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Angiogenesis in health and disease

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Blood vessels constitute the first organ in the embryo and form the largest network in our body but, sadly, are also often deadly. When dysregulated, the formation of new blood vessels contributes to numerous malignant, ischemic, inflammatory, infectious and immune disorders. Molecular insights into these processes are being generated at a rapidly increasing pace, offering new therapeutic opportunities that are currently being evaluated.

Vessel growth: modes and impact on health

Small blood vessels consist only of endothelial cells (ECs), whereas larger vessels are surrounded by mural cells (pericytes in medium-sized and smooth muscle cells (SMCs) in large vessels). Vessels can grow in several ways. Vasculogenesis refers to the formation of blood vessels by endothelial progenitors, angiogenesis and arteriogenesis refer to the sprouting and subsequent stabilization of these sprouts by mural cells, and collateral growth denotes the expansive growth of pre-existing vessels, forming collateral bridges between arterial networks. Both capillary angiogenesis and arterial growth are targets for therapy, as distal capillaries distribute the flow while proximal arterioles provide bulk flow to the tissue. When vessel growth is dysregulated, it has a major impact on our health and contributes to the pathogenesis of many disorders, some quite unexpected. Indeed, a long list of disorders is characterized or caused by excessive angiogenesis. Historically, the best known are cancer, psoriasis, arthritis and blindness, but many additional common disorders such as obesity, asthma, atherosclerosis and infectious disease are included, and the list is still growing (**Table 1**). Several congenital or inherited diseases are also caused by abnormal vascular remodeling (**Table 1**). In addition, insufficient vessel growth and abnormal vessel regression not only cause heart and brain ischemia, but can also lead to neurodegeneration, hypertension, pre-eclampsia, respiratory distress, osteoporosis and other disorders (**Table 2**). Few other processes have as daunting an impact as angiogenesis on the well-being of so many people worldwide. Recent advances in the understanding of molecular, genetic and cellular mechanisms of vessel growth and their possible implications for medicine will be discussed in this overview.

Endothelial progenitors

For many years, the prevailing dogma stated that vessels in the embryo developed from endothelial progenitors, whereas sprouting of vessels in the adult resulted only from division of differentiated ECs. Recent evidence, however, indicates that endothelial progenitors contribute to vessel growth both in the embryo and in ischemic, malignant or inflamed tissues in the adult, and can even be therapeutically used to stimulate vessel growth in ischemic tissues, a process termed 'therapeutic vasculogenesis'[1–3] (**Fig. 1**). ECs differentiate from angioblasts in the embryo[4] and from endothelial progenitor cells (EPCs), mesoangioblasts, multipotent adult progenitor cells, or side-population cells in the adult bone marrow[1,5]. EPCs can also contribute to vessel growth by releasing angiogenic growth factors[6]. ECs may also share a common origin with blood cells in the embryo and arise from the hemangioblast[4]. Endothelial and hematopoietic progenitors and their descendents share common markers, are affected by common signals, and influence each other. For instance, hematopoietic stem cells (HSCs) bud from hemogenic ECs in the embryo, and HSCs and leukocytes stimulate angiogenesis partly by releasing angiogenic factors or transdifferentiating to ECs[7–10]. Identification of the signals that recruit or differentiate these progenitors offers opportunities to manipulate their contributions to vascular growth. Vascular endothelial growth factor (VEGF), placental growth factor (PlGF, a homolog of VEGF), angiopoietin (Ang)-1, inhibitor of differentiation (Id) proteins, cytokines, and other signals have a role[9–12]. Overall, the functional contribution of EPCs and HSCs to pathological angiogenesis still remains largely undefined (see accompanying review in this issue[13]).

Vascular cell specification

Endothelial progenitors differentiate to mature ECs, but not all ECs are alike. One well-known anatomical and physiological distinction between vessels is that of arteries and veins. Not only do they differ in the blood pressure they sustain and the thickness of their SMC coat, but their ECs and SMCs also have a distinct identity and origin. For instance, SMCs surrounding some thoracic vessels are derived from neural crest, whereas coronary SMCs are derived from epicardium, and other SMCs arise from mesenchyme[14]. Little is known about the various pathways specifying the identity of arterial and venous SMCs, but recent genetic studies offer insight into the signals controlling arterial and venous identities of ECs. The Notch pathway, with its ligands (Delta-like-4, Jagged-1 and Jagged-2) and receptors (Notch-1, Notch-3 and Notch-4), promotes arterial fate of ECs by repressing venous differentiation[15,16]. Sonic Hedgehog and VEGF act upstream, whereas Gridlock probably acts downstream of Notch to determine arterial fate, even before the onset of flow[16,17]. ECs can differentiate into either arterial or venous ECs in embryonic development, in the neonatal retina and even in the adult heart, indicating that ECs have a remarkable phenotypic plasticity[18,19]. Selective use of arterial or venous ECs or their precursors may offer opportunities for therapeu-

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tic vasculogenesis. Notch signaling, however, is also critical for proper maintenance of arteries. Mutations of the SMC-specific Notch-3 receptor, which disrupt SMC anchorage to the extracellular matrix (ECM) and impair SMC survival, cause degeneration of cerebral arterioles, leading to cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy[20]. Besides Notch, bone-marrow tyrosine kinase and neuropilin-1 (a $VEGF_{164}$-specific receptor) also influence arterial specification[18]. By secreting VEGF, peripheral nerves codetermine arterial differentiation, providing a molecular explanation as to why arteries and nerves often run alongside each other in the body[21].

