Cellular and Molecular Mechanisms of Vasculogenesis, Angiogenesis, and Lymphangiogenesis

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What You Will Learn in This Chapter

In vertebrates, two specialized vascular systems facilitate effective fluid circulation: the blood vessels and the lymphatic system. These two tubular networks are closely linked and contribute to the homeostasis of the organism at cellular and tissue level. In this chapter you will learn about cellular and molecular mechanisms of blood and lymphatic vascular system formation. This chapter also illustrates conditions and disease states when the mechanisms of blood and lymphatic vascular system formation are not properly working.

7.1 Cellular and Molecular Mechanisms of Blood Vascular System Generation

7.1.1 Introduction

The essential components of the vascular system are the heart, blood, and blood vessels. Functioning of the blood vascular system affects numerous physiological and pathological processes. This system plays, e.g., an important role in the delivery of oxygen and nutrients to the tissues, in hormone transport, in the removal of carbon dioxide and degradation products, in blood coagulation, in warm regulation, and in immune function. In pathological conditions, the vascular system contributes to tumor growth and dissemination of cancer cells.

7.1.2 Vasculogenesis, Angiogenesis, and Arteriogenesis

The blood vessel system is generated during the embryonic development by processes referred to as vasculogenesis and angiogenesis (Fig. 7.1).

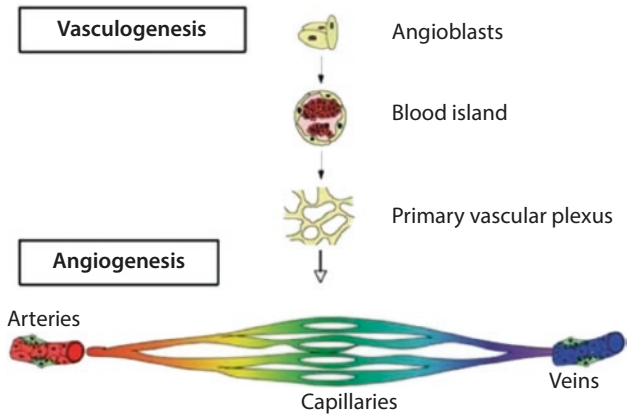

Fig. 7.1 Generation of the blood vascular system during the embryonic development. Blood vascular system is generated from precursor cells designated as angioblasts. Angioblasts together with blood cells constitute blood islands and form a primary vascular plexus before the onset of heartbeat. Veins and arteries are generated by expanding of the primitive vascular plexus in a process of angiogenesis, involving vessel remodeling and maturation. (Figure modified, and reproduced with permission, from Ref. [9] p. 32, Springer Verlag)

Vasculogenesis represents the de novo assembly of blood vessels from endothelial progenitor cells called angioblasts [1]. Angioblasts derive from mesoderm, and they share a common origin with blood cells. The formation of angioblasts is induced by fibroblast growth factors (FGFs). Although vasculogenesis is operative mainly during the embryonic development, it also contributes to blood vessel generation and remodeling during postnatal life, especially in ischemic, malignant, and inflamed tissues, by involvement of multipotent adult progenitor cells and other cell types [2].

Angiogenesis involves sprouting and subsequent stabilization of these sprouts by mural cells – pericytes in medium-sized vessels and vascular smooth muscle cells (vSMCs) in large vessels. Angiogenesis also encompasses vessel remodeling by collateral growth to a more complex and sophisticated vascular network [3].

Arteriogenesis denotes formation of arteries during the embryonic development. Endothelial progenitor cells may differentiate either into vein or arterial endothelial cells, and this process is influenced, e.g., by proteins of the Notch family [4, 5], as well as ephrin family members, specifically ephrinB2 and ephrinB4 [6–8].

7.1.3 Pro-angiogenic and Anti-angiogenic Factors Affecting Blood Vessel Formation and Regression

During vasculogenesis, vascular endothelial growth factor VEGF-A, together with its cognate VEGF receptor 2 (VEGFR2), fosters growth of endothelial cells and their survival [1, 10]. Further stimuli to this process provide cell adhesion molecules such as VE-cadherin and PECAM-1 (CD-31), as well as transcription factors (e.g., ets-1) that participate in subsequent events of endothelial cell differentiation, apoptosis, and angiogenesis [1]. However, VEGF-A does not solely act on endothelial cells, but it also affects other cell types. For example, VEGF-A acts as a chemoattractant on monocytes [11]. Other growth factors affecting angiogenesis include placental growth factor (PlGF), a homolog of VEGF-A, and angiopoietin 1 and angiopoietin 2 that bind to their receptor Tie-2. While angiopoietin 1 is important for the recruitment of vSMCs and pericytes, angiopoietin 2 acts antagonistically on angiopoietin 1 [12]. Basic fibroblast growth factor (bFGF) stimulates proliferation and differentiation of numerous cell types that are necessary for blood vessel formation, and it increases formation of vascular capillaries [13]. Platelet-derived growth factor (PDGF), and especially its isoform BB, is expressed particularly strongly in tip cells of angiogenic sprouts and in the endothelium of growing arteries, where it actively induces recruitment of pericytes and vSMCs [14, 15]. Hypoxia-inducible factor-1α (HIF-1α), rapidly synthesized under hypoxic condition, robustly increases synthesis of many pro-angiogenic factors, including VEGF-A [16]. For maintaining the integrity of established blood vessels, proper blood flow and thrombocytes are very important [17–20].

The established blood vessels may also regress. Although such regression occurs physiologically when the nascent vasculature consists of too many vessels, increased regression of blood vessels may be a hallmark and contributory factor to many pathological conditions. It can be induced, e.g., by anti-angiogenic factors secreted by cells of the connective tissue or by immune cells. Thrombospondin 1, a large glycoprotein affecting adhesion, and endostatin, a C-terminal fragment derived from type XVIII collagen, suppress proliferation and survival of vascular cells [21, 22]. Angiostatin, another anti-angiogenic factor, is a cleavage product of a coagulation protein plasmin [23]. Interferons may exert their anti-angiogenic effects by diminishing bFGF levels [24, 25].

