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Endothelial cells: a key player in angiogenesis and lymphangiogenesis

Abstract

Endothelial cells (EC) constitute the linings of the entire vascular system including blood and lymphatic vessels. The vascular and lymphatic ECs are essential for angiogenesis and lymphangiogenesis as versatile and multifunctional organs. The EC heterogeneity in different microenvironment determines the complexity and diversity of its functions in the regulation of angiogenic and lymphangiogenic processes. In fact, many molecular regulators and signaling pathways are involved in these processes. In addition to VEGF, other angiogenic factors including FGF, HGF, TSP-1, endostatin and phospholipids such as lysophosphatidic acid all act on the ECs directly or indirectly by inducing the expression of angiogenic factors. Furthermore, it should be noted that the dynamic balance between proangiogenic and antiangiogenic factors determines the angiogenic switch and process through such signaling pathways as notch and ephrin B2, in which angiogenic receptors may serve as a key axis. Up to date, many questions still remain to be answered. However, the answers to these questions will aid in understanding the cellular and molecular mechanisms of angiogenesis and lymphangiogenesis. Elucidating these mechanisms will aid in finding novel therapeutic strategies against cancer, ischemic cardiovascular diseases, cardiovascular complications of diabetes, and obesity.

Keywords: endothelial cells, angiogenesis, lymphangiogenesis, signaling, phospholipids, growth factors and inhibitors, tumor microenvironment

Volume 1 Issue 2 - 2014

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Received: October 07, 2014 | **Published:** November 24, 2014

Introduction

Blood and lymphatic vessels do more than passively carry blood or lyphatic fluid around the body. They actually play more functional roles including tissue homeostasis via the supply of gases, fluid, nutrition, and signaling molecules to the tissues, with the capillaries as the actual sites of exchange, regulation of acute and chronic inflammation. These vessels are filled with blood and other molecules, and form a complex system composed of moving mesenchymal elements (blood and lymphatic fluids), the vascular wall and endothelium. Whereas lymphatic vessels are complementary to cardiovascular system, which are lined by endothelium, and have a thin layer of smooth muscles, and adventitia that bind the lymph vessels to the surrounding tissue. The endothelium constitutes of a layer of endothelial cells (ECs) that together functions as a membrane lining blood vessels, the lymphatic system, and parts of the nervous or other systems. In fact, the capillary wall consists of a single layer of ECs, which are specialized squamous epithelial cells. In healthy tissues, ECs are quiescent and rarely migrate or divide. Most blood vessels also remain quiescent, and angiogenesis occurs only in the menstrual cycle in the women and in the placenta during pregnancy. ECs also remain remarkable capability of dividing rapidly in response to a physiological stimulus, including hypoxia for blood vessels and inflammation for lymphatic vessels.[1] ECs interact with other vascular cells such as pericytes, smooth muscle cells and circulating cells or platelets in the blood and lymphatic fluids. Moreover, they mediate important molecular and metabolic exchanges between blood and other tissues, and involve in communication with other cell populations in the surrounding target organs via a paracrine signaling.[2] In the brain the tight junctions between ECs form a highly functional blood-brain barrier, whereas the loose somewhat disorganized junctions of post capillary venular endothelium function for solute and leukocyte trafficking during inflammation. ECs are highly active in metabolism. They are essential to physiological and tumor angiogenesis, and lymphangiogenesis as well as both innate and adaptive immunity.[3,4] The vascular ECs are involved not only in health but also most disease states. Endothelial dysfunction may contribute to the initiation and/or progression of atherosclerosis,[5] and this may be associated with angiogenesis, ischemic cardiovascular diseases and vascular pathology in the diabetes.[6] The heterogeneous phenotypes of ECs existing in the body are thus an important determinant in health and disease. The endothelium consisting of ECs may be considered as a physiologically relevant organ, and ECs as a key player in angiogenesis and lymphangiogenesis demonstrate potential clinical significance in diseases associated with vascular pathology.

