Mechanisms of Endothelial Regeneration and Vascular Repair and Their Application to Regenerative Medicine

Colin E. Evans,* M. Luisa Iruela-Arispe, and You-Yang Zhao*

From the Program for Lung and Vascular Biology and Section of Injury Repair and Regeneration,* Stanley Manne Children’s Research Institute, Ann & Robert H. Lurie Children’s Hospital of Chicago, Chicago; and the Department of Critical Care, the Department of Cell and Developmental Biology, the Feinberg Cardiovascular and Renal Research Institute, the Division of Pulmonary and Critical Care Medicine, Department of Medicine, and the Department of Pharmacology, Northwestern University Feinberg School of Medicine, Chicago, Illinois

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Address correspondence to You-Yang Zhao, Ph.D., Program for Lung and Vascular Biology, Stanley Manne Children’s Research Institute, Ann & Robert H. Lurie Children’s Hospital of Chicago, 225 E Chicago Ave, Box 205, Chicago, IL 60611. E-mail: youyang.zhao@northwestern.edu.

Endothelial barrier integrity is required for maintaining vascular homeostasis and fluid balance between the circulation and surrounding tissues and for preventing the development of vascular disease. Despite comprehensive understanding of the molecular mechanisms and signaling pathways that mediate endothelial injury, the regulatory mechanisms responsible for endothelial regeneration and vascular repair are incompletely understood and constitute an emerging area of research. Endogenous and exogenous reparative mechanisms serve to reverse vascular damage and restore endothelial barrier function through regeneration of a functional endothelium and re-engagement of endothelial junctions. In this review, mechanisms that contribute to endothelial regeneration and vascular repair are described. Targeting these mechanisms has the potential to improve outcome in diseases that are characterized by vascular injury, such as atherosclerosis, restenosis, peripheral vascular disease, sepsis, and acute respiratory distress syndrome. Future studies to further improve current understanding of the mechanisms that control endothelial regeneration and vascular repair are also highlighted.

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Endothelial cells (ECs) line the inner wall of blood vessels and form a semipermeable barrier, which regulates the flux of fluid, proteins, and blood cells across the vascular wall into parenchymal tissue and maintains an antithrombotic and anti-inflammatory state of the microvascular bed.1,2 Endothelial injury leads to complications associated with inflammation, including increased vascular permeability, transmigration of inflammatory cells, exit of erythrocytes, tissue edema, and microthrombosis.3 Endothelial barrier dysfunction is a key initiating event of various vascular diseases, including atherosclerosis and in-stent restenosis.1 Evidence from humans and animals has demonstrated the central role of endothelial leakage in determining the outcome of vascular diseases, including sepsis-induced lung injury.4–10 A better mechanistic understanding of endothelial regeneration and vascular repair is therefore of pivotal importance in the development of novel therapeutic strategies to promote endothelial integrity and vascular health.

After endothelial injury, the vascular repair process involves restoration of a functional endothelial monolayer (ie, endothelial regeneration) and reestablishment of the endothelial junctions to reform a semipermeable barrier (Figure 1). Endothelial regeneration may involve resident ECs themselves (ie, endogenous) and/or cells other than the resident EC population, such as the circulating stem/progenitor cells (ie, exogenous). Mechanisms that regulate endothelial barrier development and injurious junctions...
leakage have been reviewed thoroughly by others. The current review describes the cellular and molecular mechanisms that control endothelial regeneration and recovery of endothelial barrier function after injury. Major unanswered research questions pertaining to endothelial regeneration and vascular repair are identified herein, and future studies that could improve understanding of regenerative and reparative endothelial signaling are discussed.

**Cellular Sources of Endothelial Regeneration**

Circulating Cells Fail to Contribute Directly to Endothelial Regeneration

The process of endothelial regeneration is thought to involve resident EC migration and proliferation and/or recruitment of circulating stem/endothelial progenitor cells (EPCs) that differentiate to ECs. Since the identification of circulating EPCs derived from the bone marrow (BM), investigating the role of EPCs in endothelial regeneration and vascular repair has become an active area of research. Studies have found that some circulating cells express the same surface markers and exhibit some properties in vitro similar to mature ECs. These studies indicate that circulating stem/progenitor cells may engraft into and function to regenerate the damaged endothelium. However, mounting evidence indicates that circulating EPCs (most likely monocytic) do not directly contribute to endothelial regeneration by forming part of the regenerating endothelium. Using BM cell transplantation and an EC-specific reporter, Ohle et al revealed a rare population of BM-derived cells (CD45+/CD31+/CD144–) in recipient human lungs at 1 year after transplantation. However, these cells were not found in the anatomic compartment of the pulmonary vascular endothelium, indicating no involvement in endothelium maintenance. Furthermore, the study found no contribution of BM-derived cells to the endothelium of the adult lung, heart, liver, pancreas, or kidney after hyperoxic injury and recovery. In a study of lipopolysaccharide (LPS)—induced lung injury in mice, Kawasaki et al found that pulmonary EC number markedly decreases at 1 day after LPS administration but returns to baseline by day 7. Subsequent studies with green fluorescent protein (GFP)—positive BM chimera mice revealed only a limited amount (approximately 2%) of engraftment of BM-derived ECs. However, GFP+ ECs do not proliferate with minimal changes of total GFP+ ECs, which in fact decrease approximately 0.5% from 2% during the recovery from LPS-induced injury. Studies with direct GFP+ BM cell transplantation without irradiation also found that BM GFP+ cells hardly engrafted to pulmonary vascular endothelium or proliferated during the regenerative process. The total number of circulating EPCs (Flk1/KDR+/CD133+ or Flk1/KDR+/CD34+) is below the basal levels by day 7 after lung injury, indicating no engraftment to the injured lung endothelium. Hagensen et al also attempted to determine the role of circulating cells in endothelial regeneration. Wire-injured carotid artery segments from wild-type mice were transplanted into Tie2-GFP mice, demonstrating that the neoendothelium was negative for GFP and suggesting that circulating cells, including BM-derived EPCs, do not directly engraft to the injured endothelium to form a part of the regenerated endothelium. Using pairs of genetically marked parabiotic mice with a shared anastomosed circulatory system, Purhonen et al also demonstrated that BM-derived circulating endothelial precursors do not contribute to endothelial regeneration.

