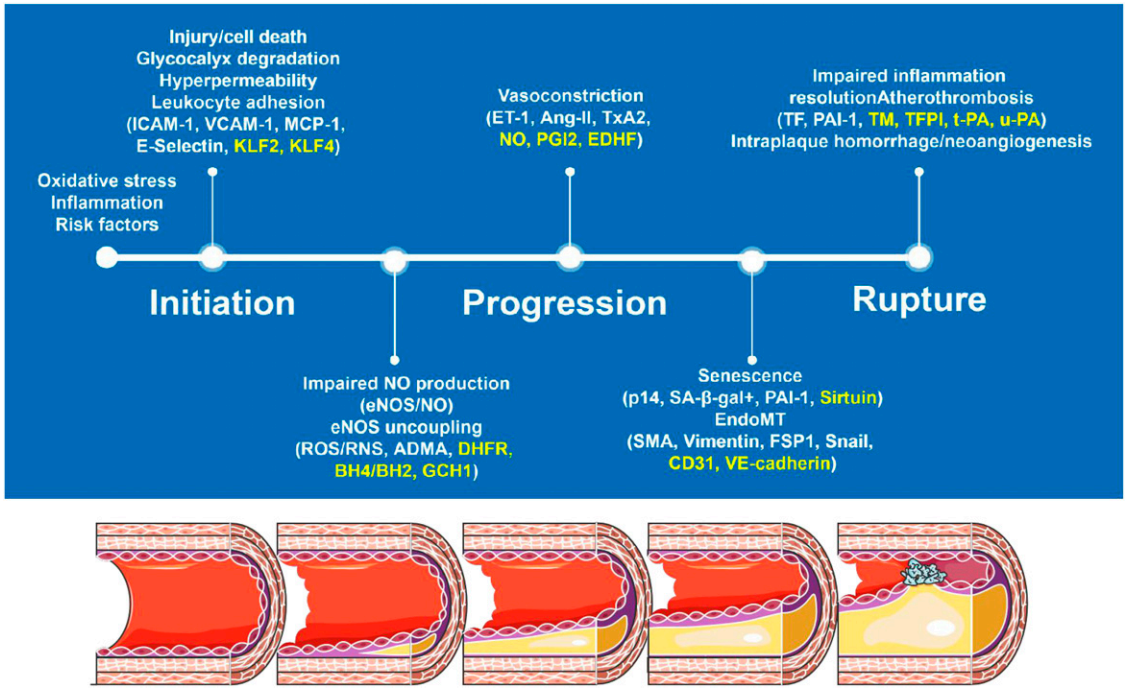

Fig. 5. Role of endothelial dysfunction in atherosclerosis. Oxidative stress, inflammation, and other risk factors play an important role in endothelial cell activation. The resulting endothelial cell injury/multiple modes of cell death, hyperpermeability, and increased expression of various adhesion molecules (such as ICAM-1, VCAM-1, MCP-1, E-selectin) and KLF2 downregulation attract leukocyte adherence to the activated endothelium and penetrate the arterial wall to initiate atherosclerosis. A reduction in endothelium-derived NO production or increased eNOS uncoupling [increased ROS, reactive nitrogen species (RNS), and ADMA and decreased DHFR, BH4/dihydrobiopterin (BH2), and GCH1] occurs as one major cause of endothelial phenotypical alterations presented as endothelium-dependent vasoconstriction [increased levels of ET-1, Ang-II, and TxA_2 and decreased levels of NO, PGI_2, and endothelium-derived hyperpolarizing factor (EDHF)] and leads to the progression of atherosclerosis. Atherorelevant stimuli also cause endothelial cell senescence (evidenced by increased senescence-associated β-galactosidase (β-gal) staining and PAI-1 secretion and decreased activity of sirtuins) and EndoMT [evidenced by increased markers of mesenchymal cells (α-SMA, vimentin, FSP1, and Snail, etc.] and decreased endothelial markers (CD31 and VE-cadherin). In addition, the exposure of endothelial cells to persistent proatherogenic stimuli further causes impaired inflammation resolution, atherothrombosis, and intraplaque hemorrhage, which lead to plaque rupture. Genes in yellow denotes that the expression of these genes was downregulated during atheroprogression. SA-β-gal, senescence-associated beta-galactosidase; TFPI, tissue factor pathway inhibitor; TM, thrombomodulin; tPA, tissue plasminogen activator; uPA, urokinase-type plasminogen activator.

Many studies have demonstrated that abnormal lipid metabolism, inflammatory responses, and hemodynamic alterations are critical players in the occurrence of endothelial dysfunction and atherosclerosis (Tabas et al., 2015). After exposure to atherogenic insults originating from oxLDL (and its atherogenic components), cholesterol crystals, inflammatory stimuli, and disturbed blood flow, endothelial cells become activated. A reduction in endothelium-derived NO is one cause of endothelial phenotypical alterations presenting as impaired endothelium-dependent vasodilation. Once activated, endothelial cells express various adhesion molecules, such as ICAM-1, MCP-1, VCAM-1, P-selectin, and E-selectin, which attract neutrophils and monocytes that attach to the activated endothelial cells and penetrate the arterial wall (Chistiakov et al., 2018). The monocytes will then differentiate into macrophages within the vessel wall and engulf oxLDL to become foam cells, which are a histopathological hallmark of atherosclerosis. Exposure of endothelial cells to various stimuli causes oxidative stress and further amplifies the vicious cycle of vascular inflammation and atherosclerosis. In addition, other types of immune cells, such as mast cells, B cells, T cells, and dendritic cells, are also involved in atherogenesis. In advanced stages of atherosclerosis, increased endothelial dysfunction evidenced by the increased platelet/endothelium interactions will render the plaques unstable and vulnerable to rupture as a result of overproduction of prothrombotic molecules (such as PAI-1 and thrombin) and ineffective antiaggregatory, antithrombotic, and fibrinolytic functions and inflammation resolution (Rajendran et al., 2013). The disruption of endothelial glycocalyx microstructure also contributes to the initiation and development of atherosclerotic plaques by regulating mechanotransduction as well as vascular tone and stability. The role of endothelial dysfunction in atherosclerosis is summarized in Figs. 4 and 5.

B. Hypertension

Endothelial dysfunction also contributes to hypertension via at least five mechanisms: 1) increased aortic stiffness, 2) altered vascular tone (imbalanced production of vasoconstrictor/vasodilator factors), 3) increased oxidative and nitrosative stress, 4) increased inflammatory responses, and 5) increased EndoMT. Lowering blood pressure with angiotensin II receptor blockers (ARBs) or angiotensin-converting enzyme inhibitors (ACEIs), but not with β-blockers, improves endothelial function, suggesting that Ang-II per se, rather than the biomechanical impacts of elevated pressure, is responsible for endothelial dysfunction. It is also likely that ET-1 overproduction plays a role in hypertension. For example, in pulmonary hypertension, increased ET-1 plasma levels were found in both human patients and in an animal model of pulmonary hypertension. The role of ET-1 is established by the clinical benefit in pulmonary hypertension of bosentan, an antagonist of ET-1 receptors A and B (Rajendran et al., 2013).

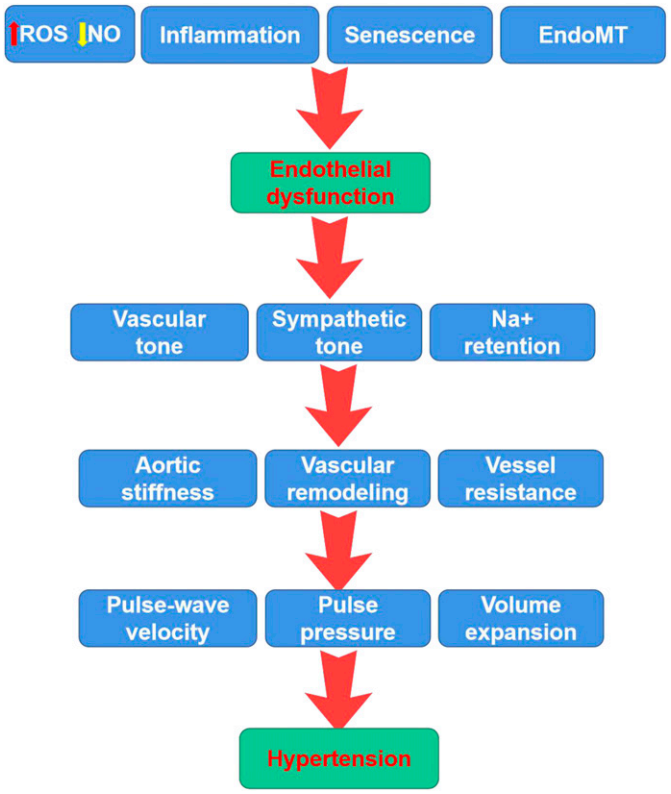

Fig. 6. Role of endothelial dysfunction in hypertension. The vascular endothelium dysfunction resulting from ROS generation, decreased NO bioavailability, inflammation, senescence, and EndoMT and may cause hypertension. Endothelial dysfunction contributes to hypertension via different mechanisms, including altered vascular tone, sympathetic tone, and Na^+ retention, which is followed by increased aortic stiffness, vascular remodeling, and vessel resistance; these events collectively lead to hypertension. Measurement of pulse wave velocity, pulse pressure, or volume expansion are frequently used as clinical assessments of the impact of chronic hypertension.

Collectively, research in the past decade has refined our understanding of the role of endothelial dysfunction in hypertension. Novel elements beyond vascular tone (such as EndoMT) are becoming better appreciated, which is crucial for devising novel therapies targeting endothelial dysfunction in hypertension. The important role of endothelial dysfunction in hypertension underscores the clinical utility of antihypertensive drugs in ameliorating endothelial dysfunction–associated diseases (Figs. 4 and 6).

C. Diabetes

Endothelial dysfunction is also associated with diabetes in animals and human patients (Fig. 4). The major forms of diabetes are type 1 diabetes, type 2 diabetes, and gestational diabetes mellitus (Colman et al., 1999). Type 1 diabetes is considered as an autoimmune disease characterized by attack of pancreatic β cells by immune cells. Type 2 diabetes is an insidious driver of CVD because the individual

multipathophysiological components, which are secondary to obesity, all have actions to worsen the development of atherosclerosis and precipitate ischemic CVD and that lead to heart attacks, strokes, and lower limb amputations (Grundy et al., 1999). Gestational diabetes mellitus is assessed as glucose intolerance during midlate pregnancy, and it occurs as a result of hormone-induced insulin resistance (Anastasiou et al., 1998; Gray et al., 2017a).

Multiple features of diabetes, including insulin resistance, impact pathologically on the endothelium, causing overt endothelial dysfunction and accelerating atherosclerosis (Nigro et al., 2006). Hyperglycemia, per se, is associated with elevated oxidative stress and inflammation, which is a biochemically hostile environment for NO and hence causes reduced vasodilator capacity and endothelial dysfunction (Sobrevia and Mann, 1997). Other factors that promote endothelial dysfunction under diabetic conditions include decreased BH4 bioavailability, increased ROS production and eNOS uncoupling, elevated glycation and expression of RAGE, increased asymmetric dimethyl arginine, increased arginase, NF-κB activation (Lee et al., 2013), inflammation, and reduced adiponectin secretion in perivascular adipose tissues (Sena et al., 2017).

D. Chronic Kidney Disease

Chronic kidney disease is a major complication of patients with diabetes and a driver of endothelial dysfunction. In a general sense, blood flow (and pressure) is a major determinant of renal function and hence glomerular filtration rate, so disturbance of blood flow associated with endothelial dysfunction impacts directly on the kidney (Fig. 4). However, the structure of the endothelium within the kidney is special in that the endothelial cells display fenestrations, which are round or ovoid transcytoplasmic holes specialized in filtration across the glomerular capillary wall (Satchell and Braet, 2009; Jourde-Chiche et al., 2019). Fenestrations and the associated glycocalyx are important in restricting protein passage from the blood to the urine (proteinuria). Regulation of fenestrations has not been comprehensively characterized because it is a difficult area to study. The most common occurrence of endothelial dysfunction in the context of deranged fenestrations occurs in pre-eclampsia. Interestingly, this condition is