Blood vessels in various tissues have specialized functions, and ECs are therefore equipped with distinct properties—there might be even as many different EC types as there are organs in the body (see accompanying review in this issue[22]). What determines this EC heterogeneity and organ-specific angiogenesis? First, the expression and activity of general angiogenic factors such as VEGF or Ang-1 varies greatly in different tissues. Low-permeability tumors overexpress Ang-1 or underexpress VEGF (or both), whereas high-permeability tumors lack Ang-1 (ref. 23). Another example is the effect of Ang-1, which stimulates angiogenesis in the skin but suppresses vascular growth in the heart[19,24]. Second, organ-specific angiogenic factors determine the angiogenic switch, but in a restricted manner in particular organs (for example, blood vessel/epicardial substance and fibulin-2 in the heart, and endocrine gland VEGF and prokineticin-2 in endocrine glands[25]). Such organ-specific molecules hold great promise for use in developing safer angiogenic therapies. Tumor vessels also change their phenotype and express new addresses ('vascular zip codes'), which are absent or barely detectable in quiescent vessels[26]. Some vessels are not even lined by ECs: cytotrophoblasts line the maternal spiral arteries during normal placentation (a process termed 'pseudovasculogenesis'), SMCs line the neointima when re-endothelialization after vessel injury is incomplete, and malignant cells line some tumor vessels (a process called 'vascular mimicry'[27]).

Vascular boundaries and polarity

After endothelial progenitors differentiate into ECs and form a primitive vascular labyrinth, further remodeling of such primitive vessels into a more complex network requires the demarcation of arterial and venous boundaries, as well as the establishment of vascular polarity (**Fig. 1**). The Eph-Ephrin system is involved in the organization of such vascular boundaries. EphrinB2 marks arterial ECs and SMCs, whereas EphB4, a receptor for EphrinB2, marks only veins. EphrinB2-EphB4 signaling is critical for the establishment of arterial and venous identities, and participates in the formation of arteriovenous anastamoses by arresting EC migration at the arterial-venous interface[28–30]. Capillaries were long considered to lack any identity, but EphrinB2 expression extends into capillaries midway between terminal arterioles and postcapillary venules, indicating that they are either arterial or venous. As development proceeds, EphrinB2 expression extends also to SMCs in arteries. In pathological angiogenesis, ECs of some new vessels also express EphrinB2, contrary to the dogma that tumor vessels arise exclusively from postcapillary venules[31,32].

Very little is known about vascular polarity, yet many vessels, such as the large thoracic vessels, develop in an asymmetric pattern and are only present in the left or right side of the body. The embryonic pharyngeal arch arteries (PAA) initially develop symmetrically, but are subsequently remodeled asymmetrically into various large thoracic arteries. Because of its complexity, this process is often derailed, giving rise to congenital vascular malformations. Hotspots of VEGF expression around the PAAs are essential for their asymmetric remodeling. When VEGF expression is dysregulated, the left-side fourth PAA abnormally regresses, whereas the right-side fourth PAA, predetermined to regress otherwise, persists as a right-side aorta, giving rise to the typical vascular malformations and birth defects found in DiGeorge syndrome[33]. A combinatorial role of Ang-1 and the Tie-1 receptor seems to be essential in establishing the right-side venous system[34]. There are many vascular malformations, especially in neural tissue, that may result from 'misguiding' and aberrant patterning, but their etiology remains largely enigmatic. Another intriguing question is whether homeobox genes determine vascular identity, boundaries, polarity and patterning.

Angiogenesis and arteriogenesis

The nascent vascular bed expands by sprouting and matures into a system of stable vessels (**Fig. 1**). Hypoxia is an important stimulus for expansion of the vascular bed. Initially, cells are oxygenated by simple diffusion of oxygen, but when tissues grow beyond the limit of oxygen diffusion, hypoxia triggers vessel growth by signaling through hypoxia-inducible transcription factors (HIFs; see accompanying review in this issue[35]). HIFs upregulate many angiogenic genes, but the induction of VEGF is perhaps the most remarkable—up to 30-fold within minutes. VEGF stimulates physiological and pathological angiogenesis in a strict dose-dependent manner and is therefore currently being evaluated for pro- and antiangiogenic therapy (see accompanying review in this issue[36]). Loss of a single allele causes embryonic vascular defects[37,38], and reduction of VEGF levels by only 25% impairs spinal cord perfusion and results in motor neuron degeneration, reminiscent of amyotrophic lateral sclerosis[39]. PlGF, which binds Flt-1, enhances angiogenesis but only under pathological conditions. It amplifies VEGF-driven angiogenesis in part through a unique cross-talk between Flt-1 and Flk-1 (refs. 12,40). The role of VEGFB in angiogenesis remains to be determined. Besides VEGF family members, numerous other molecules have been documented to regulate EC growth, including growth factors, chemokines, cytokines, lipid mediators, hormones and neuropeptides (see below).

