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Review

A systems view of the vascular endothelium in health and disease

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SUMMARY

The dysfunction of blood-vessel-lining endothelial cells is a major cause of mortality. Although endothelial cells, being present in all organs as a single-cell layer, are often conceived as a rather inert cell population, the vascular endothelium as a whole should be considered a highly dynamic and interactive systemically disseminated organ. We present here a holistic view of the field of vascular research and review the diverse functions of blood-vessel-lining endothelial cells during the life cycle of the vasculature, namely responsive and relaying functions of the vascular endothelium and the responsive roles as instructive gatekeepers of organ function. Emerging translational perspectives in regenerative medicine, preventive medicine, and aging research are developed. Collectively, this review is aimed at promoting disciplinary coherence in the field of angioscience for a broader appreciation of the importance of the vasculature for organ function, systemic health, and healthy aging.

INTRODUCTION

Cardiovascular mortality accounts for approximately one-third of human mortality in industrialized countries.[1] Although the contribution of cardiac diseases to mortality is widely acknowledged, the vascular contribution to fatal disease outcomes is much less recognized. It may be obvious that cardiac ischemia is not a disease of the heart, but a disease of blood vessels that leads to damage of the heart. Likewise, an ischemic stroke is not a disease of the brain, but a disease of the vasculature that leads to damage of the brain. However, most people consider a heart attack and a stroke as diseases of the heart and the brain, respectively. In fact, blood vessels are critically involved in almost every major life-threatening and chronic human disease. For example, without blood vessels, tumors cannot grow beyond microscopic size, and metastasis, the deadliest step of the tumor progression cascade, is critically dependent on bi-directional tumor-vessel interactions.[2,3] Inflammation—a key determinant of many human diseases—is mediated by vessel-lining endothelial cells that critically orchestrate the inflammatory trafficking of leukocytes[4] and the process of aging.[5] Preeclampsia—to this day, the most dangerous and most frequent pregnancy complication equally threatening mother and baby—is pathogenetically caused by perturbed vascular differentiation during pregnancy.[6] Although the pathological substrate of atherosclerosis may be the lumen-narrowing neointima, the inflammatory dysfunction of the monolayer of endothelial cells is causative for the pathogenesis of atherosclerosis.[7,8] Endothelial dysfunction is also implicated in the manifestation of the systemic consequences of COVID-19.[9] The list of vascular involvement in critical human disease is much longer. Taken together, it can be concluded that the official death statistics underestimate the contribution of dysfunctions of endothelial cells and the vascular system to human mortality. The same holds true for the socio-economically most important chronic diseases, including eye diseases (with vascular dysfunction being the primary cause of blindness in industrialized countries[10]), diabetes, obesity and metabolic syndrome, arthritis, and dementia.

We posit that blood vessels should be considered a systemically disseminated organ. With almost every cell of the body being within 100–150 μm of the nearest capillary—the diffusion limit of oxygen—the large surface of the microvasculature forms a critical communication interface between the circulation and the different organ compartments. Vessel-lining endothelial cells have, in this context, primarily been viewed as a responsive cell population. They execute distinct functions in response to stimuli from their microenvironment, such as an inflammatory stimulus or an angiogenesis-inducing cytokine. This conceptual view is not wrong, but it is too reductionist. Work pursued in the last decade has fundamentally changed this simplistic view of the vasculature. Instead, the strategic positioning of the vasculature between the circulation and the different organ parenchyma puts endothelial cells in a unique gatekeeper position. They actively control organ function in an instructive manner by secreting

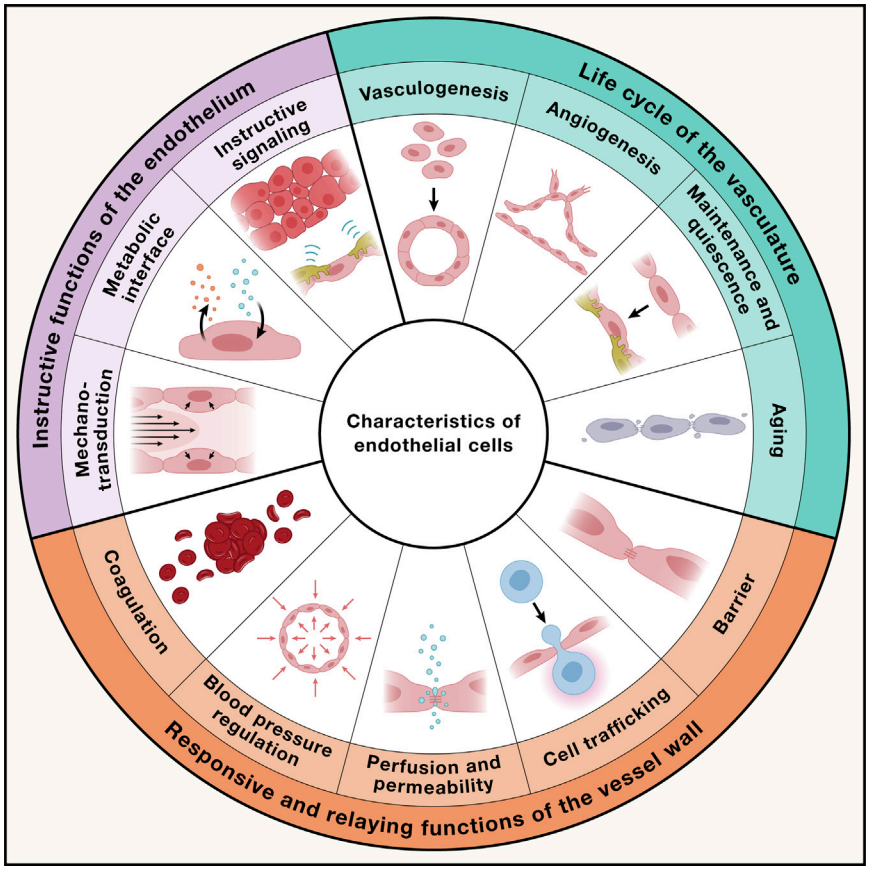

Figure 1. Key characteristics of endothelial cells

The life cycle of a vasculature involves mechanisms of vasculogenesis, angiogenesis, maintenance and quiescence during adulthood and distinct changes during aging. Vessel-lining endothelial cells exert responsive and relaying functions, acting as a barrier, controlling cell trafficking, serving as a permeability interface, regulating blood pressure, and triggering coagulation. Beyond serving as a responsive interface and largely controlled by mechanotransduction mechanisms, endothelial cells also control organ and metabolic functions in a paracrine-acting, instructive manner.

paracrine-acting growth factors (angiokines), a novel signaling paradigm that has essentially been validated for most organs.[11]

This review integrates the classical functions of the vascular endothelium with more recently emerging discoveries in the fields of mechanotransduction, metabolism, instructive signaling, and aging research. Aiming at a holistic view of the field of angioscience (Figure 1), (1) the characteristics of vessel-lining endothelial cells throughout the life cycle of the vasculature are spelled out, (2) responsive and relaying functions of the endothelium are discussed, and (3) recently discovered instructive functions of endothelial cells are reviewed. Building on the dramatic advances in deciphering endothelial cell heterogeneity at single-cell resolution, future vascular research directions and emerging translational perspectives are developed. Collectively, this review aims for a wider appreciation of the vascular endothelium as a systemically disseminated organ with vital regulatory roles and instructive gatekeeper functions for maintaining organ and systemic homeostasis as well as enabling healthy aging.

LIFE CYCLE OF THE VASCULATURE

Vasculogenesis

Endothelial cells are long-lived cells. During early embryonic development, vasculogenesis marks the *in situ* differentiation of a *de novo* forming capillary plexus from stem and progenitor cells (Figure 2A). Coalescing angioblastic cells form blood islands and a first primordial capillary plexus of differentiating endothelial cells. Differentiating endothelial cells then organize to form lumenized tubular structures, eventually enabling blood flow. Cells of the early vasculogenic lineage continue to exhibit multipotential differentiation potential, as best exemplified by the existence of so-called "hemogenic endothelium." These are specialized CD32-positive endothelial cells capable of giving rise to cells of the hematopoietic lineage[12,13] and contribute, at least in part, to later hematopoietic stem and progenitor cell heterogeneity.[14] Likewise, a subpopulation of embryonic endothelial cells is capable of reconstituting the bone marrow stromal niche upon transplantation. This occurs through an endothelial-mesenchymal transition (EndMT) mechanism and reflects the multi-lineage differentiation potential of early embryonic endothelial cells.[15]

Although vasculogenesis is firmly established as a process of embryonic development, it is, to this day, controversial whether vasculogenic blood vessel formation can also occur in the adult. The isolation of putative bone-marrow-derived endothelial progenitor cells (EPCs) more than 25 years ago[16] has stimulated much research into the nature of these cells and their use for regenerative medicine applications. Detailed analysis of adult neovascularization revealed that bone-marrow-derived mononuclear myeloid cells do not incorporate into the growing endothelial layer but rather home perivascularly to contribute to vascularization in a paracrine manner by secreting proangiogenic growth factors.[17] More recent experiments studying adult angiogenesis during liver regeneration employing parabiosis models support these findings. They suggest that the contribution of bone-marrow-derived progenitor cells to adult vascularization may be minimal unless the growing vasculature is massively damaged, e.g., as it may be induced by radiation in experimental bone marrow transplantation studies.[18] Thus, it may be most realistic to conclude that bone-marrow-derived EPCs may exist in principle but play very little, if any, significant quantitative role in vascularization processes in the adult.

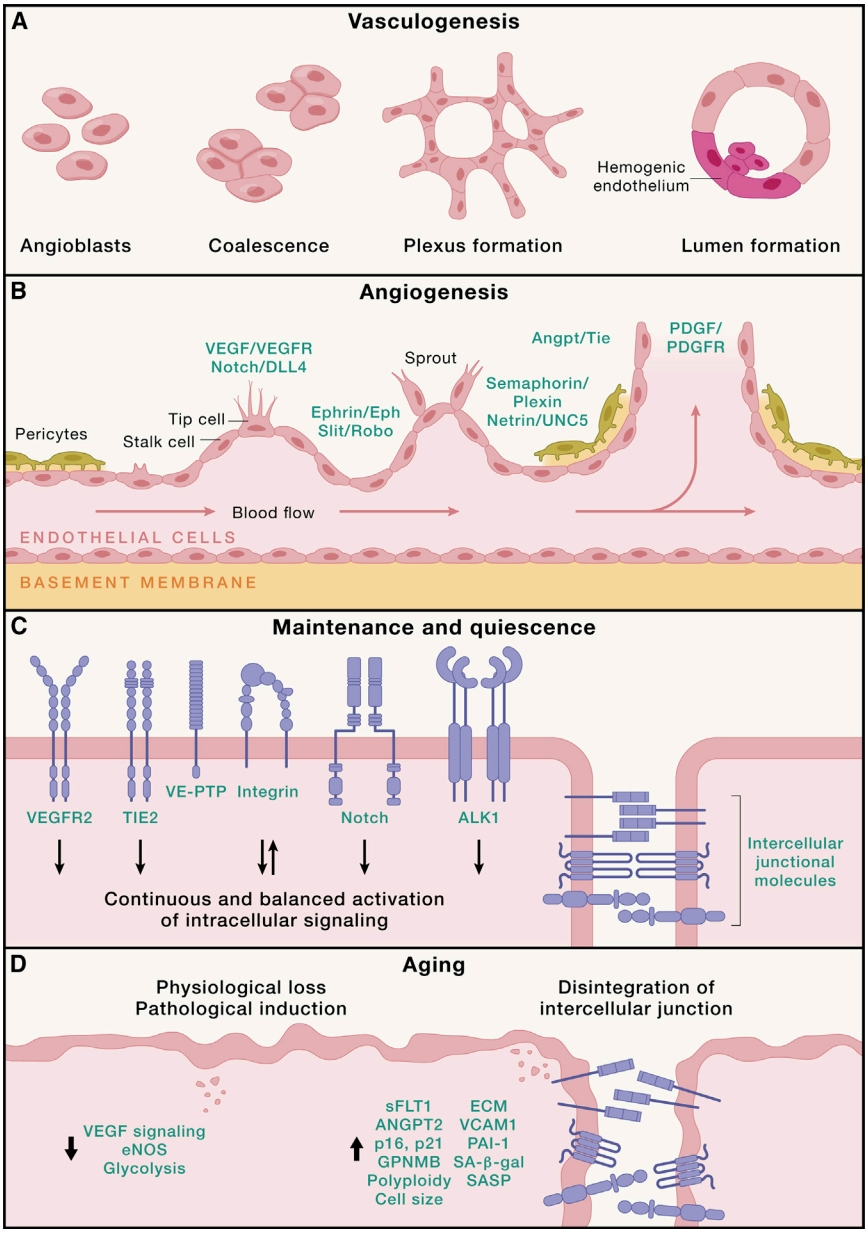

Figure 2. Endothelial cell functional and phenotypic properties during the life cycle of a vasculature

(A) During vasculogenesis, angioblastic stem cells form a primitive plexus of endothelial cells that also gives rise to the hemogenic endothelium.