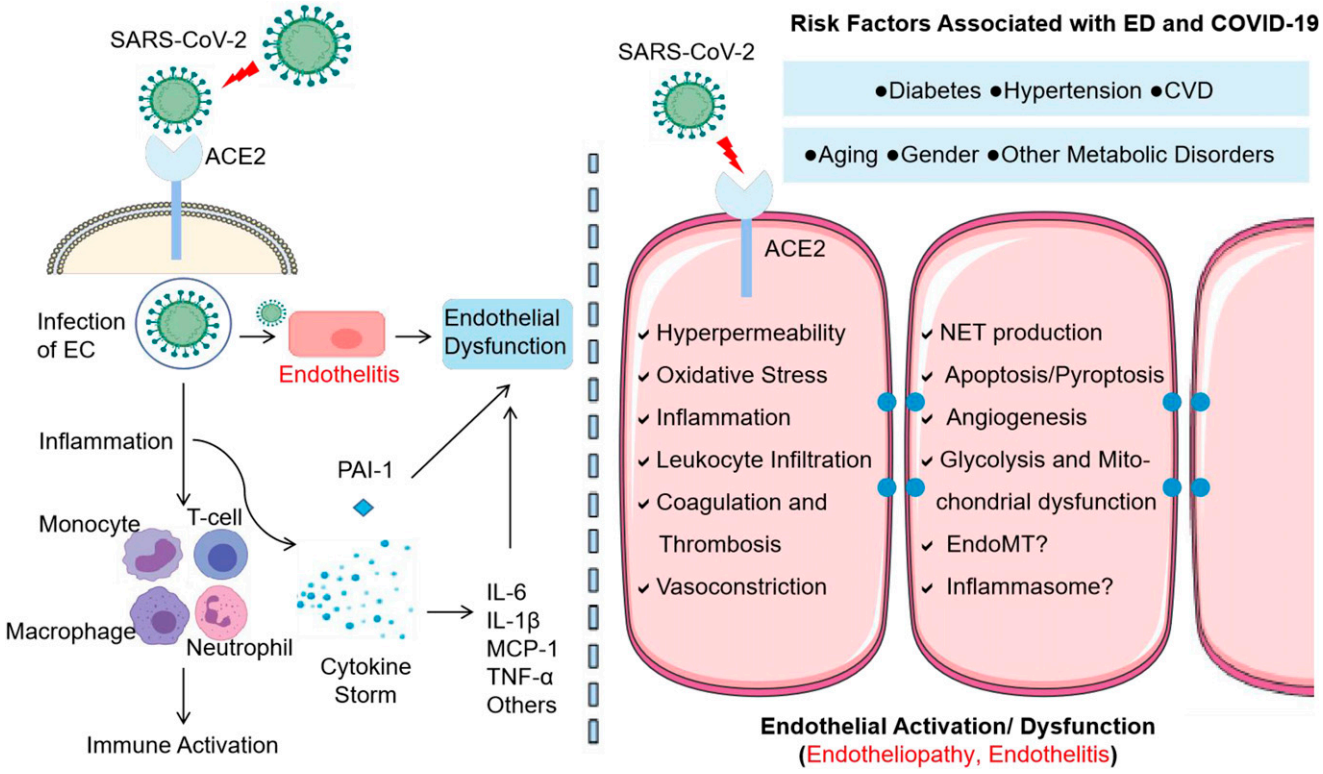

Fig. 7. Endothelial dysfunction caused by SARS-CoV-2 infection. SARS-CoV-2 enters host cells by binding to ACE2 on the cell membrane (left), which is subsequently endocytosed, potentially causing endothelitis and endothelial dysfunction. Virus infection leads to immune activation and increases the production of proinflammatory cytokines (accompanying cytokine storm), in particular IL-6, TNF-α, IL-1β, MCP-1, and PAI-1, that ultimately damage endothelial cells. Cytokine release and immune cell activation after SARS-CoV-2 infection lead to endothelial activation at multiple layers, including hyperpermeability, oxidative stress, inflammation, leukocyte infiltration, coagulation and thrombosis, vasoconstriction, NET production, apoptosis/pyroptosis, and abnormal angiogenesis. Moreover, because of the crosstalk among different layers of endothelial dysfunction, it is plausible that abnormal endothelial cell metabolism (glycolysis, fatty acid oxidation, oxidative phosphorylation, etc.), EndoMT, and inflammasome activation may also be related to endothelial dysfunction in COVID-19. Several known risk factors associated with endothelial dysfunction in COVID-19 include aging, male gender, other metabolic disorders, and comorbidities like diabetes, hypertension, and CVD. EC, endothelial cells; ED, endothelial dysfunction.

associated with reduced glomerular filtration rate accompanied by proteinuria, indicating the breakdown of the glycocalyx in the fenestrations, allowing the passage of proteins into the urine (Lafayette et al., 1998). Various drugs impact renal endothelial fenestrations. The calcium channel blocker nifedipine, for example, is used for the treatment of chronic kidney disease because of its actions both systemically and specifically with regard to its role to prevent or improve the functioning of the glomerular endothelium (Ishizawa et al., 2009).

E. Coronavirus Disease 2019

COVID-19 is an infectious disease that involves endothelial dysfunction prominent in its pathophysiology (Figs. 4 and 7). Both the micro- and macrovasculature are target sites of COVID-19, as evidenced by direct infection of the endothelium with SARS-CoV-2 virus, as well as the secondary effect due to a systemic inflammatory cytokine storm. There is accumulating evidence to indicate an interaction between SARS-CoV-2 infection and endothelial cell dysfunction, including 1) endothelial cell expression of the ACE2 receptor for SARS-CoV-2 (Hamming et al., 2004); 2) occurrence of Kawasaki disease–like symptoms (coronary vasculitis) in infant patients with COVID-19 (Toubiana et al., 2020; Viner and Whittaker, 2020); 3) evidence of endothelial infection with SARS-CoV-2 in patients with fatal COVID-19 (Evans et al., 2020); 4) impairment in COVID-19 of NO interference with the viral cellular entry via disrupting S-protein interaction with host ACE2 receptor (Green, 2020); 5) neutrophil extracellular trap (NET) production inside the microvessels in patients with severe COVID-19, with the aggregation of NETs leading to endothelial damage (Leppkes et al., 2020); 6) prevalence of systemic hyperpermeability and capillary leak syndrome in patients with COVID-19 (Case et al., 2020); 7) increased serum level of ICAM-1, VCAM-1, and vascular adhesion protein-1 in patients with COVID-19 (Tong et al., 2020); 8) intravascular coagulation, enhanced thrombosis, and D-dimer observed in patients with COVID-19, reflecting endothelial cell dysfunction (Ackermann et al., 2020; Klok et al., 2020); 9) spike glycoprotein on SARS-CoV2 causing endothelial cell dysfunction, evidenced by mitochondrial dysfunction, eNOS downregulation, and increased glycolysis (Y. Lei et al., preprint, DOI: 10.1101/2020.12.04.409144); 10) association of old age, male gender, and comorbidities (diabetes, hypertension, and CVD) with both endothelial dysfunction and COVID-19; 11) cytokine storm, cytokine release syndrome (IL-1β, IL-6, IL-8, and MCP-1), and PAI-1 overproduction may link endothelial dysfunction to COVID-19 (Wang et al., 2020b; Sims et al., 2021); 12) profound angiogenesis, coagulopathy, and thrombosis occurs in patients with severe COVID-19 (Ackermann

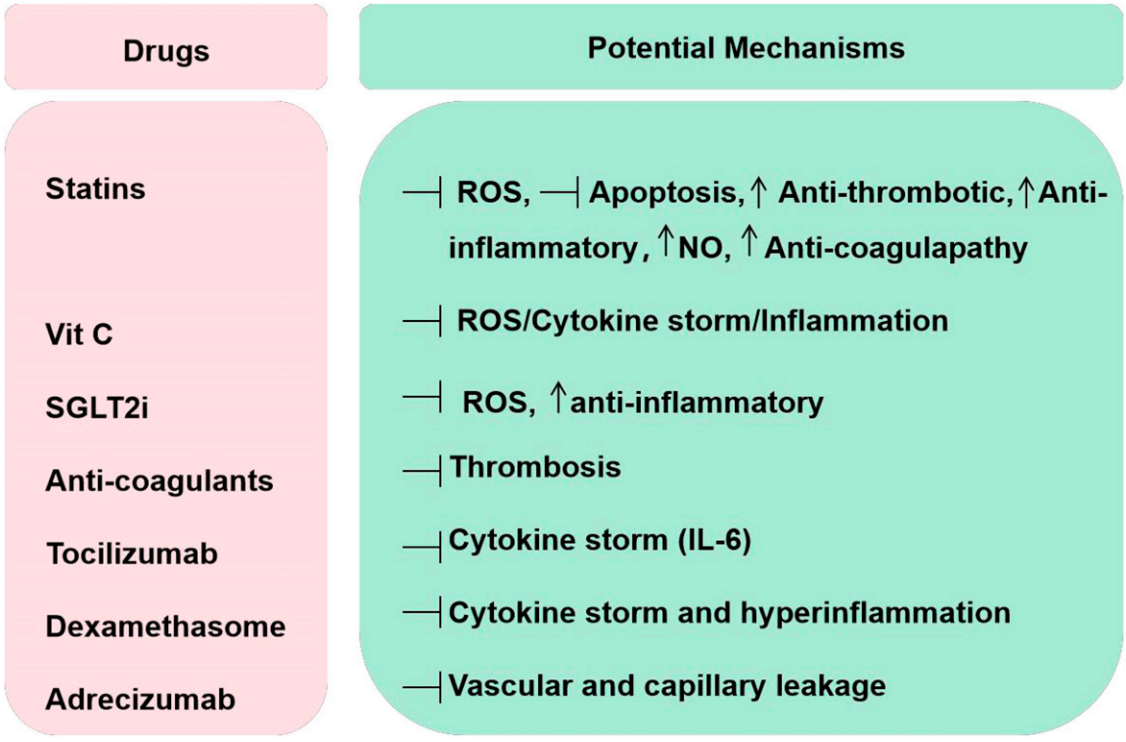

Fig. 8. Potential therapies for COVID-19–induced endothelial dysfunction. SARS-CoV-2 infection with endothelial cells causes a myriad of events, including apoptosis, coagulation/thrombosis, inflammation and vascular leakage. Several drugs that exhibit protective effects against endothelial dysfunction in COVID-19 include statins, vitamin C (Vit C), anticoagulants, tocilizumab, dexamethasone, and SGLT2i (function mechanistically by inhibiting ROS, apoptosis, cytokine storm, inflammation, and thrombosis). Adrecizumab may exert its effects via inhibition of vascular and capillary leakage during COVID-19.

et al., 2020; Katneni et al., 2020; Pine et al., 2020); 13) persistent endothelial cell injury, as evidenced by increased P-selectin marker and glycocalyx degradation in patients with COVID-19 (Fraser et al., 2020); and 14) alterations of plasma-derived extracellular vesicles of patients with COVID-19 with elevated endothelial injury factors such as vWF, TF, and proinflammatory and coagulopathy factors (B. Krishnamachary et al., preprint, DOI: 10.1101/2020.08.27.20182808). In light of the important role of inflammation in propagating NLRP3 inflammasome, EndoMT, and altered endothelial cell metabolism, it is plausible that these mechanisms may play a role in COVID-19–associated endothelial dysfunction.

These events may all lead to microvascular and endothelial dysfunction, plaque instability, myocardial injury, and infarction, as well as multiple organ failure, in patients with COVID-19, propelling investigators to deduce that COVID-19 is clinically a disease of the endothelium (Libby and Lüscher, 2020). These events also highlight the importance of stabilizing the vascular endothelium in COVID-19 (Fig. 7). Several ongoing studies are evaluating the adjuvant role of potential therapeutics for ameliorating endothelial dysfunction in COVID-19. For example, statins (Zhang et al., 2020b), a high dose of intravenous vitamin C (Liu et al., 2020a), sodium-glucose cotransporter 2 inhibitors (SGLT2i) (Das and Dutta, 2020), and renin-angiotensin-aldosterone system inhibitors (Gao et al., 2020a) have been reported to decrease the mortality rate of patients with COVID-19 via reducing CVD comorbidities. Other emerging drugs with therapeutic potential include anticoagulant drugs (Paar et al., 2021), dexamethasone (Horby et al., 2021), canakinumab (an anti–IL-1β monoclonal antibody) (Ucciferri et al., 2020), adrecizumab (an anti-adrenomedullin antibody) (Karakas et al., 2020), and NLRP3 inflammasome inhibitions (Zhou et al., 2020b), which target hyperinflammation syndrome, the cytokine storm, and pyroptosis in COVID-19 (Fig. 8).

From our perspective, designing therapies aimed to improve endothelial function and/or preventing endothelial dysfunction or by targeting the viral host factors (ACE2) could help to improve patient outcomes from this disease. It is also of interest to examine whether currently known effective anti–COVID-19 drugs are able to reverse SARS-CoV2 infection–induced endothelial dysfunction, which may provide supporting evidence to explain their clinical utility. For a detailed review of endothelial dysfunction in COVID-19, readers are referred to excellent recent reviews published elsewhere (Bermejo-Martin et al., 2020; Evans et al., 2020; Nägele et al., 2020; Teuwen et al., 2020).