Table 1 Diseases characterized or caused by abnormal or excessive angiogenesis

Organ	Diseases in mice or humans
Numerous organs	Cancer (activation of oncogenes; loss of tumor suppressors); infectious diseases (pathogens express angiogenic genes[112], induce angiogenic programs[113] or transform ECs[114]); autoimmune disorders (activation of mast cells and other leukocytes)
Blood vessels	Vascular malformations (Tie-2 mutation[68]); DiGeorge syndrome (low VEGF and neuropilin-1 expression[33]); HHT (mutations of endoglin or ALK-1 (ref. 69)); cavernous hemangioma (loss of Cx37 and Cx40 (ref. [44])); atherosclerosis; transplant arteriopathy
Adipose tissue	Obesity (angiogenesis induced by fatty diet; weight loss by angiogenesis inhibitors[115])
Skin	Psoriasis, warts, allergic dermatitis, scar keloids, pyogenic granulomas, blistering disease, Kaposi sarcoma in AIDS patients[114]
Eye	Persistent hyperplastic vitreous syndrome (loss of Ang-2 (refs. 65,116) or VEGF164 (ref. 18)); diabetic retinopathy; retinopathy of prematurity; choroidal neovascularization (TIMP-3 mutation[51])
Lung	Primary pulmonary hypertension (germline BMPR-2 mutation; somatic EC mutations[73,75,76]); asthma; nasal polyps
Intestines	Inflammatory bowel and periodontal disease, ascites, peritoneal adhesions
Reproductive system	Endometriosis, uterine bleeding, ovarian cysts, ovarian hyperstimulation[25]
Bone, joints	Arthritis, synovitis, osteomyelitis, osteophyte formation[12]

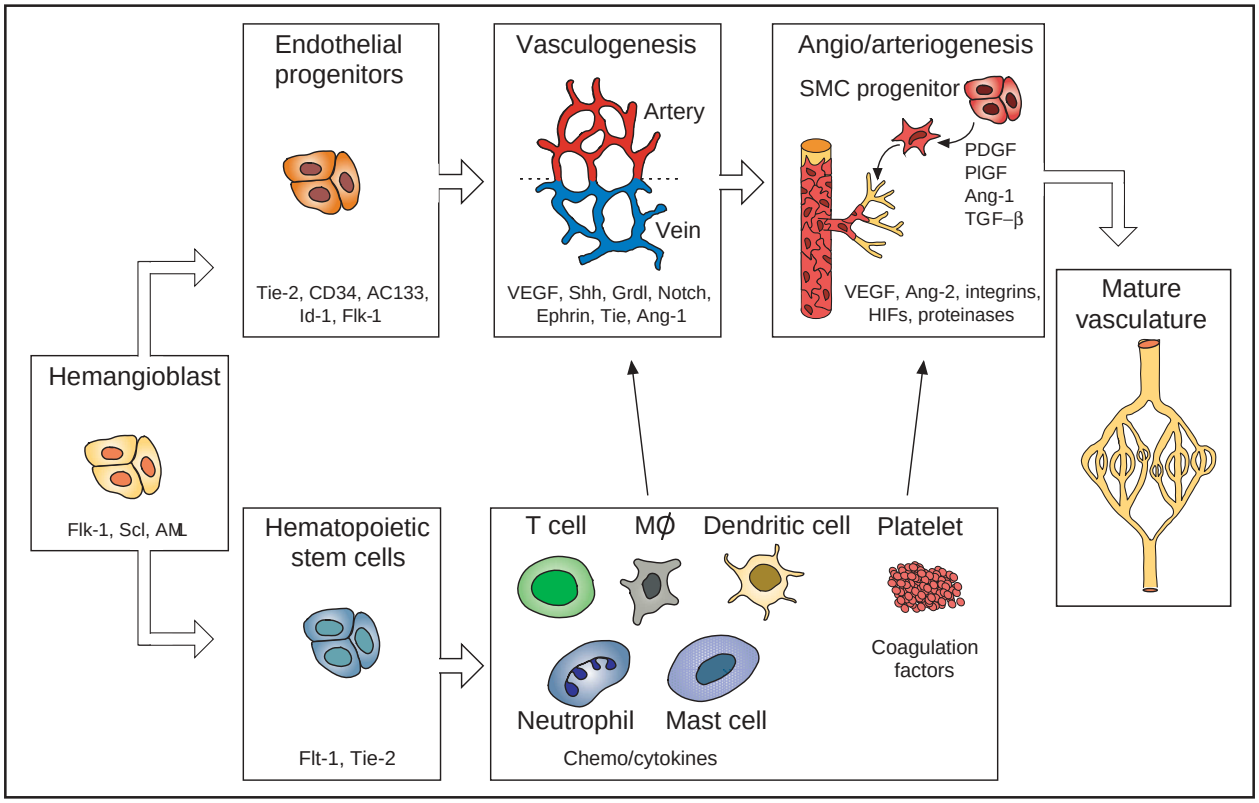

Figure 1 Formation of a vascular network. Endothelial progenitors differentiate to arterial and venous ECs, which assemble in a primitive capillary plexus. Vessels then sprout and become stabilized by SMCs, differentiating from their progenitors. HSCs contribute to angiogenesis directly and indirectly, by differentiating to leukocytes or platelets. A partial list of molecules is indicated; see text for additional information. Shh, Sonic hedgehog; Grdl, Gridlock; Mϕ, macrophage; AML, acute myeloid leukemia; Scl, stem cell leukemia.