(B) Angiogenesis marks the sprouting of new capillaries from the existing vasculature.

(C) During adult homeostasis, vascular quiescence is not default but needs to be actively maintained.

(D) During vascular aging, endothelial cells change intrinsically and as a consequence of an altered microenvironmental milieu.

Angiogenesis

Although vasculogenesis describes the *de novo* formation of new blood vessels, angiogenesis marks the growth of blood vessels from existing capillaries (Figure 2B).[19,20] Angiogenic vascular expansion occurs through the budding of capillary sprouts. There are also less-well-understood, perhaps equally important processes of non-sprouting angiogenesis, whereby network formation occurs through the longitudinal splitting of a capillary, a process known as intussusceptive angiogenesis.[21] During sprouting angiogenesis, endothelial cells respond to a gradient of angiogenic cytokines, most notably vascular endothelial (VE) growth factor (VEGF), the master regulator of the angiogenic cascade.[22] Genetically, the loss of one VEGF allele is not compatible with life and leads to haploinsufficient embryonic lethality.[23,24] The invasive sprouting of angiogenic tip endothelial cells was believed to follow a degenerate developmental program that is largely independent of the subsequent organotypic differentiation of the maturing vasculature. However, opposing this model, a Wnt7a/b-dependent differentiation program has recently been discovered that selectively controls the brain-specific angiogenic tip cell program.[25]

Angiogenic endothelial cells degrade their basement membrane to initiate directional sprouting toward the angiogenic stimulus. Controlled by Notch/Delta signaling, they do so in a coordinated manner to maintain vascular integrity during the sprouting process.[26] Endothelial migration then precedes endothelial proliferation. Eventually, growing capillary sprouts anastomose to form networks, which may be chaperoned by myeloid-derived accessory cells.[27] Network formation and arteriovenous differentiation are orchestrated by attractive and repulsive guidance cues of different classes of molecules, notably the ephrins/Ephs, Slits/Robos, semaphorins/plexins, and netrins/UNC5. Remarkably, the same classes of molecules controlling vascular network formation also govern neural network formation.[28] Finally, the growing capillary network needs to mature, which is controlled by molecules of the PDGF/PDGFR and the angiopoietin/Tie families as well as the recruitment of perivascular mural cells (pericytes).[29,30]

The historical notion that "blood vessels grow where they are needed" is molecularly translated by the finding that hypoxia is the most potent physiological trigger regulating the expression of the master regulator of the angiogenic cascade VEGF.[31] Any growth of tissue beyond the diffusion distance of oxygen requires the angiogenic growth of new capillaries. Angiogenesis is therefore, first and foremost, a physiological mechanism of embryonic development and postnatal growth. During adulthood, endothelial cells have an extremely low turnover within

months to years, as unambiguously demonstrated by radiocarbon dating experiments.[32] Recently, fate mapping experiments in mice have confirmed this low turnover of endothelial cells in the aorta, with as little as 3% of cells entering the cell cycle per month.[33] Physiologically, adult angiogenesis is largely restricted to the female reproductive system, where it occurs cyclically in the ovary and the uterus.[34] Pathologically, angiogenesis is associated with not only many different forms of tissue growth, most notably tumor growth, but also different eye diseases, which has made angiogenesis an attractive therapeutic target. In fact, anti-angiogenesis has become part of standard tumor therapy,[2,20] and intraocular injections with an anti-angiogenic drug have been approved for patients with age-dependent macular degeneration (AMD) and diabetic retinopathy.[35]

Maintenance and quiescence

Following angiogenesis, endothelial cells reach a state of quiescence. However, this quiescent phenotype is not the default but needs to be actively maintained (Figure 2C). Vascular quiescence signaling is controlled by a hitherto incompletely understood interplay of laminar-blood-flow-regulated biochemical stimuli, including PDGF/PDGFR signaling and angiopoietin/Tie signaling, as well as the recruitment of pericytes.[36] In maintaining the quiescent endothelial layer, the constitutive activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway leads to the phosphorylation of forkhead transcription factors (TFs) (FOX), which results in their exclusion from the nucleus, thereby causing their transcriptional inactivation.[37] Again, this reflects the notion that vascular quiescence is not the default but an actively maintained biological process requiring the suppression of the endothelial-cell-activation-inducing default transcriptional program.

The association of capillary endothelial cells with pericytes, which involves basement membrane penetrating pericyte foot processes and the liberation of latent transforming growth factor β (TGF-β), renders endothelial cells in a quiescent, largely growth factor refractory phenotype.[38] This explains why anti-VEGF/VEGF receptor (VEGFR) therapies prune immature, not pericyte-covered, blood vessels and spare pericyte-covered mature vessels, a process known as "vascular normalization."[39] The contextual agonist and antagonistic angiopoietin/Tie ligand angiopoietin-2 (Angpt2) plays a key role in the transition from the protected quiescent to the responsive activated endothelial cell phenotype. Angpt2 acts autocrine or paracrine, with endothelial cells being the primary source.[40] Expressed at low levels in quiescent endothelial cells, various activation stimuli lead to the rapid upregulation of endothelial Angpt2 expression, which triggers the loosening of pericyte contacts and renders endothelial cells responsive to endotheliotropic cytokines.[40]

The maintenance of endothelial cell fate and the control of endothelial function are linked processes, which depend on the constitutive cooperativity of the two ETS-TFs, ERG and Fli1. The combined endothelial deletion of ERG and Fli1 results in vasculopathy and multiorgan failure due to the loss of endothelial fate and integrity, hyperinflammation, and spontaneous thrombosis. This is associated with the transcriptional silencing of pan-vascular and organotypic vascular core genes.[41] Likewise, much has been learned in recent years through high-resolution transcriptomic analyses about the acquisition and maintenance of the quiescent endothelial phenotype and the protection from pathological challenges. The aryl hydrocarbon receptor (AHR) has been identified as an organotypic regulator of vascular quiescence. In the intestine, AHR acts as an endothelial cell sensor of dietary metabolites that promotes cellular quiescence and vascular normalcy at steady state. Endothelial AHR deficiency in adult mice results in dysregulated inflammatory responses and the initiation of proliferative pathways.[42] AHR is also highly active in lung endothelial cells, protecting against influenza-induced lung vascular leakage. Here too, the maintenance of protective AHR function requires a diet enriched in naturally occurring AHR ligands, which activate disease tolerance pathways in lung endothelia to prevent tissue damage.[43] The TGF-β-activated kinase 1 (TAK1) also serves as a regulator of endothelial survival and maintains vascular integrity upon TNF-alpha stimulation. Mice with a targeted mutation of TAK1 in endothelial cells exhibit intestinal and liver hemorrhage due to endothelial apoptosis and vascular defects in inflamed organs.[44] Likewise, simvastatin—a HMG-CoA reductase inhibitor decreasing cholesterol concentrations—has been shown to protect endothelial cells from dysfunction by reducing chromatin accessibility at transcriptional enhanced associate domain (TEAD) elements. Simvastatin thereby controls EndMT-regulating genes in a yes-associated protein (YAP)-dependent manner independent of its lipid-lowering effect.[45]

Vascular aging

Vascular aging has in the past primarily been studied in the context of macrovascular biology, as it relates to atherosclerosis and hypertension. Larger arteries lose elasticity due to extracellular matrix stiffening, which is an important contributor to aged-dependent hypertension.[46] In recent years, the mechanisms of microvascular aging and the identification of commonalities on the molecular and cellular levels between macrovascular and microvascular aging have gained increasing attention (Figure 2D).[47] On the organ level, microvascular rarefaction appears to be a commonality between different organs.[48] Several mechanisms have been proposed as possible causes of capillary rarefaction.[47] Among these, age-related VEGF signaling insufficiency may play a particularly critical role. This is thought to result from rising levels of circulating soluble VEGFR1 (sFlt1) during aging, which acts as a soluble VEGF decoy receptor.[49] In fact, reversible vascular rarefaction in different organs has also been observed in cancer patients treated for prolonged periods of time with function blocking VEGF antibodies.[50] The aging body apparently aims at compensating for the VEGF signaling insufficiency by upregulating the production of VEGF and the contextually proangiogenic Tie2 ligand Angpt2. This has been observed in the serum of aged mice[49] as well as in the plasma of aged humans.[51] Correspondingly, therapeutic low-dose systemic VEGF-overexpressing therapies (either genetically or virally) are capable of ameliorating age-dependent microvascular rarefaction in mice. As a result, this leads to a remarkable extension of the healthspan and lifespan of aging mice.[49] The strong effects of low-dose systemic VEGF overexpression on the lifespan and healthspan of aged mice are probably among the most remarkable and surprising findings in the field of angioscience in recent years. Beyond

reducing vascular rarefaction, low-dose systemic VEGF overexpression in aged mice improves systemic metabolism, fat tissue metabolism, and liver function. It also reduces sarcopenia, osteoporosis, inflammation, and aging-associated tumor burden.[49] The observed phenotype may actually reflect a state of systemic signaling whereby the vascular endothelium not only exerts angiocrine functions on the local parenchymal microenvironment but also maintains the homeostatic equilibrium in a systemic manner.

The molecular signatures and functional properties of aged and senescent endothelial cells have increasingly been analyzed in recent years. Beyond upregulated levels of soluble VEGFR1 (sFlt1), Angpt2, p16, p21, glycoprotein nonmetastatic melanoma protein B (GPNMB), vascular cell adhesion protein 1 (VCAM1), plasminogen activator inhibitor-1 (PAI-1), senescence-associated β-galactosidase (SA-β-gal), and senescence-associated secretory phenotype (SASP) are key determinants of the aging and senescent endothelium.[52,53] Cells may become polyploid and increase in size. Metabolic reprogramming may result in suppressed glycolysis, p53 acetylation, and the loss of the vasoprotective enzyme cystathionine γ-lyase (CSE). Conversely, adenoviral overexpression of CSE in aged endothelial cells maintained low p53 activity and reactivated telomerase, reverting the endothelial cell senescence phenotype.[54]

RESPONSIVE AND RELAYING FUNCTIONS OF THE VESSEL WALL

Barrier functions

The continuous endothelial lining of capillaries in most organs not only ensures laminar blood flow and provides an antithrombogenic surface but also maintains a selective barrier between the bloodstream and the surrounding tissues (Figure 3A). For example, the blood-gas barrier in the lungs, composed of a layer of endothelial cells and epithelial cells separated by a double basement membrane, may be less than 1 μm wide. This ultrathin barrier tightly separates blood carrying capillaries from the gaseous alveoli. Perturbation of this tight barrier may rapidly lead to life-threatening lung edema. Indeed, acute lung injury impairs the crosstalk between endothelial nitric oxide (NO) and pericyte soluble guanylate cyclase (sGC).[55] Endothelial-derived NO, best known for its vasodilatory effect on vascular smooth muscle cells, activates in pericytes sGC and suppresses cytoskeleton rearrangement. It inhibits vasodilator-stimulated phosphoprotein-dependent F-actin formation and myocardin-related TF A/serum response factor-dependent *de novo* synthesis of genes associated with cytoskeleton rearrangement. This leads to the stabilization of endothelial-pericyte interactions. Pharmacological activation of this crosstalk promotes vascular integrity, reduces lung edema, and ameliorates inflammation-induced lung injury.[55]

A tight vascular barrier is also critical for homeostasis and the protection of the central nervous system (CNS) from noxious substances and the spread of infections. The blood-brain barrier and the blood-retina barrier are formed by specialized tight junction rich endothelia, whose sealed quiescent phenotype is controlled not just by surrounding pericytes but also by astrocytes.[56] Inflammatory breakdown of the blood-brain barrier involves the LPS-triggered activation of the pore-forming protein gasdermin D (GSDMD) in a caspase-11-dependent mechanism, which can, in preclinical experiments, be therapeutically targeted by a GSDMD-neutralizing nanobody.[57]

Cell trafficking

As the critical interface between the circulation and the different organ compartments, endothelial cells play pivotal roles in orchestrating the trafficking of cells in and out of the circulation (Figure 3B).[58] Executed by a complex interplay of adhesion molecules, chemokines, and signaling pathways, endothelial cells regulate the physiological steady-state trafficking of cells as well as the stimulated recruitment of immune cells, e.g., following an inflammatory challenge. The leukocyte recruitment cascade comprises four distinct steps: rolling, tethering, firm adhesion, and transmigration. Leukocytes initially adhere to endothelial cells via selectin-mediated low-affinity adhesion and then slow down to roll along the surface, serving homeostatic immunosurveillance purposes. Upon further challenge, rolling leukocytes tether to the endothelial layer. This enables integrin and immunoglobulin-like adhesion-molecule-mediated firm adhesion. Eventually, this sets in motion the regulated transmigration of recruited cells while continuously maintaining the integrity of the endothelial layer. Leukocyte transmigration (diapedesis) occurs primarily intercellularly through the regulated transient opening of inter-endothelial cell junctions, but cells may also pass transcellularly through individual endothelial cells in care cases.[59] The endothelial adhesion and transmigration machinery primarily serves the trafficking of leukocytes. However, it is also exploited by metastasizing tumor cells, which intravasate at the primary tumor site and interact with the vascular endothelium as their first interface upon arrival at a distant site. Regulated by Wnt signaling and other signaling pathways, the vascular endothelium thereby plays a decisive role in controlling the fate decisions of tumor cells in the metastatic niche.[60]

Steady-state cell trafficking is part of maintaining homeostasis and also serving immunosurveillance purposes. Conversely, activated endothelial cells recruit leukocytes under conditions of inflammation. Although anti-inflammatory drugs also affect the pro-inflammatory program of endothelial cells, the full potential of selectively targeting inflammatory pathways in endothelial cells may not be exploited. This may be particularly relevant for atherosclerosis, where pathogenetic contributors converge at the endothelial lining of the arterial wall.[61] An intensified effort to exploit the endothelium as a target for future causative therapeutic intervention could be promising.

