Review

Organ/Tissue-Specific Vascular Endothelial Cell Heterogeneity in Health and Disease

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The vascular system forms the largest surface in our body, serving as a critical interface between blood circulation and our diverse organ/tissue environments. Thus, the vascular system performs a gatekeeper function for organ/tissue homeostasis and the body’s adjustment to pathological challenges. The endothelium, as the most inner layer of the vasculature, regulates the tissue microenvironment, which is critical for development, hemostatic balance, inflammation, and angiogenesis, with a role as well in tumor malignancy and metastasis. These multidimensional functions are primarily mediated by organ/tissue-specifically differentiated endothelial cells, in which heterogeneity has long been recognized at the molecular and histological level. Based on these general principles of vascular-bed heterogeneity and characterization, this review largely covers landmark discoveries regarding organ/tissue microenvironment-governed endothelial cell phenotypic changes. These involve the physical features of continuous, discontinuous, fenestrated, and sinusoidal endothelial cells, in addition to the more specialized endothelial cell layers of the lymphatic system, glomerulus, tumors, and the blood brain barrier (BBB). Major signal pathways of endothelial specification are outlined, including Notch as a key factor of tip/stalk- and arterial-endothelial cell differentiation. We also denote the shear stress sensing machinery used to convey blood flow-mediated biophysical forces that are indispensable to maintaining inert and mature endothelial phenotypes. Since our circulatory system is among the most fundamental and emergent targets of study in pharmacology from the viewpoint of drug metabolism and delivery, a better molecular understanding of organ vasculature-bed heterogeneity may lead to better strategies for novel vascular-targeted treatments to fight against hitherto intractable diseases.

Key words  endothelial cell; heterogeneity; genetic and epigenetic regulation; tissue specific regulation; blood brain barrier; tumor endothelium

1. INTRODUCTION

The endothelium is the innermost cell layer of all blood vessels, and as such plays a role in a multitude of physiological processes involving the regulation of leukocyte trafficking and circulation, the maintenance of vasomotor tone with blood pressure, maintaining blood fluidity or permeability, as well as in angiogenesis. Endothelial cells differentially regulate according to their different structural and functional levels in time and space. As a general rule, endothelial cell phenotypes change: 1) between different organs; 2) between different areas of blood circulation within the same organ; and 3) between neighboring endothelial cells of the same organ and blood vessel subtypes. For example, the von Willebrand factor (vWF) gene is expressed predominantly within the endothelium of veins, but is not expressed in the microvasculature of the heart.1 Endothelial nitric oxide synthetase (eNOS) and tyrosine kinase; Bmx gene, is a mainly expressed in larger vessels,2,3 whereas thrombomodulin is universally expressed in the vasculature of all organs except the brain.4 Recent genome-wide screening techniques have unveiled a wide array of genes that are expressed in specific vascular beds of developmental embryos, normal adult organs and tumors.5,6 Indeed, while endothelial cells’ complexity and diversity have long been recognized, little is known about the underlying molecular mechanisms that mediate the phenotypic heterogeneity of epigenetically different endothelial cell populations.

Endothelial cells’ diversity may arise from signals residing in the extracellular microenvironment, or from factors inherent in the cell.7 The genetically predetermined hypothesis predicts that organ-specific phenotypes are fixed before they migrate from the mesenchymal to specific vascular beds. This concept is largely supported via fate-mapping studies in mammalian embryos, showing that endothelial cell precursors, or angioblasts, from the vasculogenesis stages are able to differentiate along several genetically pre-programmed lines. In contrast, the epigenetically variable hypothesis holds that the site-specific properties of endothelial cells are regulated by local microenvironmental cues that exist within the resident tissues.8 Most recently, there has been growing evidence of the importance of the local environment in determining endothelial heterogeneity.5 Endothelial cells may react to soluble extracellular mediators, to cell–cell interaction, and to the expression and organization of matrix proteins through its interaction with the microenvironment. In the progressing analysis, phenotypic heterogeneities of the endothelium are likely reversible, but irreversible in several pathological cases, which are determined by a combination of genetic/epigenetic and microenvironmental factors.