7.1.4 Process of Angiogenesis Affects Numerous Physiological and Pathological Processes

Numerous animal and human studies demonstrated that angiogenesis plays an important role in wound healing and menstruation as well as in disease states, such as cardiovascular disease, stroke, psoriasis, age-related blindness, and vasculitis, and the list of known conditions where angiogenesis plays an important role has been constantly growing [2]. Augmented angiogenesis occurs in the retina of diabetic patients [26], in the intestine of patients with inflammatory bowel disease [27], in the bones and joints of patients with arthritis or synovitis [28, 29], and in the lung of patients with pulmonary hypertension or asthma [30]. Furthermore, insufficient angiogenesis or vessel regression may also affect organ function. Such condition may cause heart and brain ischemia due to an impairment in collateral blood vessel growth, e.g., in diabetic patients [31, 32]. Collateral blood vessel growth is also impaired in patients with atherosclerosis [33], and it decreases with age, as seen e.g., in older experimental animals upon the exposure to limb ischemia [34] or arterial injury [35]. Reduced angiogenesis also contributes to preeclampsia [36] and osteoporosis [37].

The elaboration of a technique of gene knockout, based on the use of embryonic stem cells and gene inactivation via homologous recombination, has allowed to elucidate the role of different genes in blood vessel formation. Remarkably, in many cases it was found that functionality of blood vessels may be severely impeded by mutations in single genes. For example, disruption of only one allele of VEGF-A led to embryonic lethality of mice due to insufficient vascular development [38]. Likewise, inactivation of VEGFR2 was early embryonic lethal due to defects in the vasculature [39]. Mice deficient for PDGF-B and PDGFR-β continued to develop only until embryonic days E16–E19, at which time massive hemorrhage and edema occurred. In these animals, the lack of pericyte and vSMC was observed already at the onset of angiogenic sprouting at around E10 [14, 40, 41]. In addition, clinical studies revealed low expression levels of VEGF-A and neuropilin-1 (co-receptor of VEGF-A and semaphorin) in patients with DiGeorge syndrome, characterized by congenital heart problems and other symptoms [42]. Mutations in Tie-2 gene were found to cause venous malformations [43], and mutations in Notch-3 gene resulted in the hereditary arteriopathy and increased susceptibility to stroke [44].

Furthermore, angiogenesis has been shown to play a crucial role in tumor development, supporting primary tumor growth, and later on fostering its metastatic spreading [45]. Specifically, tumor vascularization was found very important for the initiation of tumor growth beyond certain size, as advocated in a theory of "angiogenic switch" by Judah Folkman [46]. In the initial avascular phase of tumor growth, when the tumor is still small, normal levels of interstitial nutrients are sufficient for tumor survival. However, such avascular phase ends after the tumor has reached a size of 1–2 mm. At that stage, the hypoxia in the inner mass of the tumor induces production of HIF-1α that robustly stimulates VEGF-A secretion, leading to sprouting of existing vessels, thus supplying the tumor with blood and nourishment (Fig. 7.2).

In addition to secretion of HIF-1α and VEGF-A, tumors have developed remarkable ways to further stimulate their growth. For example, they release chemotactic cytokines, thus attracting immune cells that secrete angiogenic factors, further facilitating tumor vascularization. Furthermore, tumors not only utilize the existing blood vessel system for their modification by angiogenesis, but they also generate new blood vessels by vasculogenesis or use the mechanisms of "vascular mimicry." In the former process, tumor cells act as pluripotent stem cells that are able to differentiate into endothelial cells and contribute to the

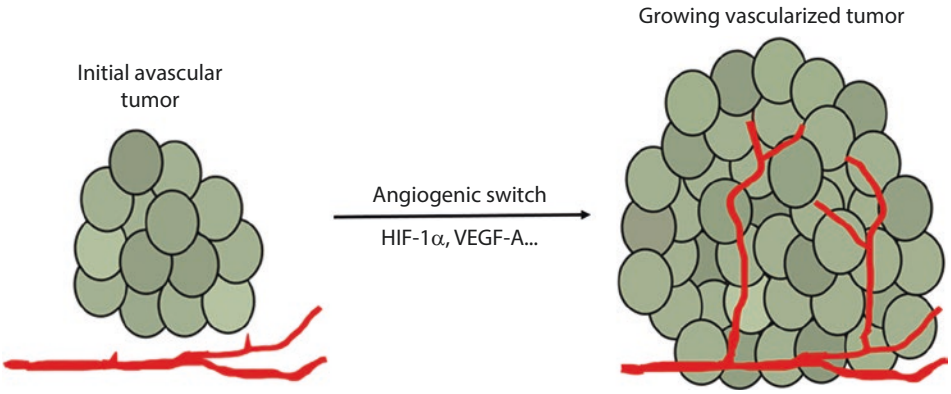

Fig. 7.2 Upon reaching a certain size, the hypoxic conditions within the tumor stimulate the production of HIF-1α leading to subsequent VEGF-A release from tumor cells, thus fostering sprouting of existing vessels. The generated blood vessels promote tumor growth

formation of new vessels. The latter process denotes the generation of microvascular channels by aggressive and deregulated tumor cells. All these mechanisms promote tumor vascularization and subsequent tumor dissemination to distant locations [47].

In agreement with the assumption that angiogenesis in tumors can be inhibited, and this would keep them small and clinically not relevant, drugs targeting VEGF or VEGF receptors have been developed. Avastin (bevacizumab), an antibody against VEGF-A, and ramucirumab, an antibody against VEGFR2, as well as other VEGF pathway inhibitors (including small molecule tyrosine kinase inhibitors sunitinib, sorafenib, and pazopanib), are in clinical use in combination with immunotherapy or cytostatic drugs for treatment of a variety of cancer types. However, latest findings argue that the effectiveness of the current anti-angiogenic therapies focused on targeting VEGF in cancer might be limited due to intrinsic tumor resistance or developed drug resistance [48]. Therefore researchers are developing alternative ways of tumor inhibition that are based, e.g., on pharmacological suppression of angiogenesis using metabolic targeting. As sprouting of endothelial cells is utterly glycolysis-dependent [49], transient suppression of glycolysis, e.g., by inhibiting the glycolytic activator PFKFB3, seems to be promising for defeating pathological angiogenesis without causing systemic effects [50, 51].