Vascular permeability

Endothelial cells are endowed with the molecular machinery enabling the regulated passage of various substances, such as water, nutrients, and gases, between the circulation and surrounding tissues. Vascular permeability thereby maintains physiological tissue function (Figure 3C).[62] Basal permeability refers to the steady-state passage of small molecules such as water, gases, and certain ions through intracellular specific channels and transporters or intercellular junctions to pass through the endothelial cell layer. This may occur by passive intercellular diffusion or transcellular aquaporin-composed water-filled channels. Various physiological and pathological stimuli may induce vascular permeability by regulating the intercellular adhesion

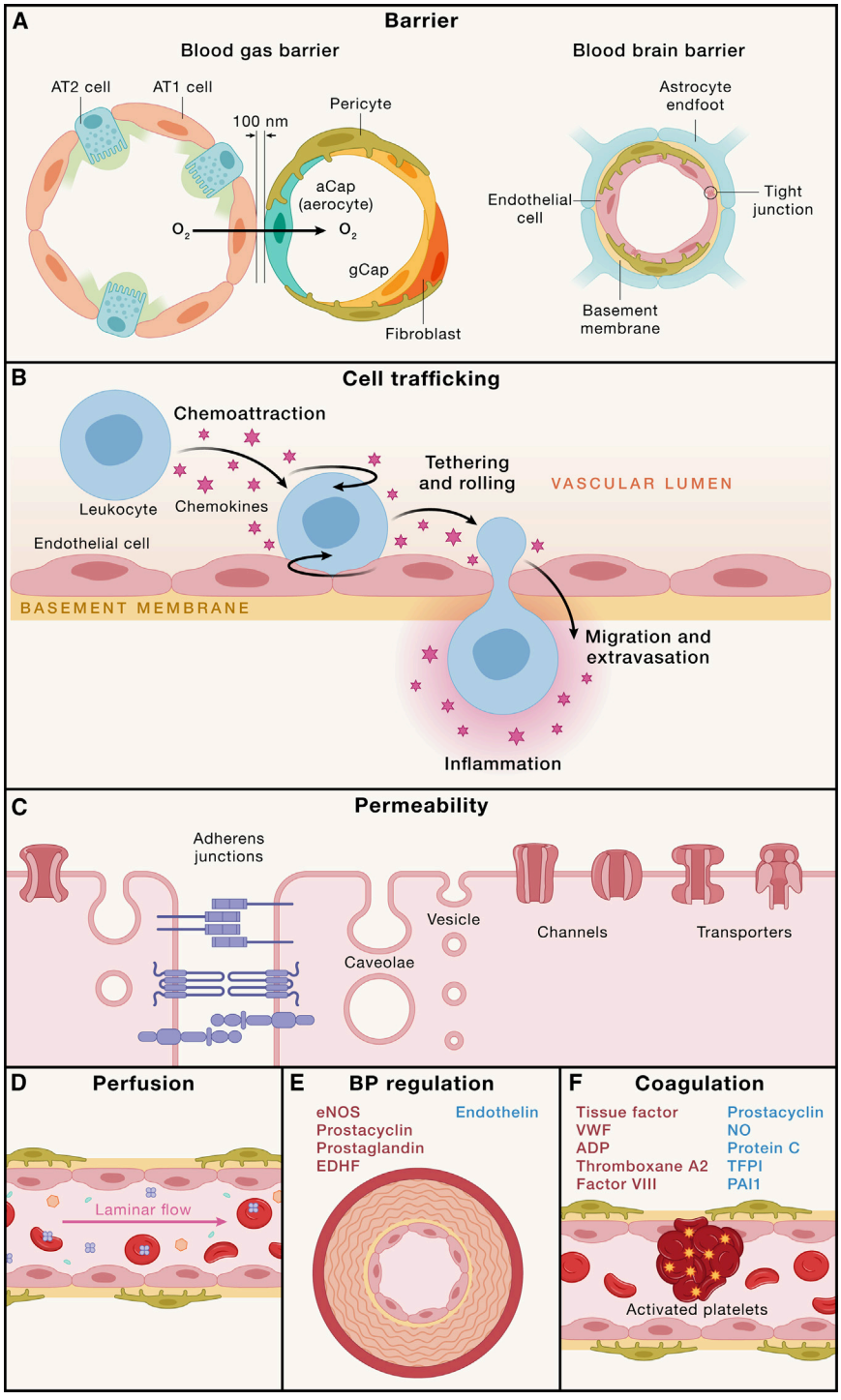

Figure 3. Responsive and relaying functions of endothelial cells

(A) Barrier-forming endothelial cells secure the function of protected organs, such as the lungs and the brain.

(B) Endothelial cells interact with circulating cells and serve crucial functions as regulators of cell trafficking.

(C) Endothelial cells are less (e.g., brain and lungs) or highly permeable (liver and kidney) depending on their spatial organ location.

(D) Perfusion with laminar or turbulent flow exposes endothelial cells to biophysical forces, which dynamically control endothelial cell function.

(E) Endothelial cells locally regulate blood pressure by secreting vasodilators as well as vasoconstrictors.

(F) Endothelial cells control coagulation by balancing pro-coagulant and anti-coagulant factors.

molecule machinery. These include vasoactive factors, such as histamine, bradykinin, and prostaglandins, as well as different inflammatory mediators, such as cytokines and chemokines.[63] Of note, the key angiogenic factor VEGF also acts as a potent vascular permeability factor (a property that was, in fact, discovered years prior to the discovery of its angiogenesis-regulating capacity[64]). The activation of endothelial cells by these cytokines opens junctional complexes consisting of cell adhesion molecules formed between adjacent endothelial cells. Tight junctions seal the endothelial lining in the brain, arteries, and continuous capillaries. Organotypic and vessel-type-specific variations in claudin-5 expression contribute to varying degrees of maintaining barrier function in continuous vascular beds.[65] In contrast to sealing tight junctions, much more plastic adherens junctions regulate venous and capillary permeability. Adherens junctions are formed by VE-cadherin and associated catenins. In response to cytokine activation, VE-cadherin is rapidly internalized, leading to the transient opening of intercellular gaps. VE-cadherin turnover, adherens junction stability, and vascular leakage involve the phosphorylation of VE-cadherin through differential functions of the cytoplasmic tyrosine kinases Yes and Src.[66] Given the critical importance of continuously maintaining vascular homeostasis and integrity, hyperpermeability is associated with many pathological conditions. It can exaggerate disease severity and can lead to edema, reduced vascular perfusion, and impaired drug delivery.[62]

Tissue perfusion and blood pressure

The regulation of vascular permeability is tightly linked to tissue perfusion (Figure 3D), which, in turn, is tightly coupled to the regulation of blood pressure (Figure 3E). Blood pressure is systemically regulated in a neuroendocrine manner (short term) and through the body's total fluid volume regulation (long term). Importantly, the dysregulation of blood pressure

leading to hypertension is one of the most critical risk factors for cardiovascular diseases.[67] Endothelial cells play a pivotal role in controlling the peripheral vascular tone by releasing vasoactive mediators that act on the contractility of executing smooth muscle cells.[68] Endothelial NO synthase (eNOS)-driven generation of NO acts in a vasodilatory manner and regulates blood flow. The short-lived and short-distance-acting gas NO activates smooth muscle cell guanylate cyclase, which results in a reduction of intracellular calcium and subsequent relaxation of smooth muscle tone. Other EC-derived vasodilatory molecules are prostaglandin (notably, prostaglandin E2), the prostaglandin derivative prostacyclin, and the putative endothelium-derived hyperpolarizing factor (EDHF). In turn, the endothelium also produces vasoconstrictor molecules such as endothelin. Endothelin plays a critical role in the pathogenesis of pulmonary hypertension, for which endothelin receptor antagonists are widely used as therapeutic standard of care.[69,70]

Coagulation

Blood needs to be constantly maintained in a fluidic state since clotted blood may lead to the formation of life-threatening blood flow perturbing emboli and thrombi. In turn, any perturbation of vessel integrity needs to be rapidly sealed in order to avoid dangerous bleeding. This inherent conundrum probably explains why coagulation is, on the one hand, so intricately regulated and, on the other hand, the culprit of many diseases (Figure 3F). The role of the endothelium in this context is to always maintain a continuous antithrombogenic surface—even during processes of vascular activation or remodeling—and, in turn, to instantly trigger the coagulation cascade when needed.

Endothelial cell pro-coagulant activities include the expression of Tissue Factor (triggers the clotting cascade), the release of von Willebrand factor (controls initial steps of clot formation by promoting platelet adhesion), the release of platelet-activating mediators such as adenosine diphosphate and thromboxane A2, and the production of factor VIII (by liver sinusoidal endothelial cells).[71,72] Endothelial pro-coagulant activity is modulated by the cell surface externalization of phosphatidylserine triggered by the phospholipid scramblase TMEM16, which may make TMEM16 a theapeutic target for thrombotic vascular diseases.[73] Counteracting excessive coagulation, endothelial cells also have different anti-coagulation activities. These include (1) the release of prostacyclin (PGI2) and NO, which act vasodilatory and inhibit the activation and aggregation of platelets; (2) the expression of the receptor for protein C, which is a natural anti-coagulant protein that inhibits factors Va and VIIIa; and (3) the release of tissue factor pathway inhibitor (TFPI), which acts as regulator of the Tissue-Factor-initiated coagulation cascade by inhibiting the activation of factor X and VIIa. Endothelial cells also act as regulators of fibrinolytic blood clot resolution. Notably, they express PAI-1, which inhibits the breakdown of clots by interfering with plasminogen activation.[71,74] Collectively, endothelial cells contribute through pro-coagulant and anti-coagulant activities to maintaining the delicate balance that prevents abnormal clot formation while ensuring proper wound healing and response to injury.[71]

INSTRUCTIVE FUNCTIONS OF THE VASCULAR ENDOTHELIUM

The perception of the vascular endothelium as primarily structure-building, vessel-lining cells has dramatically changed in recent years. The endothelial cell layer is today widely recognized as a highly dynamic interface that exerts instructive gatekeeper functions on its surrounding microenvironment. Largely regulated by mechanotransduction-mediated signaling, the vascular endothelium thereby controls organ and metabolic functions in a paracrine manner, also designated as "angiocrine signaling" (Figure 4).

Mechanotransduction

Blood flow, either in the form of laminar or turbulent flow, exerts physical mechanotransduction forces on vessel-lining endothelial cells (Figure 4A).[75] This physical force maintains the physiological homeostasis of the quiescent endothelium (an important limitation of static endothelial cell culture models). Too little or too high shear stress can lead to dysfunctional deviations from normal homeostasis. Physical forces are also exerted onto the endothelium by the blood-pressure-mediated cyclic stretching of the endothelium, which—together with the varying stiffness of the underlying extracellular matrix (which is strongly affected by aging)—also critically influences endothelial phenotype and function.