An update on heterogeneity of endothelial cells

EC heterogeneity is an important area in vascular biology, which was elegantly reviewed.[7] The heterogeneity of structure, function and gene expression exists in the vascular and lymphatic ECs from different tissues and organs. The endothelium may be considered as a consortium of distinct smaller organs located within blood vessels that are uniquely adapted to meet the demands of the specific underlying tissues from an angle of genomics and proteomics,[8] which should be similar in lymphatic vessels. ECs sense and respond to their environmental signals to maintain their functional heterogeneity along with a temporal and spatial phenotypic change. The site-specific properties of ECs depend on extracellular signals integration and transmit into the nucleus to regulate related gene transcription for adapting this change. Due to the change of cellular microenvironment, it is important to consider this phenotypic change when we interpret the *in vitro* study results whether using two dimensional or three

dimensional cultured ECs. EC heterogeneity also occurs during angiogenic sprouting, where tip cells lead the way followed by morphologically and functionally distinct stalk cells. During this process Notch/VEGFR-signaling regulates differential dynamics of VE-cadherin junctions and drives functional EC rearrangements.[9] It is reasonable to speculate that certain ECs might inherently have genetic traits of the tip cells, which can be induced to show the tip cell phenotype via an epigenetic regulation of defined gene expression under specific temporal and spatial conditions. The endothelial tip cell may coordinate with stalk cell for the regulation of angiogenesis and lymphangiogenesis.

The tumor endothelium also demonstrates the phenotypic heterogeneity, which may be coupled with cancer cell heterogeneity and heterogeneity of the tumor microenvironment. In fact, ECs lining the tumor vessels are structurally and functional abnormal. They are highly proliferative and can grow on top of one another and project into the vessel lumen.[10] The phospholipids, phosphatidylethanolamine and phosphatidylserine are shown to be redistributed from the inner to the outer membrane leaflet of the tumor-associated ECs (TAECs).[11] The increased fenestrations and widened intercellular junctions or gaps significantly increase the permeability in the tumor endothelium that is covered by morphologically abnormal pericytes.[12] TAECs are exposed to a unique microenvironment along with cancer and stromal cells including tumor associated macrophages[13] and hypoxia.[14] The cells in the tumor microenvironment may release soluble growth stimulators and inhibitors including VEGF, FGF-2, thromobspondin-1 (TSP-1) and endostatin as well as other cytokines. These complex factors cooperate to regulate the behavior of TAECs to promote angiogenesis. The tumor vessels may thus be more dilated and tortuous, show excessive branching morphogenesis, form arteriovenous shunts, and lack the normal artery–capillary–vein hierarchy.[15] Unlike what researchers originally thought, TAECs are not genetically stable but unstable, show different gene expression profile and respond differently to growth factors compared to normal ECs.[16] For example, ECs can be derived from a population of glioblastoma stem-like cells and intriguingly show a neoplastic origin in glioblastoma.[17] This kind of tumor cell differentiation into ECs might occur in other types of cancers including breast and lung cancer as well as malignant melanoma through trans-differentiation. In addition, tumors may "educate" ECs in the existing blood vessels and alter the TAEC phenotype via an epigenetic mechanism. The abnormal TAECs, in turn, release a variety of factors and cytokines to affect the tumor growth[18–20] and possibly metastasis. Cancer cells, stromal vascular cells including ECs and immune cells in the tumor microenvironment may cooperate to promote angiogenesis and lymphangiogenesis, leading to malignant progression.