7.2 Cellular and Molecular Mechanisms of Lymphatic Vascular System Generation

7.2.1 Introduction

Although the blood vascular system has been recognized for centuries, the lymphatic system was initially described only in 1627. At that time Gaspare Aselli observed in dogs fed with lipid-rich meal structures that he designates as the "lacteae venae," i.e., milky veins. The lymphatic system is composed of a vascular network of thin-walled capillaries and larger lymphatic collecting vessels and the thoracic duct. In addition, the lymphatic system also comprises lymphoid organs such as the lymph nodes, tonsils, Peyer's patches, the spleen, and the thymus, which all play an important role in the immune response.

Lymphatic capillaries, in contrast to blood vessel capillaries, do not have a continuous basement membrane, and this enables them to continuously receive interstitial fluid containing macromolecules, cells, and lipids. Larger collecting lymphatic vessels are covered by vSMCs and supporting pericytes and have a basement membrane. In addition, they have luminal valves, and this helps them to prevent backflow of the lymph [52, 53]. The lymphatic system pervades almost the entire body, with the exception of some partially avascular structures such as the epidermis, cornea, nails, and hair [54]. Until recently, the central nervous system was considered as an organ devoid of lymphatic vasculature. However, recent work showed the presence of a lymphatic vessel network in the dura mater of the mouse brain [55]. The lymphatic system enters the blood circulation through the thoracic duct into the right subclavian vein [53]. Lately, also other communication routes between the lymphatic and venous systems – specifically in the axillary and subiliac regions – were reported [56].

7.2.2 Lymphangiogenesis and Factors and Conditions Affecting Lymphatic Vessel Formation

Lymphangiogenesis, the generation of the lymphatic system, starts shortly after the vascular system has been formed by vasculogenesis and angiogenesis. In mice lymphangiogenesis begins in ~ E9.0–9.5 [54] and in human in the 6th to 7th embryonic week [57].

Two models of lymphangiogenesis were presented in the beginning of the twentieth century. At around 1902 Florence Sabin injected ink into the skin of pig embryos, which allowed her to visualize lymph sacs and lymph vessels. She concluded that lymphatics originate from embryonic veins [58]. At Sabin's times Huntington and McClure proposed that mesenchymal cells are responsible for the generation of lymph sacs and lymphatic vasculature [59]. Although contemporary studies using, e.g., a detailed "line tracing approach" in mouse knockout models fully supported Sabin's hypothesis [60], it was also shown that mesenchymal cells to a certain extent contribute to the generation of lymph sacs and lymphatic vasculature [61].

Discovery of specific markers of the lymphatic endothelium, e.g., Lyve-1 and podoplanin [62, 63], as well as the extensive use of mouse knockout models in the last two to three decades, enabled researchers to elucidate the mechanism of lymphangiogenesis. It was shown that the development of the lymphatic system is governed by the polarized expression of a master control gene of lymphatic development, the Prox1 gene [64]. Subsequent formation of lymph sacs is achieved via migration and sprouting of cells derived from the cardinal vein, and this process is under the control of VEGF-C [65]. The lymphatic system further develops "centrifugally" under the influence of angiopoietin-2, ephrinB2, FOXC2, and podoplanin genes, as reviewed [57]. In addition, integrin-α9 is responsible for the proper lymphatic valve morphogenesis [66].

During the embryonic development, the lymphatic system gets separated from the blood vessel system, and until recently it was not clear how this process takes place [57]. The "nonseparation phenotype" of the blood vessels and lymphatic system was firstly described in a pivotal study of a group of Mark Kahn, who reanalyzed previously generated mice deficient in SLP-76, Syk, or phospholipase-Cγ2 genes [67]. The cause for this "nonseparation" has remained, however, enigmatic. At the Medical University of Vienna, we identified the "nonseparation phenotype" upon the disruption of podoplanin gene in mice. We showed that podoplanin and activated platelets, both present on sprouting lymph sacks (Fig. 7.3), are critically responsible for the separation of the blood and lymphatic circulation during the embryonic development [68, 69]. This "platelet hypothesis" was corroborated soon by

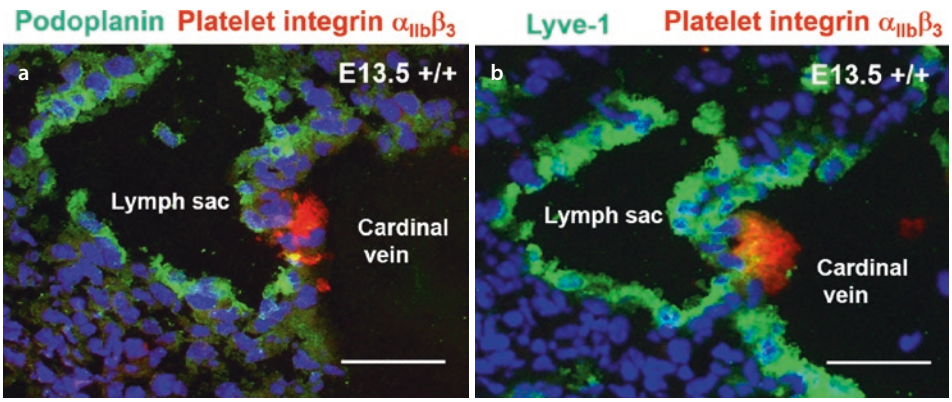

Fig. 7.3 Platelet aggregation driven by podoplanin is linked to separation of the lymph sacs from cardinal veins. In the mouse, the lymphatic system starts to develop from the cardinal vein at around ~ E9.0–9.5. This process of lymphangiogenesis is initiated by polarized expression of Prox1 and Lyve-1 on the cardinal vein and later on followed by formation of the lymphatic sac under the control of VEGF-C. Pictures show the presence of platelet thrombi (labeled for integrin $\alpha_{IIb}\beta_3$ in red) on sprouting podoplanin- **a** and Lyve-1- **b** positive cells, facilitating the developmental separation of lymphatic and blood vessels, as previously reported [69]. Scale bars in panels equal 50 μm

other studies, as the "nonseparation phenotype" was found also upon disruption of the homeodomain transcription factor Meis1 in mice completely lacking platelets [70]. Further proof for the critical role of podoplanin and platelet aggregation/activation in the developmental separation of blood and lymphatic system was provided by the group of Mark Kahn. The authors showed that disruption of the CLEC-2, podoplanin's receptor expressed mainly on the platelets, induced in CLEC-2-deficient mice the "nonseparation phenotype," thus recognizing platelets as the cell type in which SLP-76 signaling is required to regulate lymphatic vascular development [71].