ECs are elongated, thin and fragile cells, yet they build channels that do not collapse and that efficiently distribute blood to the various parts of the body. They also have long half-lives of several years, but when triggered are capable of rapidly sending out sprouts in a coordinated and directional manner. How can they possess all these qualities? It is partly because cells within the vessel wall communicate with each other and with cells inside and outside the vessel lumen. They sense changes in blood flow and pressure, and dynamically interact with the internal cytoskeleton and surrounding ECM, all in an integrated manner. Vascular cells are equipped with a set of molecules that allow them to perform these functions (see below). In quiescent vessels, vascular endothelial cadherin in adherens junctions and claudins, as well as occludin and JAM-1 in tight junctions, provide mechanical strength and tightness and establish a permeability barrier. These molecules do not only serve as 'mechanical zippers', but also transmit crucial signals for endothelial survival and other functions[41]. When ECs migrate during vessel sprouting, these contacts are transiently dissolved but later re-established, once ECs assemble a new sprout. Interrupting this cycle disrupts vessel assembly in tumors[42]. VEGF loosens, whereas Ang-1 tightens these contacts; the therapeutic potential of the latter is currently being evaluated in conditions of sepsis, inflammation, injury, stroke and cancer[43]. Homotypic ECs contacts through CD31 (PECAM) and intercellular communication through connexins (Cx) in gap junctions are also crucial for vessel formation and maintenance, as the loss of both Cx37 and Cx40 causes cavernous hemangiomas, and deficiency in Cx43 dysregulates coronary artery formation[44].

The ECM provides necessary contacts between ECs and the surrounding tissue, and thus prevents vessels from collapsing. In quiescent vessels, a basement membrane of collagen IV, laminin and other components encases vascular cells; pericytes and ECs are even embedded in the same basement membrane. An interstitial matrix of collagen I and elastin between vascular cells further provides visco-elasticity and strength to the vessel wall. The ECM also regulates the formation of new vessel sprouts. When vascular cells migrate to form new sprouts, this matrix network is not only proteolytically broken down, but its composition is also altered. Proteinases expose new cryptic epitopes in ECM proteins (such as in collagen IV) or change their structure (fibrillar versus monomer collagen), which induce EC and SMC migration[45]. In addition, a provisional matrix of fibronectin, fibrin and other components provides a support scaffold, guiding ECs to their targets. Integrins are cell-surface receptors of specific ECM molecules that, by bidirectionally transmitting information between the outside and inside of vascular cells, assist vascular cells to build new vessels in coordination with their surroundings[46,47]. The $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins have long been considered to positively regulate the angiogenic switch, because their pharmacological antagonists suppress pathological angiogenesis. Genetic deletion studies suggest, however, that vascular integrins inhibit angiogenesis by suppressing VEGF- and Flk-1-mediated EC survival, by *trans*-dominantly blocking other integrins or by mediating the antiangiogenic activity of thrombospondins (TSPs) and other angiogenesis inhibitors (such as tumstatin, endostatin, angiostatin and PEX). It remains to be determined whether and under what conditions integrins have positive or negative roles in angiogenesis.

Remodeling of the ECM during vessel sprouting requires breakdown by proteinases, including plasminogen activators (such as urokinase plaminogen activator (uPA) and its inhibitor, PAI-1), matrix metallopro-

teinases (MMPs and tissue inhibitors of metalloproteinases (TIMPs)), heparinases, chymases, tryptases and cathepsins[48–50]. Proteinases also facilitate EC sprouting by liberating matrix-bound angiogenic activators (basic fibroblast growth factor (FGF), VEGF and transforming growth factor (TGF)-β) and proteolytically activating angiogenic chemokines (such as IL-1β). Their activity is, however, not always related to proteolysis, as shown for uPA receptor and TIMP-3 (refs. 51,52). When considering the critical role of the ECM in vessel growth and maintenance, it is conceivable that proteolytic remodeling of the ECM must occur in a balanced manner. Insufficient breakdown prevents vascular cells from leaving their original position, but excessive breakdown removes critical support and guidance cues for migrating ECs and, in fact, inhibits angiogenesis[50,53]. Proteinases can also have a role in the resolution of angiogenesis, as they liberate matrix-bound inhibitors (TSP-1, canstatin, tumstatin, endostatin and platelet factor (PF)-4) and inactivate angiogenic cytokines (such as stromal cell–derived factor-1). These pleiotropic activities may explain why proteinases and their receptors and inhibitors often have activities that are context- and concentration-dependent. It may also explain why an inhibitor such as PAI-1 is a predictor of poor, not good, clinical outcome for many cancers[50,53].

