

THE ROLE OF ANG/TIE SIGNALING IN LYMPHANGIOGENESIS

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ABSTRACT

The angiopoietin/Tie system plays a key role in remodeling and maturation of blood vessels as well as lymphatic vessels. The angiopoietin family includes four ligands (Ang-1, Ang-2 and Ang-3/4) and two corresponding tyrosine kinase receptors (Tie1 and Tie2). The best characterized angiopoietins are Ang-1 and Ang-2. Ang-1 acts as an obligate agonist of the Tie2 receptor. Binding of Ang-1 to Tie2 induces its autophosphorylation and promotes vascular stability and integrity. Ang-1 induces lymphatic vessel enlargement, sprouting and proliferation in a VEGFR-3-dependent manner. In contrast, whether Ang-2 is agonistic or antagonistic is dependent on dose and context. Ang-2 modulates angiogenesis in a cooperative manner with another important angiogenic factor, vascular endothelial growth factor A. In the presence of VEGF-A, Ang-2 promotes vascular sprouting. When in the absence of VEGF-A, Ang-2 induces vascular regression. However, genetic studies have revealed that Ang-2-deficient mice exhibit more severe defects in the lymphatic vasculature than in blood vessels. Ang-2 seems to be involved in the remodeling and stabilization of lymphatic vessels. Although the Ang/Tie system is essential for blood and lymphatic vessel remodeling and maturation, defining its precise role in blood and lymphatic development has been a major challenge. This review provides an update on our current under-

standing of the angiopoietin/Tie system in lymphangiogenesis.

Keywords: lymphatic, Ang/Tie2 signaling, lymphangiogenesis

The lymphatic vascular system, as the body's second vascular system present in vertebrates, has been identified to play a crucial role in normal and pathological processes particularly in the past half-century. It is involved in the maintenance of normal tissue fluid homeostasis, immune surveillance, and absorption of fatty acids and lipid soluble vitamins in the gut. Moreover, the role of the lymphatic system has been highlighted in the pathogenesis of various human diseases, such as lymphedema, inflammatory conditions, tumor metastasis, transplant rejection, and autoimmune disease (1-4). In contrast to extensive research on angiogenesis related to the blood vasculature (i.e., hemangiogenesis), lymphangiogenesis research has long been hampered due to lack of specific molecular markers and difficulties in observing lymphatic vessels *in vivo* and performing genetic and experimental manipulation of the lymphatic system. Recently, identification of several lymphatic endothelial specific markers and development of gene-targeted animal models, together with technological advances such as high-resolution imaging and genome-wide approaches have provided significant insight into the biology of the

lymphatic vasculature. Key molecules including members of the vascular endothelial growth factors (VEGF) and angiopoietin families and their receptors have been described to regulate lymphatic development (5). While the VEGFs are reported to play a potent and primary role in the regulation of blood and lymphatic vascular growth via their specific receptors on the endothelial cells (6), angiopoietins are presumed to contribute to maturation and remodeling of the blood vessels as well as lymphatic vessels (7-13). This review focuses on updates to our current understanding of the angiopoietin/Tie system in lymphangiogenesis.

Ligands and Receptors

The angiopoietin signaling system consists of four ligands and two receptors. The ligands, including Ang-1, Ang-2, Ang-3 and Ang-4, basically consist of a carboxy-terminal fibrinogen-like domain that is responsible for receptor binding, N-terminal coiled-coil domain that serves to dimerize Angiopoietin monomers and a short amino-terminal domain that superclusters these oligomers into variably-sized multimers (8,14-17). The best characterized angiopoietins are Ang-1 and Ang-2, as the roles of Ang-3 (the murine orthologue of Ang-4) and Ang-4 are much less clear (18). Interestingly, these ligands seem to have opposing actions in endothelial cells as Ang-1 and Ang-4 appear to act as an agonist of Tie2, whereas Ang-2 and Ang-3 can act as context dependent antagonists (14,19,20). Moreover, despite the great similarity in structure between Ang-1 and Ang-2, they have distinct physiological functions in angiogenesis, lymphangiogenesis, and inflammation (8,11,21-23). Ang-1 is expressed by smooth muscle cells and other perivascular cells (19). It acts in a paracrine manner on the endothelium to promote angiogenesis as well as vascular stabilization by enhancing endothelial integrity (16,24). Ang-1 has also been reported to exert a vessel-sealing effect (25-28), acts as an anti-

