Recent Highlights of ATVB

Endothelium

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Endothelial cells form a subtle monolayer covering the inner surface of the vascular tree. This unique localization allows them to integrate physical and neurohumoral signals from the blood and surrounding tissues to regulate vascular tone, cellular adhesion, inflammation, smooth muscle phenotype and proliferation as well as thromboresistance. Alterations of endothelial atheroprotective functions pave the way for the development of atherosclerotic lesions and their clinical manifestations. Therefore, better understanding the molecular mechanisms shaping up endothelial biology will help design future therapeutic strategies to prevent the development of vascular diseases. Recent publications in Arteriosclerosis, Thrombosis, and Vascular Biology have contributed to understanding several of these important processes.

Endothelial Phenotype

Molecular mechanisms that control the induction of arterial and venous endothelial identity include upstream and downstream effectors of Wnt, Sox, and Notch pathways, but little is known on the determinants that maintain this specific identity of the endothelium. van Geemen et al bring convincing evidence that in vivo endothelial phenotype of human arteries and veins is determined by F-actin-anchored focal adhesion and biomechanical properties of extracellular matrix. In adult arteries, prominent F-actin fibers follow the orientation of fibronectin and anchor to focal adhesions recruiting integrin binding to paxillin and focal adhesion kinase, whereas in venous endothelium, F-actin fibers mostly localize cortically at cell boundaries.

Endothelial Epigenetics

Epigenetic modifications in cardiovascular diseases have raised considerable interest as so far, genome wide association studies account for only 10% of coronary artery disease inheritability. These alterations in chromatin, which do not result from changes in DNA sequence, involve DNA methylation, RNA-based mechanisms and histone variants, and post-translational modification, such as acetylation, methylation, or phosphorylation. Epigenetic modifications taking place in endothelial cells have been elegantly reviewed in a recent mini-series. Current epigenetic studies focus on protein coding-genes, and it remains unknown if such modifications also regulate nonprotein-coding genes and whether or not long noncoding RNAs play a role.

Better understanding of endothelial epigenetic modifications, in particular under different flow conditions, will certainly improve the identification of new therapeutic targets in cardiovascular disease. Two studies recently summarized how disturbed proatherogenic flow upregulates DNA methyltransferases in vivo and in vitro, resulting in alterations of genome-wide DNA methylation and changes in gene expression. For instance, the promoters of the mechanosensitive genes HoxA5, Klf3, and Klf4 are hypermethylated in disturbed flow conditions, highlighting how flow controls epigenomic methylation patterns, in turn, regulating atherosclerosis development. shear-stress dependent regulation of endothelial noncoding RNA, in particular microRNA, as well as their transfer to neighboring cells after the release of endothelial extracellular vesicles, was recently summarized in the context of atherosclerosis. Histone posttranslational modification by acetylation results from the balance between acetylase and deacetylase enzyme activities and involves the large family of histone deacetylases (HDAC), which includes sirtuins and HDAC. Further studies will determine how proatherogenic and atheroprotective flow conditions regulate endothelial HDAC and NAD-dependent HDAC sirtuin-1 that protects against oxidative stress and multiple other parameters.

Deciphering endothelial epigenetic modifications will also help biologists understand more fundamental observations such as molecular basis of endothelial cell-specific gene expression. Okada et al have tested this hypothesis to decipher the specific endothelial expression of Roundabout (Robo4), a transmembrane receptor involved in migration, proliferation, angiogenesis, and stabilization of the vasculature. The ubiquitous expression of Robo4 transcription factors GABP and S1P, which regulate its promoter activation, cannot explain Robo4 specific endothelial localization. Okada et al demonstrate that the endothelial specific DNA methylation pattern of Robo4 proximal promoter during cell differentiation regulates Robo4 endothelial specific expression. This finding might be important as other endothelial specific genes, including von Willebrand Factor (vWF), E-selectin, VEGF receptors 1 and 2, and platelet endothelial cell adhesion molecule are also regulated by non–cell-type specific transcription factors.