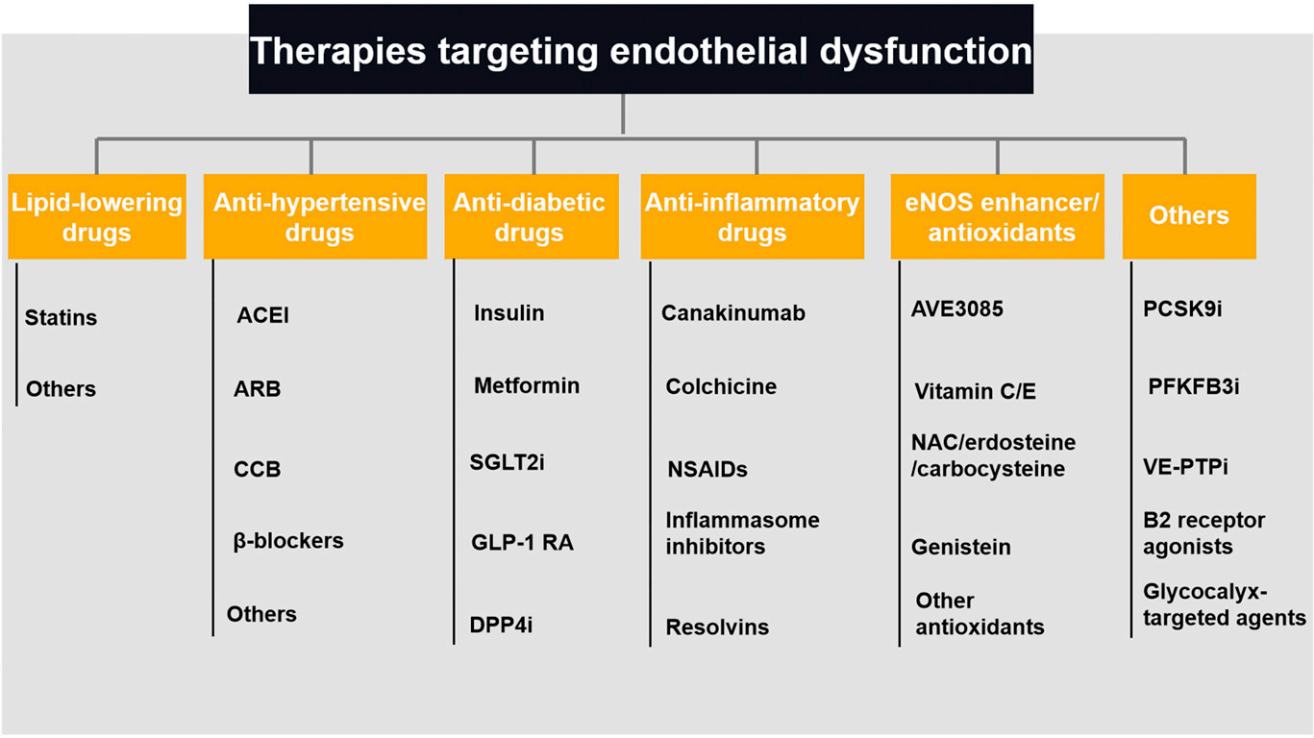

Fig. 9. Pharmacotherapies of endothelial dysfunction. There are different pharmacotherapies that target endothelial dysfunction, such as lipid-lowering drugs (statins); antihypertensive drugs (including ACEI, ARB, CCB, and β-blockers); anti-diabetic drugs [insulin, metformin, SGLT2i, GLP-1 receptor agonists (GLP-1RA), DPP-4 inhibitors (DPP-4i)]; anti-inflammatory drugs [canakinumab, colchicine, inflammasome inhibitors, nonsteroidal anti-inflammatory drugs (NSAIDs), resolvins]; eNOS enhancer (AVE3085)/antioxidants (vitamin C/E, NAC, erdosteine, carbocysteine and genistein etc); and some other therapies, including PCSK9 inhibitors, IL-1β mAb, PFKFB3 inhibitors, VE-PTP inhibitors, glycocalyx-targeted agents, and many others.

F. Other Diseases

In addition to the diseases reviewed above, endothelial dysfunction is also associated with other disease conditions such as obesity and insulin resistance (Caballero, 2003; Engin, 2017), acute lung injury (Huertas et al., 2018), erectile dysfunction (Elesber et al., 2006; Cui et al., 2017), sepsis (Boisramé-Helms et al., 2013; Ince et al., 2016), and peripheral arterial disease (Igari et al., 2016) (Fig. 4). The importance of endothelium in various tissues/organs underscores the rationale for treating human panvascular diseases by targeting endothelial dysfunction (Ge and Wang, 2018).

VI. Pharmacotherapies for Endothelial Dysfunction

Evidence from experimental, clinical, and translational studies has demonstrated that clinically used drugs and drug candidates with different structures and mechanisms of action can ameliorate multiple aspects of endothelial dysfunction (Fig. 9). Most of these drugs have shown promising cardiovascular protective effects in preclinical and clinical studies.

A. Lipid-Lowering Statins

Statins, pharmacological inhibitors of hydroxymethylglutaryl-coenzyme A reductase, are first-line pharmaceutical agents to treat hypercholesterolemia and CVD. Statins have both lipid-lowering effects and additional cholesterol-independent or pleiotropic effects (Oesterle et al., 2017). The cardiovascular protective actions of statins include the improvement of endothelial function; stabilization of vulnerable plaques; and antioxidant, anti-inflammatory, and antithrombotic effects (Oesterle et al., 2017).

Mechanistic investigations revealed that the following mechanisms were responsible for statin-mediated benefits (Ii and Losordo, 2007): 1) LDL-lowering effect; 2) protective effects against oxLDL (Hofnagel et al., 2007); 3) increased NO bioavailability by promoting eNOS phosphorylation at Ser1177 via the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway (Kureishi et al., 2000); promoting agonist-stimulated eNOS-HSP90 interaction (Brouet et al., 2001); and recoupling eNOS by increasing vascular BH4 level (Zhang et al., 2012), reducing vascular NOX-dependent O_2^- production, and increasing GCH1 expression and activity (Zhang et al., 2012). Furthermore, statins upregulate eNOS expression via increasing eNOS mRNA stability. Statins increase eNOS mRNA polyadenylation through Rho-mediated changes in the actin cytoskeleton (Kosmidou et al., 2007) and regulate eNOS gene transcription by upregulating KLF2 (the master regulator of vascular homeostasis) through inhibition of protein geranylgeranylation (Parmar et al., 2005) and activation of the MEF2 (myocyte enhancer factor 2) pathway (Sen-Banerjee et al., 2005). Overall, statin-mediated elevation of eNOS expression may be responsible for their long-term effects on endothelial function; 4) statins also exert anti-inflammatory effects by blocking NF-κB–dependent expression of proinflammatory mediators (IL-1β, IL-6, ICAM-1, and VCAM-1) (Hölschermann et al., 2006; Greenwood and Mason, 2007). The anti-inflammatory effects of statins contribute to statin-mediated inhibition of leukocyte adherence to activated endothelium; 5) statins have antiapoptotic effects; rosuvastatin prevents $CoCl_2$-induced apoptosis of human coronary artery endothelial cells by activating the Janus kinase 2/signal transducer and activator of transcription 3 (STAT3) signaling pathway (Geng et al., 2020; Wang et al., 2020c); 6) statins exert protective effects against endoplasmic reticulum (ER) stress (Geng et al., 2020). A recent high-throughput screening study has shown that various statins can ameliorate tunicamycin-induced ER stress in human coronary artery endothelial cells (Haas et al., 2020); 7) statins exert inhibitory effects on EndoMT. Recent studies demonstrated that atorvastatin suppressed the production of connective tissue growth factor and EndoMT via inducing KLF4/miR-483 axis in endothelial cells exposed to serum from patients with Kawasaki disease (He et al., 2017). In addition, lovastatin suppressed high-glucose–induced oxidative stress, EndoMT, and TGF-β1 signaling in glomerular endothelial cells, underlying its protective effects against diabetic nephropathy (Ma et al., 2017). Furthermore, similar to atheroprotective laminar flow, statins can mitigate EndoMT induced by oscillatory shear stress (Lai et al., 2018). Recent studies have shown that statins have prominent epigenetic modulatory effects by affecting DNA methylation and histone modification, thereby regulating endothelial gene expression and function (Allen and Mamotte, 2017). In addition, statins regulate anti-inflammatory responses in endothelial cells by upregulating long noncoding RNA MANTIS expression (Leisegang et al., 2019).

Together, the cardiovascular protective actions of statins on endothelial function are multifaceted. Beneficial transcriptional, post-transcriptional, and epigenetic regulation of endothelial gene expression may contribute to the overall antiatherogenic and endothelial cell protective actions of statins. Further dissection of the transcriptomic program mediated by statins is required to reveal novel therapeutic targets for treating CVD.

B. Antihypertensive Drugs

Several key components of the renin-angiotensin-aldosterone system, such as Ang-II and its type 1 receptor (AT1R), are upregulated in atherosclerotic vessels. Ang-II promotes endothelial dysfunction via activating AT1R-dependent NOX, the expression of which is upregulated in vitro by increased levels of LDL. Antihypertensive drugs such as ARBs, calcium channel blockers (CCBs), and ACEI have shown

multiple protective actions in ameliorating endothelial dysfunction–associated diseases. It is well established that ARBs, CCBs, and ACEIs can improve endothelial function in patients with CVD and increase eNOS expression, enhance eNOS phosphorylation at Ser1177, decrease NOX expression, augment GCH1-dependent vascular BH4 levels, and recouple eNOS to increase NO bioavailability (Silva et al., 2019). Other protective effects of ARBs, CCBs, and ACEIs are related to decreasing Ang-II levels and Ang-II receptor signaling, concurrent with bradykinin accumulation. ACEI and ARBs also attenuate ROS production and cyclooxygenase 2 (COX-2)–derived vasoconstrictor generation, contributing to pleiotropic endothelial protective actions. ARBs, CCBs, and ACEIs also exert protective effects by indirect antioxidant effects by enhancing the activity of extracellular superoxide dismutase (superoxide dismutase 3). Furthermore, treatment of human aortic endothelial cells with high glucose triggered EndoMT via increasing the expression of FSP1 and α-SMA in an Ang-II–dependent manner. However, irbesartan treatment reversed EndoMT (Tang et al., 2010). The effects on EndoMT can also be observed with other ARBs, such as telmisartan (Hu et al., 2014) and losartan (Wylie-Sears et al., 2014). Amlodipine, as with other common clinically used CCBs, diminishes monocyte adhesion to TNF-α–activated endothelial cells by reducing Ninjurin-1 expression through reducing NOX and mitochondria-dependent oxidative stress, ER Stress, and NF-κB activation (Toma et al., 2020). Similarly, telmisartan ameliorates hyperglycemia-induced monocyte adhesion to TNF-α–stimulated endothelial cells, partially through decreasing IKKβ/NF-κB–dependent VCAM-1 expression, supporting its use in the treatment of diabetes mellitus–associated vascular inflammation and CVD (Song et al., 2016). ACE inhibition by ramipril also reverses Ang-II–dependent expression of cell adhesion molecules such as P-selectin, VCAM-1, and ICAM-1. THP-1 monocyte adhesion to activated endothelial cells is attenuated by reducing the translocation of p65 subunit of NF-κB and AT1R expression as well as by activating bradykinin 2 receptor (Soehnlein et al., 2005). In addition to ARBs, CCBs, and ACEIs, β-blockers also improved endothelial function, as evidenced by increased FMD, an effect similar to ARBs, CCBs, or diuretics, albeit these agents were found to be inferior to ACEIs (Peller et al., 2015).

C. Antihyperglycemia Drugs

It has long been recognized that hyperglycemia is associated with endothelial dysfunction. There are multiple drugs with many different mechanisms of action used in the treatment of hyperglycemia and other metabolic disturbances of diabetes that impact endothelial dysfunction (Triggle and Ding, 2017; Triggle et al., 2020). When a drug is associated with reducing the level of a risk factor (such as hyperglycemia) but its favorable pharmacological effects exceed expected effects from the risk factor reduction, then this drug is usually described as being pleiotropic (Eriksson and Nystrom, 2015). It is also interesting to consider whether the pleiotropic action arises directly and intrinsically from the mechanistic action of the drug or from a secondary effect related to a different known or otherwise unknown target of the index drug (Eriksson and Nystrom, 2015; Lovshin and Cherney, 2015). In this context, we consider below the actions of modern antidiabetes drugs on endothelial dysfunction, atherosclerosis, and CVD, focusing on the major current therapeutic agents used for the treatment of hyperglycemia, including insulin, metformin, SGLT2i, glucagon-like peptide-1 (GLP-1) receptor agonists, and DPP-4 inhibitors. The actions of some of these agents on endothelial dysfunction have recently been reviewed (Triggle et al., 2020).

1. Insulin. Insulin therapy is the mainstay of the treatment of type 1 diabetes and an essential treatment in the later decompensated stage of type 2 diabetes. Insulin impacts the endothelium (Rask-Madsen et al., 2001; Arcaro et al., 2002; Rask-Madsen et al., 2010), and insulin treatment is intimately involved with the phenomenon of insulin resistance, which is a risk factor for atherosclerosis (Nigro et al., 2006). Insulin stimulates the release of NO via a PI3K/Akt pathway involving direct Akt phosphorylation of Ser1177 on eNOS; insulin also increases the release of the vasoconstrictor endothelin from endothelial cells via the MAPK signaling pathway. In control subjects, insulin has a net vasodilatory effect. In subjects with diabetes, the vasodilatory effect of insulin with the associated increase in glucose uptake in muscle, fat, and liver contributes approximately one-third of the hypoglycemic action of insulin. These actions are blunted in insulin resistance and hence contribute to increased hyperglycemia (Nigro et al., 2006). Hyperglycemia increases ROS and contributes to endothelial dysfunction, so the impact of insulin is determined by the balance of its direct effect and the hypoglycemic action (Vincent et al., 2002).

2. Metformin. Metformin has been generally recognized as the mainstay of the contemporary treatment of type 2 diabetes. The hypoglycemic action of metformin is a result of improved insulin sensitivity and favorable effects on the incretin system. Metformin has a protective effect on the endothelium because of its hypoglycemic actions, and it also has direct AMPK-dependent and -independent pleiotropic effects, which have very recently been reviewed by us and others in detail (Triggle and Ding, 2017; Nafisa et al., 2018; Triggle et al., 2020).

3. Sodium-Glucose Cotransporter 2 Inhibitor. SGLT2i block the renal reabsorption of glucose and have major effects that increase glucose excretion and, thereby, decrease hyperglycemia. SGLT2i reduced cardiovascular events and death in several randomized clinical trials (Zinman et al., 2015). Emerging data demonstrate that SGLT2i also, somewhat surprisingly,

have a multitude of favorable actions on the cardiovascular system.

Canagliflozin, dapagliflozin, and empagliflozin are the most well studied SGLT2i with cardiovascular actions. Canagliflozin (30 mg/kg, oral gavage, 8 weeks) was recently reported to prevent endothelial dysfunction by improving endothelium-dependent relaxation, which reduces vascular oxidative stress and inflammation. These beneficial effects eventually translate into reduced atherogenesis in diabetic apolipoprotein E knockout ($ApoE^{-/-}$) mice (Rahadian et al., 2020). This study extends the observation from a previous study showing that canagliflozin reduced plaque size and increased plaque stability in $ApoE^{-/-}$ mice by reducing vascular inflammation (Nasiri-Ansari et al., 2018).