2. GENETIC PRE-DETERMINANTS FOR SHOWING THE ENDOTHELIAL HETEROGENEITY

2.1. Endothelial Differentiation from Stem Cells...
tial endothelial cells are mainly derived from hemogenic angioblasts, which are believed to originate from hematopoietic and blood vascular endothelial stem cells. Our in vitro differentiation study of embryonic stem cells revealed that vascular endothelial growth factor (VEGF) is a unique and essential differentiation factor. Subpopulations of VEGF receptor (VEGFR) 2-positive mesenchymal stem cells autonomously expressed an endothelial lineage committed pioneer factor, ETS variant (ETV) 2, via possibly turning on the epigenetic control. ETV2 and GATA2 specifically interacted in endothelial, but not hematopoietic, cell progenitors that induced the endothelial master regulators: Friend leukemia integration (FLI) 1, and Sry-related hydroxymethylglutaryl (HMG) box (SOX) on 7/17/18. These genetic circuits ultimately determine the expression of the endothelial maintenance ETS factor: the Ets-related gene (ERG). SiRNA-mediated knockdown analysis revealed that these transcriptional cascades are critical not only for proper endothelial cell differentiation, but also in blocking the commitment to other closely aligned lineages.

Indeed, loss of ERG and FLI1 in fully differentiated endothelial cells led to endothelial-mesenchymal cell conversion (Fig. 1).

2.2. Arteries versus Veins Specification of endothelial tube formation as arteries and veins would be one of the most

Fig. 1. Schematic Model of the Genetical Regulation of the Endothelial Cell Commitment

a) Mesenchymal cells are differentiated to endothelial cells in the presence of VEGF following the expression of the pioneer factor-like Etv2, and the master regulators: Gata2, Flil, Sox7 and 18. These transcription factors activate the endothelial markers but repress the other lineage markers. b) Even if the fully differentiated endothelial cells, once loss of Erg and Flil, they initiate EndMT process following to the loss of MicroRNA-126 and BMP/Smad1 pathway. (Color figure can be accessed in the online version.)

Biography

Takashi Minami; Graduated from the Faculty of Pharmaceutical Sciences, Osaka University (Ph.D.) in 1998, continue to study abroad as the Postdoctoral fellow at the Dept. of Biology, Massachusetts Institute of Technology (MIT) until 2000, and also belonging to the Instructor at the Harvard Medical School, U.S.A. until 2002. After discovering the new theory of 'Existence of organ-vascular bed heterogeneity by using the promoter knock-in mouse system,' he was invited from the Div. of Laboratory for Systems Biology and Medicine, at the University of Tokyo, as an associate professor (2002–2012). After that, promoted to the Principal Investigator (PI) as the professor of Vascular Medicine Unit of the University of Tokyo (2012–2015). From the beginning of Winter 2016 (before attacking severe earthquake), move to Kumamoto, and became full professor and PI, Div. of Molecular and Vascular Biology, Dept. of Life Science, Kumamoto University.

Currently, Minami’s Lab studies the pathophysiology of vascular diseases involving coagulation, atherosclerosis, and angiogenesis in malignant cancer. Welcome to the graduated medical and pharmaceutical students who is interested in genome-wide and epigenetic study of the endothelial cell activation.
critical events during vascular development. Until recently, it was widely considered that the phenotypic differences between arterial and venous endothelial cells were attributable to environmental cues, such as differences in hemodynamic forces, blood flow direction, oxygen levels, and interactions with neighboring smooth muscle or mural cells. This classical recognition has now been challenged by recent advanced findings of certain molecules that are specifically and exclusively expressed in arterial or venous endothelial cells during the early developmental stage before the onset of blood circulation. As the result of these findings, there is great interest in delineating the molecular basis for artery or vein identity.

2.2.1 Notch Signaling

Notch receptors belong to the larger type I single-pass transmembrane protein family. Four of these Notch receptors, Notch 1 to 4, have been described in mammalian cells. The extracellular domain of Notch encoded up to 36 tandem-repeats of an epidermal growth factor (EGF)-like motif. Notch receptors interact with ligands that are also single-pass type I transmembrane proteins. This unique Notch signaling pathway transduces between physically adjacent cells, therefore the Notch only activated the juxtaunique signaling. The Notch ligands are encoded by the Jagged (JAG1 and 2) and Delta-like (DLL1 to 4) gene families in mammals.

Notch 1 and 3 are localized to the endothelium and smooth muscle layer of descending aortas in embryonic days (E) 13.5 mouse embryos, respectively. The expression of JAG1 and DLL4 are restricted to the arterial endothelium, suggesting that Notch ligand and receptor interactions are involved in site-specific signal transduction. DLL4 is believed to be the most critical Notch ligand required for vascular development in mice, since DLL4−/− homozygous embryos indicated embryonic lethality due to vascular defects and did not express arterial markers. Moreover, direct evidence of Notch signaling in regulating the expression of important artery-expressed genes has been reported. For example, DLL4-mediated Notch signaling induced ephrinB2 expression in cultured endothelial cells, and the ephrinB2 gene was demonstrated to be a direct Notch target.