Regulation of Vascular Tone

Because the seminal work from Furchgott and Zawadzki demonstrating the obligatory role of the endothelial cell in the control of vascular tone, the contribution of different mediators, including nitric oxide (NO), to endothelium-dependent responses has been extensively investigated. Schuler et al have established a noninvasive method in mice to assess flow- and NO-dependent vasodilation of murine femoral arteries.
This approach might be extremely useful to investigate in vivo endothelial function in murine models of vascular diseases and in genetically modified mice. In humans, endothelium-dependent regulation of vascular tone seems to be affected by ethnic origin. In particular, Ozkor et al\textsuperscript{16} report ethnic differences in the availability of NO and endothelium-derived hyperpolarizing factor. They show that the contribution of basal and stimulated release of NO to regulation of vascular tone is greater in white than in healthy black individuals. In addition, endothelium-derived hyperpolarizing factor partially compensates for the reduced NO contribution in black subjects. Vascular tone is also regulated by adipokines and yet unidentified factors that are released from perivascular adipose tissue. These relaxing factors stimulate potassium channel opening in vascular smooth muscle cells and could help fight vascular dysfunction in obesity and hypertension.\textsuperscript{17} Melanocortin-1 receptor (MRC-1), which regulates melanin pigmentation in the skin, has been also identified in endothelial cells where it increases NO availability. Rinne et al\textsuperscript{18} report that alterations in MRC-1 signaling in mice and in humans leads to impaired endothelial-dependent vasodilation and NO availability and to increased arterial stiffness. These findings highlight the role of MRC-1 in the regulation of vascular tone.

Increasing high-density lipoprotein levels after inhibition of cholesteryl ester transfer protein activity improves endothelial repair and function in a model of balloon vascular injury.\textsuperscript{19} In normcholesterolemic rabbits, cholesteryl ester transfer protein inhibition reduced intimal thickening and regenerates a functional endothelium through mechanisms involving scavenger receptor B1 and phosphatidylinositol-4,5-bisphosphate 3-kinase/Akt. Whether or not this beneficial effect also occurs in humans following treatment with cholesteryl ester transfer protein inhibitors remains an open question.

Using mice specifically deficient in endothelial AMP-activated protein kinase (AMPK) isoform α-1 or -2, Enkhjargal et al\textsuperscript{20} demonstrate that AMPK-α-1, the major AMPK isoform expressed in endothelial cells, mediates endothelium-dependent hyperpolarizations in resistance arteries, and regulates blood pressure and coronary flow in vivo. Presence of MRC-1 in endothelial cells increases NO availability.\textsuperscript{18} This receptor is abundantly expressed in the skin where it regulates melanin pigmentation. Alterations in MRC-1 signaling impair endothelial and NO-dependent vasodilation in mice and in humans, leading to increased arterial stiffness.\textsuperscript{18} These findings highlight the role of endothelial MRC-1 in the regulation of vascular tone. Expression of endothelial nitric oxide synthase (eNOS) is regulated by several exogenous stimuli, including tumor necrosis factor-α, which destabilizes eNOS mRNA leading to decreased eNOS expression. Tumor necrosis factor-α increases the expression of the cystolic peptide polypyrimidene tract-binding protein 1, which specifically binds to the eNOS 3′ untranslated region. This results in decreased eNOS expression and impaired endothelial vasodilatory responses.\textsuperscript{21} Therefore, modulation of tract-binding protein 1 expression might be of interest in treating endothelial dysfunction. Angiotensin II stimulates the endothelial expression and occupancy of histone H3K4 trimethyltransferase SET1 on the endothelin-1 promoter, activating the transcription of this potent vasoconstrictor.\textsuperscript{22} Specific-endothelial deficiency in SET1 prevents angiotensin II-induced release of endothelin-1 and abrogated hypertrophy of cultured cardiomyocytes. These findings reinforce the interest for SET1 inhibitors for treatment of cardiomyopathy.

### Angiogenesis, Arteriogenesis

Efficient endothelial repair after endothelial damage or injury is a critical step to prevent adverse arterial remodeling, thrombosis, and atherosclerosis.\textsuperscript{23,24} In this regard, microRNA seem as an interesting tool for improving angiogenesis.\textsuperscript{24} Cao et al\textsuperscript{25} investigated the therapeutic angiogenic effect of miR126-3p using ultrasound targeted microbubble destruction, a noninvasive technique for targeted vascular transfection of plasmid DNA, including microRNAs. In their study, transfection of miR-126-3p before and after rat left femoral artery ligation improved perfusion, vessel density, enhanced arteriolar formation, pericyte coverage, and phosphorylated Tie2 levels with no effect on miR-126-5p. Altogether, these findings support the potential for ultrasound targeted microbubble destruction for microRNA delivery, in particular for therapeutic angiogenesis. Manipulating insulin-like growth factor-1 receptor expression in bone marrow cells seems also of interest to improve endothelial repair after arterial wall injury.\textsuperscript{26} Insulin-like growth factor-1 receptor haploinsufficiency in bone marrow cells accelerates endothelial regeneration in a murine model of vascular wire injury and does not alter the formation of atherosclerotic lesions. Despite their lower number, angiogenic progenitors display enhanced adhesion, increased release of insulin-like growth factor-1 and enhanced angiogenic capacity. The platelet glycoprotein Ibα (GPIiba) has been identified as a critical mediator of transient platelet adhesion to endothelial cells and of leukocyte accumulation in the perivascular space of collateral vessels.\textsuperscript{27} These findings underline the role of GPIiba in arterial remodeling and arteriogenesis. In addition, inhibition or loss of function of GPIiba jeopardizes reperfusion recovery. Using a murine model of arteriolar ligation, Bruce et al\textsuperscript{28} demonstrate the unexpected and rapid entry of circulating CX3CR1+ monocytes at the postcapillary venous site to promote arteriogenesis.