Similar antiatherosclerotic effects of dapagliflozin have been observed in a rabbit model of atherosclerosis (Lee et al., 2020) and in a mouse model of diabetic atherosclerosis (Al-Sharea et al., 2018). Clinical data from the DEFENSE study reveal that dapagliflozin improves endothelial function and glycemic control in patients with T2DM (Shigiyama et al., 2017). Mechanistic studies identified that dapagliflozin reduced endothelial activation and increased endothelium-dependent relaxation (Gaspari et al., 2018). More recently, dapagliflozin treatment was shown to cause a small decrease in systolic blood pressure in patients with heart failure and was well tolerated across the range of systolic blood pressure in the DAPA-HF (dapagliflozin and prevention of adverse-outcomes in heart failure) clinical trial (Serenelli et al., 2020).

The cardiovascular protective actions of empagliflozin have been observed in the EMPA-REG outcome trial (Inzucchi et al., 2020). The possible mechanisms of benefit include increased NO production and endothelium-dependent vasodilation, reduced vascular inflammation and oxidative stress, restored structural integrity of the glycocalyx, attenuated endothelial cell senescence, and antiatherosclerotic effects (Han et al., 2017; Cooper et al., 2019; Ganbaatar et al., 2020; Khemais-Benkhiat et al., 2020; Uthman et al., 2019). The favorable effects of empagliflozin on cardiovascular functions was followed up by two studies of the effect of empagliflozin on FMD (Lunder et al., 2018; Sawada et al., 2020). It was found that a 6-month treatment with empagliflozin improves endothelial function by increasing FMD associated with reduced levels of triglyceride (Sawada et al., 2020). In addition, a 12-week treatment of patients with type 1 diabetes with empagliflozin on top of metformin improved endothelial function, as evidenced by improved arterial stiffness and FMD (Lunder et al., 2018).

For a detailed review of the cardiovascular benefits and mechanisms of SGLT2i, readers are referred to several reviews published recently by us and others in detail (Alshnbari et al., 2020; Cowie and Fisher, 2020; Li et al., 2020b; Liu Z et al., 2021).

4. Glucagon-like Peptide 1 Receptor Agonists. GLP-1 receptor agonists (GLP-1RA) act on G protein–coupled receptors, which are coupled to $G\alpha s$, thus leading to the stimulation of adenylate cyclase and generation of cAMP (Arora et al., 2016). Glucagon-like peptide 1 (GLP-1) activation was first associated with insulin release from the pancreas but was later found to have numerous actions, including effects on gastrointestinal motility and food intake, that enhance their hypoglycemic actions. These agents show antioxidant and anti-inflammatory effects and are efficacious in preventing cardiovascular events and death in clinical trials (Fonseca et al., 2014; Marso et al., 2016b).

Endothelial protective actions and mechanisms of GLP-1RA include 1) maintaining endothelial barrier integrity (Xu et al., 2019a); 2) preventing oxLDL-induced monocyte adhesion to endothelial cells via KLF2 activation (Chang et al., 2019; Yue et al., 2019); 3) eNOS upregulation and increased NO production, as well as endothelium-dependent vasorelaxation via glucagon-like peptide-1 receptor (GLP-1R) and AMPK in human arterioles (Koska et al., 2015); 4) preventing oxLDL-induced endothelial monolayer permeability (Yue et al., 2019); 5) preventing free FA–induced ROS production and NF-κB–mediated endothelial inflammatory response (Zhao et al., 2019); 6) inhibiting EndoMT by AMPK activation (Tsai et al., 2019); 7) blocking NLRP3 inflammasome activation via SIRT1 activation (Luo et al., 2019); 8) antithrombotic effects (Cameron-Vendrig et al., 2016); and 9) increasing endothelial ATP-binding cassette A1 expression and cholesterol efflux, thereby alleviating lipid accumulation and endothelial inflammation as well as endothelial cell injury (Yin et al., 2016).

Despite the preclinical endothelial protective effects, the actual effects of GLP-1RA therapy on endothelial dysfunction and atherosclerosis in human patients are unclear. Nomoto et al. (2015) compared the effects of insulin and the GLP-1 analog, liraglutide, assessed as FMD in a multicenter prospective randomized parallel group trial in 31 patients with diabetes over 14 weeks. Although there was no effect on FMD, there was an improvement in glycemia and other favorable effects on β cell function and cardiovascular protection. Despite the cardiovascular benefits of GLP-1RA being confirmed in several large-scale randomized clinical trials involving patients with diabetes (Marso et al., 2016a,c; Hernandez et al., 2018; Gerstein et al., 2019), direct evidence that GLP-1RA exerts pharmacological effects via improving endothelial function is lacking. Further studies are warranted to validate the efficacy of GLP-1RA therapy on endothelial function in patients with diabetes with or without CVD.

5. Dipeptidyl Peptidase-4 Inhibitors. DPP-4 inhibitors, also known as gliptins, are a class of oral antidiabetic drugs that decrease blood glucose by increasing incretin levels (GLP-1 and gastric inhibitory

polypeptide), thereby increasing insulin secretion and inhibiting glucagon release. They are usually prescribed for patients with T2DM who do not respond well to first-line drugs, such as metformin. Mounting evidence suggests that DPP-4 inhibitors possess endothelial protective and antiatherosclerotic functions (Liu et al., 2020b). For example, sitagliptin protects against endothelial cell injury induced by hypoxia/reoxygenation (Fan et al., 2019), and pharmacological inhibition of DPP-4 by saxagliptin (10 mg/kg per day) or small interfering RNA-mediated DPP-4 silencing ameliorated H_2O_2-induced endothelial senescence in vitro and in aging sprague-dawley (SD) rats by regulating the AMPK/SIRT1/Nrf2 pathway (Xin et al., 2019; Chen et al., 2020c). Vildagliptin (50 mg/kg per day), another DPP-4 inhibitor, improves endothelium-dependent relaxation and prevents vascular inflammation as well as reduces atherosclerotic plaques in nondiabetic ApoE$^{-/-}$ mice (Aini et al., 2019). However, the precise mechanisms underlying vildagliptin-mediated vasodilator effects remain obscure.

To address the molecular targets of vildagliptin in vasoprotection, Gao et al. (2020c) recently observed that vildagliptin improved endothelium-dependent vasodilation in diabetic mice by an action dependent on SIRT1. Mechanistic studies reveal that vildagliptin binds and activates transient receptor potential channel vanilloid 4/AMPK/SIRT1 pathway to evoke endothelial calcium intake and counteracts hyperglycemia-induced endothelial dysfunction. It remains uncertain as to whether GLP-1 contributes to vildagliptin-mediated vasoprotective effects by improving metabolic parameters. Other endothelial protective functions of DPP-4 inhibitors include 1) inhibition of free fatty acid–induced NLRP3 inflammasome activation via AMPK and SIRT1 activation (Jiang et al., 2019; Qi et al., 2019); 2) reduction of endothelial inflammation via suppressing activator protein 1 (AP-1) and NF-κB (Ma et al., 2019; Oliveira et al., 2019); 3) reduction in monocyte adhesion to activated endothelial cells (Ma et al., 2019); 4) increase in endothelial regenerative capacity after vascular injury via activating the stromal cell-derived factor-1/chemokine receptor type 4 (SDF1/CXCR4) axis (Ma et al., 2019); and 5) inhibition of EndoMT via suppressing TGF-β pathway (Xu et al., 2018a). Since most of the evidence supporting endothelial protective effects of DPP-4 inhibitors was obtained in preclinical studies, the therapeutic effects and safety of DPP-4 inhibitors in patients with diabetes with or without CVD remain to be clarified in large-scale randomized clinical trials.

D. Antioxidants

As described earlier, oxidative stress is a critical driver of endothelial dysfunction and atherosclerosis by promoting LDL oxidation and the ensuing oxLDL-mediated atherogenic events, as well as promoting eNOS uncoupling. The net effects of antioxidants improve endothelial function and normalize vascular homeostasis (Nedeljkovic et al., 2003; Silva et al., 2012). Several antioxidants bearing different structures and properties, such as vitamin C and E, *N*-acetylcysteine (NAC), erdosteine, carbocysteine, and genistein, exert endothelial protective effects. Vitamin C has vasodilator effects in patients with elevated chronic cardiovascular risk factors. Vitamin C also protects the endothelium by scavenging ROS, which prevents myeloperoxidase/H_2O_2-mediated oxLDL (Samsam Shariat et al., 2013), peroxynitrite formation, and monocyte adhesion to activated endothelium (May and Harrison, 2013). Similarly, Vitamin E also inhibits oxLDL and protects against endothelial dysfunction in patients with a history of smoking and hypercholesterolemia, despite controversial effects in diabetes (Jialal et al., 1995).

NAC is an effective antioxidant. It preserves glutathione levels, thereby reducing the impact of ROS production and associated inflammatory response. NAC can improve endothelium-dependent relaxation in patients with or without CVD. The effect of NAC on endothelial dysfunction is related to the inhibition of leukocyte adhesion, reduced NOX expression and inflammatory cytokine secretion, improved endothelial barrier integrity, and attenuation of premature endothelial senescence (Zhang et al., 2017a). NAC also has vasodilator properties and improves oxygen delivery and regional blood flow while reducing organ failure and mortality in conditions characterized by microcirculatory derangement such as that encountered in patients with sepsis (Chertoff, 2018).

Erdosteine is a mucoactive drug with anti-inflammatory and antioxidant properties. It inhibits systemic inflammation via inhibiting the expression of proinflammatory cytokines, especially those implicated in oxidative stress. It generates an active metabolite to exert its antioxidant effects that detoxify oxidants and free radicals (Dal Negro, 2008). Placebo-controlled studies demonstrated that erdosteine decreases the rate and duration of exacerbations and improves health status in patients with chronic obstructive pulmonary disease (COPD), who often suffer from cardiovascular comorbidities (Dal Negro et al., 2017; Calverley et al., 2019).

As endothelial dysfunction is an important hallmark of COPD, carbocysteine administration has been shown to exert protective effects by reducing inflammation-associated endothelial dysfunction (Macciò et al., 2009; Biswas et al., 2013). Oral administration of carbocysteine has also been reported to inhibit VEGF-induced angiogenesis and tumor growth in mice. This inhibitory function is due to the inhibition of phosphorylation of proteins associated with angiogenesis, such as protein kinase Cμ, phospholipase Cγ, and ERK1/2 (Shinya et al., 2015). Other studies have also reported the effectiveness of carbocysteine in COPD with cardiovascular comorbidities (Biswas et al., 2013).

Genistein, an isoflavone from soybeans, exerts multiple cardiovascular benefits through phytoestrogen and potent antioxidant activities (Yamagata, 2019). Genistein reduces endothelial dysfunction in hyperhomocysteinemic and hypertensive rats. This endothelial protection results from enhanced eNOS expression and the decrease in ROS and cytokine production (Vera et al., 2007; Zhen et al., 2012). Genistein also increases endothelium-dependent vasodilatoy response in healthy women after menopause and elevates NO levels, but it reduces ET-1 levels. In addition, genistein could reverse oxLDL-induced NF-κB–dependent endothelial inflammation and monocyte adhesion to endothelial cells through the miR-155/SOCS1 (suppressor of cytokine signaling 1) pathway, as well as by repressing the expression of proadhesive molecules (E-selectin, P-selectin, MCP-1, ICAM-1, and VCAM-1) in HUVECs. Therefore, genistein may hold promise for treating endothelial dysfunction–associated disorders such as atherosclerosis and hypertension (Zhang et al., 2017a). In addition, a more recent study has demonstrated that genistein attenuates endothelial senescence by accelerating autophagy via the SIRT1/liver kinase B1 (LKB1)/AMPK pathway (Zhang et al., 2019a).

Many antioxidants, including those that target mitochondria, have consistently been shown to prevent endothelial dysfunction in cultured endothelial cells and in animal models of diseases. These findings indicate that antioxidants appear to be promising drug candidates for improving endothelial function and the management of CVD, despite controversial findings from past clinical trials. Higher-efficacy antioxidants from natural sources with precise molecular targets and tissue distribution might be the preferred direction of antioxidant therapy. Moreover, further large-scale randomized clinical trials evaluating the effectiveness of novel antioxidants in endothelial dysfunction and CVD are warranted.

E. Anti-Inflammatory Drugs

The important role of inflammation in atherosclerosis indicates that potential therapies directed against inflammation may reverse endothelial dysfunction, reducing cardiovascular risk and the incidence of recurrent cardiovascular events in patients with atherosclerosis. Anti-inflammatory therapies, including nonselective (aspirin) and selective COX-2 inhibitors (COXIBs), have been reported to improve endothelial function (endothelium-dependent dilation) in patients with heightened cardiovascular risk factors and established atherosclerosis (Huang and Vita, 2006).

Isolated observations of COX inhibition and vascular function need to be considered in the broader context of prostacyclin and other COX-directed agents in reducing cardiovascular risk. Use of COXIB drugs increased myocardial infarction and stroke (Patrono, 2016). Long-term administration of several members of the COXIBs has dose-dependent increases in cardiovascular events. Thus, any improvement in endothelium-dependent vasodilation appears to be more than offset by loss of the vasoprotective effects of prostacyclin in patients taking COXIBs. In contrast, recent data from clinical trials on cardiovascular mortality reinforce the long-established evidence of secondary cardiovascular protection by low-dose aspirin in patients with CVD (McNeil et al., 2018).