In contrast to DLL4, Dll1 expression in the vasculature has been detected at approximately E13 in arterial, but not venous, endothelial cells. In Dll1 loss of function mutant embryos, the expression of arterial markers involving neuropilin1, VEGFR2, and ephrinB2 were lost, despite the fact that and Jag1 are continued to be expressed in the arterial endothelium. These results suggest that DLL1 is also a functional Notch ligand required for maintaining the arterial identity of endothelial cells during mouse embryonic development, and these Notch signaling pathways are transduced in a context dependent cross-regulatory manner.

2.2.2. COUP-Transcription Factor (TF) II

Compared to Notch signaling mediated artery formation in the endothelium, until recently, little has been uncovered about vein identity except that it involves ephrin receptor (Eph) B3 and 4 expression. Because Notch signaling is not activated in veins, venous vessel formation is believed to be a default pathway without receiving the Notch activation signals. However, Tsai and colleagues have reported that COUP-TFII, a member of the orphan nuclear receptor superfamly also known as NR2F2, is specifically expressed in venous but not arterial endothelium. Knockout of COUP-TFII in endothelial cells enables veins to acquire arterial characteristics, with increased expression of the arterial marker genes Neuropilin1 and ephrinB2, and the generation of hematopoetic cell clusters. In contrast, ectopic expression of COUP-TFII in endothelial cells from transgenic embryos results in the fusion of veins and arteries. These results suggest that COUP-TFII plays a critical role in repressing Notch signaling in order to maintain vein identity. Vein identity is now considered under genetic control, and would not be derived via a previously considered default pathway.

2.3. Tip versus Stalk Cells

New capillaries generate via sprouting from previously existing blood vessels during the angiogenic stage. Tip cells are uniquely specialized endothelial cells localizing at the ‘tip’, leading edge of filopodia of vascular sprouts. Such tip cells extend and react to the local extracellular microenvironment, and guide the direction of these sprouts following VEGF gradients. In contrast, proliferated endothelial cells adjacent to the tip cell are termed ‘stalk’ cells. Tip and stalk cell interactions are critical for the stabilization of vessel branch formation. The Notch pathway has a primary role in endothelial tip and stalk cell identification and functional vessel sprouting. In the mechanism of tip and stalk cell determination, it is well recognized that the VEGF receptor-mediated signal reception in the tip cell stimulates DLL4 expression as the Notch ligand. Such tip cell signals via DLL4 are passed to the adjacent Notch receptor-expressing stalk cells, which in turn downregulate the VEGF receptor expression. Thus, the tip cell’s fate is fixed following VEGF gradients, and subsequent Notch signal-mediated lateral suppression leads to the determination of tip-adjacent endothelial cells becoming as stalk cells. More interestingly, it is reported that Fringe proteins mediating the glycosylation of Notch1 stimulate the DLL4, whereas they attenuate the Jag1-Notch signaling. Therefore, both Notch ligands DLL4 and JAG1 react in an antagonistic fashion to each other during endothelial tip cell selection and angiogenic sprouting. DLL4, expressed more highly on tip cells, suppresses the adoption of the tip cell fate and angiogenic sprouting in stalk cells. By contrast, JAG1, which is expressed more highly in stalk cells, oppositely promotes tip cell fate determination and sprouting behavior by antagonizing DLL4-Notch suppression signaling among the stalk cells. Summarizing studies of various null mutations or knockdown experiments for Notch signaling reveal that the signal inhibition unexpectedly leads to increased sprouting and branching of blood vessels. Reduced DLL4/Notch signaling leads to the over-proliferation of retinal vasculature, with striking defects in the early postnatal stage. The observed defects are concordant by assessing DLL4−/− heterozygous mice or mice with temporally-regulated Notch1 deletion in the retinal vasculature, or by administering anti-DLL4 blocking reagents or γ-secretase inhibitors. The retinal vasculature in these mice failed to form functionally mature capillaries, with severe patterning defects. These vasculatures exhibited increased capillary density and diameter, with prolonged filopodia-extensions, both at the growing vascular front, and in the interior of the plexus. Consistent with these findings, anti-DLL4 (also termed DLL4 blockade) treatment increased blood vessel sprouting and branch formation, especially in tumor vessels. Paradoxically, despite an increase in blood vessel density, tumor growth was strongly inhibited. The vascular network in the anti-DLL4-treated tumors formed inappropriately, termed dysfunctional angiogenesis, causing...
poor perfusion and increased hypoxia, which finally led to an overall inhibition of tumor growth. In contrast, a transgenic mouse model of endothelial-specific Dll4 overexpression reduced retinal vessel angiogenesis and xenografted tumor growth by reducing VEGF-induced endothelial proliferation and overall blood supply relative to the tumor. In addition, Dll4 overexpression consistently improved vascular maturation and functionality, as indicated by increased vessel caliber and enhanced mural cell recruitment. Both Dll4 knockdown and overexpression would inhibit proper vessel growth due to the imbalance of tip and stalk cell formation, leading to dysfunctional angiogenesis and reduced vessel density, respectively.