7.2.3 Proper Functioning of the Lymphatic System Affects Numerous Physiological and Pathological Processes

The physiological role of the lymphatic system is to return proteins and fluids that have seeped into the extracellular space from the blood vessels (extravasation) back into the blood circulation. The lymphatic system functions as a conduit for lymphocytes and antigen-presenting cells that are part of the lymph fluid [72]. In addition, this system enables the absorption of triglycerides from the intestine [53]. Lymphatic system is also important for obesity, inflammation, and regulation of salt storage during hypertension [73]. The failure of the lymph transport leads to *lymphedema* – an accumulation of lymphatic fluid in the tissue, a harmful, disabling, and occasionally life-threatening disease [74]. Lymphedemas can be classified as primary and secondary.

Primary lymphedemas are caused by gene mutations. In patients with Milroy disease characterized by the absence or reduction of lymphatic vessels seen at birth [75], mutations in vascular endothelial growth factor receptor 3 (VEGFR3) have been identified [76–78]. In persons with lymphedema-distichiasis syndrome characterized by distichiasis (a double row of eyelashes) at birth, and bilateral lower limb lymphedema at puberty, mutations in the transcription factor FOXC2 were found [79, 80]. In these patients, in spite of the normal number of lymphatic vessels, their lymphatic draining is not functioning,

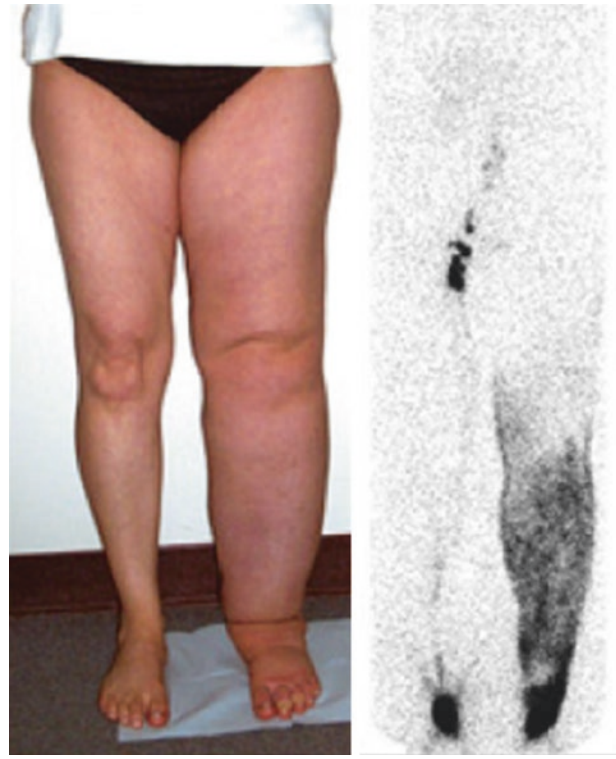

Fig. 7.4 A 46-year-old woman after surgery and radiation for uterine cancer. Lymphedema in her left leg was confirmed by the lymphoscintigram, which shows marked dermal backflow. (Figure reproduced, with permission, from Ref. [92], p. 14, Springer Verlag. Photos courtesy Emily Iker)

probably because of the lack of the lymphatic valves and impaired coverage of lymphatic capillaries with mural cells [81]. In human affected with hypotrichosis-lymphedema-telangiectasia (a syndrome characterized by hair loss and underdeveloped eyebrows and eyelashes, lymphedema, and dilated blood vessels on the skin), mutations in the transcription factor SOX18 were detected [82, 83]. Another cause for primary lymphedemas can be mutations in integrin-α9 gene detected in human fetuses with severe chylothorax [84].

Secondary lymphedemas represent worldwide most cases of lymphatic dysfunction, and they are instigated by some kind of external damage to the lymphatic vasculature. Secondary lymphedema can be caused by infections (e.g., filariasis), surgery, or radiotherapy. Filariasis itself is the most common secondary lymphedema affecting more than 100 million people, especially in tropical areas [85]. Filariasis is the result of a parasitic infection by mosquito-borne parasitic worms (*Wuchereria bancrofti* or *Brugia malayi*) which are located in the lymphatic system, where an inflammatory response stimulates the production of VEGF-A, VEGF-C, and VEGF-D. It often causes hyperplasia, obstruction, and extensive damage to the lymphatic vessel, finally leading to a chronic lymphedema of the lower limbs or genitals, which leads to permanent disability [86–88].

In the industrial world, secondary lymphedemas are primarily initiated by lymph node dissection or radiation therapy [88]. Impaired lymphatic transport causing edemas occurs in 15–20% of cases after breast cancer surgery [89], but it may be caused also by surgery and radiation of other cancer types (Fig. 7.4). Regrettably, treatment of lymphedema is still mainly restricted to conservative therapies such as compression garments, massage, manual drainage, liposuction, and modifications of the diet (primarily focused on minimizing the consumption of long-chain fatty acids) [88, 90, 91].