Endothelial cells in angiogenesis and lymphangiogenesis

Angiogenesis refers to a physiological or pathological process from which new blood vessels form from pre-existing vessels. Angiogenesis plays an essential role in growth and development, in wound healing as well as in ischemic cardiovascular disease, cardiovascular complications of diabetes and malignant tumors. The angiogenic processes involve sprouting, branching, splitting, and differential growth of vessels from the primary plexus or existing vessel into a circulation system.[21,22] A *dynamic* balance between pro-angiogenic and anti-angiogenic factors is essential for the regulation of angiogenesis including tumor angiogenesis (Figure 1).[23,24] Most researchers focus on the soluble growth inhibitors and stimulators such as the first discovered endogenous antiangiogenic factor TSP-1 and an important vascular endothelial cell growth factor (VEGF). Angiogenic factors including proangiogenic and antiangiogenic factors indeed play a crucial role in the regulation of angiogenesis. VEGF has been shown to up regulate Notch1 and DLL4 expression via a PI3kinase/Akt rather than a MAPK/Erk signaling pathway in human iliac and femoral artery ECs, which suggests the involvement of Akt signaling in arteriogenesis and angiogenesis.[25] However, using aortic EC and hindlimb ischemia model in a specific synectin gene deficient mice and Zebra fish model, Ren et al. have shown that MAPK/Erk signaling in the EC is an important player in the regulation of arteriogenesis and angiogenesis in a synectin deficient mouse or zebrafish model,[26] in which MAPK/Erk signaling may be more important in EC differentiation via regulation of angiogenic and arteriogenic gene transcription. Akt-Erk signaling crosstalk determines the angiogenic differentiation while Erk signaling defect may be responsible for impaired arteriogenesis.[26–28] These seemingly contradictory results may be explained by a phenotype change in different microenvironments[7] and also implicate the EC plasticity and plasticity of postnatal angiogenesis and arteriogenesis.

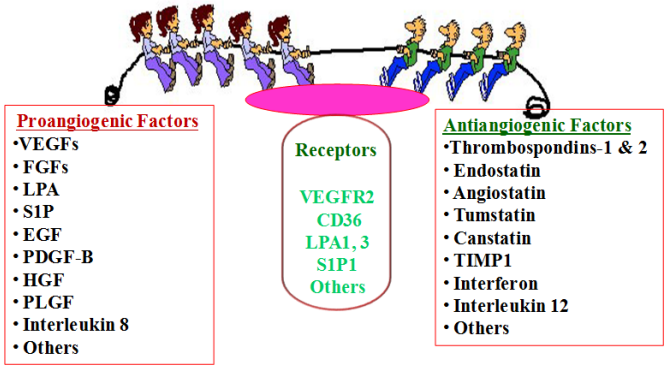

Figure 1 Molecular Regulators in Angiogenesis

Many angiogenic factors are involved in the regulation of angiogenesis. A dynamic balance exists between proangiogenic and antiangiogenic factors. The angiogenic receptor may serve as a pivotal axis to regulate the functions of its ligand accordingly, and the dominant signaling determines the angiogenic switch. VEGFs: vascular endothelial growth factors; FGFs: fibroblast growth factors; LPA: lysophosphatidic acid; S1P: Sphingosine-1-phosphate; EGF: epidermal growth factors; LPA: platelet-derived growth factor B; HGF: hepatocyte growth factor; PLGF: placental growth factor; TIMP1: tissue inhibitor of metalloproteinases-1.

Regulators of angiogenesis and lymphangiogenesis beyond VEGF

VEGF originally known as vascular permeability factor due to its function in tumor vasculature[29] is a well studied angiogenic molecule in angiogenesis, arteriogenesis and lymphangiogenesis, and extensively reviewed. However, many other growth factors are also known to be involved in the regulation of angiogenesis.[3] Among them fibroblast growth factor (FGF) stimulates EC proliferation and differentiation, Ang1 and Ang2 as well as plasminogen activator inhibitor-1 involve the stabilization of blood vessels, PDGF BB-homodimer and PDGFR regulate vascular remodeling, TGF-β, endoglin and TGF-β receptors promote extracellular matrix production, integrins avβ3 and a5β1 are able to bind matrix macromolecules and proteinases, VE-cadherin regulates endothelial junction, and ephrinB2 determines the identity of blood vessel in addition to regulation of angiogenesis.[30–32] Notch and ephrin-B2/EphB4 pathways in the endothelium are critical for a balanced arteriovenous development during blood vessel formation.[33] The expression of PDGF-B is only seen at sites where