2.4. Endothelial Phenotype Changes in Organs

The distinct embryonic origins of coronary endothelium and endocardium were previously determined using retroviral cell lineage studies in the chick embryo. The coronary endothelium, but not the endocardium, has been shown to derive from the proepicardial organ in the dorsal mesocardium. Moreover, these progenitor cells seem to be multipotent, since they give rise not only to endothelial cells, but also to vascular smooth muscle cells and fibroblasts of the coronary vessels. Heart valves are formed through a unique developmental process called endothelial/endocardial-mesenchymal transformation, in which endothelial cells delaminate from the endocardial layer, transdifferentiate into mesenchymal cells, and finally become endocardial cushions. The nuclear factor for activated T cells (NFAT) 2 predominantly regulated in this cell conversion process, and the Down syndrome critical region (DSCR)-1, as a NFAT feedback modulator, was also specifically upregulated in this cushion area. NFAT dysregulation was shown to directly lead to the pathogenesis of Down syndrome. Several Down syndrome patients have been diagnosed with this cardiac malformation. Thus, vascular dysfunction mediated by the low NFAT activity and high DSCR-1 dosage have been suggested to lead to cardiac malformation in developmental stages.

Similar to the case of different origins in heart vessels, there is also increasing evidence in the lung that endothelial cells from microvascular and large vessel segments are derived from different origins during embryogenesis. For example, microvasculature in the lung is derived from blood islands through a process that involves vasculogenesis, whereas large vessel endothelium originate from the pulmonary truncus; then, beginning at E15.5-E16.0, the primary lymphatic vasculature undergoes remodeling and maturation to form a hierarchical lymphatic vascular network composed of lymphatic capillaries, precollectors, and collecting lymphatic vessels. Lymphatic capillaries are blind-ended and highly permeable, because their basement membrane is discontinuous and they are not covered by pericytes or smooth-muscle cells, whereas collecting lymphatic vessels are surrounded by intact basement membranes and smooth muscle cells and contain intraluminal valves. The morphological changes associated with lymphatic remodeling and maturation also continue after birth.

2.5. Lymphatic Vessels versus Systemic Blood Vessels

As part of the circulatory system, the lymphatic vasculature is responsible for the maintenance of tissue fluid homeostasis, lipid absorption/transport from the digestive system, and immune surveillance. It also plays a key role in such pathological processes as inflammatory disease and the metastatic spread of tumor cells. Deficiencies in lymphangiogenesis or lymphatic valve function can impede lymph drainage, which causes lymph to accumulate in the interstitial tissue and can lead to primary (hereditary) or secondary (acquired) lymphedema. However, the nature of lymphatic vessel abnormalities and disease in humans is complex and poorly understood; therefore, investigations into the molecular and cellular mechanisms that support the formation and function of lymphatic vessels will have critical implications for the development and optimization of treatments for lymphedema, cancer, and many other disease states and conditions.

2.5.1. Lymphatic Cell Specification and Differentiation

The murine lymphatic vascular system is developed shortly after blood circulation is established. At approximately E9.5, a subpopulation of lymphatic endothelial progenitors located in the anterior cardinal vein become competent for differentiation into lymphatic endothelial cells. Competency is triggered when extracellular signal-regulated kinase (ERK) signaling induces Sox18 expression, and Sox18 and COUP-TFII then activate the expression of Prospero homeobox (Prox) 1, the master regulator of the lymphatic vascular phenotype. Prox1 physically and functionally interacts with COUP-TFII to regulate lymphatic gene regulation. (Once the identity of lymphatic endothelial cells is specified (at approx. E10.0), Prox1 lymphatic endothelial progenitors bud off and migrate dorsolaterally from the cardinal and intersomitic veins, creating chains of interconnected cells that subsequently form the primary lymph sacs and superficial lymphatic vessels by E12.5. This process is dependent on the signaling pathway of VEGF-C expressed by the surrounding mesenchyme and its receptor (VEGFR3) in Prox1 lymphatic endothelial cells.

