[Frontiers in Bioscience 16, 1413-1427, January 1, 2011]

Vasculogenensis, angiogenesis and special features of tumor blood vessels

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1. ABSTRACT

The circulatory system comprises a tubular network of blood vessels including arterioles, capillaries, venules, and lymphatic vessels. This circulatory system is essential for the embryonic development and maintenance of all tissues, which requires the transportation of oxygen, carbon dioxide, and nutrition. The system regulates the movement of fluid into and out of organs with high level of efficiency. "Tumor angiogenesis" describes the rapid growth of certain components of the circulatory system in an abnormal fashion that is both heterogeneous and dysregulated. The aberrant flow between abnormal tumor vessels and normal vessels poses a high risk for seeding of potentially metastatic cancer cells. Moreover, it has also been reported that premetastatic distant organ vessels already undergo specific changes due to the presence of a remote primary tumor. Therapeutic strategies aimed at targeting tumor vessels have the potential to suppress tumor growth, and also influence the effects of tumor-derived cytokines and circulating tumor cells. Furthermore, focusing on vessels in a premetastatic organ that have responded to a primary tumor may be one possibility for reducing metastatic risk.

2. INTRODUCTION

The circulatory closed system is conserved amongst all vertebrates, and certain key molecular regulators (such as the vascular endothelial growth factor [VEGF]-VEGF receptor [VEGFR] system axis) are conserved between fish and human beings. In embryogenesis and physiological phase, many molecules regulate normal vasculogenesis and angiogenesis. Solid tumors as well as normal tissues require blood vessels for oxygen and nutrition. However, tumor vessels have a number of special features, which distinguish them from normal vessels. Mechanism for tumor vessels might be partially in common with developmental blood vessels, but could be different in their mechanisms and architectures. Here, we firstly discuss the physiology of normal angiogenesis before presenting a discussion on tumor angiogenesis.

3. PHYSIOLOGICAL BLOOD VESSELS

Under normal physiological conditions, blood vessels are created through one of two processes, i.e.,

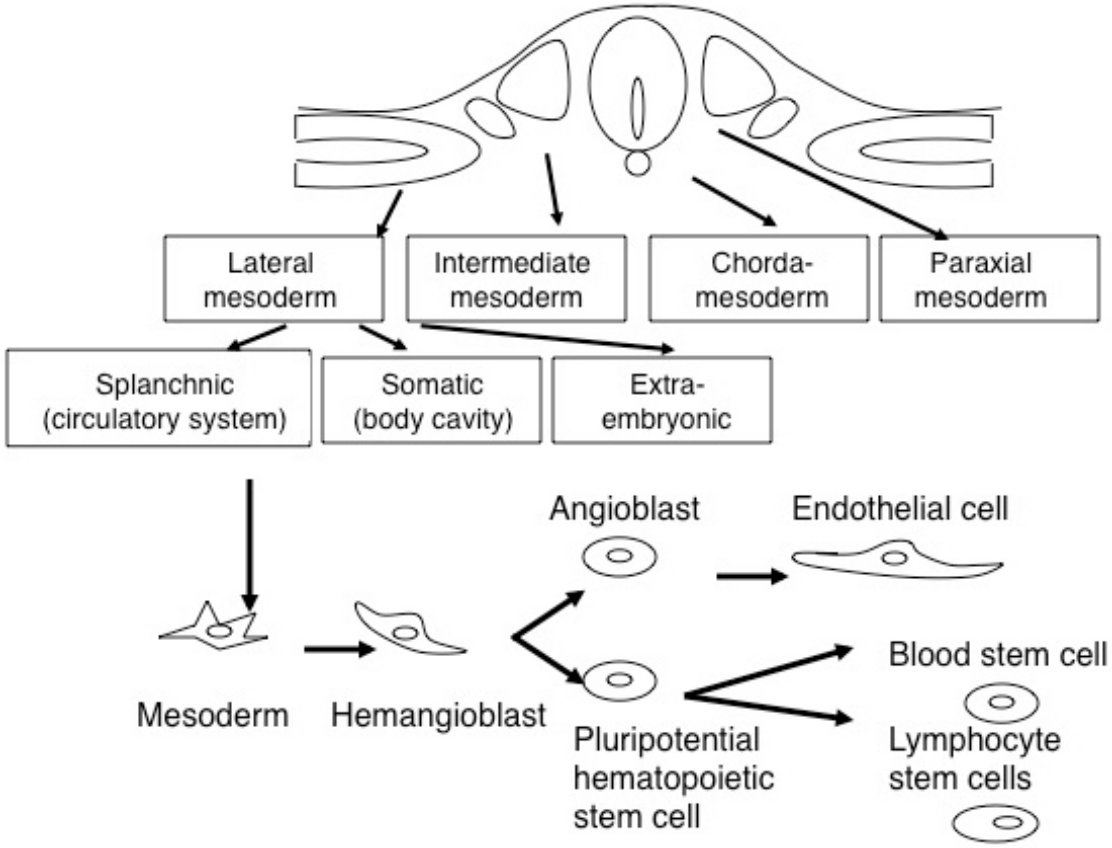

Figure 1. The lineage of the mesoderm, hemangioblasts and endothelial cells**.**

vasculogenesis and angiogenesis.

Vasculogenesis describes the formation of blood vessels from blood islands. Angiogenesis involves sprouting of blood vessels and remodeling of vascular beds. Initially, the hemangioblast, which is believed to be a common precursor of blood vessels and blood cells, is derived from the mesoderm (Figure 1). The hemangioblast subsequently differentiates to become an angioblast, i.e., the precursors of vascular endothelial cells (ECs), and pluripotential hematopoietic stem cells that generate the blood cells and lymphocyte.

In the first phase of vasculogenesis, the splanchnic mesoderm cell population gives rise to hemangioblasts, after which outer cells of blood islands become angioblasts. In the second phase, angioblasts differentiate into endothelial cells. In the third phase, these endothelial cells participate in tube formation and construction of a capillary network. Some growth factors such as fibroblast growth factor 2 (FGF2, also named basic fibroblast growth factor), VEGF and Angiopoietin are required for vasculogenesis (Figure 2). In particular, FGF2 generates hemangioblasts from the mesoderm. The VEGF-VEGFR system is important for the generation of hemangioblasts and tube formation (see VEGF system), and Angiopoietin-1 regulates the connection between ECs and pericytes.