Intriguingly, Singhal et al provided evidence of the contribution of BM-derived circulating cells to vascular repair in a murine hepatectomy model, but these cells did not directly contribute to endothelial regeneration by forming the regenerating endothelial layer. Using a partial hepatectomy model as well as chronic liver damage models where the liver vascular endothelium remains intact, Singhal et al observed no BM-derived cell incorporation into the new vessels. After irradiation-induced EC damage,
BM-derived mononuclear cells were recruited and incorporated into the vasculature, but these cells failed to differentiate to ECs. Together, the recent studies using genetic lineage tracing and experimental parabiosis as well as advanced imaging techniques provide convincing evidence that BM-derived cells and circulating EPCs are not the major source of ECs that make up the regenerating endothelium. Nevertheless, endothelial regeneration and vascular repair seems to benefit from factors released from circulating progenitor cells, and such factors could play a role in these regenerative and reparative processes (Figure 1).

These exogeneous paracrine mechanisms are described later in the article.

**Regeneration of the Endothelium by Tissue Resident ECs**

To gain insights into the role of tissue resident ECs in the mechanism of endothelial regeneration, approaches such as genetic lineage tracing have been used. Using Tie2Cre-GFP mice, Itoh et al. found that recovery of the EC-denuded later in the article. These exogeneous paracrine mechanisms are described later in the article.

**Specialized Tissue-Resident ECs Responsible for Endothelial Regeneration**

Heterogeneity is a characteristic feature of ECs. Recent studies have tried to identify and define potential specialized tissue-resident ECs responsible for endothelial regeneration. In a study mentioned above, Kawasaki et al. found that a subpopulation of lung resident ECs (CD34+/Flk-1/KDR+/c-kit+) with progenitor cell characteristics is largely responsible for lung endothelial regeneration after acute lung injury in mice. Experiments using BM chimeric mice revealed that most of the regenerating pulmonary vascular ECs are tissue-resident cells, whereas BM-derived cells barely engraft as pulmonary vascular ECs. The regenerating pulmonary vascular ECs are characterized by high colony-forming and vasculogenic capacities with high expression of CD34, Flk-1/KDR, and c-kit and low expression of Prom1/CD133 on the cell surface when compared with nonproliferating pulmonary vascular ECs. In a study by Wakabayashi et al., a subpopulation of CD157-expressing regenerative tissue-resident endothelial stem cells was identified in the arteries and veins but not capillaries of numerous mouse organs, including liver, lung, heart, skeletal muscle, skin, retina, and brain. These cells were able to regenerate entire vasculature structures after injury and maintain large vessels in the healthy liver for >12 months. As discussed above, McDonald et al. identified two subpopulations of resident ECs that contribute to endothelial regeneration in the injured aortic endothelium, and the Cdh5+/Atf3+ subpopulation is the dominant subpopulation (approximately 80%) responsible for endothelial regeneration.

These studies have defined the predominant role of resident endothelial cell proliferation in the mechanisms of endothelial regeneration. Genetic lineage tracing studies have found that >95% of the regenerative ECs are derived from resident ECs. Both BM-derived and circulating progenitor cells rarely engraft and differentiate into ECs. The relative contribution of the cells other than resident ECs should be defined by genetic lineage tracing. In addition, there is an interesting subpopulation called mesoangioblasts. These vessel-associated multipotent cells often express endothelial and/or pericyte markers, such as platelet-derived growth factor receptor β, CD271, CD73, EMCN, and delta-like 1 (DLK1) and are able to form vessel-like structures. Although the contribution of mesoangioblasts to cardiac regeneration has been well studied, the role of mesoangioblasts in endothelial regeneration and vascular repair remains unclear.
Transcription Factors That Mediate Endothelial Regeneration

FoxM1

FoxM1 is a member of the mammalian forhead box family of transcription factors that share homology in the winged helix or forhead DNA binding domain. FoxM1 expression is markedly induced in lung ECs in the recovery phase but not in the injury phase after endotoxia. The role of this transcription factor in the recovery of the pulmonary endothelium after injury has been investigated using mice that lack endothelial FoxM1. EC-specific Foxm1 knockout mice exhibited impaired EC proliferation and persistent increases in lung vascular permeability despite a similar degree of peak injury compared with that seen in wild-type mice. Overexpression of FoxM1 enhanced endothelial proliferation and thus facilitated the recovery of endothelial barrier integrity and promoted survival.