These primary lymphatic structures further develop into the lymphatic vascular network through the proliferation, sprouting, and survival of lymphatic endothelial cells (i.e., the process of lymphangiogenesis). By E14.5, the network extends throughout the mouse embryo, then, beginning at E15.5-E16.0, the primary lymphatic vasculature undergoes remodeling and maturation to form a hierarchical lymphatic vascular network composed of lymphatic capillaries, precollectors, and collecting lymphatic vessels. Lymphatic capillaries are blind-ended and highly permeable, because their basement membrane is discontinuous and they are not covered by pericytes or smooth-muscle cells, whereas collecting lymphatic vessels are surrounded by intact basement membranes and smooth muscle cells and contain intraluminal valves. The morphological changes associated with lymphatic remodeling and maturation also continue after birth.

2.5.2. Notch Signaling

Interacting with Prox1 and COUP-TFII, Notch signaling participates in a feedback loop that maintains the balance between venous-lymphatic and endothelial-cell fate. Recent studies also indicate that a loss of Notch activity increases the number of Prox1 lymphatic progenitors in the cardinal vein and lymph sacs of mice, whereas the lymphatic-specific deletion of Notch1 in mice leads to morphogenic abnormalities and excessive growth in lymphatic vessels. Low-rate laminar flow reduces Notch activity in cultured lymphatic endothelial cells, thereby inducing lymphatic sprouting. During lymphatic valve development, Notch signaling is required for the expression of integrin α9 and its ligand fibronectin,
EIIIA. Thus, Notch signaling regulates several aspects of lymphatic vessel formation.

2.5.3. Metabolic Pathways Regulating Lymphatic Function

Much attention has recently been given to the mechanisms of cellular metabolism in lymphatic vessels. Prox1 regulates the expression of CPT1A, a rate-controlling enzyme of fatty acid β-oxidation leading to the production of acetyl-CoA, which then interacts with the histone acetyltransferase p300 to acetylate histones in lymphangiogenic genes such as VEGFR3. Moreover, fibroblast growth factor receptor signaling controls the expression of c-Myc that then increases glycolysis by regulating hexokinase 2 expression. Similar to blood endothelial cells, ATP production through the glycolytic pathway enhances sprouting lymphangiogenesis.

2.5.4. Origins and Heterogeneity of Organ-specific Lymphatic Vasculature

Emerging evidence shows that lymphatic vessels have diverse cellular origins, as well as heterogeneity in several organs, reflecting organ-specific lymphatic functions in the adult. As described above, genetic tracing of the Prox1+ cell lineage supports the venous origin of lymphatic progenitors, postulated by Florence Sabine in 1902. However, recent lineage tracing studies using the endothelial-restricted Tie2-Cre mouse line indicate that dermal lymphatic vessels in the lumbar, but not cervical and thoracic, region are formed by Tie2-lineage negative, non-venous lymphatic progenitors.

While lymphatic vessels in the lumbar skin are derived from clusters of lymphatic endothelial cells, their cellular source remains unclear. Although mesenteric lymphatic vessels descend from vascular endothelial cells, some of them develop from the non-venous, hemogenic endothelium, based on lineage analyses of the hemogenic endothelial platelet derived growth factor (Pdgf)b-CreER2 and cKit-CreER2 lines. Importantly, the phosphatidylinositol (PI)-3 kinase signaling pathway acting downstream of VEGFR3 is selectively required for the development of the mesenteric lymphatic vasculature. Part of the cardiac lymphatic vasculature is also derived from a non-venous endothelial origin, and Islet1 progenitors in the pharyngeal core mesoderm have been identified as a new source of cardiac lymphatic vessels.