Angiogenesis involves the formation of new blood vessels through remodeling from existing vessels. In this phase, VEGF mediates endothelial cell proliferation and sprouting from the points at which capillaries have loose cell contacts and extracellular matrix. The nascent capillary network then matures through the actions of TGF-β and platelet derived growth factor (PDGF). The final primary capillary plexus contains two types of ECs. The arterial and venous ECs contain Ephrin B2 and its receptor EphB4 in their cell membranes. This expression pattern allows correct fusion between arterial vessels and venous vessels. In embryonic vasculogenesis and angiogenesis, lots of molecules contribute to generate blood vessels, and tumor vessels might be formed by the mechanism similar to the embryonic molecular mechanism.

3.1. VEGF-VEGFR system

3.1.1. VEGF family

The VEGF family belongs to PDGF/VEGF supergene family with a homodimeric structure (1, 2). The mammalian VEGF members are VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor (PlGF). Other members have been discovered as being encoded by viruses (VEGF-E) or as a component of the venom of some snakes (VEGF-F)(3, 4). VEGF-A is an important player in the proliferation and migration of ECs. It also acts upon a number of other cell types including

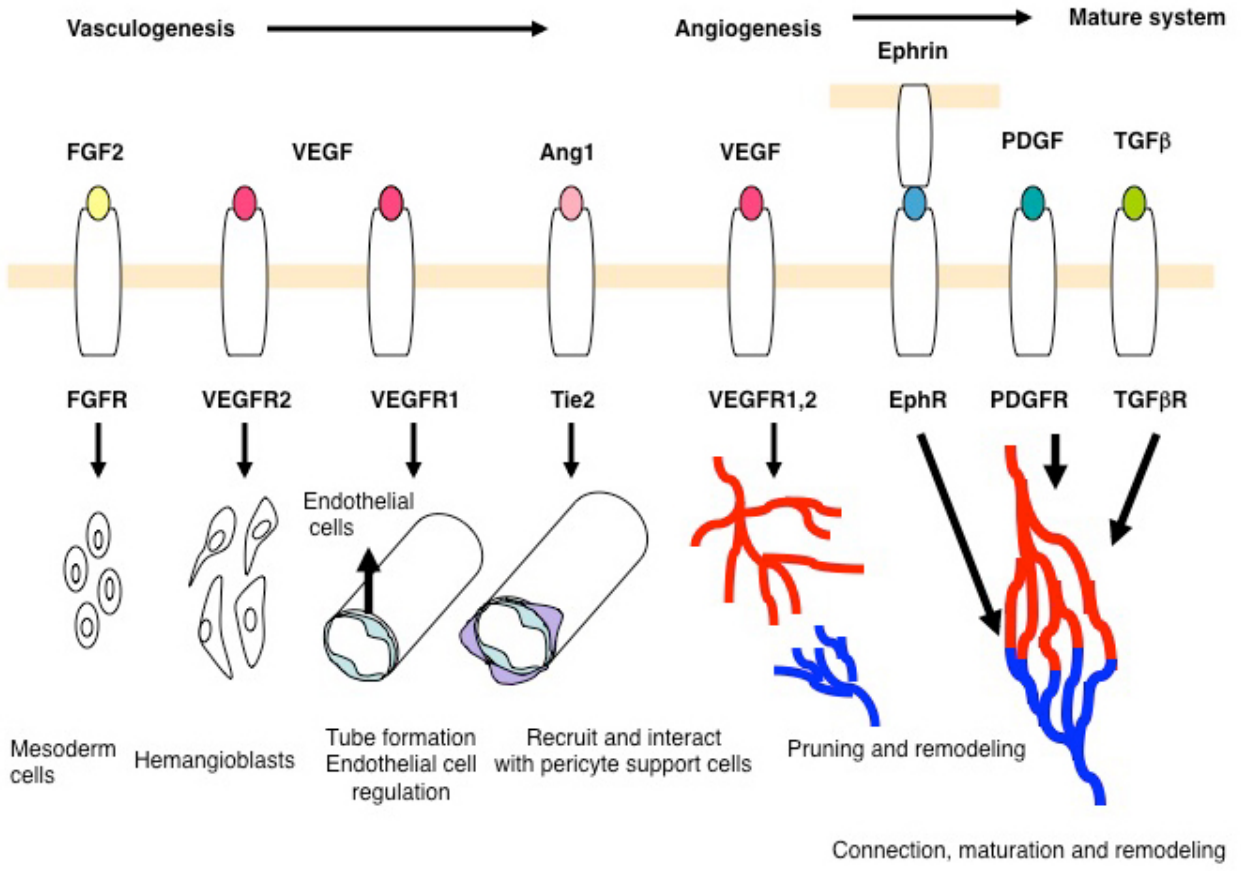

Figure 2. Vasculogenesis and angiogenesis. FGF-FGFR, VEGF-VEGFR and Angiopoietin-Tie system mainly regulate vasculogenesis. PDGF-PDGFR, TGFβ-TGFβR and ephrin-EPhR system organize angiogenesis.

monocytes and macrophages (influencing their migration), neurons, cancer cells, and kidney epithelial cells. VEGF-A increases microvascular permeability and when first discovered, was originally referred to as vascular permeability factor (VPF). Surprisingly, not only homozygotes but also heterozygotes for the *VEGF-A* gene (*VEGF-A*$^{+/-}$) mice show embryonic lethal phenotypes indicating that the VEGF-A protein level is an important determinant for completion of vessel formation (5, 6).

3.1.2. VEGF receptors

The VEGFR family consists of three members - VEGFR1, VEGFR2 and VEGFR3 - and is structurally distantly related to the PDGFR family. The PDGFR family members have five immunoglobulin.like (Ig) domains in the extracellular region, whereas the VEGFRs have 7 Ig domains (7,10) (Figure 3).

VEGFR1 and VEGFR2 bind VEGF-A and regulate angiogenesis, whereas VEGFR3 tightly binds VEGF-C and -D, and stimulates lymphangiogenesis (11, 12). The VEGF family mediate cellular responses by binding to the VEGFRs, which are tyrosine kinase (TK) receptors, in turn stimulating their dimerization and activation through transphosphorylation. The VEGF receptors have an extracellular portion (containing the seven Ig domains), a single transmembrane (TM) domain, and an intracellular portion containing the TK domain.

3.1.3. VEGF-A

VEGF-A binds to VEGFR1 (Flt-1) and VEGFR2 (KDR/Flk-1). Alternative splicing of the *VEGF* gene results in a number of VEGF-A isoforms, which differ in their heparin-binding affinity and amino acid number (in humans: VEGF121, VEGF145, VEGF165, VEGF189, VEGF206; the rodent orthologs of these proteins contain one fewer amino acids). The major subtypes of VEGF-A in humans are the 121, 165, and 189-amino acid types (2). VEGF165 is the dominant subtype and mediates interaction with neuropilin co-receptors on the cell surface, enhancing their ability to bind and activate the VEGF receptors (8). VEGF164 is essential in rodents. The mice carrying only the 164 allele (*VEGF-A164/164* mice) are viable but those with the other two genotypes (*VEGF120/120*, and *VEGF188/188* mice) show embryonic lethality (13). *VEGF-A* gene expression is induced when cells are not receiving enough oxygen. Under low oxygen, hypoxia-inducible factor (HIF) mediates the release of VEGF.