The anti-inflammatory glucocorticoids have pleiotropic actions relevant to endothelial function and cardiovascular health. Indeed, the development of anti-inflammatory glucocorticoids has been guided by a skin blanching assay called the McKenzie test, which measures slow-onset vasoconstrictor activity of topical glucocorticoids in the skin. Given the importance of the prostacyclin/thromboxane A_2 balance in the cardiovascular benefit from low-dose aspirin and in the detrimental effects of COXIBs, a similar analysis of glucocorticoid actions is warranted. Glucocorticoids have a multifaceted impact on eicosanoid metabolism, including repression of COX-2 and inducible forms of phospholipases A2, thereby limiting the availability of prostaglandins endoperoxides to convert to active prostacyclin, an idea advanced and tested in the mid-80s (Axelrod, 1983). Glucocorticoid-induced annexin A1 release further reduces phospholipases A2 activity. The absence of a nucleus in platelets precludes these regulatory effects, theoretically creating an unfavorable imbalance of prostacyclin/thromboxane A_2, as glucocorticoids have no effect on platelet thromboxane A_2 synthesis (Moraes et al., 2005). Whether the thromboresistance and vasomotor consequences of usage of glucocorticoids is net detrimental is a question of mechanistic complexity that could only be addressed with new clinical investigations. The inconsistent history of clinical use of glucocorticoids in adult respiratory distress syndrome (Matthay et al., 2019) provides good support for the reluctance to predict the benefits of steroids, even though there are clear suppressive effects on cytokine activation that preserve endothelial function.

The anti-inflammatory effects of glucocorticoids on cytokine/chemokine production and endothelial activation have been well documented because of the suppressive effects on trafficking of blood-borne lymphocytes and leukocytes. These effects are most extensively researched in asthma and chronic obstructive airways disease, and the molecular mechanisms have been recently reviewed (Keenan et al., 2015, 2016). However, when considering impacts of glucocorticoids on cell trafficking through vessel walls, the multiplicity of sites of action engenders complexity. For example, the defining feature of steroid-sensitive asthma is a reduction in airway eosinophilia. The actions of glucocorticoids include suppression of the production and release of eosinophilotactic cytokines from inflammatory and structural cells of the airways, reducing circulating eosinophil numbers. Eosinophil

trafficking by the direct endothelial response to glucocorticoids and through the reduction in intensity of the inflammatory cytokine milieu leads to a reduction in adhesion coligand molecules. These mechanisms are of current interest in selecting patients for new asthma biologic therapies, with several studies showing that blood eosinophilia is not as predictive as sputum eosinophilia for identifying steroid insensitivity. The locus of the steroid insensitivity is not clear. The epithelial barrier to eosinophil transmigration into the airspaces may also explain the less-than-expected predictive value of circulating eosinophils. More recently, glucocorticoids have also been documented to control noninflammatory leukocyte trafficking (Cavalcanti et al., 2007). The indirect effects of glucocorticoids via mobilization of annexin A1 should also be considered, as annexin A1 has a role in mediating the anti-inflammatory actions of hydrogen sulfide on IL-1β–mediated vascular inflammation (Brancaleone et al., 2014). Deactivation of leukocyte trafficking is achieved not only through multiple glucocorticoid effects, including transrepression of ICAM-1 (Wheller and Perretti, 1997) and VCAM-1 (Simoncini et al., 2000), but also indirectly through the actions of annexin A1 and glucocorticoid-induced leucine zipper protein (Cheng et al., 2013).

In addition to nonsteroidal anti-inflammatory drugs, drugs targeting NLRP3 inflammasome, such as colchicine from traditional Chinese medicine, have entered clinical trials in treating endothelial dysfunction and CVD (Kajikawa et al., 2019). Although it appears that the NLRP3 inflammasome has a pivotal role in driving atherosclerosis, it remains uncertain as to whether emerging NLRP3 inflammasome inhibitors [such as MCC950 (van der Heijden et al., 2017)] can be exploited as novel therapies for endothelial dysfunction and associated CVD. Recently, evidence has demonstrated an important role for endogenously derived specialized proresolving mediators (such as resolvins derived from the metabolism of the polyunsaturated fatty acid docosahexaenoic acid and eicosapentaenoic acid in the resolution of pre-existing inflammation (Fredman and Tabas, 2017). Moreover, endothelial cells can produce resolvin D1 (RvD1) on their own (Dufour et al., 2018). In particular, oxLDL produced from myeloperoxidase can induce a proresolution effect by induction of RvD1 from endothelial cells (Dufour et al., 2018). RvD1, via its receptors *N*-formyl peptide receptor 2 and G protein–coupled receptor 32, reduces LPS-induced endothelial-monocyte interaction via blocking H_2O_2-mediated inactivation of protein phosphatase 2A, NF-κB activation, and ICAM-1 and VCAM-1 expression (Chattopadhyay et al., 2018). Similar protective effects of RvD1 have been observed in endothelial cells treated with cholesterol crystal, a pathogenic agent found in a cholesterol-rich diet (Pichavaram et al., 2019). These studies suggest that the anti-inflammatory actions of specialized proresolving mediators underpin their therapeutic potential for endothelial dysfunction and CVD.

More clinically relevant data from several large-scale randomized clinical trials have shown that anti-inflammatory therapies with colchicine therapy [colchicine cardiovascular outcomes trial (COLCOT) (Tardif et al., 2019) and low-dose colchicine trial (LoDoCo2) (Nidorf et al., 2020)] or canakinumab [blockade of the cytokine IL-1β, canakinumab anti-inflammatory thrombosis outcome study trial (CANTOS) (Ridker et al., 2017)] reduce the risk for cardiovascular events in patients with CVD or coronary artery disease. This body of seminal work provides the proof of concept that anti-inflammatory therapies are effective in the prevention and treatment of CVD. However, much more work needs to be done to balance the risk of increasing infections and the prevention of major adverse cardiovascular events in patients with residual inflammatory risk (Liberale et al., 2021). The emergence of anti-inflammatory drugs as a potential new category of cardiovascular drugs will facilitate the translation of bench studies into clinical practice for effective cardiovascular therapeutics.

F. Bradykinin Receptor B2 Agonists

Bradykinin and kallidin are short-lived peptide mediators that signal via two GPCRs, designated B1 and B2, that mediate a diverse spectrum of physiologic and pathophysiological actions in the vasculature. The B2 receptor is abundantly expressed in the endothelium, whereas B1 is absent in healthy tissues but rapidly expressed under pathologic conditions. ACEIs prevent the degradation of bradykinin (Manolis et al., 2010; Ancion et al., 2019). An increase in the availability of bradykinin is associated with an improvement in vascular tone and structure and protects the endothelium against activated platelets and leukocytes.

Signaling downstream of the B2 receptor activates VE-cadherin and catenin, resulting in the degradation of VE-cadherin and contraction of the cytoskeleton, leading to an increase in pore size between the endothelial cells and an increase in vascular permeability (Dejana and Giampietro, 2012; Bisha et al., 2018). The activation of the B2 receptor in endothelial cells can also induce the release of NO, tissue plasminogen activator, and endothelium-derived hyperpolarizing factor to regulate vascular tone, fibrinolysis, and coagulation (Komarova and Malik, 2010). Agonists of B2 receptors lead to protection and improved functioning of the endothelium. Although several peptide and nonpeptide mimetics (Messadi-Laribi et al., 2008; Bisha et al., 2018) have been investigated for their cardioprotective properties, this effort has not yet translated into clinical use.

G. Endothelial Nitric Oxide Synthase Targeting Enhancer AVE3085

Homocysteine (Hcy) is an independent risk factor for endothelial dysfunction and CVD. AVE3085, a specific

eNOS targeting enhancer, prevents Hcy-induced endothelial dysfunction by increasing NO production and reversing Hcy-induced downregulation of eNOS expression in human endothelial cells. The protective effects of AVE3085 translate into improved endothelium-dependent vasorelaxation in Hcy-exposed human internal mammary artery (Hou et al., 2018) and asymmetric dimethylarginine–exposed human internal thoracic aorta (Xuan et al., 2012). Mechanistic experiments indicate that AVE3085 prevents Hcy-induced endothelial dysfunction in coronary arteries by preserving NO bioavailability via increasing eNOS expression and PI3K/Akt pathway–dependent eNOS phosphorylation at Ser1177 and by suppressing oxidative stress and arginase activity (Yang et al., 2013a). AVE3085 also significantly improved endothelium-dependent relaxation in the aorta of spontaneously hypertensive rats. Moreover, the blood pressure–lowering effect was not observed in hypertensive $eNOS^{-/-}$ mice, indicating the direct eNOS dependence of this drug (Yang et al., 2011). AVE3085 improves endothelium-dependent relaxation in aortae from db/db mice or C57BL/6J mice (exposed to high glucose) by increasing eNOS-dependent NO production and reducing vascular oxidative stress. The cardiovascular benefits of AVE3085 were eNOS-dependent, as pharmacological effects of AVE3085 were abolished by actinomycin D (a transcription inhibitor) and N(G)-nitro-L-arginine methyl ester (L-NAME, the NOS inhibitor) as well as in $eNOS^{-/-}$ mice (Cheang et al., 2011). Thus, AVE3085 showed great potential to improve endothelial dysfunction and associated diseases by enhancing eNOS activity and NO bioavailability. However, the efficacy and safety profile of AVE3085 in clinical settings remain to be demonstrated.

H. Other Potential Endothelial Cell Protective Drugs

1. Proprotein Convertase Subtilisin/Kexin Type 9 Inhibitors. Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors (alirocumab and evolocumab) have emerged as novel therapies to reduce cardiovascular inflammation and atherosclerosis (Liberale et al., 2017). As a major hepatic serine protease involved in cholesterol homeostasis, PCSK9 induces intracellular degradation of LDLR, thereby reducing serum LDL clearance (Liberale et al., 2017). Studies in cultured cells and animals have shown that PCSK9 is associated with inflammation and endothelial cell apoptosis. Knockdown of PCSK9 with PCSK9 short hairpin RNA reduced oxLDL-induced endothelial apoptosis via suppressing $p38^{MAPK}$ and c-Jun N-terminal kinase pathways (Li et al., 2017b). In addition, PCSK9 expression is relevant to different types of shear stress, as low–shear stress regions are associated with increased PCSK9 expression and NOX-dependent ROS generation, whereas high laminar shear stress is related to decreased PCSK9 expression (Ding et al., 2015). PCSK9 inhibition by evolocumab has cytoprotective effects against oxidative damage in endothelial cells exposed to H_2O_2 (Safaeian et al., 2020). However, the effects of PCSK9 inhibition on human endothelial function such as FMD remain to be explored. Characterization of small-molecule PSCK9 inhibitors such as berberine (Dong et al., 2015) and compound 7030B-C5 (Wang et al., 2020d) has yet to clarify whether PCSK9 inhibition has direct, LDLR-independent, protective (pleiotropic) effects in endothelial cells (Karagiannis et al., 2018) or influences FMD in patients with impaired vasodilation.

2. 6-Phosphofructo-2-Kinase/Fructose-2,6-Bisphosphatase 3 Inhibitors. PFKFB3 is an important glycolytic enzyme in endothelial glycolysis and angiogenesis (Li et al., 2019d). PFKFB3 regulates endothelial cell proliferation, filopodia/lamellipodia formation, and directional migration (De Bock et al., 2013). Mechanistic investigations revealed a lower level of phosphorylated AKT and a significant decrease of intracellular lactate in PFKFB3-deficient endothelial cells (Xu et al., 2014). In agreement with this finding, pharmacological inhibition of PFKFB3 by the small-molecule 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one reduced vessel sprouting in endothelial spheroids via inhibition of Notch or VEGF receptor (VEGFR) 1. These studies indicate that PFKFB3 is a promising target for reducing endothelial glycolysis, related pathologic angiogenesis, and vascular disorders such as acute lung injury (Wang et al., 2019a) and pulmonary artery hypertension (Cao et al., 2019b). PFKFB3 can be upregulated by TNF-α, and PFKFB3-dependent glycolysis also drives endothelial inflammation and monocyte adhesion (Zhang et al., 2019b). In agreement with this finding, PFKFB3-dependent glycolysis underlines the missing link between cardiovascular risk factor lipoprotein (a) [Lp(a)] facilitated endothelial inflammation and leukocyte extravasation (Schnitzler et al., 2020). The above cardiovascular actions of PFKFB3 inhibitor may explain the recently reported protective effects of PFKFB3 inhibitor in stabilizing atherosclerotic plaques in $LDLR^{-/-}$ mice (Poels et al., 2020). It should be noted that some inhibitors may have pharmacological actions irrelevant to the target. For example, a recent study has demonstrated that 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one prevented IL-1β– and TNF-α–induced NF-κB and c-Jun N-terminal kinase signaling, leading to reduced expression of adhesion molecules VCAM-1 and E-selectin probably via a PFKFB3-independent mechanism, since silencing of PFKFB3 or treatment with the alternative PFKFB3 inhibitor YN1 failed to mimic these anti-inflammatory effects (Wik et al., 2020). Therefore, PFKFB3-dependent and PFKFB3-independent pharmacological actions need to be considered when used in vitro and in vivo.