A study in mice showed that delivery of VEGF-C/VEGF-D via adenovirus stimulates regeneration of collecting lymphatic vessels, including the formation of intraluminal valves, after the excision of lymph nodes and the adjacent collecting lymphatic vessels [93]. These results suggested that VEGF-C/VEGF-D delivery might provide a basis for therapy of lymphedema, especially in cases of injury and restoration of primary lymphedemas but also in other conditions [94–96]. At present, a phase I multicenter clinical study in patients with early breast cancer-related upper extremity lymphedema is currently in progress. It should assess the safety and efficacy of using a VEGF-C adenoviral vector in combination with vascularized lymph node transplantation [97].

Lymphangiogenesis also plays an important role under pathological conditions in cancer. Kaposi sarcomas in AIDS patients, as well as angiosarcomas, express both lymphatic and blood vascular endothelial markers [62]. Lymphangiogenesis also facilitates primary tumor growth and cancer cell dissemination, and the presence of cancer cells in the tumor-adjacent "sentinel" lymph node has been shown to correlate with survival of patients [98, 99]. Recently, by directly injecting cancer cells into the lymphatic vessels of mice and following up their migration into the lymph nodes, researchers demonstrated that blood vessels of the lymph nodes, designated as "high endothelial venules," serve as an exit route for rapid systemic dissemination of cancer cells [100]. Currently it remains to be determined if such form of tumor cell spreading also occurs in human cancer patients.

Take-Home Message

- Blood vessel formation is controlled by a complex interplay of pro-angiogenic and anti-angiogenic factors. Vasculogenesis represents the de novo assembly of blood vessels by endothelial progenitor cells. Angiogenesis involves sprouting and subsequent stabilization of these sprouts by pericytes in medium-sized and vSMCs in large vessels. Arteriogenesis denotes formation of arteries.
- The status of blood vessels affects many conditions, including cardiovascular disease, diabetes, stroke, age-related blindness, psoriasis, osteoporosis, as well as wound healing and menstruation. Angiogenesis also contributes to primary tumor formation and metastasizing.
- The lymphatic system starts to develop shortly after the vascular system has been formed by vasculogenesis and angiogenesis, and this process is referred to as "lymphangiogenesis."
- The physiological role of the lymphatic system is to return proteins and fluids that have seeped into the extracellular space from the blood vessels (extravasation) back into the blood circulation. The lymphatic system functions as a conduit for lymphocytes and antigen-presenting cells that are part of the lymph fluid. In addition, this system enables the absorption of triglycerides from the intestine. The lymphatic system is also important for obesity, inflammation, and regulation of salt storage during hypertension. In pathological conditions, it also contributes to dissemination of cancer cells.
- Primary lymphedemas are caused by genetic mutations. Secondary lymphedemas represent worldwide most cases of lymphatic dysfunction and include, e.g., filariasis as well as lymphedemas caused by lymph node dissection or radiation therapy. Treatment of lymphedema is still mainly restricted to conservative therapies such as manual emptying, massage, compression garments, liposuction, and diet modification, and new treatment strategies are being developed.

References

1. Risau W, Flamme I. Vasculogenesis. Annu Rev Cell Dev Biol. 1995;11:73–91.
2. Carmeliet P. Angiogenesis in health and disease. Nat Med. 2003;9:653–60.
3. Risau W. Mechanisms of angiogenesis. Nature. 1997;386:671–4.
4. Lawson ND, Scheer N, Pham VN, Kim CH, Chitnis AB, Campos-Ortega JA, Weinstein BM. Notch signaling is required for arterial-venous differentiation during embryonic vascular development. Development. 2001;128:3675–83.
5. Fischer A, Schumacher N, Maier M, Sendtner M, Gessler M. The Notch target genes Hey1 and Hey2 are required for embryonic vascular development. Genes Dev. 2004;18:901–11.
6. Wang HU, Chen ZF, Anderson DJ. Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. Cell. 1998;93:741–53.
7. Adams RH, Wilkinson GA, Weiss C, Diella F, Gale NW, Deutsch U, Risau W, Klein R. Roles of ephrinB ligands and EphB receptors in cardiovascular development: demarcation of arterial/venous domains, vascular morphogenesis, and sprouting angiogenesis. Genes Dev. 1999;13:295–306.
8. Gerety SS, Wang HU, Chen ZF, Anderson DJ. Symmetrical mutant phenotypes of the receptor EphB4 and its specific transmembrane ligand ephrin-B2 in cardiovascular development. Mol Cell. 1999;4:403–14.
9. Marmé D, Fusenig N, editors. Tumor angiogenesis: basic mechanisms and cancer therapy. Berlin/Heidelberg: Springer; 2008.
10. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. Endocr Rev. 1997;18:4–25.
11. Clauss M, Gerlach M, Gerlach H, Brett J, Wang F, Familletti PC, Pan YC, Olander JV, Connolly DT, Stern D. Vascular permeability factor: a tumor-derived polypeptide that induces endothelial cell and monocyte procoagulant activity, and promotes monocyte migration. J Exp Med. 1990;172:1535–45.
12. Tait CR, Jones PF. Angiopoietins in tumours: the angiogenic switch. J Pathol. 2004;204:1–10.
13. Stegmann TJ. FGF-1: a human growth factor in the induction of neoangiogenesis. Expert Opin Investig Drugs. 1998;7:2011–5.
14. Hellstrom M, Kalen M, Lindahl P, Abramsson A, Betsholtz C. Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. Development. 1999;126:3047.
15. Gerhardt H, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, Abramsson A, Jeltsch M, Mitchell C, Alitalo K, Shima D, Betsholtz C. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. J Cell Biol. 2003;161:1163.
16. Pugh CW, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: role of the HIF system. Nat Med. 2003;9:677–84.
17. Murakami M, Simons M. Regulation of vascular integrity. J Mol Med (Berlin, Germany). 2009;87:571–82.
18. Hahn C, Schwartz MA. Mechanotransduction in vascular physiology and atherogenesis. Nat Rev Mol Cell Biol. 2009;10:53–62.
19. Ho-Tin-Noe B, Boulaftali Y, Camerer E. Platelets and vascular integrity: how platelets prevent bleeding in inflammation. Blood. 2018;131:277–88.
20. Ho-Tin-Noe B, Demers M, Wagner DD. How platelets safeguard vascular integrity. J Thromb Haemost. 2011;9(Suppl 1):56–65.
21. O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, Flynn E, Birkhead JR, Olsen BR, Folkman J. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell. 1997;88:277–85.
22. Jimenez B, Volpert OV, Crawford SE, Febbraio M, Silverstein RL, Bouck N. Signals leading to apoptosis-dependent inhibition of neovascularization by thrombospondin-1. Nat Med. 2000;6:41–8.
23. O'Reilly MS. Angiostatin: an endogenous inhibitor of angiogenesis and of tumor growth. EXS. 1997;79:273–94.
24. Singh R, Bucana C, Llansa N, Sanchez R, Fidler I. Cell density-dependent modulation of basic fibroblast growth factor expression by human interferon-beta. Int J Oncol. 1996;8:649–56.
25. Sasamura H, Takahashi A, Miyao N, Yanase M, Masumori N, Kitamura H, Itoh N, Tsukamoto T. Inhibitory effect on expression of angiogenic factors by antiangiogenic agents in renal cell carcinoma. Br J Cancer. 2002;86:768–73.
26. Capitao M, Soares R. Angiogenesis and inflammation crosstalk in diabetic retinopathy. J Cell Biochem. 2016;117:2443–53.