inflammatory agent (29-31), and protects against cardiac allograft arteriosclerosis (32) (*Fig. 1*). Ang-2 is produced in ECs and stored in Weibel-Palade bodies (WPBs) (33,34) and released from WPBs by exocytosis upon stimulation (34). It acts in an autocrine manner on the Tie2 receptor. Ang-2 serves more versatile and dynamic functions depending on its environment (22). For instance, Ang-2 destabilizes and regresses blood vessels in the absence of vascular endothelial growth factor (VEGF), but promotes robust angiogenesis in the context of the simultaneous exposure of VEGF, specifically in the setting of tumor angiogenesis (19,35), transient vascular network formation in the developing eye (36), and subcutaneous wound healing (23). Moreover, Ang-2 sensitizes endothelial cells to TNF- α and has a crucial role in the induction of inflammation (23,37,38). Similarly, results from *in vitro* experiments also suggest a role of Ang-2 in the induction of inflammation by disrupting protective Ang-1/Tie2 signaling and facilitating endothelial inflammation in a dose-dependent fashion (39). Ang-2 acts as an antagonist for Ang-1 in the setting of blood vessels, but it doesn't seem to be the case in lymphatic vessels. On the contrary, Ang-1 and Ang-2 have redundant roles in lymphatic development (11). However, the underlying molecular and cellular mechanisms leading to these distinct roles of Ang-1 and Ang-2 are still poorly understood.

The receptors, consisting of Tie1 and Tie2 (40-42), are endothelial cell-specific tyrosine kinase receptors with Ig-like and EGF-like homology domains (8) (*Fig. 1*). Tie1 is almost exclusively expressed by endothelial cells. The fact that Tie1 is expressed in the Prox1-positive venous LEC progenitors and lymphatic vessels throughout embryonic and postnatal life as well as in initial and collecting lymphatic vessels in adult mice raises the possibility that it serves a function in the development or maintenance of lymphatic vasculature (13,43-45). Although Tie1, originally identified as an orphan