Recent findings indicate that the Jagged1/Notch signaling regulates not only developmental angiogenesis but also plays an active role in adult angiogenesis.\textsuperscript{29} By blocking DLL4 signaling through Notch1, Jagged1 allows endothelial cell growth by activating VEGF. In addition, Jagged1 binds to Notch4 and triggers maturation of the newly formed vessels. Furthermore, VEGF receptor internalization and signaling contribute to the angiogenic growth of blood vessels. Full VEGF receptor signaling and recycling of VEGFR2 to the endothelial cell surface requires the clathrin-associated sorting proteins numb and numb-like.\textsuperscript{30}

Finally, Pi et al\textsuperscript{31} bring strong evidence that nicotinamide adenine dinucleotide phosphate (NADPH) oxidases are novel positive regulators of endothelial migration and angiogenesis induced by stromal cell–derived factor 1α, a potent angiogenic chemokine. Reactive oxygen species generated by NADPH oxidases oxidize and inhibit the protein tyrosine phosphatases MKP7, thereby regulating stromal cell–derived factor 1α–dependent JNK3 activity and angiogenesis. Therefore,
intracellular redox balance is critical for stromal cell–derived factor 1α–induced endothelial migration and angiogenesis.

**Hypoxia/Reoxygenation**

Although mitochondrial content in endothelial cells is rather modest when compared with other cell types, recent investigations suggest that mitochondrial dynamics affect endothelial cell function. Both studies from Haslip et al and He et al bring new evidence regarding the impact of endothelial mitochondrial function in vascular pathologies associated with hypoxia.

First, the interaction between mitochondria and the endoplasmic reticulum plays an important role in endothelial cells during reperfusion-injury. Mitochondria serve as calcium buffer sites and regulate calcium uptake and release by the endoplasmic reticulum. During cell stimulation, continuous flux of calcium through mitochondria is needed for store-operated entry and endoplasmic reticulum calcium store refilling. Acetylcholine, a neurotransmitter released during vagal nerve stimulation, decreases both intracellular and mitochondrial Ca²⁺ overload, and protects endothelial cells from hypoxia/reperfusion injury. This effect likely results from disruption of the endoplasmic reticulum/mitochondria crosstalk by limiting the interaction between mitofusin-2 and the complex voltage-dependent anion channel-1/glucose-regulated protein 75/inositol 1,4,5-trisphosphate receptor 1. Second, exposure to intermittent hypoxia of mice deficient in endothelial mitochondrial uncoupling protein-2 leads to the development of pulmonary hypertension. This effect results from excess phosphatase and tensin homolog (PTEN)–induced putative kinase 1-induced mitophagy, inadequate mitochondrial biogenesis, and subsequent endothelial apoptosis. Inhibiting PTEN–induced putative kinase 1 pathway in mice deficient in endothelial uncoupling protein-2 prevents pulmonary hypertension. As endothelial cells from patients with pulmonary hypertension have a similar phenotype to uncoupling protein-2-deficient endothelial cells, targeting the uncoupling protein-2-PTEN–induced putative kinase 1 pathway might be of therapeutic interest in this pathology.