3. Vascular Endothelial Protein Tyrosine Phosphatase Inhibitors. Receptor-type vascular endothelial protein tyrosine phosphatase (VE-PTP) dephosphorylates Tie-2 and the three components of a mechanosensitive

complex made up of CD31, VE-cadherin, and VEGFR2. As VE-PTP expression is increased under diabetic conditions, the inhibition of VE-PTP may improve eNOS activity and vascular reactivity and lower blood pressure in patients with diabetes. A recent study by Siragusa et al. (2020) showed that VE-PTP inhibition (using AKB-9778) enhances eNOS activity and NO production to improve endothelial function and decrease blood pressure via activation of Tie-2 and the CD31/VE-cadherin/VEGFR2 mechanosensitive complex. AKB-9778 also promotes the phosphorylation of eNOS at Tyr81 and Ser1177. The Piezo1 activator Yoda1, a shear stress-mimetic compound, also increased eNOS Tyr81 phosphorylation. Most importantly, AKB-9778 lowered systolic and diastolic blood pressure in patients with diabetes. VE-PTP inhibitors, therefore, represent an attractive therapeutic option for diabetes-induced endothelial dysfunction and hypertension (Siragusa et al., 2020) and herald a mechanopharmacological approach to the correction of endothelial dysfunction (Krishnan et al., 2016).

4. Glycocalyx-Targeted Therapies. Another promising therapeutic target that has demonstrated its importance in regulating endothelial cell function is the endothelial glycocalyx. Because of the structure's mechanotransductive ability, combined with its association with endothelial health, numerous therapies have been identified that either prevent initial glycocalyx degradation or restore the expression of a partially degraded glycocalyx layer. These include, but are not limited to, sulodexide (Broekhuizen et al., 2010; Li et al., 2017c), heparin (Yini et al., 2015; Lipowsky and Lescanic, 2017), metformin (Eskens et al., 2013; Targosz-Korecka et al., 2020), and empagliflozin (Cooper et al., 2019), which have been shown to both maintain or restore glycocalyx expression while also restoring aspects of proper endothelial function, including permeability regulation, leukocyte adhesion/transmigration, and vasodilation. Using a balloon-injury model of glycocalyx degradation in rat carotid arteries, Li et al. (2017c) demonstrated that sulodexide treatment restores glycocalyx expression while increasing nitric oxide synthase expression and reducing both platelet aggregation and leukocyte transmigration. In addition to the aforementioned therapeutics, others have also been shown to restore glycocalyx expression and endothelial function through the administration of exogenous glycosaminoglycans (Constantinescu et al., 2003; Mensah et al., 2017). For example, Mensah et al. (2017) found that the in vitro administration of a combination of exogenous heparan sulfate and sphingosine-1-phosphate restored both endothelial heparan sulfate expression and endothelial cell-cell communication. Future investigations utilizing in vivo models of cardiovascular diseases associated with endothelial dysfunction will likely further support the glycocalyx as a therapeutic target while validating previous studies.

VII. Novel Regulators of Endothelial Dysfunction

A. Micro RNA

miRNAs are a class of highly conserved, small, and noncoding RNAs involved in post-transcriptional regulation of gene expression. miRNAs represent the best-studied noncoding RNA with regard to effects on the vasculature. miRNAs function by binding to mRNAs, thereby causing gene silencing, inducing mRNA degradation, or by blocking translation. Mounting evidence in the past decade has revealed that miRNAs regulate multiple aspects of endothelial function and dysfunction.

1. Micro RNA and Endothelial Inflammation. Specific miRNAs function as regulators and mediators of the inflammatory responses. TNF-α induces miR-31 and miR-17-3p, which target adhesion molecule E-selectin and ICAM-1, respectively. Gain- and loss-of-function analysis of both miRNAs indicates that miR-31 and miR-17-3p serve as negative-feedback controls on TNF-α–induced inflammation and neutrophil adhesion to cultured endothelial cells (Suárez et al., 2010). Similarly, transfection with miR-126 mimics decreased TNF-α–induced VCAM-1 expression and leukocyte adhesion to endothelial cells. Conversely, treatment with an miR-126 inhibitor amplifies TNF-α–stimulated VCAM-1 expression and increases leukocyte adhesion to endothelial cells (Harris et al., 2008).

In contrast, the expression of miR-181b was decreased by TNF-α treatment. Administration of miR-181b mimics reduced expression of VCAM-1 and E-selectin, contributing to reduced leukocyte extravasation in the vascular endothelium (Sun et al., 2012b). In human aortic endothelial cells, depletion of of miR-10a increased expression of MCP-1, IL-6, IL-8, VCAM-1, and E-selectin (Fang et al., 2010), suggesting that miR-10a protects against endothelial dysfunction and atherosclerosis. Increased expression of miR-146a leads to the downregulation of IL-6 and TNF-α, as well as ICAM-1, VCAM-1, and E-selectin, whereas inhibition of miR-146a resulted in the opposite response. Mechanistically, miR-146 protects against inflammation by inhibiting LPS-induced NF-κB activation in endothelial cells (Feng et al., 2019). A recent study has shown that upregulation of miR-200a, an anti-inflammatory and antiatherosclerotic miRNA, reduces VCAM-1–dependent monocyte adhesion to endothelial cells and atherosclerotic plaque size in ApoE$^{-/-}$ mice. Elevated miR-200a also protects against oxLDL-induced inflammation and apoptosis by reducing enhancer of zeste homolog 2 (EZH2)-dependent STAT3 methylation and activity (Wang et al., 2020a).

2. Micro RNA and Vascular Tone. eNOS and eNOS-derived NO production are critical regulators of vascular tone. miRNAs that regulate vascular tone and activity via targeting eNOS are intensively investigated. One study

has shown that miR-222/221 regulates eNOS protein level and activity (Rippe et al., 2012). Mechanosensitive miRNA miR-101 represses EZH2 expression and thus mediates flow-induced protective effects in endothelial cells (Xu et al., 2018c). eNOS, as a key factor for endothelial differentiation, is transcriptionally repressed by EZH2 (Descamps et al., 2018). Thus, it is plausible that miR-101 could be involved in regulating eNOS expression and eNOS-dependent vasodilation. In contrast, miR-155 reduced NO production by decreasing eNOS expression, impairing endothelium-dependent vasorelaxation in human internal mammary arteries (Sun et al., 2012a). Furthermore, expression of miR-155 was upregulated by TNF-α but downregulated by statins (Sun et al., 2012a; Jing et al., 2017), aspirin (Kim et al., 2017b), and carbon monoxide (Choi et al., 2017). A recent study demonstrates that the oxLDL-induced miR-652-3p expression is blocked by statins. The upregulation of miR-652-3p decreased eNOS activation and decreased NO production (Liang et al., 2020). In addition to modulating NO-derived vasodilation, miRNAs also play a role in regulating vasoconstriction by regulating the production of vasoconstrictor molecules such as ET-1 and Ang-II. A recent report (Li et al., 2010) has shown that miR-125a/b-5p can suppress oxLDL-induced ET-1 expression by suppressing prepro-ET-1 mRNA expression. Similarly, miRNA-26a-5p is an miRNA downregulated in patients with CVD and endothelial cells isolated from $ApoE^{-/-}/LDLR^{-/-}$ mice. Treatment with a miRNA-26a-5p inhibitor significantly upregulated ET-1, TxA_2, and Ang-II and downregulated eNOS and PGI_2, which may lead to altered vascular activities (Jing et al., 2019). In contrast, inhibition of miR-199a-3p and -5p independently increases NO bioavailability by promoting eNOS activity and reducing eNOS degradation, thereby generating a vasodilator tone (Joris et al., 2018).

In addition, some miRNAs regulate mRNA stability. For example, miR-31-5p is an miRNA upregulated in sera from patients with pre-eclampsia and TNF-α–treated endothelial cells. Transfection with miR-31-5p mimics decreased eNOS mRNA stability without affecting eNOS promoter activity, leading to eNOS downregulation, suppressed NO production, and impaired vasodilation (Kim et al., 2018).

3. Micro RNA and Kruppel-like Factor 2. KLF2 is a master regulator of vascular homeostasis in endothelial cells. Previous studies have shown that KLF2 is a downstream target of miRNA-92a, an atherogenic miRNA in endothelial cells, the expression of which is upregulated by low shear stress and oxLDL via a STAT3-dependent manner (Wu et al., 2011; Fang and Davies, 2012; Loyer et al., 2014). A more recent study has shown that downregulation of miR-363-3p ameliorates acute myocardial infarction–related endothelial damage by targeting KLF2, indicating that miR-363-3p has the potential to treat acute myocardial infarction (Gao et al., 2020b).

4. Micro RNA and Endothelial Nitric Oxide Synthase Uncoupling. Reduced expression or activity of GCH1 drives eNOS uncoupling and endothelial dysfunction. Li et al. (2016) have recently shown that miR-133a was strongly induced by proinflammatory cytokines and oxidative stress in endothelial cells. GCH1 is a target of miR-133a. However, lovastatin inhibited miRNA-133a expression and upregulated GCH1, thereby recoupling eNOS in stressed endothelial cells. In vivo, lovastatin or miR-133a antagomir reversed hyperlipidemia- or hyperglycemia-induced ectopic miR-133a and reduced GCH1 protein expression in the vascular endothelium and impaired endothelial function (Li et al., 2016). Another recent study has shown that miR-21 silences the expression of dimethylarginine dimethylaminohydrolase-1, an effect that reduces ADMA levels and increases eNOS phosphorylation and NO production in cultured HUVECs. In the vascular endothelium of atherosclerotic lesions, endothelial miR-21 inhibited dimethylarginine dimethylaminohydrolase-1-ADMA-eNOS-NO pathway to promote the pathogenesis of atherosclerosis (Yang et al., 2020a).

5. Micro RNA and Endothelial-to-Mesenchymal Transition. EndoMT has emerged as a novel aspect of endothelial dysfunction. miRNA-455 regulates TGF-β–induced EndoMT by targeting zinc finger E-box homeobox 1 expression (Zhang et al., 2020c). Another microRNA, miR-204, was downregulated by hypoxia in rat pulmonary arterial intima and human pulmonary artery endothelial cells, and it is further downregulated by an miR-204 inhibitor, which suppresses hypoxia-induced EndoMT (Liu et al., 2019). Overexpression of miR-448-3p in endothelial cells inhibits EndoMT by blocking the TGF-β/Smad pathway, thereby reducing endothelial cell damage. Mechanistically, miR-448-3p negatively regulates DPP-4 expression, and overexpression of DPP-4 rescued the effect of miR-448-3p overexpression on EndoMT. More importantly, adeno-associated virus type 2-mediated overexpression of miR-448-3p rescued the weight loss and hyperglycemia caused by streptozotocin injection. This evidence indicates that miR-448-3p represents a promising therapeutic modality for ameliorating endothelial dysfunction as occurs in patients with diabetes (Guan et al., 2020).

6. Micro RNA and Endothelial Injury/Apoptosis. oxLDL-induced endothelial cell apoptosis contributes to endothelial dysfunction and atherosclerosis. miR-106a-5p expression is an angiogenesis-associated miRNA that is increased in atherosclerotic plaques. miR-106a-5p is involved in oxLDL-elicited apoptosis and oxidative damage of HUVECs by regulating STAT3 activation (Hu et al., 2020). Similarly, miR-106b, another miRNA that is associated with endothelial apoptosis, showed decreased expression in oxLDL-treated human aortic endothelial cells. However, overexpression of miR-106b inhibited endothelial cell apoptosis by activating the PI3K/AKT pathway (Zhang et al., 2020d).

7. Micro RNA and Endothelial Cell Senescence. Deregulated expression of miRNAs also leads to endothelial cell senescence. For example, the expression of miR-34a was increased in senescent endothelial cells. Forced expression of miR-34a caused senescent phenotypes and decreased SIRT1 expression in endothelial cells (Ito et al., 2010). In contrast, decreased expression of miRNA-130a augments endothelial senescence and reduces angiogenic functions. However, forced expression of exogenous miR-130a (by injection of miRNA-130a mimics) in aged endothelial cells reduces endothelial cell senescence and improves angiogenesis in a mouse model of hindlimb ischemia. Mechanistic experiments indicated that miR-130a directly targets homeobox A5 and mesenchyme homeobox 2, key antiangiogenic homeobox genes significantly increased in ischemic muscles of aged mice (Dhahri et al., 2020). Therefore, microRNAs could be exploited as important therapeutic targets for endothelial dysfunction and CVD.

Many miRNAs are conserved among different species and regulate multiple aspects of endothelial dysfunction, including endothelial inflammation, vascular activity, aging, and EndoMT, indicating that miRNAs could be exploited therapeutically by using miRNA mimics or inhibitors. Also, circulating miRNAs in exosomes can be used as suitable diagnostic biomarkers for endothelial dysfunction and CVD, although the direct association between circulating miRNA levels and FMD remains to be evaluated. For a detailed analysis of the role of miRNA in endothelial dysfunction, we refer to a recent review article published elsewhere (Fernández-Hernando and Suárez, 2018).

B. Long Noncoding RNAs

Distinct from short noncoding RNAs (miRNAs), long noncoding RNAs (lncRNAs) (>200 nucleotides) also regulate endothelial function (Xu et al., 2019b). lncRNAs constitute a large portion of the transcriptome and play an important role in regulating pathophysiological functions in vascular biology and atherosclerosis (Xu et al., 2018b, 2019b). The functions of lncRNAs can be mediated by RNA-RNA, RNA-DNA, and RNA-(RNA-binding) protein interactions. In the following sections, we provide an overview of the important roles of lncRNAs in endothelial pathophysiology and CVD.

1. Long Noncoding RNA and Endothelial Inflammation. Endothelial inflammation, a key initiating event in the vascular pathologic process, can lead to endothelial dysfunction and various forms of cardiovascular disorders. Emerging evidence has shown that lncRNAs play an important role in the inflammatory response. For example, hepatocellular carcinoma upregulated long noncoding RNA (HULC) expression in cardiac microvascular endothelial cells was decreased under hypoxia-induced inflammation (Chen et al., 2020d). However, overexpression of HULC in HUVEC could inhibit the expression of IL-1β, IL-6, and IL-8 and promote angiogenesis. Mechanistic studies indicate that HULC could directly target miR-29b, the overexpression of which reversed the effects of HULC on cell viability, proinflammatory cytokine expression, and angiogenesis (Chen et al., 2020d).