HIF-1α

Hypoxia-inducible factors (HIFs) are critical mediators of the adaptive response to hypoxia and ischemia. These heterodimeric proteins consist of a constitutively expressed β-subunit and an oxygen-labile α-subunit that has three isoforms (HIF-1α, HIF-2α, and HIF-3α). Huang et al found that HIF-1α but not HIF-2α is rapidly induced and stabilized in murine pulmonary vascular ECs after sepsis challenge. Mice with EC-specific disruption of Hif1α exhibited impaired lung endothelial regeneration and vascular repair in contrast to wild-type mice, despite similar levels of peak lung injury. Forced expression of FoxM1 in lung ECs of conditional Hif1α knockout mice normalized EC proliferation and vascular repair, indicating that endothelial HIF-1α is required for endothelial regeneration and vascular repair via FoxM1-mediated EC proliferation after sepsis-induced lung injury.

Sox17

Sox17 is a member of the Sry-related high-mobility group domain family F (SoxF) transcription factors and a key developmental regulator of endothelial and hematopoietic lineages. After sepsis challenge in mice, Sox17 is markedly induced in pulmonary vascular ECs in as little as 6 hours. EC-specific deletion of Sox17 resulted in decreased EC proliferation and a persistent increase in pulmonary vascular permeability in response to endotoxia. Conversely, overexpression of Sox17 in lung ECs enhanced lung EC proliferation and regeneration and promoted survival. Promoter analysis identified Sox17 as a transcriptional target of HIF-1α.

Atf3

In the regenerating endothelium of large murine blood vessels, the stress-inducible transcription factor, ATF3, is a key transcription factor. ATF3 is a member of the ATF/CREB family of transcription factors involved in many human diseases, including cancer and atherosclerosis. ATF3 is highly expressed in proliferating ECs after mechanical injury of the murine aortic endothelium imposed by a clamp. Global Atf3 knockout mice have a significant reduction in EC proliferative capacity and defective endothelial regeneration, resulting in impaired wound closure after mechanical pressure—induced injury of the aortic endothelium.

Signaling Pathways Responsible for Endothelial Regeneration and Vascular Repair

P110γPI3K Signaling

Huang et al found that endothelial FoxM1 induction after sepsis-induced lung injury in mice was mediated by the G-coupled protein receptor (GPCR)—dependent p110γ isoform of phosphoinositide 3-kinase (PI3K). Global Pik3cg knockout mice had defective pulmonary endothelial proliferation and persistent inflammatory injury after sepsis challenge despite similar levels of peak lung injury. Restoration of EC-specific FoxM1 expression in Pik3cg global knockout mice promoted EC regeneration and normalized the repair process. Furthermore, Huang et al observed diminished expression of p110γ in pulmonary vascular ECs taken from patients with acute respiratory distress syndrome (ARDS). The authors also found that this regenerative pathway is not limited to the pulmonary vasculature but is also operative in the systemic vasculature. Defective vascular repair was also observed in the cremaster muscle venules in Pik3cg-null mice, which was normalized in Pik3cg-null mice with transgenic expression of FoxM1. Mechanistically, p110γ induced FoxO phosphorylation and translocation out of the nucleus, which resulted in FoxM1 induction. The study by Huang et al further found evidence that stromal cell—derived factor 1 is a possible upstream GPCR-mediated ligand responsible for p110γ activation after sepsis challenge. These studies found the crucial role of the endogenous EC-specific p110γPI3K-FoxM1 signaling axis in endothelial regeneration and vascular repair after inflammatory injury.

miRNA-126 Signaling

The autocrine and paracrine roles of miRNAs in EC regeneration by resident ECs have also been studied. Schober et al found that EC recovery after denudation was impaired by lack of miRNA-126-5p in global knockout mice, which reduced EC proliferation through activation of the Notch1 inhibitor, Dlk1 homolog. Down-regulation of miR-126-5p by disturbed flow abrogated EC proliferation at athero-prone sites in response to hyperlipidemic stress through up-regulation of Dlk1 expression, whereas administration of miR-126-5p rescued EC proliferation at
endothelial proliferation and regeneration. On the other hand, Jansen et al.42 found that coronary artery ECs can promote EC repair in mice through microparticle-mediated transfer of miRNA-126, which increases EC migration and proliferation. The miR-126 from damaged ECs was acquired by recipient human coronary artery ECs via endothelial microparticles and functionally regulated the expression of the target protein sprout-related EVH1 domain-containing protein 1 (SPRED1). Knockdown of miR-126 in EC microparticles abrogated the EC microparticle—mediated effects on human coronary artery EC migration and proliferation. Intriguingly, miR-126 expression was reduced in circulating microparticles from patients with diabetes.42 In a separate study, miR-126 enhanced the angiogenic actions of vascular endothelial growth factor (VEGF) and fibroblast growth factor and promoted neovessel formation by suppressing the expression of SPRED1 in mice, which reduces EC migration and proliferation by inhibiting Ras/mitogen-activated protein kinase signaling in vitro.43

Notch1 Signaling

As described above, miRNA126-regulated Dlk1 inhibits endothelial proliferation and regeneration.41 Recent studies have also revealed that discrete types of non-ECs can drive EC regeneration. In a study using smooth muscle cell (SMC)—EC co-cultures, researchers have found that SMC-EC contact is required for the activation of Notch1 by bone morphogenetic protein receptor 2 (BMPR2) in both ECs and SMCs and for EC regeneration after wire-induced carotid artery injury in mice.44 BMPR2 is necessary to produce collagen IV, which in turn activates integrin-linked kinase directing phospho-c-Jun N-terminal kinase to the EC membrane, which stabilizes presenilin1 and releases notch1 intracellular domain(NICD1). NICD1 increased mitochondrial mass and induced expression of 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (a key metabolism regulator), which itself is necessary for citrate-dependent acetylation of H3K27. Thus, BMPR2-mediated activation of Notch1 coordinates EC metabolism with histone acetylation to induce expression of the Notch1 target Myc, and in turn, Myc-regulated genes, such as apelin and heme oxygenase 1, ultimately leading to EC proliferation and regeneration.