The consequences of perturbed endothelial biomechanical signal transduction have primarily been studied in the context of large vessel biology, particularly as it relates to the pathogenesis of atherosclerosis.[76,77] However, mechanosensing and mechanotransduction play an equally important, hitherto less-well-studied role in shaping the functional properties of endothelial cells in the microvasculature.[78] Endothelial cells convert mechanical stimuli into biochemical signals through receptor tyrosine kinases (e.g., VEGFR2 and VEGFR3), ion channels, integrins, and junctional proteins (e.g., PECAM-1 and VE-cadherin). VEGFR3 plays a role in the mechanosensory complex to determine a preferred level of fluid shear stress, or "set point," of different types of vessels (arteries, veins, and lymphatics).[79] Piezo1 and syndecan 4 are flow sensors of endothelial cells.[80] PI3K/AKT and mitogen-activated protein kinase (MAPK)/ERK signaling pathways activate flow-dependent TFs Krüppel-like factor 2 (KLF2) and nuclear factor erythroid 2-like 2 (NRF2) to maintain endothelial cell phenotypes[81,82] and metabolic state.[83] Likewise, KLF4 promotes through epigenetic mechanisms the expression of homeostatic endothelial genes.[84] Among these, SMAD4 maintains the fluid shear stress set point to protect against arterial-venous malformations.[85] Hippo-signaling-mediated transcriptional activities of YAP and transcriptional coactivator with PDZ-binding motif (TAZ) have emerged as endothelial regulators of laminar vs. turbulent flow.[86] Accordingly, the atheroprotective effects of unidirectional laminar flow are mediated by integrin-dependent inhibition of YAP/TAZ-JNK signaling.[87,88] Finally, the guidance receptor plexinD1 has also been discovered as mechanosensor in endothelial cells.[89] PlexinD1 forms a mechanocomplex with neuropilin-1 and VEGFR2 that is necessary and sufficient for conferring mechanosensitivity upstream of the junctional complex and integrins. It does so by adopting two

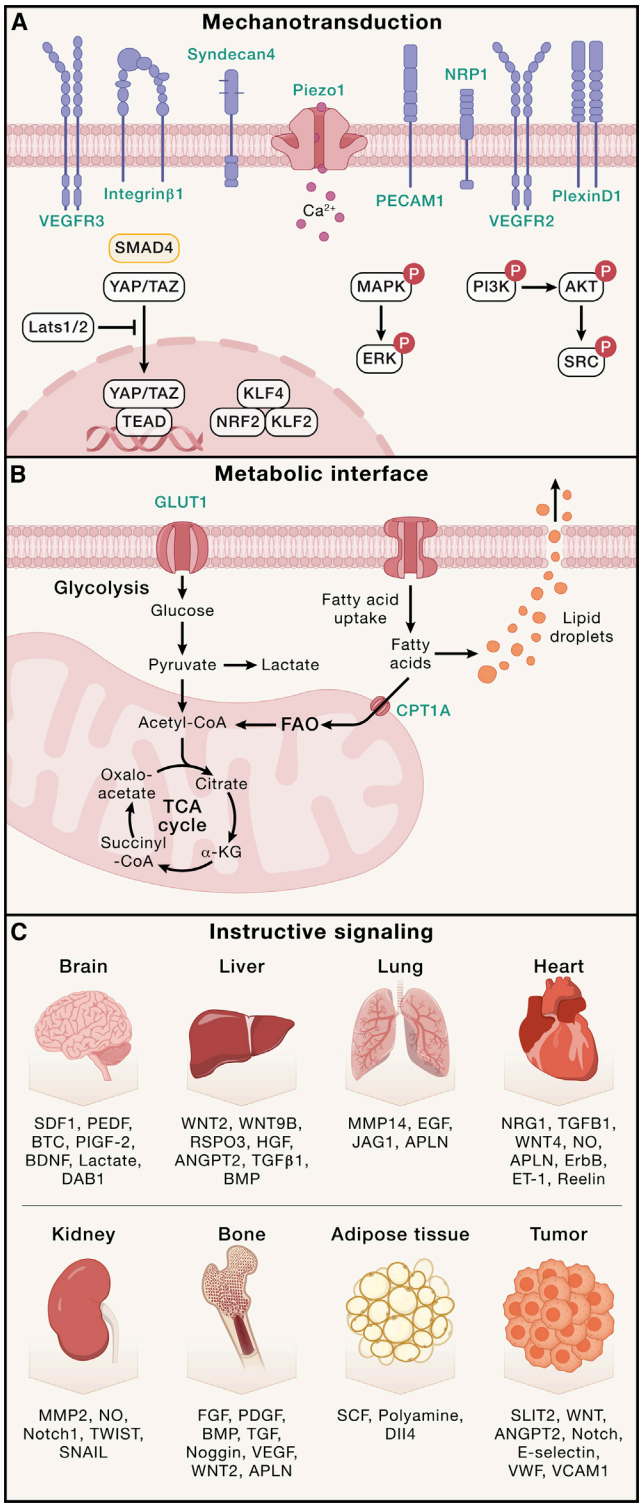

Figure 4. Instructive functions of the endothelium

(A) Endothelial cell mechanoreceptors and mechanosensitive channels act as mechanotransducers translating biophysical forces into instructive signals.

(B) Endothelial cells have distinct metabolic properties depending heavily on anaerobic glycolysis to maintain their energy metabolism.

(C) Organotypic endothelial cells secrete paracrine-acting factors to control organ function during health and disease in an instructive, angiocrine manner.

distinct molecular conformations to serve either as a ligand or a force receptor.[89]

Metabolic interface

Directly exposed to the circulating blood, endothelial cells serve as a metabolic interface (Figure 4B) with remarkable differences between organs[90] and even within a distinct organ.[91] Oxidative phosphorylation in mitochondria contributes to endothelial cell energy metabolism. However, despite being closest to the bloodstream with its high oxygen content, endothelial cells rely in their energy production primarily on anaerobic glycolysis.[92] This may serve, on the one hand, as the advantage of avoiding to contribute to a radical oxygen-rich environment and, on the other hand, aid an efficient oxygen transport across the endothelial cell layer. Likewise, endothelial activation, e.g., during angiogenesis, relies on anaerobic glycolysis, which enables endothelial cells to invade into low oxygen areas that need to be vascularized. Beyond the exceptional role of anaerobic glycolysis, distinct metabolic properties control the energy production, biomass synthesis, and redox homeostasis of endothelial cells. This includes the utilization of fatty acids (e.g., fatty acid oxidation for nucleotide synthesis) as well as glutamine, asparagine, and serine metabolism.[93] Metabolic regulation also contributes to regulating blood vessel maturation. Laminar shear stress and blood flow maintain oxidative pentose phosphate pathway activity in endothelial cells, which promotes matrix production and mural cell recruitment.[94]

The vascular endothelium also participates in the control of organ metabolism by controlling the influx of hormones such as insulin, lipids, and glucose.[95] For example, decreased transport of fatty acids into the heart by manipulating endothelial Notch signaling in mice is sufficient to cause congestive heart failure,[96] whereas dysregulated trans-endothelial fatty acid transport into skeletal muscle driven by 3-hydroxyisobutyrate, a catabolic intermediate of the branched-chain amino acid valine, contributes to insulin resistance in obesity.[97] Interestingly, the metabolism of brain endothelial cells is interconnected with the expression of the major glucose transporter GLUT1 of the blood-brain barrier, and its deletion from brain endothelial cells strongly impairs brain function.[98] Collectively, work pursued in recent years has established the endothelial contribution to organ metabolism and function by regulated trans-endothelial transport of nutrients.

Local instructive signaling mechanisms

It is increasingly recognized that capillary endothelial cells are not merely passive conduits for the delivery of oxygen or nutrients but also support organ development and adult organ regeneration through the elaboration of tissue-specific instructive signaling mechanisms exerted by paracrine-acting "angiokines" (Figure 4C).[11] The concept of angiocrine signaling has been pioneered in the liver and the pancreas. During embryonic development, paracrine signals from endothelial cells control the early steps of organogenesis.[99,100] These pioneering experiments set the stage for the discovery of vascular instructive functions in the adult. Following partial hepatectomy, liver regeneration occurs in two distinct phases: first, the hepatocyte proliferative phase and, later, the proliferation of the non-parenchymal stromal cells. It is only during this later phase that endothelial cells

support liver regeneration by executing the angiogenic program. Despite this distinct temporal order of events, endothelial cells change their transcriptome within hours of partial hepatectomy to express molecules through which they actively control the proliferation of hepatocytes.[101,102] Today, it is clear that liver endothelial cells control essentially all parenchymal liver functions in an instructive, angiocrine manner. This includes not only organogenesis and regeneration but also adult homeostatic lobular zonation (thereby regulating the hepatocytic division of labor through an angiocrine Wnt signaling axis) as well as the response to pathologic insults, including liver fibrosis, liver tumorigenesis, and the metastatic colonization of the liver.[103]

Instructive angiocrine signaling mechanisms are required to maintain the metabolic zonation of hepatocytes. Liver zonation refers to the division of labor of hepatocytes along the axis of the liver lobule. The zonated phenotype of peri-central vein hepatocytes is completely lost in mice with the endothelial deletion of the secreted Wnt signaling enhancer Rspondin3, identifying a critical role of angiocrine Wnt signals in maintaining liver homeostasis.[104] Liver endothelial angiocrine Wnt signaling in the liver is downstream of angiopoietin/Tie signaling, as was established in a spatial endothelial multiomics study that comparatively analyzed the transcriptome, proteome, and phosphoproteome of endothelial cells along the axis of the liver lobule.[105] Angiocrine liver Wnt signaling was recently also reproduced and extended in mosaic experiments *in vivo*, in which human liver tissue was grafted into mouse livers.[106] Finally, angiocrine Wnt signaling is not restricted to the liver, as evidenced by the observation that Rspondin3 is induced in lung endothelial cells upon lung injury to direct interstitial macrophages into an anti-inflammatory and wound healing program.[107]

The liver has in recent years emerged as a prototypic model organ to study angiocrine signaling mechanisms. However, the angiocrine signaling paradigm has also been solidly established for other organs and disease states. Endothelial cells control brain development[108,109] and homeostatic brain functions in the adult, including the maintenance of the blood-brain barrier,[110] neural-glia interactions,[111] and cognitive functions.[112] They control lung alveolarization[113] and lung repair following damage.[114] Myocardial growth and repair is controlled by angiocrine and lymphangiocrine signaling mechanisms.[115–117] The same holds true for kidney function,[91,118] bone function,[119] skin function,[120] hematopoiesis,[119] and pathological conditions such as fibrosis[121,122] and cancer.[123–126]

Systemic regulation

Beyond the local instructive control of organ function, endothelial cells have in recent years also been shown to systemically control the homeostatic maintenance of metabolism in an angiocrine manner. For example, endothelial-derived stem cell factor upregulates gene expression and protein levels of the enzymes for *de novo* lipogenesis and promotes lipid accumulation by activating c-Kit in adipocytes. The stem cell factor/c-Kit signaling axis thereby acts as a regulator of lipid accumulation through the increase of lipogenic enzymes in brown adipose tissue when thermogenesis is inhibited.[127] Likewise, endothelial polyamines stimulate adipocyte lipolysis and regulate white adipose tissue homeostasis. This is caused by enhanced fatty acid β-oxidation in endothelial cells concomitant with a paracrine lipolytic action on adipocytes.[128] The crosstalk between endothelial cells and adipocytes is bi-directional and may also involve the transfer of extracellular vesicles. Caveolin-expressing vesicles from endothelial cells were found to incorporate into adipocytes, and, conversely, adipocyte-derived vesicles affect endothelial cell metabolism. This transfer is physiologically regulated by fasting/refeeding and obesity, suggesting that extracellular vesicles participate in the tissue response to changes in the systemic nutrient state.[129] Finally, endothelial lipoprotein processing also affects adipose tissue remodeling during thermogenic adaptation. In cold-exposed mice, endothelial cells in adipose tissues endocytose and process triglyceride-rich lipoproteins particles. This leads to endothelial and adipocyte precursor proliferation via β-oxidation-dependent production of reactive oxygen species, which, in turn, stimulates hypoxia-inducible factor-1α-dependent proliferative responses.[130]

Pathophysiological metabolic challenge during obesity deregulates endothelial cell gene expression. Extensive multiorgan single-cell RNA sequencing (scRNA-seq) endothelial profiling of obese mice with the integration of human genome-wide association study (GWAS) data identified altered lipid handling, metabolic pathways, and AP1 TF as well as inflammatory signaling in an organ- and endothelial-subtype-specific manner. Interestingly, dietary intervention attenuated endothelial dysregulation in the liver, but not in the kidneys, suggesting possible organ-specific vulnerabilities of the vascular obesity phenotype.[131]

DECONVOLUTING AND MECHANISTICALLY EXPLOITING VASCULAR HETEROGENEITY

The vascular endothelium is structurally and molecularly differentiated in an organotypic manner, enabling it to execute organ- and microenvironment-specific functions. Continuous endothelia with firmly sealing tight junctions are found in organs, in which the endothelium forms a barrier, e.g., in the brain, the lungs, or muscle tissue. Discontinuous endothelia are present in organs with filtration function (e.g., kidney) or that release material into the bloodstream (endocrine glands). Sinusoidal endothelia form a discontinuous layer with intracellular and intercellular sieve-like gaps. They are found in immune active organs such as the bone, the spleen, and the liver. These anatomically distinct types of endothelia have long been known. What has only much more recently become amenable to scientific study is the specific molecular repertoire that shapes organ- and caliber-specific phenotypic and functional traits of endothelial cells.