HOXA cluster antisense RNA 2 (HOXA-AS2) is another lncRNA that serves as a critical repressor of endothelial inflammation by suppression of the NF-κB signaling pathway. HOXA-AS2 is inversely regulated by NF-κB by recruiting bromodomain-containing protein 4/the positive transcription elongation factor (P-TEFb) complex to HOXA-AS2 promoter, thus activating transcription elongation (Zhu et al., 2019c). oxLDL-induced endothelial dysfunction is a driver of atherosclerosis. HOXA-AS3 positively regulates NF-κB activity through regulating the expression of NFKB inhibitor alpha (IκBα) and lysine acetylation p65 at K310. HOXA-AS3 expression has a significant positive correlation with the progression of atherosclerosis. Therefore, HOXA-AS3 may serve as a promising biomarker for inflammatory vascular diseases and CVD (Zhu et al., 2019a).

Another study has shown that H19 knockdown suppressed oxLDL-induced inflammation (upregulation of IL-1β, IL-6, TNF-α, VCAM-1, ICAM-1, and E-selectin), apoptosis, and oxidative stress in HUVECs by decreasing periostin expression via interfering with let-7 (Cao et al., 2019a). H19 is an lncRNA upregulated in patients with diabetes. However, inhibition of H19 profoundly inhibits high-glucose–induced endothelial ROS production and inflammation via increasing miR-29b and downregulating VEGFA through the AKT/eNOS signaling pathway (Cheng et al., 2019).

Endothelial cell–enriched LINC00341 was recently identified as a mechanosensitive, statin-responsive, and anti-inflammatory lncRNA that suppresses TNF-α or disturbed flow–induced monocyte adhesion to endothelial cells by repressing VCAM-1. Mechanistically, LINC00341 recruits EZH2, a core histone methyltransferase in the polycomb repressive complex 2, to VCAM-1 gene promoter, thereby suppressing VCAM-1 expression (Huang et al., 2017b).

NF-κB interacting long noncoding RNA (NKILA) was recently identified as a negative regulator of endothelial inflammation. Mechanistically, NKILA positively regulates the expression/activity of KLF4, a master regulator of endothelial anti-inflammatory and atheroprotective responses. Further studies showed that NF-κB recruits DNA methyltransferase 3A to the CpG island (cytosine guanine dinucleotide repeats) of KLF4 promoter, promoting KLF4 promoter DNA methylation and transcriptional silencing. Moreover, KLF4 can also repress NF-κB transcriptional activity under the control of NKILA. These studies revealed that NKILA represents a novel lncRNA that protects the endothelial cells from

inflammatory insults and atherogenic events (Zhu et al., 2019b).

Furthermore, air pollution, PM2.5 exposure in particular, causes endothelial inflammation and dysfunction. A recent study has shown that PM2.5 treatment of HUVECs leads to upregulated expression of lncRNA NONHSAT247851.1 and genes regulating immune response. Further studies revealed that NONHSAT247851.1 depletion reduced the expression of IL-1β, a potent proinflammatory cytokine, in driving endothelial inflammation and NLRP3 inflammasome activation. Mechanistic investigations revealed that NONHSAT247851.1 interacts with raf-1 to regulate p65 phosphorylation (Zhou et al., 2020a).

2. Long Noncoding RNA and Vascular Homeostasis. It has been well established that disturbed flow is associated with endothelial dysfunction and atherosclerosis, whereas unidirectional laminar flow is associated with vascular homeostasis and atheroprotection (Xu et al., 2016a; Niu et al., 2019). However, it remains largely unclear how the endothelial barrier function is regulated by blood flow. A recent study (Yang et al., 2020b) has shown that endothelial cells exposed to unidirectional laminar flow have an increased expression of ZO-1 and occludin, which is dependent on metastasis associated lung adenocarcinoma transcript 1 (MALAT1) and β-catenin/nesprin interaction. This study suggests that pharmacological activation of MALAT1 could lead to increased vascular homeostasis by stabilizing ZO-1 and occludin expression to counteract endothelial barrier disruption and hyperpermeability (Yang et al., 2020b). By the same token, SENCR (smooth muscle and endothelial cell enriched migration/differentiation-associated lncRNA) (Bell et al., 2014), another vascular cell–enriched lncRNA, also regulates vascular homeostasis in vitro and in vivo (Lyu et al., 2019).

In addition, MANTIS (Leisegang et al., 2017), a shear stress and statin-responsive angiogenesis-related lncRNA downstream of KLF2 and KLF4, repressed endothelial inflammation by decreasing ICAM-1 gene expression (Leisegang et al., 2019). Furthermore, MANTIS was essential for flow-induced endothelial cell alignment to the direction of blood flow and decreased monocyte adhesion to endothelial cells. Mechanistically, MANTIS decreased ICAM-1 expression by reducing the binding of brahma-related gene-1 (BRG1) to ICAM-1 gene promoter (Leisegang et al., 2019). In human occluded atherosclerotic carotid arteries, MANTIS was downregulated, which was reversed in patients receiving statin therapy (Leisegang et al., 2019). These studies reveal that MANTIS is an essential lncRNA that mediates the beneficial effects of statin and laminar flow.

In addition, another mechanosensitive lncRNA, LINC00520, stabilizes adherens junctions in endothelial cells. Silencing of LINC00520 in endothelial cells decreased the stabilization of adherens junction protein component VE-cadherin, cell viability, and cell alignment. Mechanistic experiments indicate that LINC00520 interacts with CD31 and filamental protein nestin (Stanicek et al., 2020). These studies provide novel insights into LINC00520 in regulating endothelial barrier integrity. LINC00520 was also identified as an enhancer-associated lncRNA that boosts the expression of eNOS. Gain- and loss-of-function of LINC00520 differentially regulate eNOS expression and endothelial function (NO production and monocyte adhesion to endothelial cells). Mechanistic data indicate that LINC00520 facilitates the binding of RNA polymerase II to the eNOS gene promoter, thereby enhancing nascent eNOS RNA transcription, promoting NO production and anti-inflammation (Miao et al., 2018).

It is well established that KLF4 is a homeostasis-associated transcription factor that protects against endothelial dysfunction. A recent study (Lu et al., 2019) has shown that knockdown of RNA AF131217.1 (a newly described lncRNA upregulated in shear stress–treated endothelial cells) reverses laminar flow–induced anti-inflammatory effects by acting as a competing endogenous RNA for miR-128-3p and upregulating KLF4 expression.

3. Long Noncoding RNA and Endothelial Senescence. lncRNAs have also been shown to regulate endothelial senescence. The expression of H19, an lncRNA enriched in the endothelium of human atherosclerotic plaques, is reduced in endothelial cells isolated from aged mice. Loss of H19 leads to the upregulation of p16 and p21, halted endothelial proliferation, and increased endothelial cell senescence. Moreover, specific deletion of H19 in endothelial cells elevated blood pressure and leads to reduced capillary density after hindlimb ischemia surgery. Mechanistic experiments indicate that H19 depletion leads to enhanced endothelial inflammation (IL-6, ICAM-1, and VCAM-1 upregulation) via promoting STAT3 phosphorylation at tyrosine 705. Accordingly, STAT3 inhibition reversed the effects of H19 silencing on endothelial senescence and inflammation. Therefore, H19 depletion leads to premature senescence of endothelial cells, endothelial cell dysfunction, and inflammation through STAT3 signaling.

The expression of maternally expressed 3 (MEG3) was decreased in aged blood vessels from mice and humans. Decreased MEG3 expression leads to increased expression of miR-128 and decreased expression of Girdin, leading to endothelial cell senescence probably via reduced platelet phagocytosis activity in HUVECs (Lan et al., 2019). Of interest, another study observed that MEG3 expression was elevated in senescent endothelial cells (passage 16–18) and that MEG3 inhibition prevented the aging-mediated inhibition of sprouting angiogenesis in HUVECs (Boon et al., 2016). It is possible that MEG3 regulates endothelial senescence in a context- and stage-dependent manner. Therefore, the precise role of MEG3 in the endothelium from aged subjects remains to be evaluated.

More recently, to depict the novel lncRNA landscape in regulating endothelial function, Haemmig et al. (2020) profiled lncRNAs highly expressed in the vascular endothelium by evaluating differentially expressed lncRNAs in aortic intima of $Ldlr^{-/-}$ mice that were fed an atherogenic diet during plaque development and regression. The authors identified the evolutionarily conserved lncRNA small nucleolar host gene (SNHG) 12 was decreased during plaque progression. SNHG12 depletion enhanced atherosclerotic plaque formation in $Ldlr^{-/-}$ mice by increasing DNA damage and endothelial cell senescence, leaving the lipid profile or vascular inflammation intact. Gain of function of SNHG12 protects against DNA damage in the endothelium and atherosclerosis. Mechanistic studies revealed that SNHG12 interacts with DNA-dependent protein kinase, whereas SNHG12 deficiency reduces the interaction between DNA-dependent protein kinase and Ku70/Ku80, eliminating efficient repair of DNA damage. From a therapeutic perspective, the effects of SNHG12 on DNA damage, cell senescence, and atherosclerosis were reversed by nicotinamide riboside, a NAD^+ precursor with DNA damage–repairing capacities (Haemmig et al., 2020). These studies indicate that lncRNAs participate in the regulation of endothelial cell senescence and aging-related dysfunction by different pathways, pinpointing future directions of antiaging therapies by targeting the senescent endothelial cells.

4. Long Noncoding RNA in Regulating Endothelial Injury/Apoptosis. Endothelial cell injury and subsequent apoptotic cell death is a key feature of endothelial dysfunction and CVD. Silencing of MEG3 downregulates HIF-1α by sponging miR-135a, thereby inhibiting endothelial damage and apoptosis in mice subject to chronic intermittent hypoxia, identifying MEG3 as a potential therapeutic target for chronic intermittent hypoxia (Ding et al., 2020). A previous study has found increased expression of LINC00657 in the serum of patients with CVD and oxLDL-treated HUVECs. However, LINC00657 silencing reduced oxLDL-induced endothelial injury, EndoMT, inflammation, and cell apoptosis by regulating miR-30c-5p/Wnt7b/β-catenin (Wu et al., 2020). Similarly, MALAT1 expression was significantly increased by treatment with high glucose. Knockdown of MALAT1 effectively inhibits high-glucose–induced inflammatory response and apoptosis by decreasing the expression of TNF-α, IL-1β, and IL-6 in HUVECs. Mechanistic studies indicate that MALAT1 directly sponges miR-361-3p to derepress suppressor of cytokine signaling 3 (SOCS3) expression and suppresses the NF-κB signaling pathway (Huang et al., 2020; Lin et al., 2020). In addition, MALAT1 was upregulated in both patients with adult respiratory distress syndrome and LPS-treated human endothelial cells. MALAT1 increased endothelial inflammation by upregulating ICAM-1 expression via competitively binding to miR-150-5p. MALAT1 knockout suppresses endothelial inflammation (reduced levels of IL-6, IL-1β, and TNF-α and E-selectin) (Yao et al., 2020). Interestingly, in vivo, MALAT1-deficient $ApoE^{-/-}$ mice show increased plaque size and infiltration of $CD45^+$ cells compared with control $ApoE^{-/-}$ mice. Further bone-marrow transplantation studies revealed that hematopoietic cell–derived MALAT1 played a key role in atheroprotection. The anti-inflammatory effects of MALAT1 were attributed to a reduction of the miR-503. MALAT1 expression was decreased in human atherosclerotic plaques compared with normal arteries, supporting the clinical relevance of this lncRNA. Decreased expression of MALAT1 was observed in symptomatic versus asymptomatic patients (Cremer et al., 2019). These studies indicate that cell type–specific roles of MALAT1 may exist, making it necessary for the targeting of endothelial cell–specific MALAT1 in treating endothelial dysfunction.

In addition, nuclear paraspeckle assembly transcript 1 (NEAT1) levels were elevated in the serum of patients with atherosclerosis and in human endothelial cells exposed to oxLDL. NEAT1 silencing decreased cell proliferation and stimulated endothelial apoptosis, which could be rescued by the inhibitor of miR-638. Additional experiments suggest that NEAT1 competes with endogenous RNA of miR-638 (Zhang et al., 2020a). lncRNA RNA X-inactive specific transcript expression was increased upon oxLDL treatment. However, X-inactive specific transcript knockdown ameliorates oxLDL-induced endothelial injury, inflammation, and oxidative stress by targeting miR-204-5p/TLR4 (Lu et al., 2020). Air pollution, PM2.5 inhalation in particular, is associated with an increased risk of endothelial dysfunction and CVD. Zhou et al. (2020a) identified a new lncRNA, NONHSAT247851.1, which was significantly upregulated by PM2.5 treatment. NONHSAT247851.1 knockdown reduced the expression of IL-1β via interaction with raf-1 to control NF-κB–dependent inflammatory responses in endothelial cells. SNHG6 is a conventional lncRNA that is highly expressed in patients with atherosclerosis. A more recent study has shown that knocking out SNHG6 can reduce oxLDL-induced endothelial cell damage, and miR-135a-5p inhibitors can partially reverse this effect. Therefore, SNHG6 drives atherogenic events by targeting the miR-135a-5p/ROCK (Rho associated coiled-coil containing protein kinase 1) axis in endothelial cells exposed to oxLDL (Shan et al., 2020). Similarly, the depletion of lncRNA AK094457 attenuates the Ang-II–induced decrease of endothelial cell viability as well as reduced levels of eNOS and eNOS phosphorylation and subsequent NO production. In addition, Ang-II–induced proinflammatory gene expression (TNF-α, IL-1, IL-6, VCAM-1, ICAM-1, and MCP-1), ROS level, and endothelial cell apoptosis were also decreased after AK094457 depletion. Altogether, these results indicated that lncRNAs represent promising targets in ameliorating endothelial injury and apoptosis (Xu et al., 2020a).