Apelin Signaling

A recent study by Masoud et al.15 found that apelin directs EC differentiation and vascular repair after immune-mediated injury. They also found that human and mouse heart allografts with alloimmune-mediated vasculopathy had increased expression of apelin in arteries and myocardial microvessels. Loss of apelin expression in the donor heart promoted graft immune cell infiltration, impaired vascular repair, and increased occlusive vasculopathy in mice. In contrast, an apelin receptor agonist induced endothelial nitric oxide synthase activation, promoted endothelial monolayer wound repair, and reduced immune cell adhesion. Treatment with the apelin receptor agonist also reduced progression of arterial occlusion in mice. A separate study found that hypoxia-induced expression of apelin modulates EC proliferation and regeneration in zebrafish.46 These authors found that apelin is induced by hypoxia in human pulmonary vascular lung ECs and SMCs. Furthermore, miRNA against apelin or APJ receptor knockdown inhibited hypoxia-induced EC proliferation in vitro and vessel regeneration in zebrafish. Together, these data identify apelin as a key regulator of vascular endothelial regeneration.

VEGF Signaling

In rat models of nephritis, Ostendorf et al.47 found that VEGF inhibition reduced glomerular endothelial regeneration and increased EC death without altering proteinuria. In a model of balloon angioplasty-induced endothelial denudation of the rabbit carotid artery, VEGF treatment increased endothelial regeneration.48 In a coronary stent implantation model in minipigs, overexpression of VEGF receptor reduced neointimal proliferation, suggesting that VEGF signaling promotes endothelial regeneration after coronary injury.49 Similarly, in a mouse model of arterial endothelial denudation, adenoviral VEGF treatment increased luminal EC proliferation and accelerated endothelial repair, whereas VEGF inhibition had the opposite effects.50 These studies identify VEGF as an important factor for EC proliferation and regeneration and vascular repair.

Other Signaling Molecules

Endothelial-specific overexpression of insulin-like growth factor (IGF)-1 receptor (IGF1R) in transgenic mice promotes endothelial regeneration in a wire injury model of the femoral artery but reduces nitric oxide bioavailability, whereas Igf1r heterozygous knockout mice have reduced endothelial regeneration.51 EC isolated from lungs of the transgenic mice exhibit enhanced migration in vitro compared with wild-type ECs. Dimethylaminohydrolases degrade asymmetric dimethylarginine, which is an endogenous nitric oxide synthase inhibitor. Konishi et al.52 found that overexpression of dimethylaminohydrolase 1 promotes endothelial regeneration in the murine femoral artery after wire injury.

The studies summarized in the two sections above have identified the molecular mechanisms (transcriptional factors and signaling pathways) of endothelial regeneration and vascular repair in the systemic vasculature (Figure 2) and pulmonary vasculature (Figure 3). It appears that different vascular beds use different molecular mechanisms for endothelial regeneration. These differences may be ascribed to different injury models as well as different sizes of vessels (endothelial denudation in large arteries in the systemic vasculature versus inflammatory endothelial injury in...


Figure 2  Molecular and signaling mechanisms of endothelial regeneration and vascular repair in systemic arterial vessels after endothelial injury induced by mechanical or electrical denudation. Several studies that used carotid artery injury models have found an important role of endothelial Notch1 activation in the mechanism of endothelial proliferation. Microparticle-released miR-126 also plays an important role in endothelial proliferation and migration through inhibition of the Notch inhibitor delta-like 1 (Dlk1) and sprout-related EVH1 domain-containing protein 1 (SPRED1), Ras, and mitogen-activated protein kinase (MAPK) signaling. Smooth muscle cell (SMC) interaction with endothelial cells (ECs) promotes Notch1 activation through bone morphogenic protein receptor 2 (BMPR2) signaling, leading to EC proliferation. SMCs also release various factors (eg, CXCL7) to activate CCR2 signaling in the neighboring ECs and promote their migration for reendothelialization. Injured ECs and other cells, such as stem/progenitor cells, release microparticles and angiogenic and migratory factors (eg, BMPR2) signaling, leading to EC proliferation. SMCs also release various factors (eg, CXCL7) to activate CCR2 signaling in the neighboring ECs and promote their migration for reendothelialization. Injured ECs and other cells, such as stem/progenitor cells, release microparticles and angiogenic and migratory factors and therefore promote endothelial regeneration. In an aorta mechanical pressure injury model, the stress response gene ATF3 plays an important role in mediating endothelial regeneration and vascular repair. NICD1, notch1 intracellular domain; PFKFB3, phosphofructo-2-kinase/fructose-2,6-biphosphatase 3; pJNK, phospho-c-Jun N-terminal kinase; PKC-ε, protein kinase C-ε.

micovessels in the pulmonary vasculature. Zhao et al identified the important role of endothelial FoxM1 in mediating endothelial regeneration in mouse lungs after inflammatory injury induced by endotoxemia, whereas McDonald et al found that loss of endothelial FoxM1 has only delayed endothelial wound closure without affecting endothelial proliferation in injured aortic endothelium by mechanical pressure. However, in another study, p110γPI3K signaling is required for FoxM1-dependent endothelial regeneration and vascular repair in the pulmonary vasculature and cremaster muscle venules (systemic vasculature) in the same inflammatory injury model. Future studies are warranted to determine whether different vascular beds and different sizes of vessels share some of the molecular mechanisms for regeneration of the endothelium using the same injury model.