active angiogenesis occurs.[34,35] The cytokines and chemokines including IL-1, IL-8, IL-18, chemokine (C-X-C motif) ligand (CXCL) 3, and CXCL12 have been shown to exert proangiogenic and lymphangiogenic activities.[3] VEGF-C expression can be induced in response to various pro-inflammatory cytokines including TNF-α and IL-1β probably via activation of the NF-κB signaling pathway to promote lymphangiogenesis.[36,37] Macrophages also promote VEGF-C and VEGF-D expression to induce the formation of lymphatic vessels via VEGF-mediated macrophage recruitment.[38]

Phospholipid, angiogenesis and lymphangiogenesis

Recently, phospholipids including sphingosine-1-phosphate (S1P) and lysophosphatidic acid (LPA) are emerging as an interesting player in angiogenesis and lymphangiogenesis via acting on ECs. LPA is a bioactive lipid signaling mediator to induce cell proliferation, migration, differentiation and cytokine production through with its G-protein coupled receptor (GPCR). LPA and autotoxin-LPA signaling axis is involved in both angiogenesis and lymphangiogenesis.[39–41] Autotoxin is a key enzyme to produce LPA while LPA not only stimulates angiogenesis by regulating Gi/NF-kB-dependent angiogenic factor expression such as VEGF[42] but also mediates macrophage migration inhibitory factor-stimulated angiogenesis.[42,43] Moreover, this lipid signaling mediator is able to upregulate angiogenic factors by multipotent adipose-derived stromal cells (ASC), synergistically improving the proangiogenic effects of ASC in ischemia. This could be used as a novel strategy for enhancing cell-based strategies for therapeutic angiogenesis.[44] LPA has also been reported to induce proliferation, survival, migration, and tube formation, and promotes lymphangiogenesis *in vitro* in human dermal lymphatic EC (LECs), which may be associated with LPA-mediated IL-8 expression via activation of the NF-kappa B pathway in the LECs.[45] In human umbilical vein ECs (HUVECs), LPA exposure mediated VEGF-C expression through IL-1beta,[46,47] the upregulation of VEGF-C was LPA(1)- and LPA(3)-dependent and required cyclooxygenase-2 (COX-2), endothelial growth factor receptor (EGFR) transactivation and activation of nuclear factor kappa B (NF-kappa B).[48]

Another important phospholipid S1P acts as an extracellular mediator through binding to 5 highly specific S1P receptors, S1P (1-5). S1P and its GPCR, sphingosine-1-phosphate receptor-1 (S1P1), have been shown to regulate EC proliferation and vascular morphogenesis.[49–51] S1P1 is originally found to be expressed in ECs, but actually it is expressed widely in cultured cells, including both endothelial and mesenchymal cells and other nonvascular cells in adult tissues, and S1P and its receptor are important in EC function, angiogenesis and vascular development.[51–55] EC-specific knockout of *s1p1* leads to the mural sheath defects due to impaired mural vessel coverage resulting from the loss of S1P1 activity in the endothelium.[56] Importantly, S1P1 involves in vascular plexus stabilization and sprouting angiogenesis. EC-specific knockout of S1P1 results in an excessive sprouting phenotype.[51,57] S1P as a potent bioactive lipid mediates lymphangiogenesis through promoting migration, capillary-like tube formation, and intracellular $Ca^{(2+)}$ mobilization in human lymphatic endothelial cells (LECs). Furthermore, *in vivo* Matrigel plug assay shows the increased outgrowth of new lymphatic vessels in response to S1P. S1P-mediated lymphangiogenesis is achieved via stimulating S1P1/G(i)/phospholipase C/$Ca^{(2+)}$ signaling pathways.[58] Sphingosine kinase-1 (SK1) has recently shown to promote paracrine angiogenesis and lymphangiogenesis via increasing SIP levels,[59] subsequently promoting breast cancer progression.[60,61] SK1 over expression in HEK cells or its downregulation in glioma or breast cancer cells modulates extracellular S1P levels accordingly, and subsequently promotes or inhibits both migration and tube formation in co-cultured vascular or LECs. Whereas the angiogenic and lymphangiogenic switch triggered by these cancer cells is not able to be blocked via downregulating VEGF and VEGF-C. Secretion of S1P produced inside cells by SK1 in turn signals through SIP receptors in autocrine, paracrine, and/or endocrine manners and induces an "inside-out" signaling, suggesting the importance of the transport of S1P out of cancer cells and stimulating angiogenesis and lymphangiogenesis in the tumor microenvironment.[62] These studies implicate that LPA, S1P and their receptors could be new therapeutic targets against ischemic cardiovascular diseases, inflammatory diseases and vascular and lymphatic metastasis in malignant tumors.