The first molecular system robustly discriminating endothelial cells in arteries and veins was identified 25 years ago. The EphB4 receptor is preferentially expressed by venous endothelial cells and the ephrinB2 ligand by arterial endothelial cells. Genetic perturbation of either EphB4 or ephrin B2 on endothelial cells consequently leads to embryonic lethal developmental perturbation of arteriovenous differentiation.[132] Progress in molecularly defining subpopulations of endothelial cells has been slow since then. Most genetic manipulation studies on endothelial cells in mice take advantage of the VE-cadherin promoter, a pan-endothelial cell-expressed homotypic adhesion molecule. Although

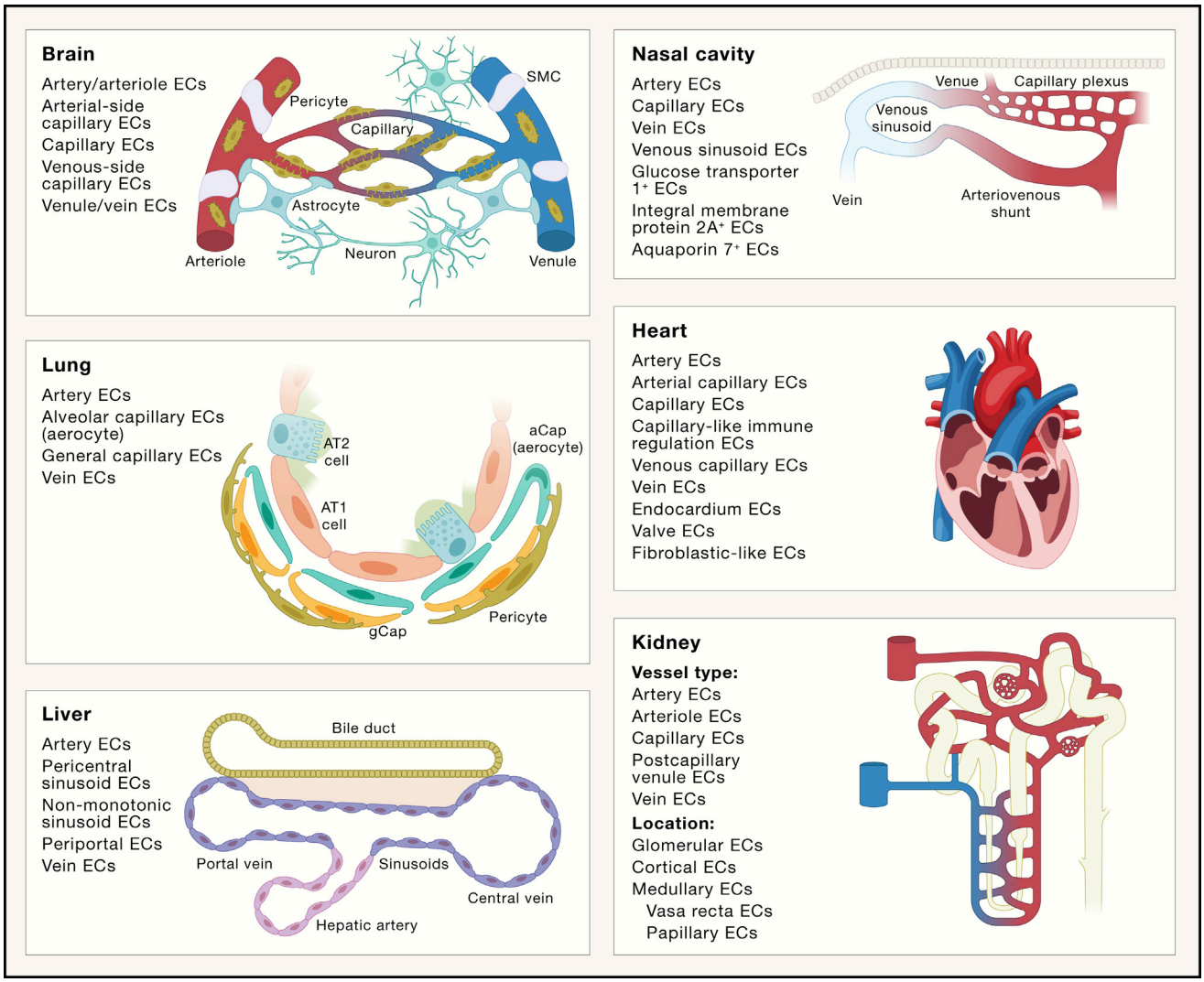

Figure 5. Dissecting vascular heterogeneity at single-cell resolution

Single-cell multiomics analyses have yielded unparalleled molecular and functional resolution into the properties of caliber-specifically and organotypically differentiated endothelial cells.

VE-cadherin-CreERT2 mice have become a universal tool to genetically delete floxed genes in the vasculature,[133] driver mice for subpopulations of endothelial cells are only slowly becoming available, e.g., BMX-Cre mice to delete genes selectively in arterial endothelial cells[134] or Stab-Cre and Clec4g-Cre mice to delete genes in sinusoidal endothelial cells.[135,136] Dual recombinase-mediated genetic approaches involving Cre-loxP and Dre-rox may help overcome the limitations of currently established conditional mutagenesis techniques. A suite of new Dre recombinase drivers has recently been presented for this purpose.[137]

The revolution in the technological advances in single-cell multiomics analyses in the last decade has enabled the molecular dissection the endothelial pedigree at the highest resolution (Figure 5). When pursued at the systems level, single-cell transcriptomic analyses provide an unparalleled level of resolution into cellular function. Nevertheless, at the level of individual molecules, single-cell transcriptomic data need to be interpreted with caution. Indeed, an incongruence between transcriptional and vascular pathophysiological cell states was recently reported by analyzing single and compound genetic mutants for all Notch signaling members. Significant differences were observed in the way ligands and receptors regulate liver vascular homeostasis, with the loss of Notch receptors unexpectedly causing endothelial hypermitogenic cell-cycle arrest and senescence.[138]

Single-cell transcriptomes of endothelial cells have been extracted from whole organ shotgun sequencing studies such as the Tabula Muris project.[139,140] Such studies provide some insight into the molecular repertoire of endothelial cells, but they lack depth and mostly underestimate the abundance of endothelial cells in different organs. This might be a

consequence of below average RNA content of endothelial cells compared with most other cell types. High-resolution transcriptomic maps from endothelial cells have, therefore, mostly been generated by sequencing pools of purified endothelial cells. Starting with a 2018 study that identified 15 and 17 unique endothelial cell types in the mouse brain and lung, respectively,[141] additional studies on various organs in mice and humans have significantly advanced the understanding of endothelial-cell-specific organotypic heterogeneity (Table 1). These mapping studies have provided an unparalleled level of molecular resolution for future functional studies. For example, deciphering mechanisms by which microvascular diversity is attained could set the stage for treating regenerative disorders in an organ-specific manner and promote the healing of organs without provoking deleterious side effects, such as fibrosis.[142]

Moving the field beyond snapshot molecular maps and atlases, comparative spatial and temporal analyses have yielded much insight into endothelial cell function, e.g., by temporally dissecting single-cell transcriptomes during development[157] or by spatially deconvoluting the adaptation to selective challenges, e.g., the response to hypoxia.[158] The response to different pathological perturbations has also been studied at single-cell resolution, e.g., the changes associated with vascular regeneration during cardiac repair.[159] Similarly, several studies have examined endothelial transcriptomic responses during tumor growth.[160–162]

Linking disease states to the transcriptomes of defined endothelial subpopulations holds great promise to yield important and oftentimes unexpected insights into disease mechanisms. For example, 30 of the top 45 genes that have been linked to Alzheimer's disease risk by GWASs were recently found to be expressed in the human brain vasculature. Functionally, these map to protein transport, adaptive immune responses, and extracellular matrix hinting at an important vascular contribution to the pathogenesis of Alzheimer's disease.[143] Likewise, only single-cell transcriptomic profiling approaches have enabled the discovery of a small subpopulation of apelin-expressing endothelial cells, representing only 0.003% of bone marrow cells that are critical for physiological homeostasis and transplant-induced bone marrow regeneration. This surprising discovery revealed critical functional roles for apelin-positive endothelial cells in hematopoiesis, suggesting potential targets for improving bone marrow transplantation.[163]

Single-cell transcriptomic analyses of specialized vascular beds have yielded unexpected functional insight. For example, the nose is an intricate organ with multifaceted functions, including olfaction; the clearance of pathogens and toxic chemicals; and the passage, humidification, warming, and filtration of air. To execute these fundamental functions, the nose has a well-equipped epithelium and a unique vascular system. The morphological and molecular heterogeneities of the mouse and human nasal vasculature identified by immunofluorescence staining of whole-mounted nasal mucosal tissue combined with scRNA-seq have recently been elucidated.[154] Venous sinusoids constitute a major portion of the nasal vasculature, and, surprisingly, the endothelium of the venous sinusoids expresses two lymphatics-specific transcriptional factors, PROX1 and FOXC2. The venous sinusoids are responsible for immune surveillance by leukocyte trafficking from the vascular lumen to the submucosa in a manner dependent on VCAM1. Notably, nasal venous sinusoids regress or are inflamed in a mouse model of allergic rhinitis and in a hamster model of peak acute COVID-19 infection. Moreover, in aged mice, nasal cavity venous sinusoids expand and enlarge with the loss of smooth muscle cells.[154]

Another example for a uniquely specialized vasculature is the venous sinuses in the dura mater of the brain. The CNS borders—meninges, choroid plexus, and skull bone marrow—constitute an immune barrier at CNS interfaces, with resident immune cells that vigilantly patrol the CNS borders.[164] Each CNS border tissue is compartmentalized and shaped as a distinct immunologic niche for CNS immunosurveillance under normal conditions. Venous sinuses in the dura mater are a group of blood channels that drain venous blood circulating from the brain parenchyma. The immune cells along the dural venous sinuses serve as an immune barrier against systemic viral invasion.[152] scRNA-seq analysis revealed that dural sinus endothelial cells are distinguished by their high expression of genes related to leukocyte adhesion and anti-viral cytokines.[152] These findings suggest that dural sinus endothelial cells serve as sites of immune cell recruitment to the peri-sinus by expressing high levels of adhesion molecules for leukocyte trafficking. This is in agreement with the emerging concept of the sinus vasculature as a strategic site for active immune-vessel crosstalk.