3.1.4. PlGF and VEGF-B

PlGF and VEGF-B bind only to VEGFR1. PlGF production is mainly from the placental trophoblast. The

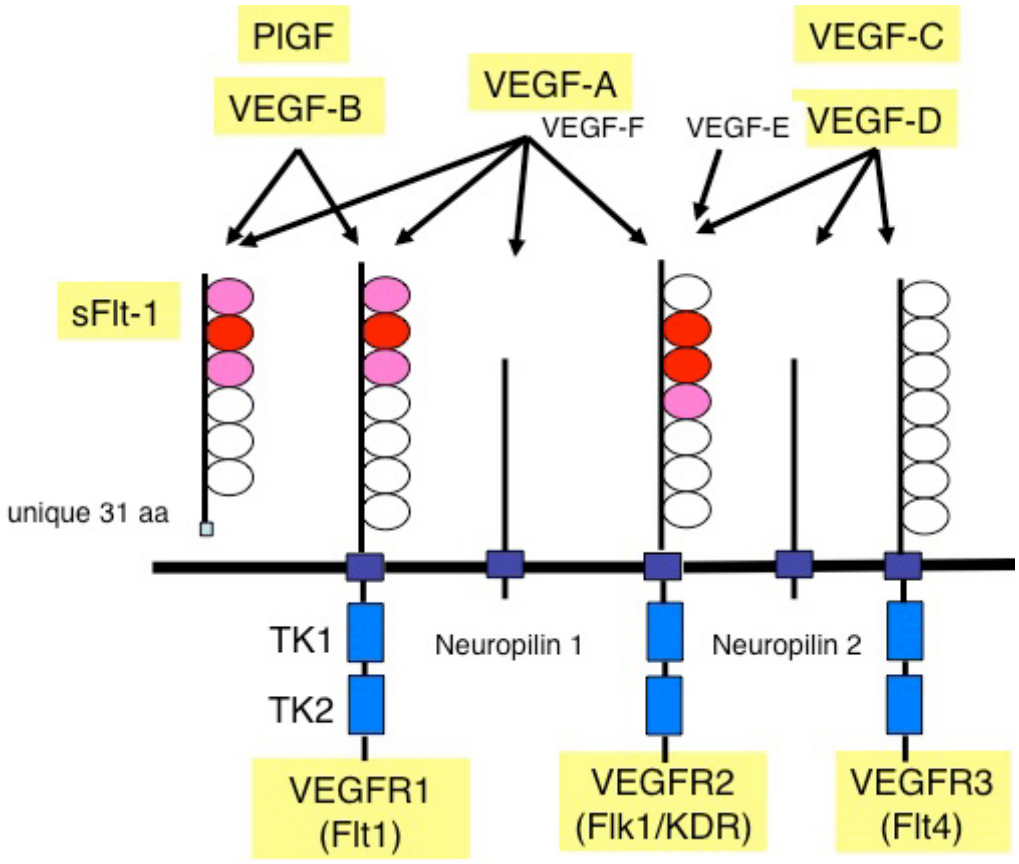

Figure 3. The interaction of VEGFs and VEGF receptors. VEGF-A stimulates VEGFR1 and VEGFR2 resulting in strong angiogenesis. VEGF-C and VEGF-D induces lymphangiogenesis via VEGFR3.

angiogenic activity mediated by PlGF and VEGF-B is usually about 10-fold weaker than that of VEGF-A. However, activation of VEGFR1 elicits the mobilization of bone marrow-derived myeloid cells into tumors or inflammatory lesions, and enhances pathological angiogenesis including tumor angiogenesis (14,17).

3.1.5. VEGF-C and VEGF–D

VEGF-C expression is seen in lymph sacs and lymph vessels during embryonic development (18, 20). VEGF-D is expressed in the heart, lung, skeletal muscle, colon and small intestine in humans (21, 23). VEGF-C and VEGF-D have a strong affinity for VEGFR3 increasing with processing, and only the mature forms of those bind VEGFR2 (22, 24). Deletion of *VEGF-C* in mice gives rise to an absence of lymph vessels and embryonic lethality. Even in *VEGF-C* heterozygous mice, lymph vessels in the skin are mostly absent and resemble the phenotype found in VEGFR3 heterozygous mice (20, 25). VEGF-D seems to be dispensable for development of the lymphatic system (26, 27). Both VEGF-C and VEGF-D induce lymphangiogenesis in various tumor models (12, 27, 34).

3.1.6. VEGFR1/Flt-1 (Fms-like tyrosine kinase-1): The role of transmembrane (TM) and tyrosine kinase (TK) in VEGFR1

VEGFR1 and VEGFR2 exist in close proximity on the endothelial cell membrane (35, 36). VEGFR1 is expressed not only on vascular ECs but also in monocyte/macrophage.lineage cells (37, 39). VEGFR1 has a very high affinity for VEGF-A at Kd= 2 to 10 pM, however, its TK activity is weak, about 10-fold lower than that of VEGFR2. Based on this weak activity, VEGFR1 only weakly stimulates endothelial proliferation. But VEGFR1 mediates migration of macrophages, which lack VEGFR2 in physiological situations.

The roles of the various domains of the VEGFR1 can be understood though the phenotypes observed in mice deficient in certain domains (Figure 4). In brief, the extracellular domain of VEGFR1 on ECs acts to absorb excessive VEGF (40). The membrane-bound domain plays an important role in the delivery of VEGF to the membrane, resulting in the appropriate quantitative regulation of the VEGF that binds to VEGFR2 (35, 36). The TK activity of VEGFR1 itself is not required, and the VEGF ligand family is recruited to the cell membrane via the TM domain of VEGFR1.