Establishment of a functional vascular network further requires that nascent vessels mature into durable vessels (**Fig. 2**). The association of pericytes and SMCs with newly formed vessels regulates EC proliferation, survival, migration, differentiation, vascular branching, blood flow and vascular permeability (see accompanying review in this issue[54]). Platelet-derived growth factor (PDGF)-BB and its receptor, PDGFR-β, have essential roles in the stabilization of nascent blood vessels by recruiting PDGFR-β-positive mesenchymal progenitors. Dropout or insufficient recruitment of mural cells results in EC growth, permeability, fragility, vessel enlargement, bleeding, impaired perfusion and hypoxia in embryos lacking PDGF-B[55], in retinas of diabetics, in tumors[56] and in hemangiomas, which are the nonmalignant vascular tumors that rapidly enlarge in infants and often spontaneously regress[57]. The subsequent increase in VEGF further aggravates vascular permeability and edema, and promotes hemangioma formation. In contrast, a combination of PDGF-BB and VEGF results in the formation of more mature vessels than monotherapy with either factor, a finding relevant for future development of therapeutic angiogenesis strategies[58]. PDGF-CC and PDGF–DD also promote angiogenesis, but their roles remain less well characterized[59].

Another signaling system involved in vessel maintenance, growth and stabilization is the Tie-2 receptor, which binds the angiopoietins (Ang-1 and Ang-2). Unlike Ang-2, which activates Tie-2 on some cells but blocks Tie-2 on others, Ang-1 consistently activates Tie-2. Even though trapping angiopoietins suppresses pathological vascularization[60], their role is pleiotropic and context-dependent. Ang-1 stimulates vessel growth in skin, ischemic limbs, gastric ulcers and in some tumors[23,61], presumably because it is an EC survival factor and mobilizes EPCs and HSCs[62]. But Ang-1 also suppresses angiogenesis in tumors and the heart[19,63]. Although it is still not entirely understood, the antiangiogenic effect of Ang-1 may relate to the fact that vessels must loosen up before ECs can migrate; if vessels are too tight, vessel sprouting may be impeded. Ang-1 tightens vessels by affecting junctional molecules[43] and by promoting the interaction between ECs and mural cells as an adhesive protein and recruiting pericytes[64]. Ang-2 has been proposed to stimulate the growth of immature (SMC-poor) tumor vessels by loosening endothelial-periendothelial cell interactions and degrading the extracellular matrix, thereby antagonizing Ang-1 (refs. 63,65). The angiogenic activity of Ang-2 seems to be contextual as well, however. Ang-2 synergizes with VEGF to stimulate angiogenesis in the heart[19] but, when insufficient angiogenic