Re-Annealing of Endothelial Junctions to Restore Endothelial Barrier Integrity

Healing of the injured endothelium involves reestablishment of endothelial junctions to restore endothelial barrier integrity. Interendothelial junctions include adherens junctions (AJs), tight junctions, and gap junctions. AJs and tight junctions form molecular connections between adjacent ECs, promoting a strong seal along the EC perimeters. In contrast, gap junctions do not contribute to homotypic attachment but instead form intracellular channels, allowing for chemical and electrical communication between neighboring ECs. Intercellular junctions that connect the continuous monolayer of ECs impose restrictions in the movement of fluid, proteins, and blood cells across the endothelium and constitute a semipermeable barrier. Weakening or loss of interendothelial junctions results in transport of proteinaceous fluid into the perivascular space, which in turn leads to tissue edema, a common characteristic of a broad range of pathologic findings. Endothelial barrier permeability is largely regulated by AJs, which respond to mechanical and chemical stimuli under physiologic and pathologic conditions. The AJ complex is composed of vascular endothelial cadherin (VE-cadherin), β-catenin or plakoglobin, α-catenin, and p120-catenin. Homotypic interaction between the extracellular domains of VE-cadherin adheres two neighboring ECs together to form the junctions, whereas its cytoplasmic domains bind to p120-catenin and β-catenin or plakoglobin. In turn, β-catenin binds with the actin-associated protein α-catenin. Transcriptional and posttranslational modifications of these functional proteins, as well as cellular and molecular signaling pathways, mediate the plasticity of the endothelial junctions and, in doing so, regulate endothelial permeability. ECs also express N-cadherin, which regulates VE-cadherin expression and recruitment to the AJs and thus endothelial barrier integrity. Although mechanisms that regulate endothelial barrier development and injurious junction leakage have been reviewed thoroughly by others, this section summarizes the mechanisms involved in the reannealing of endothelial junctions and restoration of endothelial barrier integrity after injury.

β-Catenin Regulated by FoxM1 and SHP2

Using EC-restricted disruption of Foxm1 in mice and primary human lung microvascular ECs, Mirza et al
found that FoxM1 is required for endothelial AJ recovery via its transcriptional control of β-catenin. In mouse lungs and human lung microvascular ECs, conditional FoxM1 deletion resulted in persistent increases of vascular leakage and defective endothelial barrier recovery after thrombin-induced disruption of the endothelial barrier. However, loss of endothelial FoxM1 did not affect the extent of vascular leakiness during the injury phase. Restoration of β-catenin expression in Foxm1-null ECs in a separate study, Timmerman et al. found that β-catenin is a substrate of the Src homology 2 (SHP2) domain that contains tyrosine phosphatase. SHP2 regulates the recovery of disrupted EC junctions by dephosphorylating β-catenin associated with VE-cadherin and thus stimulating VE-cadherin mobilization at the plasma membrane. These studies emphasize the important role of β-catenin as a junctional protein in mediating reannealing of the endothelial AJs and driving the recovery of endothelial barrier integrity.

Gβγ, Fyn, and FAK Signaling

The heterotrimeric G-protein complex is composed of the Gα and Gβγ subunits. On stimulation with the inflammatory mediator thrombin, the Gz subunit is dissociated from the Gβγ subunit, which signals the increase in endothelial permeability. The released Gβγ subunit is reported to play an important role in reannealing of the AJs and persistent vascular leaking, although it does not affect the permeability increase after thrombin treatment. Thrombin induces Gβ dissociation from RACK1 and subsequent interaction with Fyn. Fyn-dependent phosphorylation of focal adhesion kinase (FAK) induces association of FAK with the endothelial AJs and mediates endothelial barrier recovery. Global Fyn knockout mice exhibit...
impaired vascular repair, and endothelial expression of the FAK phosphorylation mutant results in FAK activation and normalization of vascular repair in Fyn−/− mice. These data indicate that Gβγ-mediated Fyn activation integrates FAK with the endothelial AJs and mediates vascular repair.

AMPK and N-Cadherin

AMP-activated protein kinase (AMPK) is a molecular sensor triggered by stimuli that disrupt the vascular endothelium and subsequently mediates the recovery of the endothelial barrier. In pulmonary microvascular ECs, the α1-subunit of AMPK is required for the reformation of cell-cell networks and for the repair of EC monolayers after in vitro wounding. AMPK-α1 co-localizes with the AJ protein, N-cadherin, and in isolated lungs, AMPK activation contributes to EC barrier recovery. In a subsequent study on AMPK-α1, Jian et al58 found that AMPK stimulation by metformin treatment decreased the LPS-induced increase in lung vascular permeability in rats and resolved pulmonary edema in vivo. In ex vivo perfused rat lungs and isolated pulmonary microvascular ECs, AMPK-α1 was responsible for endothelial barrier recovery after EC injury, a process coordinated by N-cadherin. Disruption of N-cadherin’s intracellular domain causes translocation of AMPK away from the membrane and attenuates AMPK-mediated restoration of barrier function in LPS-treated endothelium. Inhibition of N-cadherin impaired AMPK-mediated vascular repair in murine lungs after LPS challenge.59

CD31, SHP, and Metabolic Reprogramming

CD31 is expressed at high density at the lateral borders of ECs. CD31 deficiency has been associated with excessive vascular leakage after endothelial injury induced by histamine, thrombin, and LPS-induced endotoxemia. Using the major histocompatibility complex activation–induced endothelial barrier disruption model, Cheung et al60 found that endothelial barrier recovery requires a CD31-induced glycolytic response that sustains endothelial AJ reassembly. The metabolic reprogramming requires SHP1/2 activation, leading to Akt-mediated nuclear exclusion of FoxO1 and concomitant β-catenin translocation to the nucleus, thus inducing cMyc transcription. Pharmacologic Akt or AMPK activation normalizes vascular repair and inhibits pathologic vascular leakage in CD31−/− mice via augmented glycolytic flux in ECs. These studies indicate the requirement of glycolysis-produced ATP in reestablishing endothelial AJs and repairing the leaking vessels.