5. Long Noncoding RNA and Endothelial-to-Mesenchymal Transition. EndoMT emerges as a novel feature of endothelial dysfunction (Cho et al., 2018). Studies in the past few years have demonstrated that several lncRNAs have played significant roles in EndoMT and endothelial dysfunction. High glucose is one potent driver of EndoMT that causes endothelial dysfunction. Decreased expression of H19 was observed in retinal endothelial cells after exposure to high glucose levels (25 mM) in vitro or diabetic induction in vivo. H19 overexpression prevented high-glucose–induced EndoMT in a TGF-β1–dependent, but Smad-independent, pathway. Mechanistically, H19 controls TGF-β1 expression through an ERK1/2 MAPK pathway (Thomas et al., 2019). Another example is MALAT1, which is an lncRNA associated with cancer metastasis. MALAT1 competitively interacts with miR-145 (which inhibits EndoMT) and blocks the inhibitory effect of miR-145 and promotes EndoMT (Xiang et al., 2017) by directly targeting SMAD3 and TGF-βR2. In addition to high glucose, oxLDL is another stimulus that drives EndoMT and endothelial dysfunction. Another study demonstrates that MALAT1 expression was increased upon exposure to oxLDL and in hypercholesterolemic ApoE$^{-/-}$ mice. The upregulation of MALAT1 was associated with the process of EndoMT. Mechanistic investigations revealed that MALAT1 facilitated the oxLDL-mediated nuclear translocation of β-catenin. However, this effect was reversed by MALAT1 inhibition (Li et al., 2019b).

Recent studies have shown that EndoMT occurs in vascular aging, which is evident by the observation that vascular endothelial cells from 8-month-old mice show markers of EndoMT compared with those from 2-month-old (young) mice. EndoMT was accompanied by endothelial hyperpermeability, probably because of the decrease of endothelial marker proteins. MALAT1, as an endothelial cell–enriched lncRNA, was upregulated in aged endothelium and was involved in aging-induced EndoMT through increased Snail expression and transcriptional activity. Mechanistic studies suggest that SIRT6 directly binds to the promoter regions of MALAT1 and negatively regulates MALAT1 expression, providing novel mechanistic insights into MALAT1-driven EndoMT. This evidence collectively suggests that EndoMT is a nexus event in vascular aging and endothelial permeability (Qin et al., 2019).

In addition to MALAT1, GATA6-AS, the antisense transcript of GATA binding protein 6 is another lncRNA that positively regulates EndoMT. GATA6-AS interacts with LOXL2 (lysyl oxidase-like 2) epigenetic enzyme to control the expression of endothelial genes by changing the methylation of histone 3 lysine 4 trimethylation (H3K4me3), including genes encoding for COX-2 and periostin. Hypoxia induces GATA6-AS expression in cultured endothelial cells. However, inhibition of GATA6-AS can block TGF-β2–induced EndoMT in vitro and promote the formation of blood vessels in mice (Neumann et al., 2018). This evidence suggests that EndoMT may be explored as a new approach for preventing aging-associated vascular disorders. The potential roles of these lncRNAs in EndoMT-driven diseases such as atherosclerosis and tissue fibrosis remain to be determined.

Together, the discovery and characterization of the biologic functions of novel conserved and disease-relevant lncRNAs (those enriched in endothelium in particular) have provided meaningful insights into the pathologic mechanisms of endothelial dysfunction and have profound implications for future therapeutic intervention. Selectively targeting diseased endothelium using tissue-specific gene delivery or silencing to afford endothelial protection is a possible direction for development of more selective cardiovascular therapeutics, but many technical hurdles remain. For a detailed analysis of the role of lncRNA in endothelial biology and vascular diseases, readers are referred to excellent reviews published elsewhere very recently (Weirick et al., 2018; Xu et al., 2019b; Jaé and Dimmeler, 2020).

VIII. Conclusion and Outlook

Endothelial cells are ubiquitously present in multiple tissues and organs and regulate whole-body homeostasis. Endothelial dysfunction is the common feature, as well as potentially being the driving force for CVD, metabolic diseases, and emerging infectious diseases like COVD-19. Multiple mechanisms, such as impaired vasodilation, oxidative stress, inflammation, cell injury/death, senescence, and EndoMT, are involved in endothelial dysfunction, among which decreased NO bioavailability represents the hallmark of endothelial dysfunction. Development of drugs with endothelium protective properties may offer clinical benefits in patients. Several potentially important areas of investigation in the field are given below.

A. Complex Pathology of Endothelial Dysfunction and Endothelial Heterogeneity in Cardiovascular Disease

The molecular mechanisms underpinning endothelial dysfunction are rather complex. Single-cell RNA-sequencing (scRNA-seq) has successfully been used in atherosclerosis to identify previously uncharacterized cell populations and evaluate the heterogeneous, dynamic, and plastic nature of different plaque-resident cell populations (Winkels et al., 2018; Fernandez et al., 2019; Williams et al., 2020). scRNA-seq has also revealed that endothelial cells are metabolically plastic and active in regulating multitissue homeostasis (Kalucka et al., 2020; Rohlenova et al., 2020). scRNA-seq is also successful in revealing endothelial cellular heterogeneity in a context-dependent manner. In agreement with these findings, a recent study using scRNA-seq and scATAC-seq (single-cell sequencing assay for transposase-accessible chromatin) of mouse carotid artery undergoing partial ligation has revealed that the induction of disturbed flow

by partial carotid ligation induces phenotypic switch of endothelial cells from atheroprotective phenotypes to atheroprone phenotypes (an unexpected immune-like cell phenotype in particular has been noted) (Andueza et al., 2020). These advanced technologies allow researchers to identify novel plaque-resident cell types and new regulators of blood flow–induced biologic responses (Feng et al., 2019b).

B. Emerging Role of Endothelium as an Active Participant in Innate Immunity

Mounting evidence has suggested that inflammation and immune dysfunction is involved in the initiation and progression of CVD. In addition to regulating various physiologic processes, endothelial cells, as a novel type of immune cell, emerge as an active participant in innate and adaptive immune responses, thereby regulating inflammatory and cardiometabolic diseases (Shao et al., 2020). Intriguingly, endothelial cells have various biologic functions that immune cells assume, such as phagocytosis, cytokine/chemokine secretion, antigen presentation, sensing of pathogen-/danger-/homeostasis-associated molecular patterns, immune checkpoint, and immunometabolism (Shao et al., 2020). Understanding the diverse functions of endothelial cells in modulating inflammatory and immune responses and the involved molecular mechanisms may yield new therapeutic approaches to reduce inflammation and advance anti-inflammatory and immunomodulatory therapies (Al-Soudi et al., 2017).

C. The Application of Systems Biology and Multi-Omics Technology to Reveal Novel Regulators of Endothelial (Dys)Function

Our knowledge of regulators of endothelial (dys) function relies greatly on focused analysis of gene sets in target pathway and the use of Cre/LoxP technology to dissect the role of genes of interest in endothelial dysfunction and CVD. The emergence of systems biology and multi-omics technology allows us to understand the role of these regulators in regulating the endothelial transcriptional, translational, and chromatin landscape. For example, in a recent study, Linna-Kuosmanen et al. (2020) performed an integrated analysis from high throughput chromosome conformation capture, chromatin immunoprecipitation-sequencing, assay for transposase-accessible chromatin with high-throughput sequencing, global run-on sequencing, microRNA-sequencing, and RNA-sequencing to apprehend Nrf2 transcriptional programs in endothelial cells exposed to oxidized phospholipids. This study revealed that Nrf2 is a novel regulator of >100 endothelial pri-microRNAs, with miR-21-5p and miR-100-5p being the two hub miRNAs that regulate endothelial gene expression. More clinically relevant, the authors observed that the expression levels of miR-21-5p and miR-100-5p in the exosome are secreted by senescent endothelial cells, and levels correlate with the disease severity (Linna-Kuosmanen et al., 2020). This study exemplifies the use of advanced biotechnologies to decipher the atherogenic effects of oxidized phospholipids and other atherorelevant treatments in endothelial cells and yields novel diagnostic biomarkers of endothelial dysfunction.

D. Crosstalk among Different Aspects of Endothelial Dysfunction

Although, in clinical practice, the main index of endothelial function is FMD (in animals: endothelium-dependent vasodilation in pressure myograph), different aspects of endothelial dysfunction normally occur concurrently or serve as concomitant triggers. Lp(a) induces endothelial cell inflammation and CVD. A recent study (Schnitzler et al., 2020) has shown that Lp(a), via its oxidized phospholipid content, induces endothelial glycolysis and facilitates endothelial inflammation and leukocyte transendothelial migration. This study echoes previous reports on HIF-1α–dependent, endothelial glycolysis–driven endothelial inflammation (Feng et al., 2017; Wu et al., 2017) (hereby, we coin the descriptor "inflammetabolism" for this interaction). Similar crosstalk also occurs between cell metabolism and senescence (Sabbatinelli et al., 2019), indicating an intricate interaction network among different aspects of endothelial dysfunction, which perpetuate endothelial dysfunction and tissue damage and eventually lead to endothelial dysfunction–associated disorders.

E. The Identification of Novel Natural Products in Preventing Cardiovascular Disease by Targeting Endothelial Dysfunction

Natural products have long been perceived as an important source for cardiovascular drug discovery because of their intrinsic antioxidant and anti-inflammatory properties. For example, the chalcone derivative 1m-6 has been recently shown to attenuate TNF-α–induced upregulation of VCAM-1 and ICAM-1 via inhibiting Janus kinase/STAT3 and activating Nrf2/heme oxygenase-1 signaling pathway in HUVECs. In addition, treatment with 1m-6 significantly reduces atherosclerotic plaque formation in hypercholesterolemic mice by promoting cholesterol efflux and inhibiting endothelial inflammation and dysfunction, which suggests that 1m-6 is a promising lead compound with atheroprotective functions and opens a new avenue for atherosclerotic cardiovascular disease treatment (Chen et al., 2020a). Another recent study reports that halofuginone, an antimalarial drug, also prevents LPS-induced monocyte adhesion and endothelial dysfunction by suppressing the expression of ICAM-1, VCAM-1, and E-selectin and the production of proinflammatory cytokines (TNF-α, IL-1β, and IL-6). The effects of halofuginone were exerted through suppressing NOX2-dependent ROS production and promoting ERK5-dependent KLF2 upregulation.

This study suggests the possibility to repurpose halofuginone as an effective therapeutic agent against endothelial dysfunction in atherosclerosis (Zhong et al., 2020). It can be envisaged that additional naturally occurring bioactive compounds will be identified through high-throughput or high-content drug screening and will likely provide further templates for drug optimization.

F. The Exploration of Epigenetic Drugs in Preventing Endothelial Dysfunction

Studies in the past decade have demonstrated the important roles of DNA methylation and histone modification in endothelial dysfunction and atherosclerosis. For example, several Food and Drug Administration–approved epigenetic drugs, histone deacetylases inhibitors (suberoylanilide hydroxamic acid) (Xu et al., 2017), DNA methyltransferase inhibitors (Xu et al., 2019b), and bromodomain-containing protein 4inhibitors (Borck et al., 2020) have been shown to prevent endothelial dysfunction, such as leukocyte adhesion to activated endothelium. As these epigenetic drugs are clinically used in treating patients with various cancers, the safety profile of these drugs is somewhat established and may be acceptable for use in CVD. Further studies are warranted to validate whether these drugs can be repurposed for cardiovascular therapeutics and also whether the combination of these epigenetic drugs with different mechanisms will yield additional benefit over standard-of-care therapeutics.

G. Interleukin-1 Targeted Therapies in Preventing Endothelial Dysfunction and Reducing Residual Cardiovascular Risk

IL-1 is a disease-relevant proinflammatory cytokine produced by cells in the plaque (such as endothelial cells) and leukocytes (such as monocytes). IL-1 has two isoforms, IL-1α and IL-1β, which have functionally redundant (Beltrami-Moreira et al., 2016) and stage-dependent roles (Vromman et al., 2019) in vascular pathologies including atherosclerosis. The drugs that target IL-1 and are currently used in clinical trials are anakinra (IL-1 receptor antagonist), the monoclonal antibodies canakinumab and gevokizumab (against IL-1β), and the soluble decoy receptor rilonacept. In light of the important role of IL-1 in inflammation and CVD, their potential use in patients with CVD is anticipated in future studies (Heydari et al., 2020). Also, the differential downstream effects mediated by IL-1α and IL-1β are of interest to elucidate the therapeutic potential by targeting different aspects of endothelial dysfunction in CVD. Further, the combination therapy of anti–IL-1 drugs with traditional lipid-lowering drugs (such as statins) or anti–IL-6/IL-18 therapies can be considered as effective strategies to reduce residual cardiovascular and inflammatory risk (Ridker et al., 2020).

Taken together, despite tremendous progress in studying endothelial (dys)function in the past decades, our knowledge of endothelial dysfunction remains incomplete but is continuously expanding. This is reflected in a monograph by Dr Rudolf Altschul (University of Saskatchewan, Canada) who states, "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" (Altschul, 1954). Six decades later, this insightful remark remains highly relevant to our understanding of the pivotal role of endothelial dysfunction in CVD and beyond and also guides the potential for future drug discovery by targeting the endothelium and endothelial cell dysfunction (Gimbrone and García-Cardeña, 2016b).

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