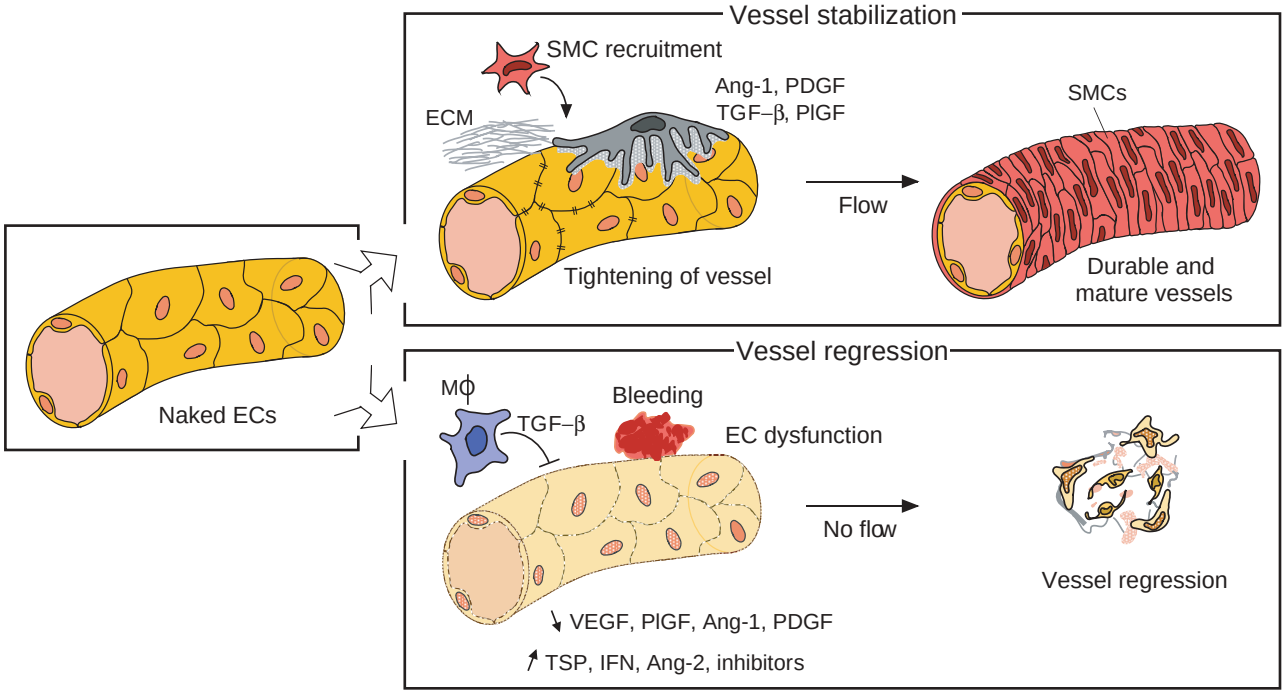

Figure 2 Vessel maintenance versus vessel regression. Nascent vessels initially only consist of ECs. Upper panel: vessel maturation requires a mix of angio- and arteriogenic factors for a sufficient duration, so that ECs can tighten up and become covered by mural cells and ECM. Flow is a critical determinant of vessel maintenance and durability. Lower panel: when insufficient angio- and arteriogenic factors are present and angiogenesis inhibitors are present, EC channels remain naked, leaky and fragile, are easily ruptured and bleed—conditions that reduce flow and result in vessel regression. A partial list of molecules is indicated; see text for additional information.

signals are present, Ang-2 causes EC death and vessel regression[66,67]. A precise balance of Tie-2 signals thus seems critical, as an activating Tie-2 mutation causes venous malformations that are composed of dilated, serpiginous endothelial channels covered by a variable amount of SMCs[68].

Additional signaling molecules, such as members of the TGF-β superfamily, contribute to the resolution and maturation phases of angiogenesis, but in a pleiotropic manner. TGF-β family ligands stimulate type II receptors that, in turn, phosphorylate type I receptors (such as activin receptor–like kinase (ALK)) and activate the downstream signaling Smads[69]. Endoglin is a type III receptor, which facilitates binding of TGF-β1 to the type II receptors. Both pro- and antiangiogenic properties have been ascribed to TGF-β1, through effects on ECs and other cell types. At low doses, TGF-β1 contributes to the angiogenic switch by upregulating angiogenic factors and proteinases, whereas at high doses, TGF-β1 inhibits EC growth, promotes basement membrane reformation and stimulates SMC differentiation and recruitment. Hereditary hemorrhagic telangiectasia (HHT), characterized by telangiectasias and arterio-venous malformations, has been associated with loss-of-function mutations of endoglin (HHT-1) and ALK-1 (HHT-2)[69]. Because interpretations of the respective roles of ALK-1 (with Smad1 and Smad5) and ALK5 (with Smad2 and Smad3) in the activation or resolution phases of angiogenesis differ, the precise mechanisms of the vascular abnormalities of HHT lesions remain uncertain[69–71]. Nevertheless, an imbalance between vessel growth and maturation seems to cause the excessive fusion of capillary plexi into cavernous vessels and the hyperdilation of large vessels[72]. Mutations in the type II bone morphogenetic protein receptor (BMPR)-2 gene, also belonging to the TGF-β superfamily, cause primary pulmonary hypertension, in which pulmonary arterioles become occluded by intravascular endothelial tumors[73]. By downregulating BMPR-1A (mediating BMPR-2 signaling), increased Ang-1 levels may further contribute to primary pulmonary hypertension by recruiting SMCs around pulmonary vessels[74]. In other primary pulmonary hypertension subjects, ECs acquire somatic mutations that lead to 'misguided angiogenesis'[75,76].

Table 2 Diseases characterized or caused by insufficient angiogenesis or vessel regression

Organ	Disease in mice or humans	Angiogenic mechanism
Nervous system	Alzheimer disease	Vasoconstriction, microvascular degeneration and cerebral angiopathy due to EC toxicity by amyloid-β[117]
	Amyotrophic lateral sclerosis; diabetic neuropathy	Impaired perfusion and neuroprotection, causing motoneuron or axon degeneration due to insufficient VEGF production[39]
	Stroke	Correlation of survival with angiogenesis in brain[118]; stroke due to arteriopathy (Notch-3 mutations[20])
Blood vessels	Atherosclerosis	Characterized by impaired collateral vessel development[119]
	Hypertension	Microvessel rarefaction due to impaired vasodilation or angiogenesis[105]
	Diabetes	Characterized by impaired collateral growth[120] and angiogenesis in ischemic limbs[121], but enhanced retinal neovascularization secondary to pericyte dropout
	Restenosis	Impaired re-endothelialization after arterial injury at old age[122]
Gastrointestinal	Gastric or oral ulcerations	Delayed healing due to production of angiogenesis inhibitors by pathogens[123].
	Crohn disease	Characterized by mucosal ischemia
Skin	Hair loss	Retarded hair growth by angiogenesis inhibitors[124]
	Skin purpura, telangiectasia and venous lake formation	Age-dependent reduction of vessel number and maturation (SMC dropout) due to EC telomere shortening[125]
Reproductive system	Pre-eclampsia	EC dysfunction resulting in organ failure, thrombosis and hypertension due to deprivation of VEGF by soluble Flt-1 (ref. 126)
	Menorrhagia (uterine bleeding)	Fragility of SMC-poor vessels due to low Ang-1 production[127]
Lung	Neonatal respiratory distress	Insufficient lung maturation and surfactant production in premature mice due to reduced HIF-2α and VEGF production[128]
	Pulmonary fibrosis, emphysema	Alveolar EC apoptosis upon VEGF inhibition[129]
Kidney	Nephropathy	Age-related vessel loss due to TSP-1 production[130]
Bone	Osteoporosis, impaired bone fracture healing	Impaired bone formation due to age dependent decline of VEGF- driven angiogenesis [131]; angiogenesis inhibitors prevent fracture healing[132]