Phospholipase D2/PTPN14

VE-cadherin recycling to AJs and dephosphorylation of tyrosine phosphorylated VE-cadherin promotes junctional reassembly and restoration of endothelial barrier function. Fu et al61 found that inhibition of phospholipase D2 (PLD2) activity resulted in prolonged phosphorylation of Tyr-658 in VE-cadherin in human lung microvascular ECs during the recovery phase after thrombin challenge and thus impaired endothelial barrier recovery via inhibition of protein tyrosine phosphatase nonreceptor type 14 (PTPN14). After thrombin challenge, PLD2, PTPN14, and VE-cadherin form a complex to facilitate PTPN14 dephosphorylation of VE-cadherin. PTPN14 depletion impaired reannealing of endothelial AJs and barrier recovery in human lung microvascular ECs. In Pld2−/− mice, lung vascular permeability failed to recover from PAR-1–activating peptide-induced injury. Together, these studies indicate that PLD2 promotes PTPN14-mediated dephosphorylation of VE-cadherin, which leads to recycling of VE-cadherin at the AJs for recovery of endothelial barrier function.

Ang2 and miRNA-150

Rajput et al62 found that miRNA-150 expression was elevated only during the recovery phase after murine lung injury. Deletion of miRNA-150 in mice resulted in a sustained increase in angiopoietin-2 (Ang2) levels, blocked repair of AJs after injury, increased vascular permeability, and promoted rapid postsepsis mortality. In contrast, restoration of miRNA-150 expression in ECs of global miRNA-150 knockout mice inhibited Ang2 production via targeting of the transcription factor early growth response 2 (Egr2), which restored endothelial barrier function. Deletion of Egr2 or Ang2 in mir-150−/− ECs restored junctional reannealing and reinstated endothelial barrier function.62 Overall, these studies found that restoring endothelial junctional integrity provides a therapeutic avenue for diseases characterized by vascular leakage.

Regulation of Endothelial Regeneration by Paracrine Factors from Other Cells

Although evidence reviewed earlier in the article suggests that nonresident/circulating cells are not a major source of regenerating endothelium, the numbers of circulating EPCs are correlated with disease risk, severity, or outcome in conditions that are characterized by vascular injury, such as cerebrovascular disease and sepsis.63,64 Exogenous administration of mesenchymal stem cells (MSCs) or BM-derived progenitor cells can inhibit inflammatory lung injury and promote survival after sepsis challenge.65–72 Importantly, MSC therapy shows promise for the treatment of patients with ARDS.73,74 It is now thought that BM-derived cells as well as exogenously administered adult stem cells (eg, MSCs) could promote endothelial regeneration and vascular repair through paracrine mechanisms, particularly given the studies described above that found that these cell types rarely engraft into the vasculature.

One such study clearly found the paracrine function of these stem/progenitor cells in promoting endothelial
| Source     | Impact | Molecule(s)                        | Model      | Tissue(s)                  | References |
|------------|--------|-----------------------------------|------------|---------------------------|------------|
| Endogenous| + ER   | Apelin/APJ                        | Allograft  | Mu heart                  | 45         |
|           |        | Activating transcription factor 3 | Clamping   | Zebrafish                 | 24         |
|           |        | dihydrodiol dehydrogenase 1      | Wire       | Mu femoral artery         | 52         |
|           |        | FoxM1                             | LPS        | Mu lung and ECs           | 9          |
|           |        | Endogenous                        | CLP        | Mu lung                   | 34         |
|           |        | Hypoxia-inducible factor-1α       | CLP/LPS    | Mu lung                   | 37         |
|           |        | miRNA-126-5p                      | Wire       | Mu carotid artery         | 41         |
|           |        | miRNA-126                         | PCL        | Mu carotid artery         | 42         |
|           |        | Myc                               | Atherosclerosis | Mu carotid artery | 24         |
|           |        | Notch1/notch1 intracellular domain| Stable CAD | Hu plasma                 | 44         |
|           |        |                                   | +/− diabetes | Hu ECs                   |            |
|           |        |                                   | Clamping   | Mu aorta                  |            |
|           |        |                                   | Wire       | Mu carotid artery         |            |
|           |        |                                   | Hypoxia    | Mu carotid artery         |            |
|           |        | p110γ isoform of phosphoinositide 3-kinase | LPS/CLP | Mu lung | 10 |
|           |        | Sox17                             | Wire       | Hu ECs                    |            |
|           |        | Vascular endothelial growth factor | LPS        | Hu ECs                    |            |
| − ER      |        | Delta-like 1                      | Wire       | Mu carotid artery         | 41         |
|           |        | Sprout-related EVH1 domain-containing protein 1 | ED     | Hu EC | 43 |
| + JR      |        | AMP-activated protein kinase α5/N-cadherin | LPS      | Rat lung                  | 57         |
|           |        | FoxM1/β-catenin                    | Scratch    | Rat ECs                   | 59         |
|           |        | miRNA-150                         | PAR1 agonist | Mu lung | 7  |
|           |        | Src homology 2/β-catenin           | Thrombin   | Mu ECs                    |            |
|           |        | Gβγ/Fyn/FAK                       | LPS/CLP    | Mu lung and ECs           | 62         |
|           |        | CD31/Src homology 2               | Thrombin   | Mu ECs                    | 55         |
|           |        | Phospholipase D2                  | Thrombin   | Mu EC, Mu skin            | 60         |
|           |        | /PTPN14                           | Thrombin   | Mu EC, Mu lung            | 61         |
| − JR      |        | Erg and angiopoietin-2            | LPS        | Mu lung and ECs           | 62         |
| Exogenous | + ER   | Bone morphogenic protein receptor 2 | Wire       | Mu carotid artery         | 44         |
|           |        | Microparticles                    | Coculture  | Mu ECs/smooth muscle cell | 42         |
|           |        | miRNA-126                         | ED         | Mu carotid artery         | 42         |
| + JR      |        | Protein kinase C6/CXCL7            | Wire       | Mu carotid artery         | 42         |
|           |        | Sphingosine-1 phosphate            | Angioplasty | Mu femoral artery | 83 |
|           |        |                                   | Coculture  | Mu carotid artery         | 75         |