Angiostatic factors thrombospondins and endostatin in angiogenesis

Angiostatic or antiangiogenic factors are important players for the control of excessive growth of new vessels through regulating EC phenotype and fate, among which thrombospondins TSP-1 and TSP-2 as well as endostatin are important members.[23,63–79] TSP-1 and its family member TSP-2 are actively involved in the regulation of angiogenesis, especially in tumor angiogenesis, via interaction with CD36 receptor on microvascular ECs[23,70,80] as an endogenous inhibitor of angiogenesis and tumor growth. TSP-1 and TSP-2 also regulate angiogenesis by a mechanism through which TSPs interact with another receptor CD47 to inhibit nitric oxide signaling.[81,82] The inhibition of VEGF receptor-2 signaling by TSP-1 can be realized by disrupting its association with CD47. Furthermore, the functional domain of TSP-1 or 3TSRs combined with TRAIL has been shown to inhibit angiogenesis and tumor progression by inducing EC apoptosis via upregulation of death receptor DR4/DR5 expression, stimulation of JNK but inhibition of Akt signaling.[65] The 3TSRs have been shown to inhibit VEGF signaling in human dermal microvascular ECs via reducing VEGFR2 phosphorylation at tyrosine-1175 in a dose-dependent fashion.[74] Intriguingly, TSP-1 was suggested to maintain the Syk-CD36-VEGFR-2 complex formation in tetraspanin-enriched microdomains for the regulation of EC function and angiogenesis.[72] Moreover, TSP-1 binding to CD36 may recruit Src homology 2 domain–containing protein tyrosine phosphatase SHP-1 to CD36-VEGFR2 complex in microvascular ECs for attenuating VEGFR2 signaling[83] and this could be associated with interaction of the functional domain of TSP-1 or TSR interaction with CLESH domain of CD36.[84] Endostatin, a naturally-occurring, 20-kDa C-terminal fragment derived from type XVIII collagen, is another important endogenous angiogenesis inhibitor similar to TSP-1, which was discovered by Judah Folkman group and showed to inhibit angiogenesis via inhibition of EC proliferation and migration, and stabilization of newly formed endothelial tubes[66,69] as well as induce EC apoptosis.[63,64,85]

A brief update on angiogenic receptors in angiogenesis and lymphangiogenesis

The receptor tyrosine kinase or angiogenic receptors on the ECs may serve as a pivotal axis to regulate the functions of corresponding ligand. They are important in the control of angiogenic switch. For example, VEGF signaling properties are regulated by the tight control of intracellular VEGF receptor-2 (VEGFR-2) localization, and VEGFR-2 is critical for upregulating VEGF signaling[86,87] while CD36 signaling may function as a critical negative regulator in

angiogenesis.[23,65,88] VEGFR-2 is expressed on both blood and lymphatic vessels with high expression on endothelial tip cells.[89] However, VEGFR-1 is expressed only on blood vessels and can trap VEGF-A to prevent excess signaling via VEGFR-2 during embryogenesis. In the adult the function of VEGFR-1 remains more elusive.[90–92] Recently, ASK1-interacting protein-1, a signaling scaffold protein highly expressed in the vascular endothelium, was shown to mediate VEGFR-3-dependent angiogenic and lymphangiogenic signaling to regulate VEGFR-3-dependent angiogenesis and lymphangiogenesis.[93]