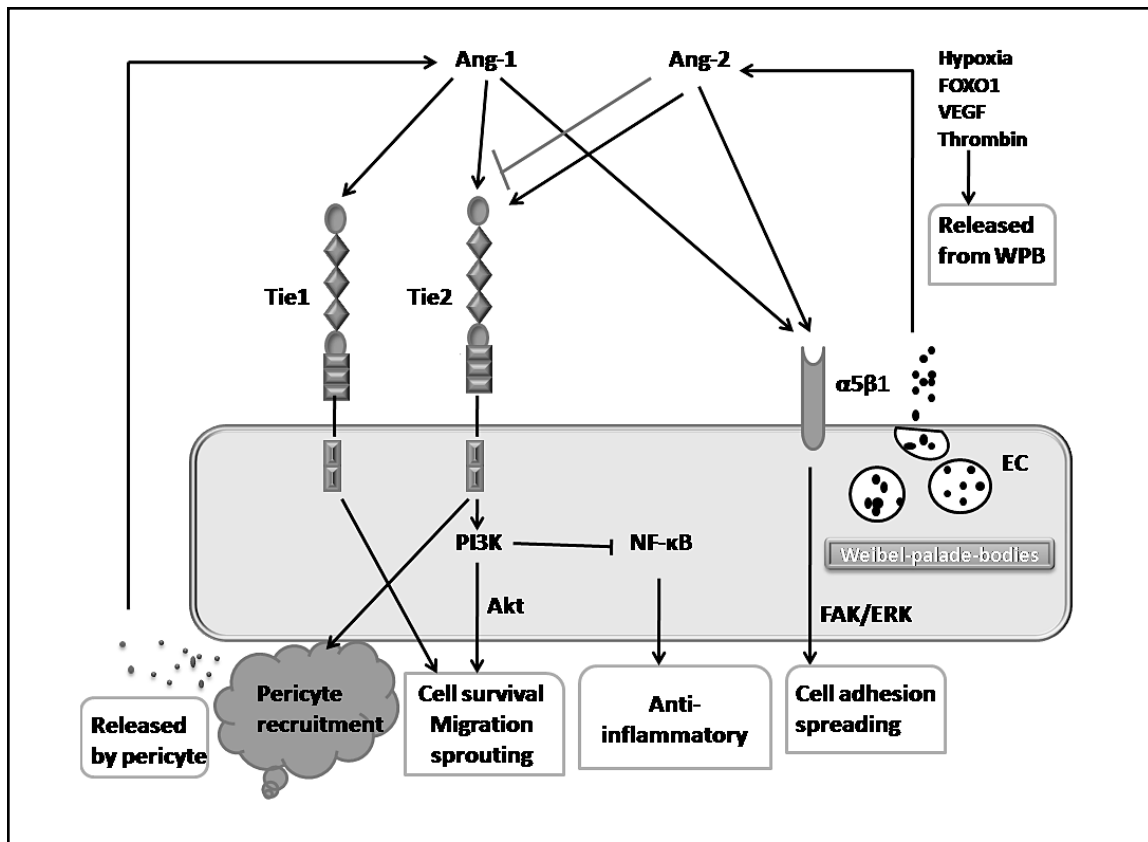


Fig. 1. A schematic model of angiopoietin signaling system. Quiescent endothelial cells are invested by pericytes that constitutively produce Ang-1, which induces phosphorylation of the Tie2 reception on endothelial cell. Tie2 activation leads to cell survival through PI3K/Akt signaling pathway. Furthermore, it suppresses inflammation by inhibiting the expression and protein production of NF-κB responsive inflammatory genes. In addition, Tie2 activation contributes to vascular maturation and stability by promoting pericyte recruitment. When endothelial cells are activated by, for example, hypoxia, VEGF, thrombin, Ang-2 can be released rapidly from the endothelial Weibel-Palade bodies. Ang-2 then interferes with Ang-1-Tie2 signaling. However, Ang-2 can act as agonist for Tie2 receptor under certain conditions. Both Ang-1 and Ang-2 can bind to integrin such as $\alpha5\beta1$, thus promoting cell adhesion and spreading. Tie1 activation also contributes to cell survival through PI3K/Akt signaling pathway.

receptor, shares a high degree of homology and is able to form heterodimers with Tie2 (44), Tie1 does not act as a transmembrane kinase. Indeed, Tie1, via its ectodomain cleavage that occurs in response to activation of protein kinase C, VEGF and inflammatory cytokines (46-48), regulates the binding of ligands to Tie2 and modulates its signaling by restricting the recruitment of signaling intermediates to Tie2 and preventing Tie2 activation (49-52). Moreover, recent work suggests that Ang-1 can induce Tie1

phosphorylation in cultured lymphatic endothelial cells (44), and blood vascular endothelial cells in a Tie2-dependent manner and recruit both Tie1 and Tie2 to cell-cell contacts (51,53). The main role for Tie1 is to modulate blood vessel morphogenesis by down-regulating Tie2-driven endothelial survival (51). Similarly, results from Tie1 gene-targeted mice also suggest a role for Tie1 in blood vessel formation during development as mice lacking Tie1 die in mid-gestation as a consequence of hemorrhage

and defective microvessel integrity (54,55). Strikingly, the lymphatic vascular defects in Tie1-deficient mice occur earlier and are more apparent during development than those of blood vessels, indicating the development of lymphatic vasculature is more sensitive to Tie1 than the development of blood vasculature (45,56). Moreover, further evidence suggests that Tie1 is required for the early stages of normal lymphangiogenesis and is also involved in the later remodeling and stabilization of lymphatic vessels in a dosage dependent manner (45).

The second angiopoietin receptor, Tie2, is mainly expressed in vascular endothelial cells (57,58), hematopoietic stem cells (59-61), tumor cells (62,63) and a subset of monocytes/macrophages (64). Tie2 mRNA and protein have been detected in cultured lymphatic endothelial cells (65,66). Moreover, Tie2 expression was recently shown by immunohistochemical staining to be present in developing lymphatics throughout embryonic and neonatal life and in LYVE-1 positive lymphatic vessels of adult mouse ear skin and small intestine (67-69). However, the role of Tie2 in development and maintenance of the lymphatic vasculature remains elusive as Tie2 could not be detected in lymphatic vessels in Tie2 GFP transgenic mice (13). The angiopoietins are all ligands for the Tie2 receptor (19,20,70), despite the fact that Tie1 and Tie2 are endothelial cell-specific receptors that share a high degree of homology (71-73). In experiments to date, Ang-1 and Ang-4 can act as agonist ligands for Tie2, whereas Ang-2 and Ang-3 appear to behave as antagonists (14,19,20).