**Flow Sensing, Shear Stress**

The regulation of endothelial function and proatherogenic phenotype by mechanical forces including shear stress was recently reviewed in ATVB. Until not too long ago, the effect of shear stress on endothelial cells had focused on changes in the steady-state levels of mRNAs. However, several new findings point out that epigenetic mechanisms of transcription, changes in mRNA stability and RNA translational efficiency, mediated by altered microRNA expression are other important mechanisms regulating endothelial gene function in different hemodynamic conditions. Furthermore, Murphy and Hynes provide new evidence that disturbed blood flow affects mRNA splicing of a functionally important endothelial cell gene product. In their study, they demonstrate that disturbed flow leads to changes in the mRNA splicing of endothelial fibronectin and that these alternatively spliced exons protect the vasculature from inflammation caused by disturbed flow. Shear stress also affects endothelial metabolism. Exposure of endothelial cells to laminar flow impairs their capacity to uptake glucose and their mitochondrial content in a Kruppel-like factor (KLF)-2 dependent manner. This effect results from the inhibition caused by shear stress of key glycolytic enzymes, and in particular, repression of 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase-3 (PFKFB3) promoter. All together these findings demonstrate that shear stress repression of endothelial metabolism is mediated by KLF2 and that PFKFB3 seems as an underestimated regulator of endothelial cell phenotype. These findings might help explain the switch of endothelial cell activation and angiogenesis back to a quiescent endothelium once blood flow is established.

Endothelial senescence has been observed in atheroprone areas of the vasculature. Warboys et al provide now a mechanistic link between endothelial shear stress and cell senescence by demonstrating that disturbed or low flow promotes endothelial senescence via the p53–p21 pathway. As this effect can be inhibited by siRNA-1, pharmacological activation of sirtuin1 may promote endothelial health by suppressing endothelial senescence in atheroprone areas. Flow-dependent outward remodeling of resistance arteries is a key adaptive process declining with age. Tarhouni et al demonstrate that estrogen deprivation rather than age impairs flow mediated remodeling and that timing for estrogen replacement is important to improve this response.

**Endothelial Inflammation**

Leukocyte recruitment at inflammatory sites is important for an appropriate inflammatory response in cardiovascular pathologies. Zuchtriegel et al identified specific-spatiotemporal expression patterns of selectins in inflammatory cells and endothelial cells. For instance, P-selectin, L-selectin, and P-selectin-glycoprotein ligand regulate neutrophil and monocyte flux, whereas endothelial E-selectin controls the rolling velocity of inflammatory monocytes. These specific-expression patterns of selectins associate with the sequential infiltration of inflammatory leukocytes and collectively enable the sequential extravation of leukocytes to the inflammed tissue. As endothelial peroxisome proliferator-activated receptor gamma receptor activation downregulates P-selectin expression and impairs leukocyte-endothelial interaction, this pathway might be an interesting therapeutic target to protect against thrombosis. On the contrary, endothelial angioptoein-like-protein-2 accelerates vascular inflammation by activating proinflammatory signaling in endothelial cells and increasing macrophage infiltration, leading to endothelial dysfunction and atherosclerosis progression. Psoriasis is an inflammatory skin disease associated with increased cardiovascular mortality. A mouse psoriasis model where interleukin-17A (IL-17A) was overexpressed in the dermis was used to test the hypothesis that increased IL-17A production in the skin may systemically cause vascular dysfunction. This model overexpressing dermal IL-17A was characterized by infiltration of myeloperoxidase-labeled GR1/F4/80 inflammatory cells, increased oxidative stress, systemic endothelial dysfunction, and hypertension. The deleterious consequences of IL-17A overexpression were improved by neutralizing cytokines downstream of IL-17A.
such as tumor necrosis factor-a and IL-6, or depleting GR1+ inflammatory cells. These finding are in favor of the causal role of IL-17A-dependent dermal inflammation in systemic vascular alterations observed in psoriasis.

Endothelial inflammation is associated with increased permeability. Near-infrared fluorescence imaging seems as an interesting new tool to monitor endothelial permeability in large vessel, as it leaves tissues intact for subsequent histological analysis of quantification of leucocytes subpopulations by flow cytometry.47 A nanoparticle-based imaging strategy was also developed to identify pathological endothelial cells in a murine model of arteriovenous fistula.48 In this model, nanoparticles labeled preferentially sites expressing high levels of VCAM-1 and characterized by increased permeability. Therefore, these nanoparticles identify areas with a pathological endothelium where neointimal hyperplasia subsequently develops in arteriovenous fistula. This strategy could be of potential interest to monitor therapeutic interventions to decrease the failure of arteriovenous fistula resulting from in flow stenosis in patients with end-stage renal disease.