VEGFR1 homozygotes (*VEGFR1*$^{-/-}$) are embryonically lethal due to an overgrowth of vascular ECs and the disorganization of blood vessels (41). Interestingly, VEGFR1 TK domain.deficient mice (*VEGFR1-TK*$^{-/-}$ mice) are basically healthy, showing that the negative role of

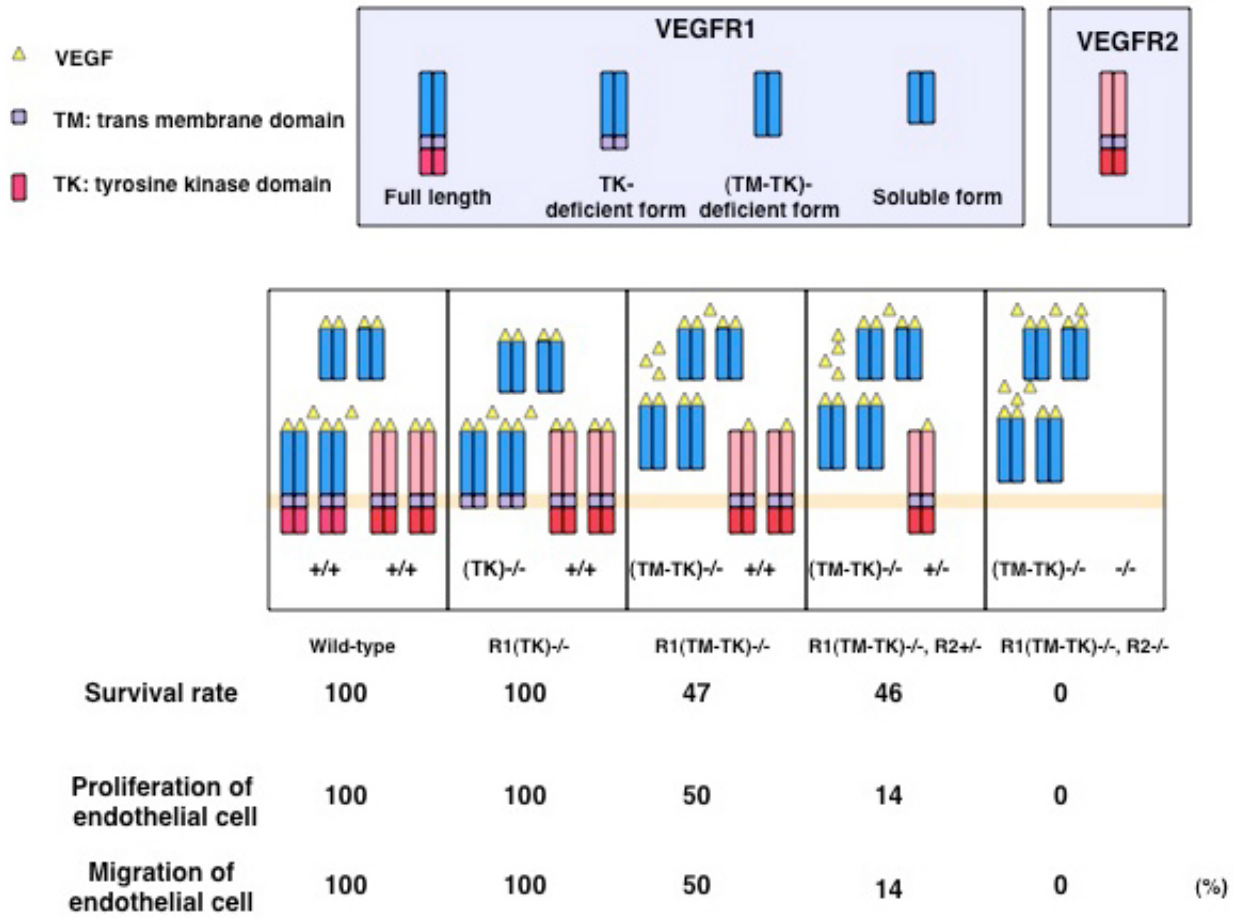

Figure 4. The roles of various domains of VEGFR1. The extracellular domain controls VEGF protein level. A transmembrane domain seems to deliver VEGF to VEGFR2 on the membrane.

VEGFR1 deletion is dependent on its extracellular region, and most likely this domain's trapping the endogenous VEGF-A to decrease its local concentration (40). In some tumor transplantation models, primary tumor growth decreased in *VEGFR1-TK*$^{-/-}$ mice, reducing macrophage infiltration and angiogenesis. The induction of matrix metalloproteinase 9 (MMP9) by a distant primary tumor increases in pre.metastatic lungs, and the degree of metastasis is significantly reduced in *VEGFR1-T*$^{-/-}$ mice compared with wild.type mice (15). TM-TK-deficient mice, leaving only the extracellular domain, can absorb VEGF but cannot induce the recruitment of VEGF to the cell membrane. About one.half of 129/C57BL6 mice lacking the TM.TK region of VEGFR1 died as embryos with abnormal blood vessel formation (35, 36).

3.1.7. Soluble VEGFR1 (sFlt1)

The *VEGFR1* gene expresses two types of mRNA, one for the full-length VEGFR1 receptor, and another for the ligand-binding region alone as a secreted soluble protein (sFlt1) (8, 42). An increase of sFlt1 protein level in serum has been reported in preeclampsia patients. In addition, serum levels of VEGF, PlGF, and sFlt1 are significantly higher in patients with pancreatic cancer. Tumor angiogenesis is the consequence of an imbalance between positive and negative angiogenic regulatory factors. The anti.Flt1 antibody (MF1) might effect on the balance between sFlt1 and VEGFR1, which regulates complicate system in local area because this antibody was reported as efficient or not in mouse tumor models (43, 44).

3.1.8. VEGFR2

VEGFR2 mediates the stimulation of vascular endothelial cell survival/growth and promotion of angiogenesis. VEGFR2 gene inactivation results in embryonic death at E8.5 and E9.0 due to a lack of vasculogenesis and very poor hematopoietic development (45). These findings suggest that VEGFR2 plays an essential role in the growth and differentiation of endothelial cell progenitors. In addition, VEGFR2 generates a variety of angiogenic signals not only for endothelial proliferation but also for cell migration and morphogenesis, including tubular formation. VEGFR2 has autophosphorylation sites on several tyrosine residues, including those at positions 951, 1054, 1059, 1175 and 1214. This receptor tyrosine kinase (RTK) activates PLCγ that stimulates protein kinase C (PKC) (46, 47). Among these tyrosine residues, a single autophosphorylation site,