Notch signaling mediates angiogenic signaling in endothelial cells:

Notch signaling in the endothelium is essential for the regulation of angiogenesis in developing mice, zebrafish embryos and tumor models.[94–97] The activation of DLL4–Notch by VEGFR-2 and the repression of VEGFR-2 expression downstream of Notch activation are also associated with endothelial sprouting and angiogenic processes.[98,99] Notch pathway may modulate VEGF signaling output through VEGF interaction with Delta-Notch, subsequently impacting on angiogenic processes.[100–102] Synaptojanin-2 binding protein (SYNJ2BP, also known as ARIP2) was recently identified as a novel inhibitor of tip cell formation that is essential for the endothelial sprouting, which inhibited EC migration, proliferation, and VEGF-induced angiogenesis via promoting Delta-Notch signaling.[103] Notch-VEGF signaling interactions in regulation of angiogenesis not only occur in tumor microenvironment but also in cardiovascular ischemic conditions. Intriguingly, angiogenesis in Notch-deficient vessels still occur in the absence of VEGF-A or VEGFR2 activity. Notch inhibition actually stimulates angiogenesis independently of VEGFR2 in that substantial level of endothelial notch signaling is maintained without VEGFR2 function but with a Notch-dependent VEGFR3 upregulation.[104] VEGFR3 has been shown to be regulated at post-transcriptional level by Notch, and VEGFR3 activity is proangiogenic in ECs with low or no Notch signaling activity,[104] demonstrating a crucial role for VEGFR3 in the regulation of angiogenic sprouting in the ECs with low Notch signaling activity and implicating its role in lymphangiogenesis. Notch signaling may actually determine the fates and behavior of the ECs via controlling VEGF pathway for developing a physiologically normal vascular sprouting.[94,104,105] Signaling crosstalk between Notch and other pathways can be occurred. As it has been shown recently, Nrarp, a NOTCH-regulated ankyrin repeat protein, coordinates endothelial Notch and Wnt signaling to control vessel density in angiogenesis.[106]

Role of endothelial ephrin-B2 in angiogenesis and lymphangiogenesis

EC-specific ephrin-B2 inactivation significantly reduces angiogenesis during vascular development, and the size and complexity of the endothelial network, tip cell number, sprout length and EC proliferation in the retinal vasculature.[107] Mechanistically, ephrin-B2 regulates the internalization and signaling activity of VEGFR2 to stimulate angiogenesis during development and tumor progression. The ephrin-B2 reverse signaling via PDZ interactions regulates vessel sprouting by promoting endothelial tip cell filopodial extension during developmental and tumor angiogenesis.[108] Furthermore, internalization of VEGFR3 and its signaling activity are dependent on ephrin-B2 activity, which is consistent to a previously reported role of ephrin-B2 reverse signaling in lymphangiogenesis.[107,109] In addition, sprouting EC in the retina have high rates of VEGF uptake, VEGF receptor endocytosis and turnover, which is associated with atypical protein kinase C activity. This kinase can phosphorylate a clathrin-associated sorting protein or Dab2 and regulates VEGF receptor endocytosis and downstream signal transduction via interaction with ephrin-B2 and the cell polarity regulator PAR-3.[110] It is reasonably speculate that the ephrin-B2 activity might integrate VEGF signaling via VEGFR-2 and/or VEGF-R3 and anti-angiogenic signaling via TSP-1-CD36 signaling axis to ensure the formation of new vasculature and arterial remodeling, in which tip cells lead the way to vascular morphogenesis. Intriguingly, VEGFR-2 and VEGFR-3 signaling drives not only angiogenesis but also the inflammatory lymphangiogenesis.[111–113] VEGF-C and VEGF-D can bind VEGFR-3 expressed on lymphatic ECs whereas VEGF-C also induces VEGFR-2 signaling after proteolytic cleavage.[114–117] Interestingly, a PDZ domain-containing scaffold protein known as synectin has been shown to regulate not only angiogenesis and arteriogenesis[26,118] but also genetically interacts with Vegfr3 and neuropilin-2a to regulate lymphangiogenesis.[119]