Recently, the complexity of the angiogenic program during tumor growth has been deconvoluted at single-cell resolution in a comprehensive atlas covering 200,000 cells from 31 different human cancer types, offering an unparalleled resource for future functional and translational studies.[165] Beyond angiogenesis, single-cell transcriptomic analyses of tumor-associated endothelial cells have yielded important mechanistic insight into the bi-directional crosstalk of tumor cells and endothelial cells that facilitate tumor progression and metastasis.[162,166,167] scRNA-seq of hepatocellular carcinoma-associated endothelial cells revealed the oncofetal reprogramming of tumor-associated vasculature that promotes an immunosuppressive phenotype of macrophages.[168] The tumor vasculature also significantly remodels to thereby affect the intratumoral trafficking and function of immune cells. Postcapillary venule-derived tumor-associated high endothelial venules (HEVs) serve as major sites of lymphocyte entry into tumors at baseline and upon treatment with anti-PD-1/anti-CTLA-4 immune checkpoint blockade.[169] In fact, antiangiogenic immune-modulating therapies evoke the transdifferentiation of postcapillary venules into inflamed HEVs via lymphotoxin/lymphotoxin beta receptor signaling. In turn, tumor HEVs boost the intratumoral lymphocyte influx and foster permissive lymphocyte niches for PD-1(−) and PD-1(+)TCF1(+) CD8 T cell progenitors that differentiate into GrzB(+)PD-1(+) CD8 T effector cells. Tumor HEVs require continuous CD8 and natural killer (NK) cell-derived signals, revealing that tumor HEV maintenance is actively sculpted by the adaptive immune system through a feedforward loop.[170] Likewise, endothelial expression and activation of the stimulator of interferon genes (STING) signaling pathway correlates with enhanced T cell infiltration and prolongs survival in human colon and breast cancer.[171] To translationally exploit these findings, STING-activating

Table 1. Single-cell transcriptomics of endothelial cells in different organs

Organ	Species	Experimental approach	Vascular resolution	Biological focus	Reference
Brain	mouse	*Cldn5*-GFP mouse (C57BL/6J strain); EC isolation: fluorescence-activated cell sorting (FACS) of brain cell suspension; scRNA-seq: plate-based (Smart-seq2)	artery (*Bmx*, *Efnb2*, *Vegfc*, and *Sema3g*); arterial-side capillary (*Jdp2*, *Rorb*, and *Zeb2*); capillary (*Mfsd2a*, *Tfrc*, *Angpt2*, and *Ivns1abp*); venous-side capillary (*Irf2* and *Slc38a5*); vein (*Vwf*, *Zc3h121b, Nr2f2*, and *Lcn2*)	endothelial arteriovenous zonation	Vanlandewijck et al.[141]
	human	postmortem brain tissue; nuclei isolation from the brain tissue lysates; single-nuclei RNA sequencing (snRNA-seq) (droplet-based)	pan ECs (*FLT1* and *CLDN5*); artery (*DKK2, VEGFC,* and *CDH13*); capillary (*BTNL9, ANGPT2,* and *INPP5D*); vein (*TSHZ2, ACKR1,* and *ADGRG6*)	downregulation of ABCB1 and ATP10A in brain capillary endothelial cells of Alzheimer's disease	Yang et al.[143]
	human	isolated EC and perivascular cells from fresh human biopsy material (fetal: abortive material; adult: surgery)	single-cell resolution of >600,000 EC and perivascular cells from human brain. Most comprehensive developmental/adult and healthy/diseased brain EC resource	brain EC during development, adulthood, and disease (brain vascular malformations, brain tumors)	Wälchli et al.[144]
Heart	mouse	adult mice (C57BL/6J); EC isolation: FACS of heart cell suspension; scRNA-seq: droplet-based (10×)	H1: artery (*Fbln5*, *Hey1*, and *Mecom*); H2: arterial capillary (*Cxcl12* and *Rbp7*); H3: capillary (*Kdr* and *Endou*); H4: venous capillary (*Vcam1*, *Fmo1*, and *Fmo2*); H5: vein (*Mgp*, *Cfn*, *Bgn*, and *Vwf*); H6: interferon response (*Isg15*, *Ifit3*, and *Ifit203*); H7: angiogenic (*Col4a2*, *Apln*, and *Sparcl1*); H8: lymphatic (*Ccl21a*, *Prss23*, and *Lyve1*)	single-cell endothelial cell atlas of healthy murine tissues	Kalucka et al.[90]
	human	transplant heart donors; magnetic cell separation (MACS) in the heart cell suspension; scRNA-seq: droplet-based (10×)	EC1: capillary (*RGCC* and *CA429*); EC2: capillary (*RGCC, CA429*, and *APQ1*); EC3: capillary (*RGCC, CA429*, *JUN,* and *FOS*); EC4: capillary-like immune regulation (*RGCC, CA429, CX3CL1, CCL2, IL-6,* and *ICAM1*); EC5: (*SEMA3G, EFNB2,* and *DLL4*); EC6: vein (*NR2F231* and *ACKR132*); EC7: endocardium (*SMOC133* and *NPR3*); EC8: lymphatic (*PROX1, TBX1*, and *PDPN*); EC9: fibroblastic-like (*PLAVP* and *CX3CL1*)	intercommunication between endothelial cells and pericytes	Litvinukova et al.[145]
Lung	mouse	processed scRNA-seq Smart-Seq2 data for adult mouse lung were obtained from the Tabula Muris resource	artery (*Gja5* and *Bmx*); alveolar capillary (aerocyte) (*Car4, Ednrb, Fibin, Tbx2,* and *Apln*); general capillary: (*Gpihbp1, Plvap, Cd93, Aplnr,* and *Lpl*); vein (*Vwf* and *Nr2f2*); lymphatic (*Pdpn* and *Prox1*)	aerocytes (alveolar capillary [aCap] ECs) are specialized for gas exchange; general capillary (gCap ECs) are the structural endothelial cells of the lungs	Gillich et al.[146]

(*Continued on next page*)

Table 1. ***Continued***

Organ	Species	Experimental approach	Vascular resolution	Biological focus	Reference
	human	analysis of publicly available datasets	pan ECs (*ENG, PCDH17, CLEC14A, CLEC1A, ESAM, ITM2A, BMPR2, FLT1, ADGRL4, VIPR1, PLXNA2, FZD4, IL-4R,* and *IL-15RA*; *SLCO2A1, SLCO4A1,* and *AQP1*; *EPAS1, GATA2, FOXF1,* and *ETS2*); artery (*EFNB2, SOX17, BMX, SEMA3G, HEY1, LTBP4, FBLN5, GJA5,* and *GJA4*); capillary (*vWF, THBD, EMCN, EDN1, CA4, PRX, RGCC, SPARC,* and *SGK1*): aerocytes: $vWF^{neg}/EMCN^{high}/EDNRB^{pos}$ population (*TBX2, FOXP2, CLEC4E,* and *SPON2*), general capillary ECs: $vWF^{pos}/EMCN^{low}/EDN1^{pos}$ population (*GPIHBP1, FCN3,* and *IL-7R*); vein (*NR2F2, COUP-TFII, VACAM1, ACKR1,* and *SLEP*); lymphatic (*PDPN* and *PROX1*)	comparison of homeostatic and hypertension endothelial cells; human validation of mouse aCap vs. gCap differentiation concept	Schupp et al.[147]
Liver	mouse	adult mice (C57BL/6J); single-cell and paired-cell (hepatocyte-EC) sorting: FACS; plate-based (MARS-seq capture)	pan ECs (*Ptprb, Aqp1, Igfbp7, Clec4g,* and *Ehd3*); zonation from the central vein to the portal vein; central vein (*Wnt9b* and *Rspo3*); pericentral sinusoid (*Wnt9b, Wnt2, Kit, Rspo3,* and *Thbd*); non-monotonic sinusoid (*Pcdhgc5* and *Ecm1*); periportal sinusoids (*Ltbp4* and *Efnb2*)	paired-cell sequencing, in which mRNA from pairs of hepatocytes and adjacent endothelial cells; bimodal expression pattern for *Rspo3, Thbd*, and *Cdh13* being highly expressed in both pericentral ECs and central vein endothelial cells	Halpern et al.[148]
	human	dissected liver tissues from normal portion of metastatic patients and lesion portion of cirrhotic patients; EC isolation: FACS of liver tissue cell suspensions; droplet-based	sinusoid-1 (*CLEC4M* and *CLEC4G*); sinusoid-2 (*CPE, CD320,* and *BMPER*); central vein (E4) (*RSPO3, WNT2, LHX6,* and *HTR2B*); artery (*AIF1L* and *PLVAP*); scar-associated[#] (*VWA1 PLVAP PDGFD, PDGFB, LOX,* and *LOXL2*); cirrhotic (*ACKR1* and *PLVAP*); lymphatic (*CD34* and *PDPN*)	endothelial cells in the cirrhotic liver	Ramachandran et al.[149]

(*Continued on next page*)

Table 1. ***Continued***

Organ	Species	Experimental approach	Vascular resolution	Biological focus	Reference
Kidney	mouse	adult mice (C57BL/6J); EC isolation: FACS of renal cell suspension; scRNA-seq: droplet-based	glomerular ECs (*Pi16, Plat, Ehd3, Cyp4b1, Tspan7,* and *Lp1*): G1: afferent arteriole (*Edn1* and *Alox12*), G2: the terminal portion of the afferent arteriole (*Efnb2* and *Cd3001g*), G3: fenestrated capillary (*Ehd3* and *Lpl*), G4: efferent arteriole expressing mixed markers (*CD9* and *Rdx*), G5: efferent arteriole (*Pde2a* and *Calca*); cortical ECs (*Plvap, Igfbp3,* and *Npr3*): C1: artery (*Mgp* and *Cdln5*), C2: arteriole (*Sox17* and *Cst3*), C3: efferent arteriole (*Kitl* and *Thbd*), C4: capillary, high ApoE (*Plpp3* and *Apoe*), C5: capillary, low ApoE (*Kdr* and *Npr3*), C6: postcapillary venule (*Tnxb* and *Jup*), C7: vein (*Bgn* and *Plavp*), C8: angiogenic capillary (*Aplnr* and *Col4a1*), C9: interferon response (*Isg15* and *Ifit1*); medullary ECs (*Igf1, Cryab,* and *Igfbp7*): M1: arteriole (*Klf4* and *Ltbp4*), M2: descending vasa recta (*Aqp1* and *Hpgd*), M3: descending vasa recta/papilla (*S100a6* and *S100a4*), M4: capillary (*Cd36* and *Npr3*), M5: postcapillary venule (*Jup* and *Il-6st*), M6: ascending vasa recta (*Fxyd6* and *Gas6*), M7: ascending vasa recta/papilla (*Cryab* and *Fxyd2*), M8: capillary/angiogenic (*Aplnr* and *Trp53i11*), M9: capillary/interferon (*Isg15* and *Ifi203*), M10: ascending vasa recta/interferon (*Ifit3* and *Ifi35*)	in response to dehydration and hypertonicity, medullary renal endothelial cells upregulate the expression of genes involved in the hypoxia response, glycolysis, and oxidative phosphorylation	Dumas et al.[150]
	human	kidney donors; nuclei isolation of renal cell suspension; snRNA-seq: droplet-based	glomerular capillary (*EMCN* and *FLT1*); afferent/efferent arteriole (*SEMA3A* and *MMRN1*); ascending vasa recta (*CD93* and *DNASE1L3*); descending vasa recta (*VIM* and *AQP1*)	similar to mouse renal endothelial cell heterogeneity	Lake et al.[151]
Meninges	mouse	adult mice (C57BL/6J); EC isolation: FACS of meningeal cell suspension after the deletion of intravascular $CD45^+$ cells; scRNA-seq: droplet-based (10×)	leptomeninges (arachnoid mater and pia mater): artery (*Cldn5* and *Slc2a1*), arteriole (*Slco1c* and *Slc2a1*), capillary (*Spock2* and *Slco1a4*), venule (*Pglyrp1* and *Itm2a*), vein (*Slc2a1* and *Ptn*); dura mater: artery (*Fabp4* and *Fosb*), arteriole (*Socs3* and *Irf1*), capillary (*Aqp1* and *Igp1*), venule (*Pim1* and *Oam*), vein (*Ptgs2* and *Cxcl10*), venous sinus (*Selp* and *Serpine 1*)	venous sinus endothelial cells are specialized to recruit immune cells via the selective expression of leukocyte adhesion molecules	Kim et al.[152]

(*Continued on next page*)