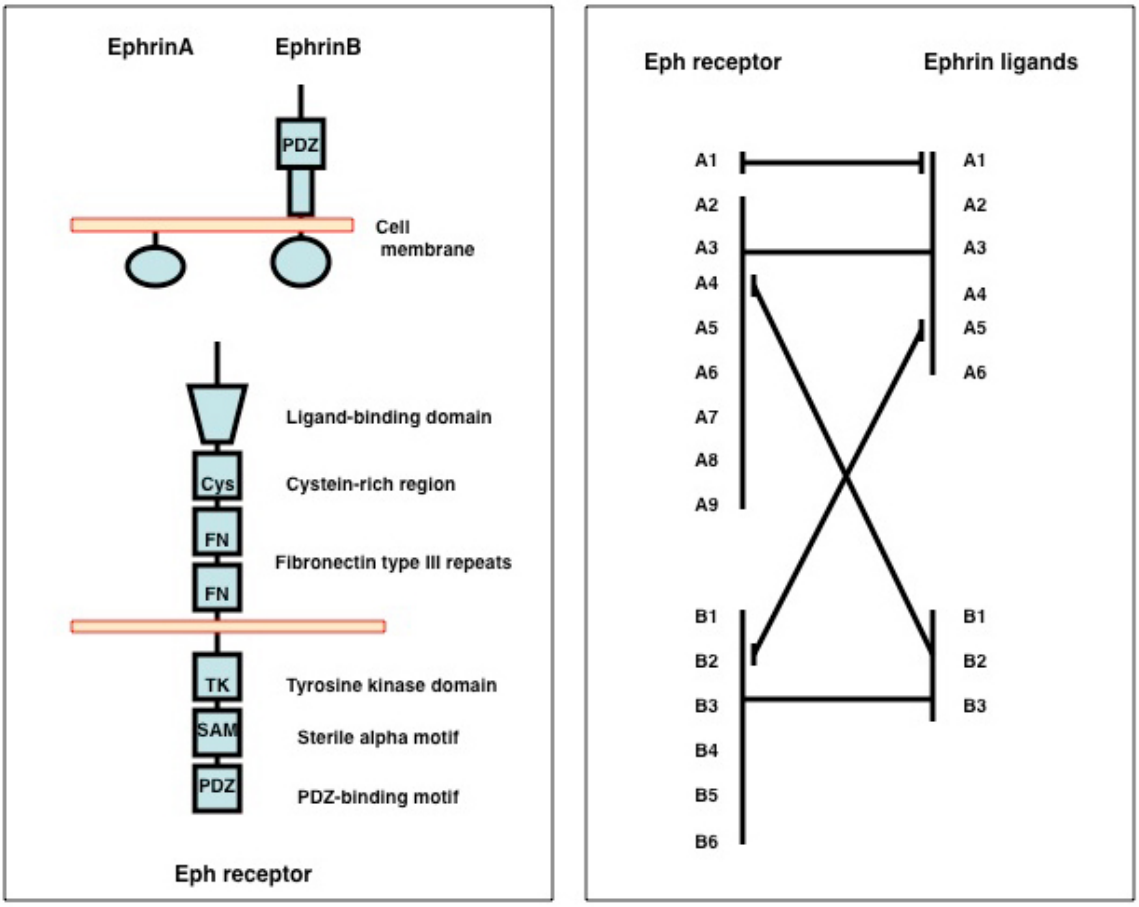

Figure 5. Structure and relationship of ephrin ligands and Eph receptors.

1175-tyrosine, is critical for the binding of PLCγ and activation of the PLCγ-PKC pathway (48). The critical role of 1175-tyrosine was shown by the finding that a point mutation at this tyrosine in mice induces embryonic lethality at E8.5-E9.0 due to a lack of development of blood vessels (49).

3.1.9. VEGFR3

VEGFR3 is expressed in ECs during murine embryogenesis, but is later largely found in the lymphatic endothelium (50, 52). The deletion of VEGFR3 results in defective remodelling of the primary vascular plexus, disturbed haematopoiesis, cardiovascular failure and embryonic death by E9.5 (52). Stimulation of VEGFR3 elicits lymphatic endothelial proliferation and migration *in vitro*, and its phosphorylation leads to PI3K.dependent Akt activation and PKC-dependent p42/p44 mitogen.activated protein kinase (MAPK) activation (53, 55). A soluble VEGFR3 fusion protein or VEGF-C/D trap leads to the regression of newly forming lymphatics. However, VEGF.C/D trap or blocking antibodies against VEGFR3 have no effect on mature lymph vessels (55).

3.2.VEGF-independent angiogenesis

The Angiopoietin-Tie family and EphrinB2-EphB4 signaling systems seem to be specific in the vascular system.

3.2.1. The Angiopoietin-Tie family

Angiopoietin 1 (Ang1) stimulates mostly the PI3K-Akt pathway as a survival signal, and stabilizes blood vessels. Ang2 acts as a partial agonist or antagonist of Ang1 signaling. When enough VEGF-A exists, the destabilizing effect of Ang2 enhances angiogenic responses, however, with little or no VEGF, Ang2 is known to decrease angiogenesis via destabilization of vessels and apoptosis of vascular ECs (56, 57).

3.2.2. Eph Receptors and Ephrin Ligands

Eph receptors are known as the largest family of RTKs. These consist of a glycosylated extracellular domain with an Ig.like ligand.binding site, followed by a cysteine.rich region and two fibronectin type III repeats, a single TM domain, the intracellular region containing a juxtamembrane domain, a TK domain, a sterile alpha motif, and a Postsynaptic density 95-Discs large.Zonula occludentes-1 (PDZ-1)-binding motif (58, 59) (Figure 5). Ephrin, the ligand for Eph receptor, have two subclasses, A and B. Class A ephrins are membrane-bound via a glycosylphosphatidylinositol anchor and class B ephrins contain a TM domain and a short cytoplasmic region with

conserved tyrosine residues and a PDZ-binding motif. There are 15 receptors and 9 ligands in this family. Basically, A-type and B-type ephrins bind class A and class B Eph receptors respectively. Eph receptor/ephrin complexes demonstrate unique properties of bidirectional, "forward" and "reverse" signaling (60, 62). When ephrins bind Eph receptors, forward signaling elicits autophosphorylation of intracellular tyrosine residues of the Eph receptor and mediates activation of downstream signaling cascades (63, 64). On the other hand, B.type ephrins mediates reverse signal that is mediated via cytoplasmic tail of the ephrin resulting in activation of different signaling cascades. The Eph receptors and the ephrin ligand system regulate cell motility, adhesion, cell-cell attachment and cell-matrix contacts in different biological processes such as embryonic and neural development and tumorigenesis. Furthermore, they also mediate physiological angiogenesis and tumor angiogenesis.