Focus on endothelial cells in lymphangiogenesis

The lymphatic vasculature is essential for maintaining tissue fluid homeostasis by draining protein-rich fluid from the interstitial space back to the general blood circulation. Its malfunctioning results in lymphedema formation and compromises immune function.[120] The lymphatic vessel network consists of lymphatic capillaries, precollecting vessels, collecting vessels, and the thoracic duct. Lymphatic endothelial hyaluronan receptor-1 (LYVE-1) and VGFR-3 are first expressed at sites where lymphangiogenesis will take place in the cardinal vein around 8.5dpc during early lymphatic vascular development.[121,122] During lymph- angiogenesis VEGF-C initiates VEGFR-3 signaling and stimulates proliferation, migration, and survival of LECs.[115,123] The venous ECs can be trans-differentiated toward the lymphatic endothelial phenotype, which is a critical step for lymphatic vascular development. LEC precursors from the cardinal vein migrate outwards and form the lymph sac, from which LECs can be generated and lymphatic vessels start to develop throughout the body, and connect with each other to form lymphatic vessels.[124] The trans-differentiated LECs subsequently undergo separation of blood and lymphatic vasculature, sprouting, remodeling and maturation of lymphatic vessels, developing into a vascular system with a highly branched network of capillaries and ducts present in most organs with the exception of the central nervous system and a vascular tissues.[120] Furthermore, VEGF-C/VEGR3 signaling is essential for the growth and survival of LECs.[116] In response to VEGF-C, LECs undergo sprouting morphogenesis via activating the VEGFR-3 and its nonsignaling transmembrane co-receptor neuropilin 2 (Nrp2).[125,126] Intriguingly, different from what was reported by Veikkola et al.,[127] postnatal development of lymphatic vessels in organs other than skin is Vegf-c/Vegfr-3 independent, and internal lymphatic capillaries can regrow in mice with mutated Vegfr3 or upon Vegf-c depletion,[128] indicating the plasticity of lymphatic vascular development. Notch/Dll4 signaling that is important in vascular development and specification is another signaling pathway involved in guiding LECs along arteries in the zebrafish.[129] This implicates that Notch signaling may bridge the crosstalk between arteries and lymphatic vessels. This is supported by the fact that loss of the arterial regulator synectin[26,118] may compromise the development of zebrafish lymphatics.[119]

The prospero homeodomain transcription factor (Prox1 or Pros1a) is also essential for the maintenance of lymphatic cell fate as a master control gene for lymphangiogenesis in mammals though the Prox1b activity has been shown not essential for embryonic lymphatic development in the zebrafish.[130] Conditional loss of Prox1 function in

the adult can induce LECs to revert to a blood vascular phenotype.[131] In addition, transcription factors including SOX18, COUP-TFII, FOXC2, TBX1 and NFATc-1d are all involved in the regulation of lymphangiogenesis.[131] Whereas SOX18 as a critical switch for lymphangiogenesis in the mouse embryo is shown to be important in tumor-induced lymphangiogenesis and melanoma metastasis.[132] Coxam et al. recently reported that a zebrafish mutant, lymphatic and cardiac defects 1 (lyc1), demonstrates the reduced lymphatic vessel development, which is attributable to a mutation in polycystic kidney disease 1a (Pkd1), the most frequently mutated gene in autosomal dominant polycystic kidney disease. The loss of Pkd1 in mice failed to show morphogenesis of the subcutaneous lymphatic network with defective LEC phenotype including defective polarity, elongation, and adherence junctions.[133]