Ang/Tie2 Signaling Pathway

Ang-1 and Ang-2 interact with their cognate receptor, Tie2. But only Ang-1 induces its autophosphorylation thereby the activation of the receptor (14). Binding of Angs to Tie2 promotes phosphorylation of Tie2 to initiate downstream effects including: 1) cell survival, migration and permeability

through phosphorylation of Akt threonine kinase and p42/44 MAPK (9,51); 2) anti-inflammation through inhibition of the expression and protein production of the NF- κ B-responsive inflammatory genes, such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin, via activation of PI3K/Akt and direct interaction between the Tie2 receptor and A20 binding inhibitor of NF- κ B activation-2 (ABIN-2) (74); 3) recruitment and association of pericytes/vascular smooth muscle cells to mature and stabilize newly formed blood vessels (75) (*Fig. 1*). The mechanisms involved in Ang/Tie-mediated SMC recruitment need to be elucidated, although several possible molecules have been implicated in Ang/Tie-mediated recruitment of mural cells, including the EC-derived heparin binding EGF-like growth factor (HB-EGF) (76), hepatocyte growth factor (77), platelet-derived growth factor B (PDGF-B) (78), transforming growth factor- β (TGF- β) (79-81). In addition, VE-PTP, vascular endothelial specific receptor tyrosine phosphatase, has recently been showed to trigger the activation of Tie2 in a ligand-independent manner by dissociating Tie2 from VE-PTP, leading to enhanced endothelial cell proliferating and enlargement of vascular structures (82). Constitutive Ang-1/Tie2 signaling contributes to the maintenance of the quiescent and stable EC phenotype. However, somatic Tie2 mutations leading to Tie2 hyperphosphorylation in a ligand-independent manner have been identified as the cause for autosomal dominantly inherited cutaneomucosal venous malformation (VMCM) (83,84), indicating excess Tie2 signaling might in turn have deleterious effects.

It is difficult to define the downstream effects of Tie1 activation due to lack of a known physiological ligand. However, the role of Tie1 in endothelial cells was recently studied using a chimeric receptor consisting of the extracellular domain of colony-stimulating factor-1 receptor and the

intracellular domain of Tie1. The activation of Tie1 can activate PI3K/Akt signaling pathway to inhibit apoptosis and promote cell survival, indicating overlapping functions of Tie1 and Tie2 (85,86). Tie1's role in promoting cell survival in developmental lymphangiogenesis was investigated by gene-targeted studies. In Tie1 hypomorphic mice with dosage-dependent reduction of Tie1, an increase in apoptosis in the lymphatic endothelium of jugular lymphatic sacs indicates that Tie1 is required for early LEC proliferation and subsequent survival of developing LEC (45).

Both *in vitro* and *in vivo* experiments support the concept that Ang-1 is an agonist of Tie2. *In vitro*, Ang-1 binding to Tie2 leads to activation of the PI3K-Akt pathway and then exerts prosurvival, antipermeability and anti-inflammatory effects on endothelial cells (ECs) (14,24,87-91). *In vivo*, the phenotype of Ang-1-deficient embryos is nearly identical to the phenotype of embryos lacking Tie2, although the Ang-1-deficient embryos die in midgestation. These findings confirm the role of Ang-1 as a Tie2 agonist (14,92). In contrast, both *in vitro* and *in vivo* experiments demonstrate that Ang-2 is a competitive antagonist to Ang-1 for the receptor tyrosine kinase Tie2 in ECs (11,12,22,35,36,38,92-95). However, under certain conditions, such as in prolonged stimulation in stressed endothelial cells or at high Ang-2 concentrations, Ang-2 was also able to induce Tie2 activation through the PI3K/Akt pathway (8,58,95,96). Moreover, recent results provide evidence that Ang-2 induces the autophosphorylation of Tie2 expressed by cultured bovine mesenteric lymphatic endothelial cells to promote survival and proliferative responses in the lymphatic endothelial cells in a more effective manner than Ang-1 (97). Interestingly, Ang-2 is found to act as an agonist when Ang-1 is absent but as a dose-dependent antagonist when Ang-1 is present, suggesting that Ang-1 and Ang-2 competitively bind to the same binding domain on Tie2 (96).