Among the Eph receptors and ligands, it is well known that the main angiogenic mechanism is mediated by EphB4 and ephrin B2, respectively. They demonstrate a unique expression pattern in that ephrinB2 is expressed on arteries and EphB4 on veins in early developmental stages (65, 67). EphrinB2/Eph signaling is critical for connection between ECs and perivascular supporting cells in vascular development (68). The several functions of endothelial ephrinB2 and EphB4 seem to mediate spatial position signaling during angiogenesis and vessel assembly (69). The importance of reverse signaling via ephrinB2 for vascular development is that phosphorylation at the intracellular domain of ephrinB is dependent on Src kinases and regulates EC and pericyte assembly into vascular structures (70, 71). In addition, the stimulation of EphB4 receptors with ephrinB2.Fc fragments leads to phosphorylation of Akt kinase resulting in proliferation and migration of the ECs (72).

EphA2 and ephrinA1 may be important for angiogenic processes because ephrinA1 expression corresponds to regions of vasculogenesis and angiogenesis. EphrinA1 is expressed in murine embryonic endocardium, dorsal aorta and primary head veins and later in intersomitic vessels and the limb bud vasculature (73). To understand the different mechanisms regulated by VEGF-VEGFR system, it has been shown that soluble EphA2-Fc receptors inhibit VEGF.induced survival, migration, and sprouting of ECs, and corneal angiogenesis (74). Furthermore, ephrinA1 with EphA2 induces activation of PI3 kinase and Rac1 GTPase and hence EC aggregation and migration (75).

In summary, ephrin and Eph receptor system contributes to angiogenesis and/or vasculogenesis using the mechanism that is independent of the VEGF-VEGFR system.

3.2.3. Other molecules

In addition to the pathways discussed above, the Delta-Notch pathway is another regulator for artery-vein differentiation and blood vessel formation. Delta-like 4 ligand (Dll4) knockout mice showed abnormal sprouting but fewer functional blood vessels, resulting in a poor circulatory system (76). Growth factors such as epidermal growth factor (EGF) and HGF are not specific to vascular ECs but stimulate a variety of cells. Recently, several molecules such as retinaldehyde dehydrogenase 2 (Raldh2), Norrin, Frizzled-4 (Fz4) and Noge-B were identified, which contribute to angiogenesis/vasculogenesis. Among them, Raldh-2 null mice fail to remodel the primary vascular plexus and do not recruit mural cells to vessels (77).

3.3. Angiogenic inhibitors

A number of endogenous inhibitors of angiogenesis have been described. The endogenous anti.angiogenic factors platelet factor-4 and interferon-α are found on ECs. Thrombospondin-1 (TSP-1), bone morphogenetic protein 4 (BMP4), chondromodulin and others are reported to have anti.angiogenic activity *in vitro* and *in vivo*. Yet other inhibitors are proteolytic fragments of collagens and other macromolecules. These include fragments of fibronectin, prolactin (78, 79), plasminogen (angiostatin) (80), platelet factor-4 (81), endothelial growth factor, and a propeptide of type 1 collagen (82).

4. TUMOR ANGIOGENESIS

Tumors depend on angiogenesis to obtain nutrients and growth factors. Hypoxia in solid tumors occurs at a distance of over 70 μm from functional blood vessels and it is generally accepted that tumors do not exceed a volume of 1-2 mm^3 without the induction of angiogenesis (83, 84). Compared to physiological angiogenesis, pathological vessels such as tumor vessels are structurally abnormal, heterogeneous and dysregulated. They are characteristically fragile, leaky and disorganized. The molecular mediators of tumor angiogenesis may be similar to those involved in physiological angiogenesis and /or vasculogenesis.

Given the dependence of tumor growth upon angiogenesis, inhibition of angiogenesis was proposed as an effective strategy to suppress growth of both primary tumors and metastases. Generally, tumor cells produce both pro- and anti- angiogenic proteins, and their relative balance leads to the angiogenic switch in a tumor (83, 84). The heterogeneous vessels formed as a result of excessive local amounts of pro.angiogenic factors derived from both tumor cells and tumor.stimulated stromal cells.

Most anti-angoiogenic therapies target pro-angiogenic molecules that contribute to angiogenesis, and hence attempt to redress the imbalance described above. Many such agents effectively reduce tumor angiogenesis and induce "normalization" of vessels (85). In addition, blockade of tumor.mediated host reactions (such as inhibition of bone marrow derived cell mobilization in tumors) may also further suppress tumor angiogenesis.

4.1. Abnormal structure and function of tumor vessels

Normal vessels are hierarchically structured and demonstrate and well-organized branching patterns. Tumor