Schlemm’s canal (SC) is a unique endothelial vessel that encircles the cornea. It drains aqueous humor from the anterior chamber of the eye and delivers it into systemic circulation via aqueous and episcleral veins. Significantly, SC has an intermediate, blood-lymphatic endothelial cell type expressing lymphatic markers (Prox1, VEGFR3, and Integrin α9) with low or no expression of other lymphatic molecules (lymphatic vessel endothelial hyaluronan receptor (Lyve)1 and podoplanin). The development of SC in mice begins postnatally by sprouting from the choroidal vein. SC progenitors subsequently express Prox1 in response to aqueous humor outflow and acquire a lymphatic phenotype. While the formation and differentiation of SC is dependent on the VEGF-C/VEGFR3 signaling pathway, prior to the onset of aqueous humor outflow, the primitive SC venous endothelial cells do not express VEGFR3, but Tie2 and VEGFR2. In fact, the angiopoietin/Tie2 signaling pathway is essential for the formation and maintenance of SC, and a deficiency of this pathway leads to glaucoma by elevated intraocular pressure. SC integrity is impaired during aging, which can be rescued by treatment of a Tie2 agonistic antibody, indicating that the stimulation of Tie2 signaling could be a new treatment option for glaucoma.

3. MICROENVIRONMENT GOVERNS ENDOTHELIAL HETEROGENEITY

Every organ has its own unique vascular bed in order to exercise its versatile and distinctive function corresponding to dynamically changing systemic demands in our body. Thus, organ-specifically differentiated endothelial cells execute common or specific needs of the vasculature in different organs. The morphological differentiation of endothelial cells has been well-recognized among each of these three barrier forming types: continuous-, fenestrated-, or sinusoidal-endothelial layer types. Nonetheless, the functional properties and underlying molecular mechanisms of organ-mediated vascular bed heterogeneities have been just begun to be identified under microenvironmental cues.

3.1. Flow-Dependent Variables of Vasculature

Rheological blood flow-mediated biophysical forces, also termed ‘shear stress,’ are critical to maintaining the quiescent, inert endothelial cell’s environment. Laminar shear stress induces NO production from the e-NOS gene into the endothelium, followed by release to the mural or smooth muscle cells that contribute to vessel homeostasis, following its function as a regulator of vasomotor tone. In contrast, turbulence flow occurs in the aortic arch, an atherosclerotic prone area, which leads to induction of the inflamed endothelial marker genes involved the vascular cell adhesion molecule (VCAM)-1, intercellular cell adhesion molecule (ICAM)-1 and E-selectin. There are increasing reports on the molecular characterization of shear stress sensing machinery. Endothelial cells switch mechanical stimuli to biochemical signals through mechanotransducers, such as integrins, receptor tyrosine kinases, ion channels, and junctional membrane proteins involving the Claudin5, CD31, and Cadherin5. Primary cilia, or the recently identified PIEZO1/2, are thought to be the flow sensors of the endothelium. Each calcium/calcineurin, ERK, and PI3 kinase signaling pathway activates flow-dependent transcription factors NFAT, Krüppel-like factor (KLF) 2 and NF-E2-related factor (NRF) 2 to maintain the endothelial phenotypes and metabolic states. More recently, it has emerged that YAP and TAZ (Hippo signaling-mediated transcriptional activators) work as divides regulators, whether of laminar or turbulent flow. Accordingly, the atherosclerosis-protective effects of unidirectional laminar flow are, at least in part, mediated by the integrin dependent inhibition of YAP/TAZ and c-Jun N-terminal kinase (JNK) signaling.

3.2. BBB Forming Vasculature

Vascular endothelial cells that separate the blood stream from the brain interior are tightly closed and usually impermeable. This tight layer of brain vasculature is termed the blood–brain barrier (BBB). The BBB features specialized tight intercellular junctions, no fenestrae, and an extremely low rate of transcytosis. As a result of these tight barrier properties, the BBB plays an extremely critical role in maintaining the homeostasis of the central nervous system by protecting neurons from fluctuations in blood composition, and from toxic blood-borne substances. These barrier properties are found throughout the entire brain, including its capillaries, arterioles, and venules, as well as its larger arteries and veins. The important caveat is that once endothelial cells are isolated from the BBB and then cultured...
in dishes, these specific protective features of the BBB are easily lost. Thus, microenvironmental cues from communication with pericytes, astrocytes, and other soluble factors would seem to play a critical regulatory role in maintaining the endothelial specific phenotypes in brain. Remarkably, it has recently been reported that maintenance of BBB integrity requires several specific receptors, including G protein coupled receptor (GPCR) 124, in the endothelium. GPCR124 works as a coactivator of Wnt7a- and b-mediated canonical Wnt signaling. A Wnt receptor, Frizzled-4, and a low density lipoprotein (LDL) receptor related protein co-receptor to control vascular development of the central nervous system and to maintain the BBB. Attenuation of Wnt/Frizzled-4 signaling results in BBB defects, which are ameliorated by stabilizing β-catenin. These special BBB features are also an obstacle for the delivery of therapeutic drugs into the brain. Thus, in the pharmacological field, the understanding of BBB formation and identification of BBB penetrable molecules which could lead to drug delivery through the BBB are topics of deep discussion. For example, endothelial cells from the BBB express several transporters and receptors, including the type I glucose transporter GLUT1, and the adenosine triphosphate-binding cassette (ABC) transporter family. Since glycolysis metabolic consumption is the highest to occur in the central nervous system through the glucose from blood circulation, nutrient transfer via the BBB is essential for organisms. Moreover, docosahexaenoic acid (DHA) is an omega-3 fatty acid essential for normal brain growth and cognitive function. DHA cannot be de novo synthesized in the brain, but must be transported across the BBB. Interestingly, a major facilitator superfamily related gene, MFSD2a, has been identified as the major transporter of DHA, as well as a negative regulator of transcytosis. These recent findings, above, offer some hints for developing drug delivery tools combining nutrient transporters and receptors currently expressed in the BBB.