VEGF is known as an important player in angiogenesis. However, in addition to its proangiogenic effect, VEGF induces tumor lymphangiogenesis and metastasis to regional and distant lymph nodes.[134,135] It can also indirectly stimulate lymphangiogenesis via recruitment of bone marrow-derived macrophages (BDMs) in a mouse model of inflammatory-induced corneal neovascularization. These BDMs in turn secrete angiogenic and lymphangiogenic factors to stimulate both angiogenesis and lymphangiogenesis.[38] Whereas antiangiogenic factor TSP-1 signaling via CD36 on monocytic cells can function as an endogenous inhibitor of lymphangiogenesis through downregulation of VEGF-C expression[136] and subsequent altered phenotype of LECs. On the contrary, LPA increases VEGF-C expression in ECs for promoting angiogenesis and lymph- angiogenesis.[47] These studies implicate the importance of the communication between LECs, vascular ECs, BDMs and macrophages in lymphangiogenesis. Other factors especially occurring in tumor microenvironment involve lymphangiogenic processes include VEGF-D, FGF-2, ephrin-B2, hepatocyte growth factor, Angiopoietin-1, -2, insulin-like growth factor-1, 2, platelet-derived growth factor PDGF-BB, growth hormone, endothelin-1, adrenomedulin, neutrin-4, and fibronectin.[131] These factors together with the relevant factors I have discussed and their related receptors constitute the potential molecule targets in lymphangiogenesis.

Summery and prospective: focus on tumor microenvironment

Up to date much still remains to be understood in intracellular endothelial signaling events that result in the appearance of microvascular, lymphatic, venous or arterial phenotypes. This understanding may reduce the bench-to-bed gap in EC biomedicine. ECs are known to be essential in angiogenesis and lymphangiogenesis. Take tumor microenvironment as an example, theoretically, targeting neovessel formation by blocking tumor derived angiogenic signals or their receptors on the surface of TAEC or targeting preexisting and established tumor vessels should provide optimal therapeutic strategies. However, current antiangiogenic therapies are not as effective as what were expected. The discovery and validation of novel tumor EC-specific markers and unique molecular signature in TAECs as well as highly effective nano-carriers could facilitate the development of molecular imaging for diagnosis and advance antiangiogenic therapy. In this regard, the phenotypic heterogeneity of ECs in the tumor microenvironment should be considered. However, we need to be cautious that targeting tumor endothelium and associated blood vessels also potentially interferes with normal angiogenic processes, including wound healing and pregnancy. Therefore, understanding the distinct feature of TAECs and discovering the unique molecular signatures in these ECs could effectively and specifically control the tumor angiogenesis and lymphangiogenesis. Many questions still remain to be answered:

i. How is a developmental proangiogenic and lymphangiogenic program reinitiated in the adult under pathological conditions?

ii. What signaling pathway is crucial for regulating the secretion of the "angiocrine" factors that may be critical to improving the treatment of malignant tumors?

iii. How can we find the key signaling axis in heterogenic ECs exposed to growth factors, growth inhibitors and a variety of cytokines in different microenvironment?

iv. How do genetic and epigenetic interactions reprograms EC for phenotypic change and differentiation via transcriptional regulation of angiogenic genes?

v. How do other cells such as immune cells, tumor associated macrophages, or pericytes and platelets communicate and interact with ECs or LECs to mediate physiological and pathological angiogenesis or lymphangiogenesis?

vi. Finding answers to these questions will not only aid in understanding how to target malignant tumors but discover potential novel therapeutic strategies against ischemic cardiovascular diseases and cardiovascular complications of diabetes as well as obesity, a 'chronic inflammatory disease" associated with angiogenesis and lymphangiogenesis.

Acknowledgements

None.

Conflict of interest

The author declares no conflict of interest.

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