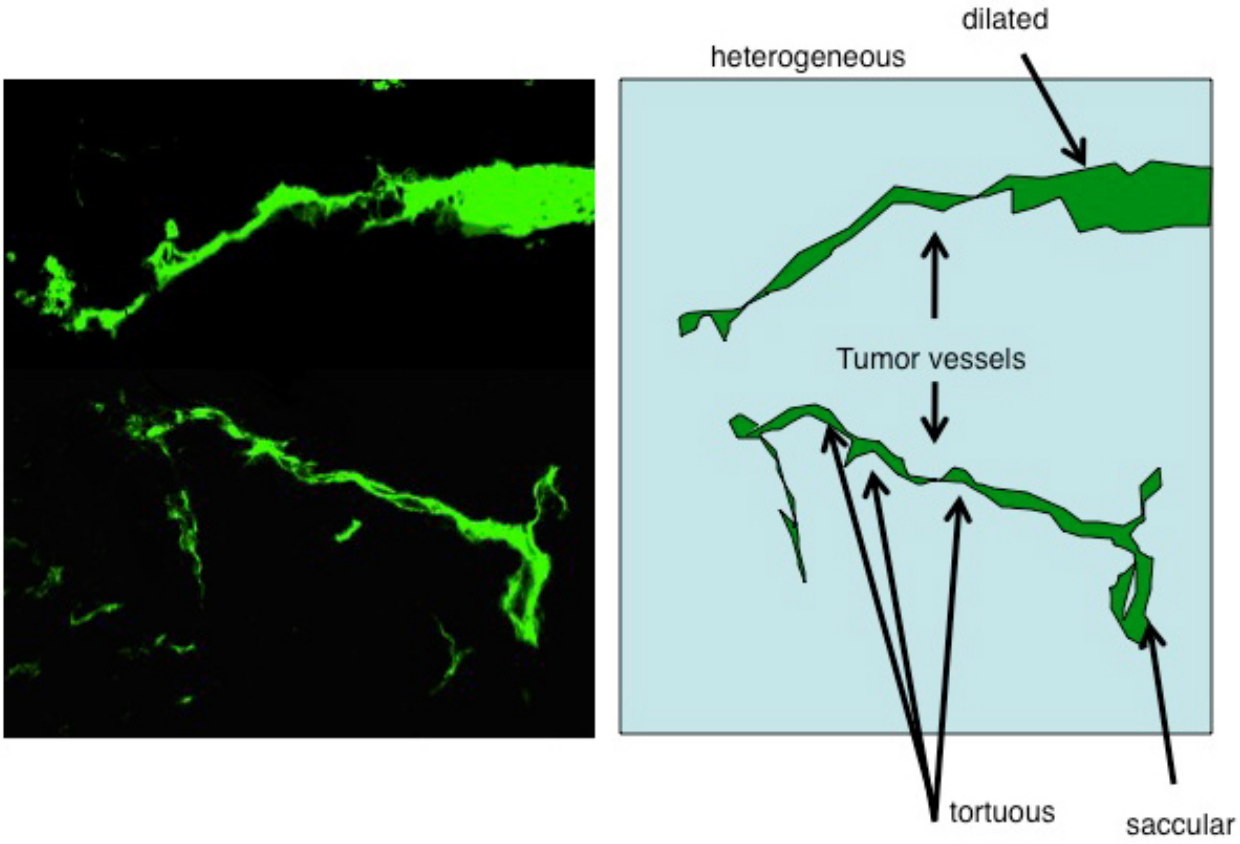

Figure 6. Tumor vessels of LLC tumors in mice. Abnormal vessels are expressed by FITC-conjugated lectin.

vessels, however, are dilated, disorganized, and heterogeneous, show branches with uneven diameters (86) (Figure 6). Hyperpermeability is also well known feature of tumor vessels. Moreover, vascular wall structure is also abnormal in tumors (87, 89). Aberrations include large inter-endothelial junctions, increased numbers of fenestrations, transendothelial channels formed by vesicles, and a lack of normal basement membrane (1, 90). Physiological small vessels are usually covered by pericytes, but perivascular cells around tumor vessels often have an abnormal morphology and heterogeneous associations. In relatively large solid tumors, mechanical stress can also compresses tumor vessels further compromising normal vessel function.

It is also important to consider transvascular exchange of fluid and solutes, both of which are primarily transported across the walls of capillaries and post-capillary venules. The movement of molecules from blood vessels occurs by diffusion, convection, or transcytosis in an exchange vessel. In tumors, diffusion is considered to be the major form of transvascular transport (91).

In a vascular network, blood flows sequentially through large arteries, small arteries, arterioles, terminal arterioles, capillaries, post-capillary venules, small veins and large veins (91). Post-capillary venules have a larger diameter than capillaries, are composed of a single layer of ECs and basement membrane, and usually lack smooth muscle cells – features which make them an important site for transvascular escape of metastatic cancer cells (92, 94).

The diffusive permeability of a molecule depends on its character as well as the transvascular transport pathway. Focal leaks are often found in tumor vessels, and perfusion rates (blood flow rate per unit volume) in tumors are lower than in many normal tissues. In addition, a normally functioning lymphatic network efficiently drains excess fluid out tissues, whereas a solid tumor compresses intra.tumoral lymphatic vessels (95), resulting in reduction of functional lymphatic vessels (96, 97).

4.2. Molecular aspects of tumor angiogenesis

4.2.1. Angiogenesis in VEGF- or PlGF-overexpressing tumors

Lewis lung carcinoma (LLC) tumors engineered to overexpress PlGF (LLC-PlGF) show morphologically different vessels when compared to those from LLC-VEGF tumors. In LLC-PlGF, relatively large vessels over 100 μm in diameter are often observed. However, LLC-VEGF tumor cells induce production of many small vessels less than 50 μm in diameter (98) (Figure 7). These results suggest that the processes of angiogenesis stimulated by PlGF and by VEGF are not identical although mechanisms are unclear. Recently, it has been reported that treatment with an anti-PlGF antibody inhibits metastasis and primary

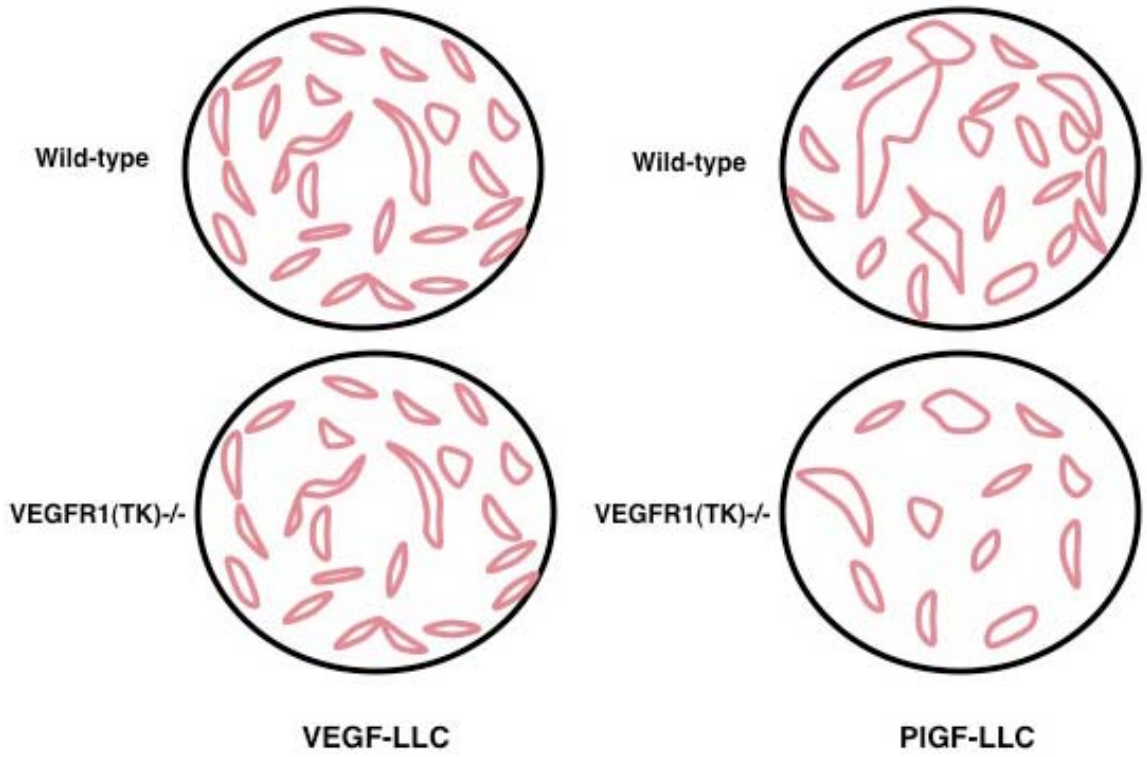

Figure 7. The morphological difference between VEGF. and PlGF.stimulated vessels The models of number and size of blood vessels in PlGF- or VEGF- overexpressing primary tumors in wild-type and VEGFR1(TK)$^{-/-}$ mice.

tumor growth (99). On the contrary, another report suggests that although anti-PlGF treatment inhibited extravasation of melanoma cells and growth of a VEGFR1-overexpressing tumor, it had no significant effect on primary tumor angiogenesis (100). Furthermore, combination of anti-PlGF with anti-VEGF-A antibodies did not result in greater anti-tumor efficacy than anti.VEGF-A monotherapy. Taken together, the PlGF-VEGFR1 cascade might regulate vessel formation and susceptibility of metastasis but not angiogenic proliferation in primary tumors.