BBB dysfunction is a hallmark of several neuronal disorders including ischemic stroke, multiple sclerosis, cerebral malaria, brain tumors, and Alzheimer’s disease. Recent research into the pathology of cerebral cavernous malformations (CCMs) have found important insights into BBB maintenance and broken mechanisms. CCMs are common inherited and sporadic vascular malformations that cause strokes and seizures in younger individuals. Loss of function in any of the three genes, CCM1-CCM3, causes familial CCMs. Mechanistically, CCM2 binds MEK kinase; MEKK3 activates KLF2/4 signaling as the downstream target, leading to early CCM lesions, perturbing the quiescent endothelial phenotype. CCM3 suppresses UNC13B- and vesicle-associated membrane protein (VAMP) 3-dependent exocytosis of angiopeptin 2 in brain endothelial cells. CCM3 deficiency in endothelial cells augments the exocytosis and secretion of angiopeptin 2, which is associated with destabilized endothelial cell junctions, enlarged lumen formation and endothelial cell-pericyte dissociation. Although the BBB presents a significant hurdle to clinical progress, from bench to bedside, further elucidation of the cellular interplay of neurovascular units in health and disease, using epigenomics and proteomics, would facilitate this medicinal and pharmacological process.

4. ENDOTHELUM IN PATHOLOGY

4.1. Aberrant Activation of Tumor Endothelium

Many solid tumors require a supply of oxygen and nutrients to maintain their excessive proliferation. In order to support tumor growth, tumor cells secrete abundant angiogenic factors to induce endothelial cell migration, proliferation and sprouting from pre-existing blood vessels, which finally leads to the tumor angiogenesis. Angiogenic factors are secreted not only from the tumor itself, but also from the surrounding tissues composed of tumor associated-fibroblasts, -macrophages and infiltrated leukocytes. An imbalanced condition between the pro-angiogenic and anti-angiogenic factors is believed to initiate the hyper-activation of endothelial cells in tumor tissue. In the pathological condition, VEGF is the most effective pro-angiogenic factor for endothelial cells. Previous studies have demonstrated that NFAT transcription factor plays a crucial role in VEGF signaling by promoting endothelial activation-related gene expression, including DSCR-1, as a feedback modulator of the VEGF signal, and EGR3, as an early response factor for further activation. The DSCR-1 gene is encoded on chromosome 21, which is well recognized as correlated to the Down syndrome trisomy. Unexpectedly, many epidemiological studies reveal that Down syndrome patients with higher DSCR-1 level due to the trisomic expression also show a significantly lower rate of solid tumors. Consistent with these findings, we have previously reported that a reason for reduced cancer risks is an increased expression of DSCR-1, which can suppress tumor angiogenesis and related pathological vascular inflammation. In addition to the VEGF-NFAT/DSCR-1 signaling axis, the tumor microenvironment can strongly influence a tumor endothelial cell’s reaction to hypoxia, low pH, and interstitial fluid pressure. Such a variable cancer microenvironment might lead to the formation of unique and disorganized vasculature exhibiting significantly different morphologies in comparison to normal blood vessels. Indeed, several reports on tumor endothelium indicate higher permeability, lower attachment (or even non-attachment) to the basement membrane, less coverage of pericytes, more dilated and tortuous circulation, and missing exclusive arterioles- or venules-identities. Abnormal vessel-branching patterns are also frequently detected in tumors, which are believed to be due to the inappropriate cell polarity of endothelial cells.