In addition to Tie2 signaling, both Ang-1

and Ang-2 are recently reported to stimulate Tie2-independent cell adhesion of ECs and fibroblasts as well as skeletal myocytes to Ang-1- or Ang-2-coated surfaces through $\alpha 5\beta 1$ and $\alpha v\beta 5$ integrin-mediated activation of ERK and focal adhesion kinase (FAK) signaling (98,99) (*Fig. 1*). Ang's interaction with integrins has been presumed to occur through the fibrinogen-like receptor-binding domain present in the Ang protein structure (100). Some groups have reported that Ang-2 promotes certain tumor cells invasion and metastasis through $\alpha 5\beta 1$ and $\alpha v\beta 5$ integrins (101-104). Similarly, even in the absence of Tie2 receptor, Ang-1 has been reported to stimulate cell adhesion to fibronectin via $\beta 1$ integrin (100,105), which is required for normal development of the lymphatic system (106). Meanwhile, integrin $\beta 1$ has also been shown to form a ligand-independent signaling complex with VEGFR-3 (107). It is thus possible that Ang signaling in endothelial cells might be modulated by 2 signaling systems, Tie2 and integrins.

Regulation of Expression of Angs

Ang-1 is mainly expressed by the myocardium during early development and restricted to perivascular cells around the developing vessels during later development and in adult tissues (14,19,92). Moreover, Ang-1 is also expressed by tumor cells (108,109) and neuronal cells of the brain (109). Ang-1 does not seem to be strongly regulated, either up or down, in many different adult tissues. In contrast, Ang-2 expression is regulated by multiple factors. Under physiological conditions, Ang-2 is expressed in regions undergoing intense neovascularization, for example during vascularization of the retina or development and endocrine function of the ovarian corpus luteum in the adult female reproductive cycle (19,110). In ECs under stress, Ang-2 mRNA expression is induced by fibroblast growth factor 2, VEGF and hypoxia through the tyrosine kinase or mitogen-activated protein

kinase pathway (111-122). The expression of Ang-2 is, amongst others, regulated by the forkhead transcription factors: FOXO1 and FOXC2. In endothelial cells, ligand binding of Tie2 leads to activation of phosphatidylinositol 3-kinase (PI3K)/Akt, which in turn results in activation of FOXO1 and subsequent nuclear export of FOXO1, thereby inhibiting Ang-2 liberation (123-124). Moreover, in adipose tissues of FOXC2 transgenic mice, FOXC2 overexpression transcriptionally upregulates expression of Ang-2 and other angiogenic factors. Thus, FOXC2 indirectly controls angiogenesis as well as remodeling and maturation of the vasculature by inducing Ang-2 expression in adipocytes, as evidenced by the observation that such vascular phenotypes could be reversed after blockade of Ang-2 activity by an Ang-2 inhibitor in FOXC2 transgenic mice (125). Additionally, estrogen, leptin, high glucose also induce Ang-2 expression under different conditions (93,126,127). Both Tie1 and Tie2 are upregulated by hypoxia, VEGF stimulation, although the latter is also modulated by interleukin (IL)-1, transforming growth factor and tumor necrosis factor (TNF) (72,128-131).

Role of Ang/Tie2 Signaling in Physiologic Lymphangiogenesis and Hemangiogenesis

Ang-1 acts as natural agonist ligand for Tie-2 by inducing its autophosphorylation and leads to activation of signaling pathways inside the cell by promoting the association with support cells. Ang-1 null mice die at E11-E12.5 with defects in endocardial differentiation and generalized vascular complexity attributable to a disruption of the interaction of the endothelial cells with adjacent support cells, as evidenced by the observation that mural cells are scarce and not associated with endothelial cells in Ang-1-deficient mice (92). Interestingly Ang-1-deficient mice have the same phenotype as mice deficient for Tie2. Both mutant mice display disorganized vessels with fewer