Collateral growth

Unlike distal capillaries, which distribute blood flow to individual cells, arteries provide bulk flow to the tissue and are therefore of utmost importance. When an artery is occluded, its vascular territory becomes ischemic. Because arterial systems are often interconnected by pre-existing collateral vessels, however, the collaterals can enlarge and salvage the ischemic region[77]. The mechanisms of angiogenesis and collateral growth differ significantly. Because of the large pressure differences between the perfusion territories, the increased shear stress activates ECs, which then recruit monocytes. These cells produce growth factors and proteinases (uPA and MMPs), which enable SMCs to migrate and divide, explaining why depletion of monocytes impairs, whereas delivery of monocytes enhances, collateral growth[78,79]. Cytokines that attract monocytes or prolong their life span (such as monocyte chemoattractant protein (MCP)-1, granulocyte-macrophage colony-stimulating factor, TGF-β1 and tumor necrosis factor-α) enhance collateral growth, whereas anti-inflammatory cytokines (such as IL-10) are inhibitory[80–83]. PlGF also enhances collateral growth, not only because it recruits monocytes, but also because it stimulates EC and SMC growth[12,84]. Delivery of acidic FGF, FGF-4 or basic FGF (together with PDGF-BB) stimulates collateral growth, in part by upregulating PDGFR expression[85]. VEGF alone seems to affect capillary angiogenesis more efficiently than collateral growth, explaining, at least in part, why results of clinical trials have not been more positive[77,86]. Coadministration of VEGF with additional molecules such as PDGF, PlGF or Ang-1 may enhance its therapeutic potential (ref. 58 and P.C., unpublished data). The identification of molecules

regulating collateral growth offers significant potential for the treatment of ischemic heart and limb disease.

Leukocytes and angiogenesis

Inflammation- and immune-driven angiogenesis affect numerous disorders (**Tables 1** and **2**), in part because most leukocyte subtypes produce a myriad of angiogenic factors such as VEGF, PlGF, PDGF, basic FGF, Ang-2, epidermal growth factor, TGF-β1, MCP-1 and various interleukins and proteinases (tryptase, chymase, MMPs, heparanase and uPA; **Fig. 1**)[87,88]. Leukocytes affect many angiogenic processes. For instance, neutrophils and natural killer cells have been implicated in cyclical uterine angiogenesis, and in abnormal angiogenesis in endometriosis[89], whereas tumor-associated macrophages promote cancer by releasing angiogenic factors and inducing tumor cells to release angiogenic factors[90]. Mast cells, when they encounter allergens and pathogens in the skin and mucosa, release vasoactive and angiogenic factors, thereby affecting autoimmune diseases in many organs. Mast cells also infiltrate skin carcinomas, where they hyperactivate angiogenesis through chymase-dependent activation of MMP-9 (ref. 91). Type I dendritic cells help eradicate tumors through immune stimulation and suppression of tumor angiogenesis[92]. Monocytes are a source of EPCs[6] and can differentiate into endothelial-like cells[93]. Because leukocytes also generate angiogenesis inhibitors, their overall role in initiating or terminating angiogenesis depends on the temporal and spatial balance of these modulators.

Leukocytes and vascular cells influence each other in other ways (**Fig. 1**). Angiogenic factors amplify the inflammatory process by recruiting leukocytes and affecting their function[12]. For instance, VEGF enhances, whereas TSP-1 and Ang-1 forestall, T-cell-dependent allograft arteriopathy by reducing leukocyte infiltration[94]. VEGF promotes cancer, not only by stimulating angiogenesis, but also by inhibiting the functional maturation of dendritic cells and enhancing adhesion of natural killer cells to tumor microvessels[95,96]. Other angiogenic molecules (such as PlGF, TGF-β1, PDGF and FGFs) also modulate leukocyte function[12]. Because of the significant involvement of leukocytes, anti-inflammatory drugs suppress pathological angiogenesis[97]. Another class of candidates are chemokines, which recruit leukocytes and directly stimulate ECs. These include growth-related oncogene, IL-8, stromal cell–derived factor-1, MCP-1 and others that bind CXCR2 and CXCR4 receptors[98].