Table 1. *Continued*

Organ	Species	Experimental approach	Vascular resolution	Biological focus	Reference
	human	postmortem leptomeningeal tissue; nuclei isolation from the leptomeningeal tissue lysates; snRNA-seq (droplet-based)	leptomeninges (arachnoid mater and pia mater): artery/arteriole (*NKAIN2* and *PLPP1*), capillary (*PLPP1* and *IL-1R1*), venule/vein (*IL-1R1*, *TLL1*, *PKHD1L1*, and *LRRC1*)	altered intercommunication between endothelial cells in the leptomeninges of Alzheimer's disease patients	Kearns et al.[153]
Nasal cavity	mouse	VE-cadherin^{ERT2}-tdTomato mice (C57BL/6J) and Prox1-GFP mice (C57BL/6J); EC isolation: FACS of nasal mucosal tissue cell suspension; droplet-based (10× Genomic) and plate-based (Smart-seq3)	artery (*Gja4* and *Sema3g*); capillary (*Ednrb* and *Igfbp3*); vein (*Ackr1* and *Sele*); venous sinusoids (Foxc2 and *Cfh*)—large caliber, Prox1^{+}: VCAM1^{hi} (*Foxc2, Nr2f2, Vwf, Vcam1*, and *Icam1*), valvular (*Foxc2, Cldn11*, and *Gja4*), cytokine like-1^{+} (*cytl1*), MHCII^{+} (*Cd74* and *H2-Ab1*), proliferating (*Stmn1*), glucose transporter 1 (Glut1^{+}) (*Glut1, Slc2a1*, and *Mfsd2a*); integral membrane protein 2A (ITM2A^{+}) (*Itm2a* and *Spock2*); aquaporin 7 (AQP7^{+}) (*Aqp7* and *Cd36*); capillary-resident regenerative population (CRP) (*Kit* and *Apln*); lymphatic (*Lyve1* and *Ccl21a*)	extensive network of venous sinus endothelial cells expresses Prox1	Hong et al.[154]
	human	dissected tissues of the nasal cavity—normal portion of the respiratory mucosa; EC isolation: FACS of nasal mucosal tissue; plate-based (Smart-seq3)	artery (*GJA4*); capillary (*EDNRB* and *IGFBP3*); vein (*ACKR1* and *SELE*); venous sinusoid 1 (*PROX1, FOXC2^{hi}, VCAM1^{hi},* and *VWF^{hi}*); venous sinusoid 2 (*PROX1, FOXC2, VCAM1,* and *VWF*); lymphatic (*LYVE1* and *CCl21a*); interferon-γ (IFN-γ)	similar findings to mouse nasal vasculature	Hong et al.[154]
Salivary gland	mouse	VE-cadherin^{ERT2}-tdTomato mice (C57BL/6J); EC isolation: magnetic cell separation (MACS) sorting of salivary gland cell suspension; scRNA-seq: droplet-based (10×)	artery (*Gja4* and *Sema3g*); capillary type 1 (*Car4*); capillary type 2 (*Cd200*); capillary type 3 (*Cd300lg*); vein (*Ptgs1*); lymphatic (*Flt4* and *Stab1*)	endothelial cells express unique genes and display the highest overlap in gene expression with other fenestrated ECs in the colon, small intestine, and kidney	Altrieth et al.[155]
Skin	human	biopsied tissues of breast skin; FACS of skin tissue cell suspensions; plate-based (Smart-seq2)	arteriole (*GJA5* and *ASS1*); post-arterial capillary (*ASS1* and *S100A4*); pre-venular capillary (*SOX17* and *PLAUR*); post-capillary venule (*ICAM1* and *SELE*); venule (*EGR2* and *LRG1*); collecting venule (*ACKR1* and *SELP*)	association of vascular remodeling program to inflammatory response	He et al.[156]

nanoparticles (STANs) were found to promote vascular normalization, reduce tumor hypoxia, and increase endothelial cell expression of T cell adhesion molecules. STING-mediated vascular reprogramming thereby enhanced the infiltration, proliferation, and function of antitumor T cells and potentiated the response to immune checkpoint inhibitors and adoptive T cell therapy.[172]

Metastasis is critically dependent on bi-directional tumor-endothelial cell interactions. Tumor cells have been shown to induce in a double-stranded-RNA-dependent manner the expression of the axon-guidance gene Slit2 in the endothelium, which promotes the migration of cancer cells toward endothelial cells and intravasation through the Robo1-expressed Slit2 receptor on tumor cells. Thus, cancer cells co-opt innate RNA sensing to induce a chemotactic signaling pathway in endothelium that drives intravasation and metastasis.[124] During cancer cell dissemination, metastatic tumor-cell-derived signals downregulate the expression of tumor-necrosis-factor-related apoptosis-inducing ligand (TRAIL) in premetastatic niche endothelial cells. This interferes with an endothelial quiescence-mediating intracrine signaling loop whereby TRAIL inhibits the activity of its cognate death receptor 5. This inhibition contributes at steady state to preserving the quiescent vascular phenotype. As a result of TRAIL downregulation, death receptor 5 activation induces endothelial cell death and nuclear factor κB/p38-dependent endothelial stickiness, compromising vascular integrity and promoting myeloid cell infiltration, breast cancer cell adhesion, and metastasis.[173] Upon arrival at the metastatic site, seeded tumor cells make further fate decisions that are critically controlled by metastatic niche endothelial cells. Controlled by endothelial cell Wnt signaling, metastatic tumor cells may extravasate, which is an essential requirement to acquire a state of metastatic latency, which may eventually lead to long-term dormancy. The ability of tumor cells to extravasate is controlled by their epigenetic state, with hypomethylation favoring the extravasation/latency route.[60]

Taken together, the dramatic advances in deconvoluting endothelial cell heterogeneity all the way to single-cell resolution has led to an enormous wealth of new knowledge. This now awaits to be mechanistically dissected and integrated. Datamining of individual novel target genes and mechanism- and pathway-specific signatures will pave the way for novel translational opportunities in both preventive medicine applications and therapeutic intervention.

PERSPECTIVES IN TRANSLATIONAL ANGIOSCIENCE RESEARCH

Vascular research has led to the development of numerous blockbuster drugs for critical human diseases involving angiogenesis, inflammation, blood pressure regulation, and coagulation. Endothelial cells are readily accessible to therapeutic intervention, given their exposed location at the systemic circulation. However, unlike many parenchymal cell populations, such as hepatocytes and cardiomyocytes, endothelial cells are notoriously difficult to selectively target by viral vectors. However, the recent knowledge on organ-, caliber-, and pathologic-situation-selective endothelial cell marker molecules could be exploited to target distinct endothelial cell populations. Likewise, endothelial cell vector research has made substantial progress in recent years.[174,175]

Regenerative medicine

Regenerative medicine applications aimed at replacing, engineering, or regenerating cells and tissues face the inherent problem that any growth of a tissue beyond microscopic size is critically dependent on vascular supply. However, microvascular tissue engineering (Figure 6A) faces several challenges and involves biological considerations (cellular source for grafting), material science challenges (suitable biocompatible scaffolds), engineering science bottlenecks (assembling cells with scaffold into tissues), and clinical limitations (grafting technique).

Suitable cell populations used for grafting should preferentially be autologous, which has driven research to use differentiated stem cells as seed source.[176] If possible, grafted cells should display organotypic traits, as was recently shown by grafting human induced pluripotent stem cell (iPSC)-derived cells into the mouse liver vasculature.[177] Similarly, it has been proposed to reprogram differentiated endothelial cells for transplantation purposes. The ETS-family TF ETV2 functions as a pioneer factor that relaxes closed chromatin and regulates endothelial development.[178] Transient reactivation of ETV2 in mature human endothelial cells "resets" these endothelial cells to adaptable, vasculogenic cells, which form perfusable and plastic vascular plexi.[179] Such reprogrammed cells obtained from patient-derived autologous differentiated endothelial cells could be an attractive source for tissue engineering purposes.

Co-transplanted mesenchymal stromal cells facilitate the *in vivo* grafting of endothelial cells. These co-grafted mesenchymal stromal cells have been shown to transfer mitochondria to endothelial cells through tunneling nanotubes, thereby enhancing endothelial bioenergetics and enabling them to form functional vessels in ischemic tissues.[180] An alternative to grafting endothelial cells is the transplantation of small organ constructs or organoids that engage the endogenous pool of endothelial cells. For example, bone engineering can be accomplished by grafting osteogenic constructs of cultured human-bone-marrow-derived mesenchymal stem cells in a fibrin hydrogel. Decorating such constructs prior to grafting with VEGF serves to attract endogenous endothelial cells that vascularize the grafted construct. VEGF dosing in such applications is critical to prime the proper balance of angiogenesis and osteogenesis.[181]

Another goal of tissue engineering is to generate *ex vivo* complex vascularized tissues that connect to the host vasculature upon grafting. 3D bioprinting has been developed toward this end, in which cells are incorporated within bioinks into a granular support material. Following gelation, the bioprinted tissue structures are cultured to mature into functional native-like tissues. A "print-and-grow" technology involving 3D bioprinting and subsequent cultivation in kappa-Carrageenan-based microgels (CarGrow) for several days enhances the long-term structural stability of the printed objects by providing mechanical support.[182] *Ex vivo* engineering of hierarchically structured vessel segments has also been pursued by interconnecting millimetric vessel-like scaffolds and 3D-bioprinted vascularized tissues to

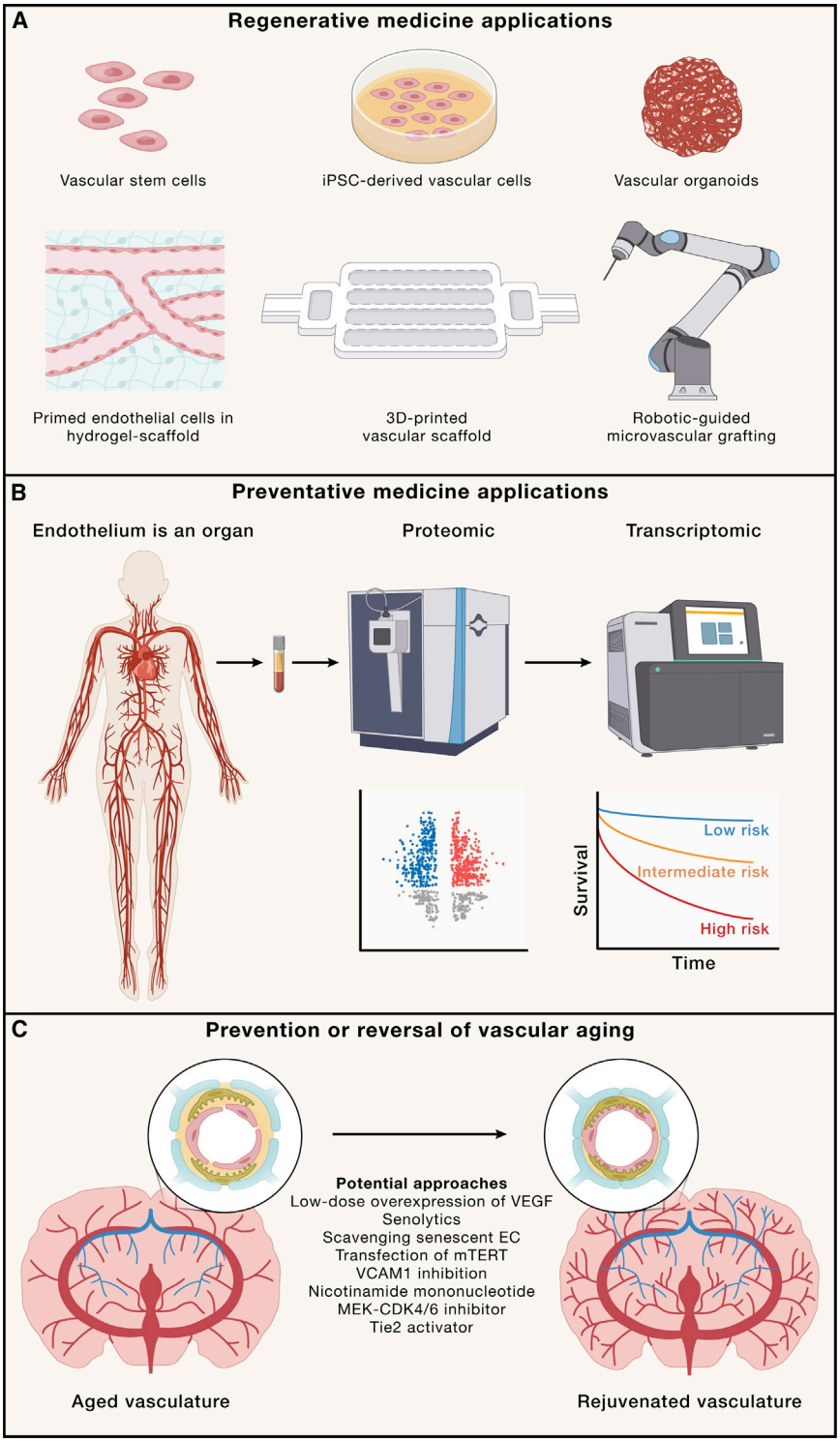

Figure 6. Translational perspectives in angioscience research

(A) Microvascular tissue engineering from vascular stem cells and reprogrammed iPSC-derived endothelial cells may have substantial translational potential in emerging regenerative medicine applications.

(B) Vasculature-related parameters and molecules may be exploited as a biomarker readout of systemic health in preventive medicine applications.