branches and poor periendothelial cells coverage (55,92,132,133). By contrast, transgenic mice overexpressing Ang-1 have normal cell-cell contacts between endothelial cells and between endothelial and perivascular cells (93). These findings suggest that the Ang-1-Tie2 system plays a key role in vascular remodeling, maturation as well as the stabilization of the cardiovascular system. Little is known about the role of Ang-1 in lymphangiogenesis, despite intensive investigation into its functions in blood vascular development. Recent studies show Ang-1 overexpression induces lymphatic vessel enlargement and sprouting, proliferation and eventually even formation of new branches in a VEGFR-3-dependent fashion (68). Furthermore, cartilage oligomeric matrix protein (COMP)-Ang-1, a chimeric form of Ang-1, not only stimulates *in vitro* colony formation of LECs, but also promotes *in vivo* lymphatic angiogenesis in mouse cornea. Most notably, these Ang-1-induced *in vivo* and *in vitro* effects on LECs were inhibited by soluble Tie2-Fc fusion protein which acted as an inhibitor by sequestering Ang-1, indicating that Ang-1 seems to regulate lymphatic vessel formation through Tie2 (67). However, more studies need to be done to define whether Ang-1 exerts its effects on the lymphatic endothelium directly through Tie2 or indirectly via the VEGF-C/VEGFR-3 pathway. Ang-2 serves more versatile and dynamic functions during the development of blood vessels and lymphatic vessels. High levels of Ang-2 expression have been found to be present in lymphatic and blood vessels undergoing remodeling (11,69). Ang-2's major role in hemangiogenesis appears to be in cooperation with VEGF-A function. Ang-2, if acting alone, appears to induce vessel destabilization and regression by interfering both with the Ang-1/Tie2 signals and with the interactions between endothelial cells and perivascular cells (19,134). However, if in cooperation with VEGF-A, Ang-2 facilitates vascular sprouting and destabilizes blood vessels by disrupting

interactions between ECs and peri-ECs, thus enhancing VEGF-A stimulation (19,35). Transgenic mice overexpressing Ang-2 had phenotypes similar to those of Ang-1- and Tie2-null mice, displaying disrupted interactions between ECs and peri-ECs, suggesting Ang-1 serves activating, agonistic function on Tie2, whereas Ang-2 acts as an inhibiting, antagonistic regulator of Ang-1/Tie2 signaling (19). Ang-2's role in destabilizing the vascular network has been validated by the observation of the high degree stability and inability of the central retinal artery to remodel vascular structures during angiogenesis in Ang-2-deficient mice (11). Further evidence shows Ang-2 functions in disrupting the association of mural cells with endothelial cells. In a retina model, Ang-2 expression was found to induce pericyte dropout of the retinal vasculature (135,136). Ang-2 was associated with the loss of pericytes from blood vessels in necrotic and hypoxic regions (137). Overexpression of Ang-2 in a rat glioma model resulted in aberrant vessels with low SMC coverage (138). In addition, recent studies show antibody or peptibody inhibition of Ang-2 increased pericyte coverage on tumor vessels, thereby reducing tumor growth and the number of blood vessels. This observation and others make Ang-2 a potential anti-angiogenic target for cancer therapy (139,140)

It is notable that Ang-2-null mice develop severe lymphatic defects, but only minor blood vascular defects. Mice deficient for Ang-2 show chylous ascites shortly after feeding and widespread edema indicating defects in the lymphatic system (11). The lymphatic vessels are able to form but fail to remodel or form normal hierarchical networks. The collecting lymphatic vessels are not properly invested by smooth muscle cells (SMCs), suggesting, unlike in the blood vessels, Ang-2 has a role in lymphatic SMC recruitment. By contrast, an aberrant recruitment of periendothelial cells to lymphatic capillaries in mice lacking Ang-2 implies that Ang-2 is requisite to keep the lymphatic

capillaries free from periendothelial cells. The discrepancy in responses to Ang-2 between the lymphatic capillaries and collecting lymphatics highlights additional mechanisms of pericyte recruitment. However, the presence of lymphatic vessels postnatally, albeit disorganized, within the Ang-2-deficient mice raises at least one possibility that the differentiation of the lymphatic endothelium does not require Ang-2 involvement. In contrast to the Ang-2-deficient phenotype, mice with loss of VEGF-C show almost complete absence of peripheral lymphatics, suggesting the primary role of VEGF-C in the development of lymphatic vessels (141). Moreover, the identification of VEGFR-3 gene mutation in human hereditary lymphedema patients also confirms the primary role of VEGF-C/VEGFR-3 signaling in lymphangiogenesis (142). These data indicate VEGF-C is essential for lymphatic development in the early stages, whereas Ang-2 plays a crucial role in the proper formation of lymphatic vessels by regulating optimal support-cell recruitment. Interestingly, the genetic replacement of Ang-2 with Ang-1 rescues the defects, viz the disorganization and disordered structure of the lymphatic capillaries, but not the blood vascular defects (11,12). Lymphatic vessel formation is highly dependent on Ang-2/Tie2 as the absence of periendothelial cells at the very beginning of vascular remodeling may lead to lack of Ang-1 which is produced by periendothelial cells. The genetic knock-in of Ang-1 into the Ang-2 locus completely restores the remodeling of lymphatic vessels and rescues the disorganization and disordered structure of the lymphatic vessels in Ang-2 knockout Ang-1 knock-in mice (Ang-2A1/A1). These findings suggest that: 1) Ang-2 acts as an agonist for the receptor Tie2 to contribute to the morphogenesis of lymphatic vessels; 2) Activation of Tie2 induces lymphangiogenesis and is crucial for normal lymphatic development; 3) Ang-2 is agonistic in lymphatic vessels but antagonistic in blood