4.2.2. Eph-ephrin system in tumor angiogenesis

It has been known that Eph receptors and ephrins are expressed in both tumor cells and tumor vasculature of many types of cancer (68).

For class B molecules, the importance of EphB4/ephrinB2 in tumor angiogenesis and tumor growth has been demonstrated. The soluble ephrinB2-Fc form can suppress tumor growth by inducing maturation of vessels in the tumor (101). Another report shows the effects of EphB4/ephrinB2 on tumor microvasculature, growth and survival, indicating that EphB4 works as positive regulator in tumors (102, 103). Class A molecules also involve tumor angiogenesis. The expression of both ephrinA1 and EphA2 was found in tumor cells and ECs in mouse and human cancers (104). In addition, soluble receptors such as EphA2.Fc and EphA3.Fc receptors suppressed tumor vascular density and tumor volume suggesting that the soluble Eph receptors can inhibit tumor vessels (105, 107). Compared to forward signaling, reverse signaling through ephrins is also important for tumor angiogenesis. Expression of truncated, soluble EphB4 receptor increases tumor angiogenesis, suggesting that soluble EphB4 stimulates tumor angiogenesis via ephrinB2 (108). Furthermore, Eph receptors might be an early cancer biomarker. In one study, an increase of EphA5 levels in plasma was found in mice bearing microscopic dormant glioblastoma. In summary, Eph and ephrin system may regulate tumor angiogenesis depending on cell types.

5. ALTERATIONS IN VESSEL FUNCTION AND ARCHITECTURE IN PREMETASTATIC LUNGS

It has been reported that the lung ECs can be "activated" by secreted factors from distant primary tumors before metastasis has occurred (Figure 8). In this pulmonary premetastatic soil, ECs secrete chemoattractants such as S100A8, S100A9 and serum amyloid A3 (SAA3) in response to cytokines and chemokines derived from primary tumors (110, 111). Primary tumors also induce matrix metalloproteinase 9 (MMP9) in lung ECs (15). These phenomena appear to provide an advantage for subsequently arriving circulating cancer cells in terms of metastasis formation. In addition, alveolar macrophages and infiltrating bone marrow-derived cells might synergistically stimulate endothelial cells in this phase. Finally, lung endothelial morphology is changed and the integrity of surrounding basement membranes in the tunica intima and media seemed to be disrupted in tumor.bearing

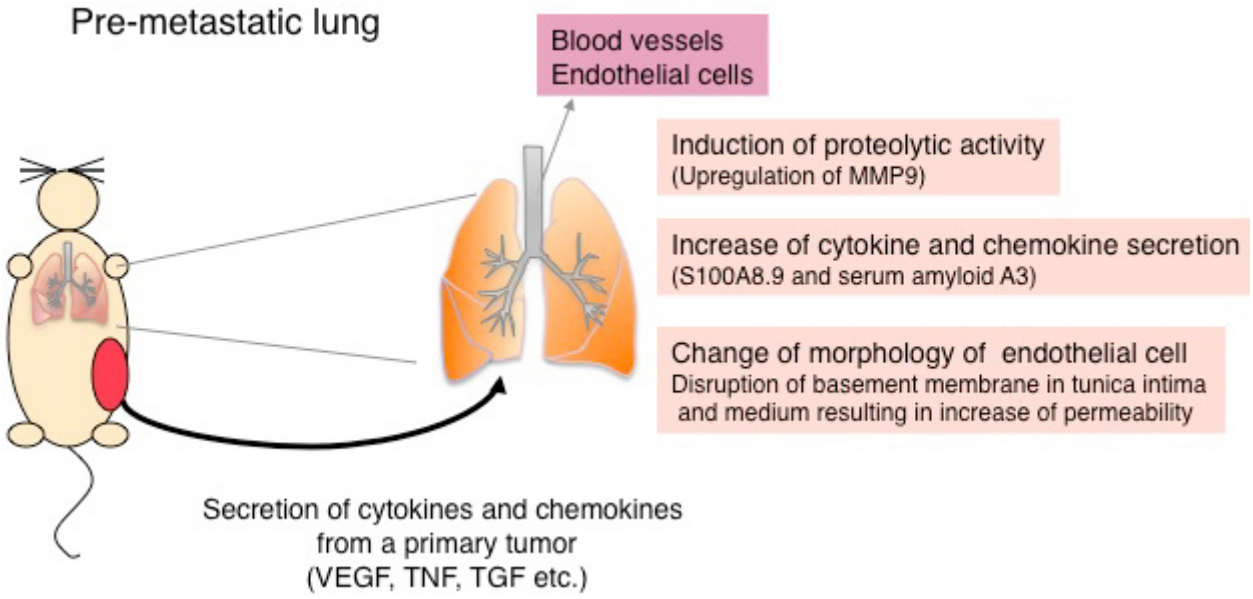

Figure 8. The change of blood vessels stimulated by a distant primary tumor in the premetastatic lung. Before metastasis, the proteolysis, cytokine/chemokine secretion and morphological changes are induced in lung endothelial cells by a primary tumor.

mice (112). Such "pre.metastatic" changes in vessels can contribute significantly to the future development of metastases.

6. PERSPECTIVE

Tumor angiogenesis is mediated by many molecules that regulate normal angiogenesis. The imbalance between angiogenic factors and anti-angiogenic factors in local area may cause the abnormality of tumor vessels. It is not known whether there exists a tumor-specific angiogenic factor, separate from those already identified and targeted, which might contribute to the profound abnormalities and heterogeneity in tumor vessels. Furthermore, it is not clear whether any specific locally acting angiogenic molecules contribute to these phenomena. Such possibilities should be considered when developing new strategies to effectively inhibit tumor vessels. In addition, it will be an important strategy to focus on the change of vessels in premetastaic organs to target more early stage in metastases.

7. ACKNOWLEDGE

I would like to express my gratitude to members in Steele laboratory and Dr. Y. Maru for critical comments.

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Key Words: Tumor vessels, VEGF, VEGFR, Ephrin, EphR, Premetastasis, Review

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