27. Scaldaferri F, Vetrano S, Sans M, Arena V, Straface G, Stigliano E, Repici A, Sturm A, Malesci A, Panes J, et al. VEGF-A links angiogenesis and inflammation in inflammatory bowel disease pathogenesis. Gastroenterology. 2009;136:585–595.e585.
28. Paleolog EM. Angiogenesis in rheumatoid arthritis. Arthritis Res. 2002;4(Suppl 3):S81–90.
29. Elshabrawy HA, Chen Z, Volin MV, Ravella S, Virupannavar S, Shahrara S. The pathogenic role of angiogenesis in rheumatoid arthritis. Angiogenesis. 2015;18:433–48.
30. Knox AJ, Stocks J, Sutcliffe A. Angiogenesis and vascular endothelial growth factor in COPD. Thorax. 2005;60:88–9.
31. Rivard A, Silver M, Chen D, Kearney M, Magner M, Annex B, Peters K, Isner JM. Rescue of diabetes-related impairment of angiogenesis by intramuscular gene therapy with adeno-VEGF. Am J Pathol. 1999;154:355–63.
32. Waltenberger J. Impaired collateral vessel development in diabetes: potential cellular mechanisms and therapeutic implications. Cardiovasc Res. 2001;49:554–60.
33. Van Belle E, Rivard A, Chen D, Silver M, Bunting S, Ferrara N, Symes JF, Bauters C, Isner JM. Hypercholesterolemia attenuates angiogenesis but does not preclude augmentation by angiogenic cytokines. Circulation. 1997;96:2667–74.
34. Rivard A, Fabre JE, Silver M, Chen D, Murohara T, Kearney M, Magner M, Asahara T, Isner JM. Age-dependent impairment of angiogenesis. Circulation. 1999;99:111–20.
35. Gennaro G, Menard C, Michaud SE, Rivard A. Age-dependent impairment of reendothelialization after arterial injury: role of vascular endothelial growth factor. Circulation. 2003;107:230–3.
36. Chaiworapongsa T, Chaemsaithong P, Yeo L, Romero R. Pre-eclampsia part 1: current understanding of its pathophysiology. Nat Rev Nephrol. 2014;10:466–80.
37. Filipowska J, Tomaszewski KA, Niedzwiedzki L, Walocha JA, Niedzwiedzki T. The role of vasculature in bone development, regeneration and proper systemic functioning. Angiogenesis. 2017;20:291–302.
38. Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, Fahrig M, Vandenhoeck A, Harpal K, Eberhardt C, et al. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature. 1996;380:435–9.
39. Shalaby F, Rossant J, Yamaguchi TP, Gertsenstein M, Wu XF, Breitman ML, Schuh AC. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature. 1995;376:62–6.
40. Soriano P. Abnormal kidney development and hematological disorders in PDGF beta-receptor mutant mice. Genes Dev. 1994;8:1888–96.
41. Hellström M, Gerhardt H, Kalén M, Li X, Eriksson U, Wolburg H, Betsholtz C. Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. J Cell Biol. 2001;153:543.
42. Stalmans I, Lambrechts D, De Smet F, Jansen S, Wang J, Maity S, Kneer P, von der Ohe M, Swillen A, Maes C, et al. VEGF: a modifier of the del22q11 (DiGeorge) syndrome? Nat Med. 2003;9:173–82.
43. Vikkula M, Boon LM, Carraway KL 3rd, Calvert JT, Diamonti AJ, Goumnerov B, Pasyk KA, Marchuk DA, Warman ML, Cantley LC, et al. Vascular dysmorphogenesis caused by an activating mutation in the receptor tyrosine kinase TIE2. Cell. 1996;87:1181–90.
44. Kalimo H, Ruchoux MM, Viitanen M, Kalaria RN. CADASIL: a common form of hereditary arteriopathy causing brain infarcts and dementia. Brain Pathol. 2002;12:371–84.
45. Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. Nat Rev Cancer. 2003;3:401–10.
46. Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med. 1971;285:1182–6.
47. Rafii S, Lyden D, Benezra R, Hattori K, Heissig B. Vascular and haematopoietic stem cells: novel targets for anti-angiogenesis therapy? Nat Rev Cancer. 2002;2:826–35.
48. Itatani Y, Kawada K, Yamamoto T, Sakai Y. Resistance to anti-angiogenic therapy in cancer-alterations to anti-VEGF pathway. Int J Mol Sci. 2018;19:1232.
49. De Bock K, Georgiadou M, Schoors S, Kuchnio A, Wong BW, Cantelmo AR, Quaegebeur A, Ghesquiere B, Cauwenberghs S, Eelen G, et al. Role of PFKFB3-driven glycolysis in vessel sprouting. Cell. 2013;154:651–63.
50. Schoors S, De Bock K, Cantelmo AR, Georgiadou M, Ghesquiere B, Cauwenberghs S, Kuchnio A, Wong BW, Quaegebeur A, Goveia J, et al. Partial and transient reduction of glycolysis by PFKFB3 blockade reduces pathological angiogenesis. Cell Metab. 2014;19:37–48.
51. Cantelmo AR, Conradi LC, Brajic A, Goveia J, Kalucka J, Pircher A, Chaturvedi P, Hol J, Thienpont B, Teuwen LA, et al. Inhibition of the glycolytic activator PFKFB3 in endothelium induces tumor vessel normalization, impairs metastasis, and improves chemotherapy. Cancer Cell. 2016;30:968–85.