Inflammatory conditions can form large vWF fibers, which immobilize on endothelial surface to form highly adhesive substrate under shear conditions. Grässle et al elegantly show that vWF directly binds and immobilizes extracellular DNA released from leucocytes (neutrophil extracellular traps). DNA-bound vWF decreases platelet binding to vWF and might be a linker for leukocyte adhesion to endothelial cells, favoring leukocyte extravasation, and inflammation.49 Cocaine consumption leads to endothelial dysfunction and accelerated atherosclerosis. Endothelial cells exposed to cocaine or plasma from chronic cocaine consumers were more proadhesive, with increased von Willebrand deposition, enhancing platelet binding, an effect prevented by statin treatment.50 Atherosclerotic stimuli increase Arginase-2 expression, leading to eNOS uncoupling. The specific HDAC2 is a critical regulator of endothelial Arginase2 transcription. HDAC2 limits Arginase2 transcription in healthy conditions, but this control is impaired in proatherogenic conditions and oxidative injury. Therefore, HDAC2 activation or overexpression could represent a new therapy for endothelial dysfunction in atherosclerosis.51 Finally, deficiency in scavenger receptor B1 in Ldlr−/− mice is associated with increased endothelial VCAM and ICAM expression in coronary arteries and augmented levels of circulating inflammatory markers, including increased proportions of Ly6C(hi) and Ly6C(int) monocytes.52

Endothelium in Diseases

Diabetes Mellitus

Diabetes mellitus affects endothelial function. Metformin, an AMPK activator used for treatment of diabetic patients, improves endothelial dysfunction by inhibiting endoplasmic reticulum stress and increasing NO bioavailability after activation of the AMPK/peroxisome proliferator-activated receptor delta pathway in a model of obese diabetic mice.53 Inhibition of the bone morphogenetic protein-4 (BMP4) pathway is also an interesting target in the treatment of diabetic endothelial dysfunction, as it has been previously established that BMP4 impairs endothelium-dependent vasodilatation and is upregulated in hypertension.54 Different strategies inhibiting BMP4 in diabetic db/db mice reduce reactive oxygen species production and rescued endothelium-dependent relaxations.55 Similar findings are observed after inhibition of activin receptor-like kinase 3, a pathway downstream of BMP4 receptor. Regulation of the BMP4 pathway might therefore represent an interesting therapeutic strategy to fight endothelial dysfunction in diabetes mellitus.

Fatty Liver Diseases

Nonalcoholic fatty liver disease, the most common chronic liver conditions, confers an increased risk to develop cardiovascular diseases.56 The study by Long et al57 identifies an association between nonalcoholic fatty liver disease and vascular function in participants from the Framingham Heart study without overt cardiovascular diseases. After multivariable adjustment, nonalcoholic fatty liver disease associates with high mean blood pressure and low peripheral arterial tonometry ratio, indicating that nonalcoholic fatty liver disease may directly contribute to microvascular dysfunction.

Atherosclerosis and Coronary Artery Diseases

Endothelial function in patients is evaluated through the measure of brachial flow-mediated dilatation, but this approach remains challenging.58 Therefore, an extensive area of research aims at identifying circulating biomarkers to assess endothelial health in patients. Plasma levels of soluble endothelial cell selective adhesion molecule were investigated in patients with stable coronary artery disease with respect to their kidney function.59 Circulating endothelial cell selective adhesion molecule levels were strongly and independently associated with reduced glomerular filtration rate in patients with coronary artery disease,60 suggesting that high endothelial cell selective adhesion molecule levels are a risk factor for a reduced kidney function in coronary artery disease.

Recent experimental evidence indicates that alterations of endothelial shear stress regulate specific mecanosensitive microRNA,8 which control key signaling pathways including cell cycle, inflammation, apoptosis, and NO pathway. Therefore, these mecanosensitive microRNA might represent potential targets for the treatment or prevention of the development of atherosclerosis.60

To decipher the specific contribution of glucocorticoid receptor in endothelial cells during the progression of atherosclerosis, Goodwin et al61 designed a mouse model of atherosclerosis with specific deletion the endothelial glucocorticoid receptor. Mice lacking the endothelial glucocorticoid receptor developed more severe plaques characterized by increased macrophage content. Altogether these data underline the anti-inflammatory role of the endothelial glucocorticoid receptor to limit atherosclerosis development.61

In conclusion, significant advances have been made in understanding the complexity of molecular mechanisms participating in the development of vascular diseases. These
original contributions recently published in ATVB provide critical basic knowledge in vascular biology and may lead to the identification of new therapeutic avenues or diagnostics to fight cardiovascular diseases in the decades to come.

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References


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