(C) Vascular aging is a major determinant of organismic aging. Therapeutic vascular rejuvenation may consequently be a strategy to promote healthspan and lifespan.

create fully engineered vascular constructs. Sacrificial molds are used to create polymeric vessel-like scaffolds and endothelial cells seeded in their lumen form native-like capillaries.[183] Such endothelialized scaffolds are assembled within vascularizing hydrogels to cooperatively create vessels, enabling tissue perfusion through the scaffold lumen. Such fully-*ex-vivo*-engineered vascular constructs have been microsurgically anastomosed to the femoral arteries of rats to give rise to durably perfused implanted tissue.[183]

Taken together, it is still a long road to making tissue engineering with fully vascularized grafts a clinical reality. However, the few examples spelled out here highlighting recent advances in cell engineering (either stem cell-derived or reprogrammed differentiated cells), biomaterial science, cell and tissue engineering (from organoid technology to bioprinting applications), and grafting techniques (from spontaneous self-organization to microsurgery) give great hopes that translational breakthroughs may become reality in the years to come for multiple clinical applications.[176]

Preventive medicine

Translational perspectives may also emerge in the field of preventive medicine applications (Figure 6B). The vascular endothelium in an adult human covers a huge surface, possibly exceeding the size of a soccer field. That is why it is possible to detect secreted endothelial molecules at the proteomic level in the circulation even when the transcriptomic changes on the single-cell level may be subtle. Interestingly, the systems map of transcriptomic changes of endothelial cells in the premetastatic niche has led to the discovery of the TGF-β pathway modifier LRG1 as an endothelium-derived secreted instructor of metastasis.[184] The detailed multiorgan vascular analysis of LRG1 expression in tumor-bearing mice revealed that the primary tumor-induced upregulation of LRG1 was not restricted to premetastatic niche endothelial cells but could be observed in endothelial cells of all analyzed organs. Concomitantly, LRG1

protein was abundantly detectable in the circulation of tumor-bearing mice. Surprisingly, even small primary tumors were capable of systemically reprogramming the vascular endothelium with detectably elevated protein concentrations in the circulation. Mechanistically, this was interpreted to mean that primary tumors probably utilize the vast surface of the vascular endothelium as an amplifier of tumor-derived instructive signals.[184] This concept could be translated to other disease states that involve systemic vascular activation with release into the circulation of a possibly disease-stimulus-specifically activated endothelial secretome. It may not be far-fetched to hypothesize that the systemic vascular endothelium could be exploited as a biomarker readout of systemic health and a warning screening system for the early identification of patients at risk of developing vascular dysfunction involving diseases.

Exploiting the vascular endothelium as a readout of health may be particularly powerful for longitudinal monitoring. Integrating digital wearable devices[185] with clinical biochemistry would require minimally invasive sampling techniques and easily measurable robust biomarkers with high stratification potential. The analysis of the endothelial secretome from a drop of blood might just serve that purpose. For example, the amino-terminal fragment of tumor-derived Angptl4 was recently identified as a potent endogenous suppressor of metastasis. High circulating concentrations of nANGPTL4 were found to correlate with better prognosis of melanoma patients compared with patients with lower circulating nANGPTL4. Remarkably, more important than the absolute concentrations of circulating nANGPTL4 was the longitudinal trend analysis of whether nANGPTL4 was going up or down in individual patients. When grouping melanoma patients into those with declining or increasing concentrations of circulating nANGPTL4, as little as an upregulation of 10% compared with a downregulation of 10% leads to a substantial dissociation of Kaplan-Mayer survival curves.[186] This simple example impressively illustrates the prospective power to translationally exploit vascular-related parameters and molecules as readout of systemic health in routine longitudinal monitoring preventive medicine applications. Beyond monitoring preventive medicine applications, future work will also address whether preventive therapeutic measures may be beneficial to improve vascular health, e.g., by promoting quiescence signaling through stimulating Tie2 activation in a mimetic approach.[187]

Preventing or reversing vascular aging

A third field of vascular research with substantial potential for translational developments may unfold in the field of vascular aging, aiming at preventing or reversing vascular aging (Figure 6C). Endothelial cells—such as other cell populations of the mesenchymal lineage—are in their maintenance and turnover pattern distinctly different from other cell populations. Epithelial and hematopoietic cells are mostly derived by asymmetric cell division from stem cells. Here, differentiating cells usually have a short half-life time, and regeneration occurs from stem cells, e.g., as can be seen in skin or intestinal epithelial cells. In turn, long-lived differentiated cells usually have a very limited proliferative capacity, e.g., cardiomyocytes or neuronal cells. In contrast, endothelial cells are long-lived cells[32] that at the same time have an enormous proliferative capacity, e.g., when challenged by an angiogenic stimulus. The long lifespan of differentiated endothelial cells—in combination with the notion that bone-marrow-derived progenitor cells probably do not contribute to the turnover of endothelial cells in the adult (see above)—could suggest that the number of differentiated endothelial cells of an organism may be the endothelial cell pool for the entire lifespan. This would be a provocative concept since it would raise the question of whether the aging of the differentiated endothelial cell pool could be a rate-limiting bottleneck of aging.

Aging research has focused, on the one hand, extensively on the elucidation of molecular mechanisms of aging and, on the other hand, on manipulations affecting lifespan and healthspan on the organismic level.[188] In contrast, aging on the cellular level and particularly comparative studies of different cell populations are not so widely pursued. Such studies could shed insight if some cell populations may be more vulnerable to aging than others. The age-dependent decline in vessel density with distinct organ-specific differences observed in mouse and human tissues could indeed suggest a particular cell-type-specific vulnerability of the vascular endothelium during aging.[48] In line with these aging-related vascular rarefaction studies, functional studies start to accumulate evidence that endothelial cells could be among the more-age-sensitive cell populations (Figure 2D). Lineage tracing of senescent cells (using p16(Ink4a) as a senescent marker) revealed that p16(high) senescent cells in mice manifested around 10–12 months of age. The majority of these cells were endothelial cells of liver sinusoids and, to a lesser extent, macrophages and adipocytes. The elimination of senescent cells disrupted blood-tissue barriers with subsequent liver and perivascular tissue fibrosis. Intriguingly, there was no replacement of senescent endothelial cells.[189] Similar findings were made in a model of Hutchinson-Gilford progeria syndrome (HGPS), in which an accelerated aging syndrome was associated with premature vascular disease and death due to heart attack and stroke. Endothelial cells differentiated from iPSCs derived from these patients exhibit hallmarks of senescence, including replication arrest, increased expression of inflammatory markers, DNA damage, and telomere erosion, supporting the notion that endothelial aging could be the disease course determining bottleneck cell population. Lentiviral transfection of mTERT in a mouse model of HGPS ameliorates the endothelial cell senescence phenotype leading to increased NO generation and acetylated low-density lipoprotein uptake, improved angiogenesis, and reduced inflammatory parameters.[190] A third study suggesting a preferential susceptibility of endothelial cells was based on the observed abundant expression of GPNMB in senescent VE cells. Senolytic vaccination of mice against GPNMB resulted in a reduction of GPNMB-positive cells with improved normal and pathological phenotypes associated with aging and extended lifespan of progeroid mice.[191] Pharmacological senolytic therapy has also been proposed as local therapy in ophthalmology. Intravitreal injection of a BCL-xL-targeting senolytic drug reduced diabetes-induced retinal vascular leakage and preserved retinal function.[192] These preclinical findings laid a foundation for subsequent early clinical trials in diabetic macular edema patients.[192]

Endothelial cells undergo pronounced transcriptional and phenotypic changes during aging, including an upregulating

innate immunity and oxidative stress response pathways. The aging phenotype is at least in part controlled by soluble factors, as evidenced by the observation that the infusion of young plasma into aged mice exerts rejuvenation effects on the capillary transcriptome.[193] Aged endothelial cells display a pro-inflammatory phenotype and contribute thereby to the inflammaging phenotype seen in aged organisms. For example, aged mouse hippocampus endothelial cells express an inflammatory transcriptional profile with focal upregulation of VCAM1. Correspondingly, soluble VCAM1 is prominently increased in the plasma of aged humans and mice. The inhibition of VCAM1 (genetically or by antibodies) is capable of reversing aspects of the aging phenotype, including microglial reactivity and cognitive deficits.[194] An impairment of an endothelial NAD^+-hydrogen sulfide (H_2S) signaling network has also been established as a reversible cause of vascular aging. The treatment of mice with nicotinamide mononucleotide (NMN) improves blood flow and increases endurance in elderly mice. NMN acts as an NAD^+ booster and promotes SIRT1-dependent increases in capillary density. This effect is augmented by exercise or increasing the levels of H_2S.[195] The consequences of endothelial cell aging have also been studied in the heart. Aged cardiac endothelial cells exhibit dysregulated expression of neuroregulatory genes. This results in altered neuronal function and reduced nerve density in the ventricle, leading to perturbed cardiac function during aging.[196] Altered angiocrine signaling mechanisms during aging are also observed in the bone marrow. Here, aged endothelial cells fail to support the young phenotype of hematopoietic stem and progenitor cells (HSPG). As a result, HSPG acquires an aged phenotype. Netrin-1 and mTOR signaling in endothelial cells have been reported as regulators of the young endothelial cell phenotype that involves an active DNA damage response machinery.[197,198]

Tumor-associated endothelial cells appear to be particularly sensitive to senescence-inducing manipulations. The induction of the SASP through a senescence-inducing therapy (combination of MEK and CDK4/6 inhibitors) in pancreatic-tumor-bearing mice preferentially affected tumor-associated endothelial cells to induce a proangiogenic phenotype, which, in turn, enhanced drug delivery and stimulated the intratumoral accumulation of $CD8^+$ T cells.[199] Accordingly, tumor angiogenic endothelial cells and senescent endothelial cells appear to have transcriptional and phenotypic commonalities.[200] Distinct functional characteristics of senescent endothelial cells have also been observed in the kidney and the lung. In the kidney, selective inactivation of PAI-1 in endothelial cells was found to protect glomeruli from lesion development and podocyte loss in aged mice. Correspondingly, the depletion of senescent cells prevents podocyte loss in old p16 INK-ATTAC transgenic mice. Glomerular PAI-1 expression in humans is predictive of poor outcomes in transplanted kidneys from elderly donors, and urinary PAI-1 was associated with age-related chronic kidney disease.[201] In the lung, dysfunctional endothelial ERG signaling drives pulmonary vascular aging and persistent fibrosis. This is associated with reduced chromatin accessibility and maladaptive transcriptional responses to injury.[202]

Thus, new concepts, approaches, methods, technologies, bio-medical and imaging machines, and equipment are emerging daily. How do we accommodate and incorporate them into angioscience research and eventually treat patients with vascular diseases? More intensive, definitive, and insightful research and clinical trials are required to make a solid bridge between the bench and the bed.

CONCLUDING REMARKS

Vascular research is the smaller branch of the broader field of cardiovascular biology and medicine. Although cardiology is scientifically and clinically a coherently developed discipline, the field of vascular research is much more fragmented, and there is a disconnect between basic and clinical research. The clinically relevant branches angiogenesis, inflammation, blood pressure regulation, coagulation, and atherosclerosis are largely pursued as monolithic pillars with little interdisciplinary synergy. Moreover, given that blood vessels are part of every organ, vascular research is pursued within every organ discipline. However, within each organ discipline, vascular research is mostly pursued within a marginalized niche. As a result, the discipline itself is fragmented, and outside the discipline, the central role of the vascular endothelium for organ function and systemic health is not widely appreciated.

The appreciation of the vasculature as a systemically disseminated organ, which we have holistically conceptualized as "angioscience," holds great prospects to advance systems physiology and the pathophysiological understanding of diseases. Moreover, developing the synergies and functional crosstalk between the heart and the vasculature will contribute to the development of the field of cardiovascular multiorgan communication biology. These developments occur at a time when the diversity of caliber-specifically and organ-specifically differentiated endothelial cells has molecularly been deconvoluted through multiomics approaches all the way to single-cell resolution. Datamining this wealth of information offers unprecedented opportunities for mechanistic studies aimed at identifying and validating disease-relevant vascular integrity pathways and novel pathogenetic vulnerabilities and targets.

An integrated systems-based structure-function analysis of the vascular endothelium holds great prospects to pave the way for the development of new clinical applications. We spelled out emerging opportunities in the fields of regenerative medicine, preventive medicine, and vascular aging. However, in the long run, much broader translational opportunities may emerge. Being directly exposed to the circulation, no cell population is in principle as readily accessible to therapeutic intervention as the vascular endothelium. As such, it may well be conceivable to develop strategies that therapeutically target select subpopulations of organ endothelial cells in order to locally deliver therapeutics or reprogram organotypic instructive angiocrine signaling mechanisms that control organ function. Substantially intensified research efforts are warranted toward this end to fully exploit the translational potential of the vascular endothelium. Likewise, better disciplinary coherence of the different branches of vascular research will benefit the recognition of the many different facets through which the cells of the vessel wall affect organ function.

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The authors declare no competing interests.

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