52. Baluk P, Fuxe J, Hashizume H, Romano T, Lashnits E, Butz S, Vestweber D, Corada M, Molendini C, Dejana E, McDonald DM. Functionally specialized junctions between endothelial cells of lymphatic vessels. J Exp Med. 2007;204:2349–62.
53. Oliver G, Detmar M. The rediscovery of the lymphatic system: old and new insights into the development and biological function of the lymphatic vasculature. Genes Dev. 2002;16:773–83.
54. Oliver G. Lymphatic vasculature development. Nat Rev Immunol. 2004;4:35–45.
55. Aspelund A, Antila S, Proulx ST, Karlsen TV, Karaman S, Detmar M, Wiig H, Alitalo K. A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. J Exp Med. 2015;212:991–9.
56. Shao L, Takeda K, Kato S, Mori S, Kodama T. Communication between lymphatic and venous systems in mice. J Immunol Methods. 2015;424:100–5.
57. Alitalo K, Tammela T, Petrova TV. Lymphangiogenesis in development and human disease. Nature. 2005;438:946–53.
58. Sabin FR. On the origin of the lymphatic system from the veins, and the development of the lymph hearts and thoracic duct in the pig. Am J Anat. 1902;1:367–89.
59. Huntington GS, McClure CFW. The anatomy and development of the jugular lymph sac in the domestic cat (Felis domestica). Am J Anat. 1910;10:177–311.
60. Srinivasan RS, Dillard ME, Lagutin OV, Lin FJ, Tsai S, Tsai MJ, Samokhvalov IM, Oliver G. Lineage tracing demonstrates the venous origin of the mammalian lymphatic vasculature. Genes Dev. 2007;21:2422–32.
61. Buttler K, Ezaki T, Wilting J. Proliferating mesodermal cells in murine embryos exhibiting macrophage and lymphendothelial characteristics. BMC Dev Biol. 2008;8:43.
62. Breiteneder-Geleff S, Soleiman A, Kowalski H, Horvat R, Amann G, Kriehuber E, Diem K, Weninger W, Tschachler E, Alitalo K, Kerjaschki D. Angiosarcomas express mixed endothelial phenotypes of blood and lymphatic capillaries: podoplanin as a specific marker for lymphatic endothelium. Am J Pathol. 1999;154:385–94.
63. Banerji S, Ni J, Wang SX, Clasper S, Su J, Tammi R, Jones M, Jackson DG. LYVE-1, a new homologue of the CD44 glycoprotein, is a lymph-specific receptor for hyaluronan. J Cell Biol. 1999;144:789–801.
64. Hong YK, Harvey N, Noh YH, Schacht V, Hirakawa S, Detmar M, Oliver G. Prox1 is a master control gene in the program specifying lymphatic endothelial cell fate. Dev Dyn. 2002;225:351–7.
65. Karkkainen MJ, Haiko P, Sainio K, Partanen J, Taipale J, Petrova TV, Jeltsch M, Jackson DG, Talikka M, Rauvala H, et al. Vascular endothelial growth factor C is required for sprouting of the first lymphatic vessels from embryonic veins. Nat Immunol. 2004;5:74–80.
66. Bazigou E, Xie S, Chen C, Weston A, Miura N, Sorokin L, Adams R, Muro AF, Sheppard D, Makinen T. Integrin-alpha9 is required for fibronectin matrix assembly during lymphatic valve morphogenesis. Dev Cell. 2009;17:175–86.
67. Abtahian F, Guerriero A, Sebzda E, Lu MM, Zhou R, Mocsai A, Myers EE, Huang B, Jackson DG, Ferrari VA, et al. Regulation of blood and lymphatic vascular separation by signaling proteins SLP-76 and Syk. Science. 2003;299:247–51.
68. Uhrin P, Zaujec J, Bauer M, Breuss J, Alitalo K, Stockinger H, Kerjaschki D, Binder BR. Podoplanin-induced platelet aggregation mediates separation of blood an lymphatic vessels. Vasc Pharmacol. 2006;45:190.
69. Uhrin P, Zaujec J, Breuss JM, Olcaydu D, Chrenek P, Stockinger H, Fuertbauer E, Moser M, Haiko P, Fassler R, et al. Novel function for blood platelets and podoplanin in developmental separation of blood and lymphatic circulation. Blood. 2010;115:3997–4005.
70. Carramolino L, Fuentes J, Garcia-Andres C, Azcoitia V, Riethmacher D, Torres M. Platelets play an essential role in separating the blood and lymphatic vasculatures during embryonic angiogenesis. Circ Res. 2010;106:1197–201.
71. Bertozzi CC, Schmaier AA, Mericko P, Hess PR, Zou Z, Chen M, Chen CY, Xu B, Lu MM, Zhou D, et al. Platelets regulate lymphatic vascular development through CLEC-2-SLP-76 signaling. Blood. 2010;116:661–70.
72. Randolph GJ, Angeli V, Swartz MA. Dendritic-cell trafficking to lymph nodes through lymphatic vessels. Nat Rev Immunol. 2005;5:617–28.
73. Kerjaschki D. The lymphatic vasculature revisited. J Clin Invest. 2014;124:874–7.
74. Wang Y, Oliver G. Current views on the function of the lymphatic vasculature in health and disease. Genes Dev. 2010;24:2115–26.
75. Milroy WF. An undescribed variety of hereditary oedema. N Y Med J. 1892;56:505–8.
76. Ferrell RE, Levinson KL, Esman JH, Kimak MA, Lawrence EC, Barmada MM, Finegold DN. Hereditary lymphedema: evidence for linkage and genetic heterogeneity. Hum Mol Genet. 1998;7:2073–8.