Molecules that have either a beneficial (+) or inhibitory (−) effect on ER or JR have been identified by experimental studies of isolated ECs and multiple organs and species.

CLP, cecal ligation and puncture; EC, endothelial cell; ED, electric denudation; ER, endothelial regeneration; Hu, human; JR, junction reannealing; LPS, lipopolysaccharide; Mu, mouse; PCL, partial carotid ligation.
regeneration and vascular repair.\textsuperscript{75} The study found that induction of endogenous FoxM1 expression in ECs is required for the reparative effects of transplanted BM progenitor cells in mice. Transplantation of cultured BM progenitor cells induced resolution of inflammatory lung injury and survival in wild-type mice, but these effects were abrogated in FoxM1-EC knockout mice after inflammatory injury. This study found that progenitor cell treatment failed to induce pulmonary EC proliferation and thereby endothelial regeneration and vascular repair in FoxM1-EC knockouts. Intriguingly, Foxm1 deletion in ECs did not affect the early protective effects of exogenous progenitor cells, consistent with the selective role of endothelial FoxM1 in mediating endothelial regeneration and vascular repair. Furthermore, this study found that treatment of wild-type but not FoxM1-deficient EC monolayers with BM-derived progenitor cells resulted in enhanced endothelial barrier function, whereas restoration of β-catenin in Foxm1-null ECs reenabled progenitor cell–induced enhancement of endothelial barrier function. Thus, transplantation of exogenous adult progenitor cells promoted endothelial regeneration and reannealing of endothelial AJ for restoration of endothelial barrier integrity through paracrine activation of endothelial expression of FoxM1.

One of the potential key paracrine factors from the adult stem/progenitor cells is sphingosine-1 phosphate, which promotes FoxM1-dependent endothelial junction recovery.\textsuperscript{77} Furthermore, adult stem/progenitor cells can release several proangiogenic factors, such as keratinocyte growth factor, IGF-1, basic fibroblast growth factor, and VEGF.\textsuperscript{76–78} Future studies should investigate the individual role of these angiogenic factors in activating endothelial regeneration and vascular repair.

Recent studies also found that adult stem/progenitor cells (as well as ECs themselves) can release microparticles or microvesicles that induce endothelial regeneration by promoting proliferation of tissue-resident ECs.\textsuperscript{79–82} Microparticles are small membrane fragments released from cells and may contain DNA, RNA, or miRNA. These microparticles can, in turn, be uptaken by resident ECs to activate the endothelial regeneration program.\textsuperscript{80} Jansen et al\textsuperscript{12} found that injected endothelial microparticles promote endothelial regeneration after electric endothelial denudation of carotid artery in mouse via transfer of miRNA-126, which increases EC migration and proliferation. In their study, mir-126 functionally regulated the target protein SPRED1. Furthermore, knockdown of mir-126 in endothelial microparticles abrogated their effects on human coronary artery EC migration and proliferation \textit{in vitro} and reendothelialization \textit{in vivo}.

Other studies have revealed that SMCs can also drive EC regeneration. As described above, Miyagawa et al\textsuperscript{40} recently found that SMC-EC contacts promote EC regeneration after wire-induced carotid artery injury in mice. BMPR2 was required by both cell types to produce collagen IV, which activates integrin-linked kinase and directs phospho-c-Jun N-terminal kinase to the EC membrane, where it stabilizes presenilin1 to activate Notch1 to promote EC proliferation. Ren et al\textsuperscript{13} also recently investigated whether SMCs can modulate EC regeneration in a paracrine manner. Using a mouse model of wire-induced arterial injury and a rat model of carotid angioplasty, the authors found that inactivation of protein kinase C (PKC)-δ in SMCs impairs EC regeneration, whereas gene transfer of PKC-δ to the SMCs of these animals promoted EC regeneration. Conditioned medium taken from SMCs infected with PKC-δ adenoviruses could be used to induce EC migration but not proliferation. CXCL7 was expressed in SMCs in a PKC-δ/STAT3–dependent manner. Blockade of CXCL7 or its receptor (CXCR2) attenuated EC migration and inhibited endothelial wound closure. Thus, SMCs stimulate EC regeneration via PKC-δ–dependent expression of CXCL7, which likely leads to recruitment of ECs from uninjured endothelium for vascular repair. Taken together, it seems that although circulating cells are not a major source of regenerating ECs, these cells may regulate EC regeneration and vascular repair through the release of reparative paracrine factors that act on the vascular endothelium.