4.2. Genetic and Epigenetic Control of Tumor Endothelium

Many specific aspects of tumor vessels are likely to be based on the different gene expression patterns of the tumor endothelium compared to inert and stable endothelial cells. Such unique tumor endothelium expressions would be affected according to cancer cell type, their location, as well as the tumor roots, whether from primary or metastasized cells. For example, lung microvascular endothelium respond with high sensitivity to VEGF secreted from the primary tumor, which results in increased angiopeptin 2 expression as a VEGF-NFAT downstream target. Such premetastatic niche formation leads to further endothelial pathological activation before the tumor cells arrive. Epigenetic regulation is believed to play a key role in causing endothelial cells’ heterogeneity in tumors. When we consider that tumor vessels invade different organs, their original site-specific epigenetic marks will be preserved, even though endothelial cells are
There are several reports that consider the possibility that tumor endothelial cells have aneuploidy and multiple centrosomes. However, this is still under debate. Tumors may co-opt existing blood vessels, resulting in chromosomal abnormalities in the endothelium, reflecting aneuploidy from the tumor cells. A recent study reported that certain populations of glioblastoma mimic endothelial cells of partially formed tumor blood vessels. It is not clear whether bone marrow-derived endothelial progenitor or tumor cells contribute to neovascularization in tumor endothelial cells. What we can conclude at this point is that tumor vessels obtain unique characteristics to indicate the existence of endothelial cell heterogeneity, even though there are several controversial ideas about the genetic and epigenetic regulation and origins of this heterogeneity.

4.3. Anti-angiogenic Therapy

Tumor angiogenesis plays a key role not only in tumor growth at a primary site, but also in metastasis to distant organs, thought to be the main cause of cancer malignancy. Around half a century ago, Folkman, a famous surgeon and medical scientist, proposed the concept of a novel anti-cancer therapy targeting tumor angiogenesis, called anti-angiogenic therapy. To date, many anti-angiogenic drugs have been developed and investigated regarding their therapeutic effect on various tumor types. As reported above, VEGF have been recognized as the most effective endogenous angiogenic factor under both physiological and pathological conditions. In fact, VEGF deficient mice show embryonic lethality at an early developmental stage due to a lack of endothelial cell generation. Clinical databases reveal that tumor tissues secreting high levels of VEGF accelerate neovascularization into the tumor, effectively feeding it, which results in a poor tumor-free and/or metastasize-free survival rate. Bevacizumab, a humanized monoclonal antibody for targeting VEGF, is now preferentially applied for the clinical treatment of colorectal cancer, non-small cell lung cancer, glioblastoma multiforme, and ovarian cancer. Although such anti-VEGF treatment is a unique method for targeting the tumor endothelium, several side effects have been reported, such as hypertension, a heightened risk of bleeding, and bowel perforation. Moreover, a current limitation for this anti-angiogenic therapy is that there is no significant elongation of overall survival in certain types of cancer patients. In order to eliminate or improve these current difficulties, a promising course of study would to deeply understand endothelial cell heterogeneity in tumors.

5. CONCLUSION

It is widely appreciated that endothelial cells are heterogeneous in structure and function (Fig. 2). Recent sophisticated studies using single cell analysis and lineage tracing techniques have provided insights into the importance of genetic and epigenetic determinants in mediating phenotypic differences between endothelial cells. Indeed, most existing data support a dual role of both lineage determination and microenvironmental cues in mediating organ/tissue-specific phenotypes. Endothelial cells are initially created within a transcriptional factors circuit. Prox1 positive cells are further converted to lymphatic endothelial cells from the fate of a venous cell. Epigenetic modifications of these regulatory promoter regions of the endothelial committed transcription factors are quickly changed via VEGF treatment. It is still unknown, and remains to be determined, how histone modification patterns are controlled, whether via cell-cell communication or in a cell-autonomous manner. Once fully differentiated endothelial cells reach their tissue residence, their ultimate phenotype is largely controlled by signals from the microenvironment. Finally, from a pathological perspective, we focused on the tumor endothelium, which is likely to shown unique phenotypes, as mediated by a combination of genetic and microenvironmental factors. The most recent advanced genome-wide screening approach would assist in uncovering the mechanisms of different organ/tissue mediated vascular bed heterogeneity in health and disease, which would in turn be useful as a foundation for novel diagnostic or tissue specific angiogenesis mediated therapies.
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