77. Irrthum A, Karkkainen MJ, Devriendt K, Alitalo K, Vikkula M. Congenital hereditary lymphedema caused by a mutation that inactivates VEGFR3 tyrosine kinase. Am J Hum Genet. 2000;67:295–301.
78. Karkkainen MJ, Ferrell RE, Lawrence EC, Kimak MA, Levinson KL, McTigue MA, Alitalo K, Finegold DN. Missense mutations interfere with VEGFR-3 signalling in primary lymphoedema. Nat Genet. 2000;25:153–9.
79. Fang J, Dagenais SL, Erickson RP, Arlt MF, Glynn MW, Gorski JL, Seaver LH, Glover TW. Mutations in FOXC2 (MFH-1), a forkhead family transcription factor, are responsible for the hereditary lymphedema-distichiasis syndrome. Am J Hum Genet. 2000;67:1382–8.
80. Finegold DN, Kimak MA, Lawrence EC, Levinson KL, Cherniske EM, Pober BR, Dunlap JW, Ferrell RE. Truncating mutations in FOXC2 cause multiple lymphedema syndromes. Hum Mol Genet. 2001;10:1185–9.
81. Petrova TV, Karpanen T, Norrmen C, Mellor R, Tamakoshi T, Finegold D, Ferrell R, Kerjaschki D, Mortimer P, Yla-Herttuala S, et al. Defective valves and abnormal mural cell recruitment underlie lymphatic vascular failure in lymphedema distichiasis. Nat Med. 2004;10:974–81.
82. Irrthum A, Devriendt K, Chitayat D, Matthijs G, Glade C, Steijlen PM, Fryns JP, Van Steensel MA, Vikkula M. Mutations in the transcription factor gene SOX18 underlie recessive and dominant forms of hypotrichosis-lymphedema-telangiectasia. Am J Hum Genet. 2003;72:1470–8.
83. Bastaki F, Mohamed M, Nair P, Saif F, Tawfiq N, Al-Ali MT, Brandau O, Hamzeh AR. A novel SOX18 mutation uncovered in Jordanian patient with hypotrichosis-lymphedema-telangiectasia syndrome by whole exome sequencing. Mol Cell Probes. 2016;30:18–21.
84. Ma GC, Liu CS, Chang SP, Yeh KT, Ke YY, Chen TH, Wang BB, Kuo SJ, Shih JC, Chen M. A recurrent ITGA9 missense mutation in human fetuses with severe chylothorax: possible correlation with poor response to fetal therapy. Prenat Diagn. 2008;28:1057–63.
85. Wynd S, Melrose WD, Durrheim DN, Carron J, Gyapong M. Understanding the community impact of lymphatic filariasis: a review of the sociocultural literature. Bull World Health Organ. 2007;85:493–8.
86. Pfarr KM, Debrah AY, Specht S, Hoerauf A. Filariasis and lymphoedema. Parasite Immunol. 2009;31:664–72.
87. Taylor MJ, Hoerauf A, Bockarie M. Lymphatic filariasis and onchocerciasis. Lancet. 2010;376:1175–85.
88. Rockson SG. Lymphedema. Am J Med. 2001;110:288–95.
89. Vignes S, Arrault M, Bonhomme S, Spielmann M. Upper limb lymphedema revealing breast cancer. Rev Med Interne. 2007;28:631–4.
90. Brorson H. Liposuction in arm lymphedema treatment. Scand J Surg. 2003;92:287–95.
91. Rockson SG. Lymphedema. Vasc Med. 2016;21:77–81.
92. Tretbar LL, Morgan CL, Lee BB, Simonian SJ, Blondeau B, editors. Lymphedema: diagnosis and treatment. London: Springer; 2008.
93. Tammela T, Saaristo A, Holopainen T, Lyytikka J, Kotronen A, Pitkonen M, Abo-Ramadan U, Yla-Herttuala S, Petrova TV, Alitalo K. Therapeutic differentiation and maturation of lymphatic vessels after lymph node dissection and transplantation. Nat Med. 2007;13:1458–66.
94. Zhou Q, Guo R, Wood R, Boyce BF, Liang Q, Wang YJ, Schwarz EM, Xing L. Vascular endothelial growth factor C attenuates joint damage in chronic inflammatory arthritis by accelerating local lymphatic drainage in mice. Arthritis Rheum. 2011;63:2318–28.
95. Bouta EM, Bell RD, Rahimi H, Xing L, Wood RW, Bingham CO 3rd, Ritchlin CT, Schwarz EM. Targeting lymphatic function as a novel therapeutic intervention for rheumatoid arthritis. Nat Rev Rheumatol. 2018;14:94–106.
96. D'Alessio S, Correale C, Tacconi C, Gandelli A, Pietrogrande G, Vetrano S, Genua M, Arena V, Spinelli A, Peyrin-Biroulet L, et al. VEGF-C-dependent stimulation of lymphatic function ameliorates experimental inflammatory bowel disease. J Clin Invest. 2014;124:3863–78.
97. Schaverien MV, Aldrich MB. New and emerging treatments for lymphedema. Semin Plast Surg. 2018;32:48–52.
98. Alitalo A, Detmar M. Interaction of tumor cells and lymphatic vessels in cancer progression. Oncogene. 2012;31:4499–508.
99. Ma Q, Dieterich LC, Detmar M. Multiple roles of lymphatic vessels in tumor progression. Curr Opin Immunol. 2018;53:7–12.
100. Brown M, Assen FP, Leithner A, Abe J, Schachner H, Asfour G, Bago-Horvath Z, Stein JV, Uhrin P, Sixt M, Kerjaschki D. Lymph node blood vessels provide exit routes for metastatic tumor cell dissemination in mice. Science. 2018;359:1408–11.