Coagulation and angiogenesis

Fibrin-rich clot formation and platelet aggregation precede infiltration of blood vessels into a wound. Not surprisingly, therefore, hemostasis and angiogenesis are closely linked[99–101] (**Fig. 1**). Upon activation, platelets release large stores of angiogenic factors such as VEGF, PDGF, TGF-β, IL-6, thrombin and sphingosine-1-phosphate. The latter stimulates the growth and stability of nascent vessels by tightening their junctions and recruiting mural cells[102]. Platelets also contain antiangiogenic factors (TSP-1, PF-4 and others) that may have a role in the resolution of angiogenesis once the wound has healed. The link between angiogenesis and hemostasis also has implications for cancer. Thromboembolism is a common cause of death in cancer patients. By covering tumor cells, platelets protect tumor emboli from immune surveillance and promote their lodging at distant metastatic sites. In many tumors, production of tissue factor, initiation of coagulation, and microvessel density are closely associated[101]. Tissue factor upregulates VEGF, downregulates TSP-1 and, by initiating coagulation, generates additional angiogenic pathways that are dependent on factor Xa, thrombin, the protease-activated receptors (PAR-1, PAR-2, PAR-3 and PAR-4) and fibrin[100]. The incidence of thrombosis in cancer patients treated with angiogenesis inhibitors may be attributable to EC dysfunction and death, platelet activation, the release of tumor procoagulants and cytokines upon tumor lysis, and an inflammatory response[99].

Vessel regression

Vessel regression, a physiological mechanism to match perfusion with metabolic demand, occurs when the nascent vasculature consists of too many vessels. Vessel regression also constitutes the basis of many antiangiogenic therapeutic strategies. Abnormal vessel regression also contributes to the pathogenesis of numerous disorders, however. Several mechanisms shift the angiogenic switch from 'on' to 'off' (**Fig. 2** and **Table 2**). Removal of angiogenic stimuli causes vessels to regress, as in tumors[103] and the heart[104], especially when vessels have only been recently assembled and are still immature. When angiogenic stimuli are provided for a sufficient length of time, new vessels mature and persist for months, even after the angiogenic stimulus is withdrawn[104]. Flow may have an important role in determining whether neovessels regress or persist. By affecting several factors (including MMPs, PDGF, basic FGF, integrins and nitric oxide), flow stimulates hyperplasia of ECs and SMCs, and induces the reorganization of endothelial junctions and the deposition of ECM—all of which contribute to vessel maturation. Thus, insufficient perfusion may lead to regression, whereas sufficient perfusion promotes vessel persistence. An abnormal sensitivity of small arterioles to vasoconstrictor stimuli may lead to functional constriction and subsequent structural rarefaction of nonperfused 'ghost arterioles' in hypertension[105]. Pericytes also determine the susceptibility of vessels to regression. Indeed, once vessels are surrounded by pericytes, they become resistant to oxygen-induced regression[103]. Delivery of PlGF or VEGF with PDGF-BB causes vessel maturation and results in the persistence of stable, durable vessels for more than a year[12,58]. In contrast, disruption of endothelial-pericyte associations results in the regression of vessels[106].

Angiogenesis inhibitors also contribute to vessel regression. TSP-1 inhibits angiogenesis through direct effects on ECs and indirect effects on growth factor mobilization or activation[107]. Upregulation of endogenous TSP-1 and TSP-2 contributes to the resolution of angiogenesis and vessel stabilization after ischemia, and forced overexpression of TSP-1 or TSP-2 in cancer cells results in reduced tumor vascularization and tumor growth[107]. There are more angiogenesis inhibitors, however. When VEGF levels are low, Ang-2 marks regressing vessels[108]; interferons exert angiostatic effects by lowering the expression of basic FGF and VEGF. Macrophages (such as hyalocytes in the eye) contribute to vessel regression by releasing TGF-β1 (ref. 109). Inhibitory PAS domain protein, a splice variant of HIF-3α, functions as a dominant-negative regulator of hypoxia-induced angiogenesis to maintain an avascular phenotype in certain tissues[110]. Additional inhibitors include chemokines binding CXCR3 (such as PF-4, Mig, interferon-inducible protein-10 and others)[98], soluble receptors (Flt-1 and Tie-2), clotting antagonists and others. A growing list of inhibitors is being discovered, including cleavage products of matrix components (such as arresten, canstatin and tumstatin from collagen IV; vastatin from collagen VIII; restin from collagen XV; and endostatin from collagen XVIII), proteinases or enzymes (such as PEX from MMP2; mini-TrpRS from tryptophanyl-tRNA synthetase) or plasma proteins (such as angiostatin from plasminogen; 16K prolactin from prolactin; and fragments of several serpins)[111]. The endogenous roles of many of these cleavage products in physiological and pathological angiogenesis remain enigmatic. Nevertheless, they offer opportunities to suppress tumor angiogenesis and growth when administered.

Conclusion

Historically, angiogenesis was initially only implicated in cancer, arthritis and psoriasis. In recent years it has, however, become increasingly evident that excessive, insufficient or abnormal angiogenesis contributes to the

pathogenesis of many more disorders. Ongoing clinical trials reveal that both pro- and antiangiogenic treatments with single angiogenic molecules is more challenging than anticipated, and monotherapy with a single angiogenesis inhibitor may not suffice to combat the myriad of angiogenic factors produced by cancer cells. This may not be surprising, however, when one considers that building new, functional and durable vessels requires a complex interplay of multiple molecular signals. The challenge for the coming years is thus to define the molecular basis and pathways of angiogenic disorders in greater detail and in a more integrated manner, so that the excitement of the science can be converted into the development of efficient, safe therapies.

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