\textbf{Future Perspectives}

Improved understanding of the cellular and molecular mechanisms that control the intrinsic endothelial regenerative and reparative processes could lead to the development of novel and effective treatments for diseases characterized by EC injury and dysfunction. As described in this review, multiple transcription factors and signaling pathways that control the intrinsic endothelial regeneration and vascular repair process have been identified (Figures 2 and 3 and Table 1). Although intuitive, it has been convincingly demonstrated that endothelial regeneration is significantly impaired with aging, at least in large vessels.\textsuperscript{28} Additional studies that aim to further dissect the mechanisms of aging-impaired endothelial regeneration and vascular repair and elucidation of potential organ-specific regulation would be of high value. It would be interesting to see which of the reparative factors/pathways summarized in this review are impaired by aging and other risk factors for EC dysfunction and to study whether restoration of their functions could reactivate the intrinsic regenerative process in aged animals and elderly patients after vascular injury. Given the drastic increases in severity and mortality of elderly patients with sepsis and ARDS derived from pneumonia, flu, and coronavirus 19 (COVID-19), this line of research is of high relevance.\textsuperscript{84–87} Future development of small molecule drugs that target regenerative and reparative pathways, such as the GPCR-dependent p110γPI3K pathway, and/or development of cell-based gene therapy to activate the regenerative and reparative mechanisms for treatment of vascular diseases is warranted. Indeed, it is important to know whether vadadustat, a HIF prolyl hydroxylase
inhibitor, is effective to promote recovery and survival of patients with severe COVID-19 (NCT04478071) because it may activate HIF-1α—dependent endothelial regeneration and vascular repair.

The metabolism of ECs in diseased vasculature is markedly aberrant, given that cell proliferation and endothelial junction reannealing are metabolically demanding processes that require substantial energy and biomass. Characterization of EC metabolism in regenerating endothelium is appealing, which could reveal novel reparative pathways and facilitate the targeting of endothelial metabolism in endothelial regeneration and vascular repair. The potential of modulating epigenetic enzyme activities to accelerate EC recovery and vascular repair remains to be fully explored. The metabolic and epigenetic control of endothelial regeneration and vascular repair after injury therefore represents another area of research that warrants further attention.

Studies could also aim to identify the discrete EC subpopulation(s) with the greatest proliferative potential, for instance, by comparing venous and arterial ECs and by studying ECs across different tissue types. One potentially productive approach would be to use single-cell RNA-sequencing analysis to define the subpopulations of regenerative ECs or putative tissue-resident vascular endothelial stem/progenitor cells. Genetic lineage tracing and genetic depletion approaches could be used to define the roles of specific EC subpopulations in mediating endothelial regeneration. Defining such a regenerative subpopulation of ECs would be a crucial first step for their isolation, expansion, and use in regenerative medicine.

In future studies, the effect of different types of non-ECs, including adult stem/progenitor cells (e.g., MSCs), on EC regeneration and vascular repair could also be investigated. Adult stem cells release various paracrine factors, including microparticles/microvesicles and miRNAs, to stimulate endothelial regeneration and vascular repair. SMCs also release multiple reparative factors, such as CXCL1 and CXCL7, that stimulate EC migration or activate regenerative signaling pathways in ECs. Future studies should aim to elucidate the mechanisms by which exogenous progenitor cells activate the intrinsic regenerative and reparative programs, which could lead to the exploitation of cell-based gene therapy to activate the intrinsic endothelial regeneration and vascular repair processes.

It also remains to be seen whether other vascular cell types, including fibroblasts, could be directly converted into endothelial progenitors or even ECs at the injury sites for in situ endothelial regeneration. The direct conversion of fibroblasts into functional ECs could hold great promise for the inhibition of atherosclerosis and restenosis after endothelial denudation by angioplasty treatment. Although adult stem cells, including MSCs and BM-derived progenitor cells, are rarely engrafted into the damaged vasculature, recent studies have found that induced ECs or EPCs differentiated from embryonic or induced pluripotent stem cells or directly reprogrammed from fibroblasts and other cell types can engraft into local neovascularature of ischemic tissue to improve tissue perfusion. Thus, it would be interesting to determine whether and under what conditions transplantation of induced ECs or EPCs can engraft into injured endothelium for endothelial regeneration and vascular repair. Finally, future studies of the EC response to changes in mechanical flow could be of interest, given that decreased flow can impair endothelial repair and that the injury and repair phases of vascular conditions, such as restenosis, atherosclerosis, and thrombosis, are characterized by changes in flow forces.

Conclusions

Endothelial regeneration and vascular repair after vascular injury is predominantly dependent on tissue-resident ECs through migration and proliferation. Future studies should aim to characterize the regenerative EC subpopulation(s), identify intrinsic regenerative and reparative signaling pathways, and develop pharmacologic and/or cell-based gene therapies. These strategies would enable harnessing the intrinsic regenerative and reparative processes for the treatment of vascular disorders characterized by endothelial injury, such as atherosclerosis, restenosis, sepsis, and ARDS, including severe COVID-19.

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