vessels. On the other hand, hyaloid blood vessels in the eye's lens persist for 10 days after birth in Ang-2-deficient mice while they largely regress at the same age in WT mice. This reflects a role of Ang-2 in blood vessel remodeling and vascular regression (11,143). Defective remodeling of the blood and lymphatic vasculatures in Ang-2 null mice raises the possibility that Ang-2 exerts similar functions in hemangiogenic and lymphangiogenic remodeling, although molecular mechanisms need to be further explored and clarified. Interestingly, Ang-2 and Foxc2 (a member of the Forkhead transcription factor family) mutant mice exhibit some similarities in lymphatic phenotype, i.e., improper recruitment of smooth muscle cells into lymphatic capillaries (144). FOXC2, linked to human hereditary lymphedema (145) has been found to inhibit SMC coverage of initial lymphatic vessels through the suppression of PDGF-B expression (79,144,146). The injection of recombinant Ang-1 has been shown to almost completely rescue pericyte dropout of the retinal vasculature caused by the blocking of PDGFBR antibodies (147), suggesting coordinated activities of Ang-1 and PDGF-B. It is thus possible that Ang-2, as an agonist for Ang-1 in the lymphatic vessel setting, could potentially function in collaboration with FOXC2 or PDGF-B in the remodeling and maturation of the lymphatic vasculature. In addition, the lymphatic capillary phenotype with aberrant recruitment of SMCs in both Ang-2 and Foxc2 mutant mice suggests that Ang-2 and Foxc2 may be involved in keeping the lymphatic capillaries free from periendothelial cells.

It is interesting to note that Tie1 mutant mice have a similar phenotype to Ang-2-null mice, displaying subcutaneous edema and dilated and disorganized lymphatic vessels with mild blood vessel defects (45). Ang-2 did not induce Tie1 phosphorylation (44,105). Ang-1 can induce Tie1 phosphorylation in cultured lymphatic endothelial cells in a Tie2-dependent manner (44,51). Different lines of evidence have suggested Tie1 inhibits Tie2

signaling by forming heterodimers with Tie2 (44,51,52,148). Further analysis shows the association between Tie1 and Tie2 inhibited Ang-2-mediated Tie2 activation in endothelial cells and are differentially modulated by Ang-1 and -2 (49,52,148,149). On the other hand, Tie1 is present in the endothelium of the same lymphatics as Ang-2 throughout embryonic and neonatal development (43,69,150). In this light, it is tempting to speculate that Ang-2 might affect Tie2 signaling via modulation of Tie1 in the lymphatic ECs.

In addition, Ang-2 has also recently been shown to be involved in inflammatory lymphangiogenesis. In Ang-2 knockout mice, lymphangiogenesis and inflammation are significantly reduced in inflammatory bowel disease model compared to WT mice (151). This finding suggests that Ang-2 may play a key role in inflammatory lymphangiogenesis, although the molecular mechanisms need to be elucidated.

CONCLUSION AND OUTLOOK

Accumulated studies on the role of the Ang/Tie system in lymphangiogenesis using gene targeted animal models and cell culture have delineated functions of this system in lymphangiogenesis. It is well recognized that the Ang/Tie system regulates lymphangiogenesis in cooperation with the VEGF family. The VEGF system appears to initiate vascular formation, whereas the Ang system promotes subsequent vascular remodeling, maturation, and stabilization, perhaps, in part, by supporting interactions between endothelial cells and surrounding support cells. However, the challenge of studying lymphangiogenesis is to understand the underlying mechanisms and interacting pathways in the process within its larger biologic context. As described in this article, the complexity of Ang/Tie system functions and its regulation are highly cell context dependent. A better understanding of the system in lymphangiogenesis should lead to

future advances in effective pro- and anti-lymphangiogenic therapies for a spectrum of lymphatic-related diseases.

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