

Angiogenesis and lymphangiogenesis: highlights of the past year

Aernout Luttun and Peter Carmeliet

Purpose of review

The purpose of this review is not to provide an extensive overview of well-established mechanisms of angiogenesis and lymphangiogenesis but rather to highlight several recent key studies that constituted a significant conceptual or medical advancement to the field during the past year or so. The authors apologize for their inability, because of space restrictions, to reference all other relevant work of the past or previous years.

Recent findings

In 1993, fewer than 400 studies on angiogenesis were published. During the past year alone, more than 4000 angiogenesis studies were reported, making angiogenesis one of the most rapidly growing fields. Moreover, the first studies on lymphangiogenesis were published only a couple of years ago. A milestone in the field in the past year has been the first successful report that the angiogenesis inhibitor bevacizumab (Avastin), an antibody against vascular endothelial growth factor, prolonged the survival of colorectal and renal cancer patients in phase 3 clinical trials. This remarkable achievement provides great promise and hope for the future development of therapeutic strategies to inhibit or stimulate angiogenesis.

Summary

The intensive search for antiangiogenic and proangiogenic mechanisms during the past decade is starting to translate into clinical promise. Further discovery of novel pathways and concepts in angiogenesis may lead to the optimization and refinement of current strategies to improve the clinical benefit and therapeutic safety for a vast number of patients with angiogenesis-related disease.

Keywords

angiogenesis, vasculogenesis, collateral growth, placental growth factor, vascular endothelial growth factor

Abbreviations

EC	endothelial cell
EPC	endothelial progenitor cell
FGF	fibroblast growth factor
HIF	hypoxia-inducible factor
MMP	matrix metalloproteinase
PDGF	platelet-derived growth factor
PIGF	placental growth factor
PH	prolyl hydroxylase
TIMP	tissue inhibitor of matrix metalloproteinase
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor

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Introduction

Disease is increasingly associated with abnormal vascularity, affecting the blood and/or lymphatic vessels, and therefore the understanding of how vessels form has become a largely studied topic. Many efforts have been taken to translate this knowledge into clinical benefit, with variable success. Improving therapeutic efficacy may depend on identifying new concepts, mechanisms, and molecular and cellular players in (lymph)angiogenesis. The mechanisms of angiogenesis and lymphangiogenesis have been recently reviewed [1•–4•]. The following overview highlights some of the most recent findings in (lymph)angiogenesis that may help to design effective and safe therapies.

Endothelial progenitors

The existence of circulating endothelial progenitor cells (EPCs) in the adult has been repeatedly demonstrated, but their origin, molecular identity, and actual contribution to new vessel growth in ischemia, wound healing, or cancer still remain controversial [5•,6]. For instance, the contribution of EPCs to the newly formed tumor vasculature has been reported to be negligible or as high as 40 to 50% [7•–9•,10]. Tumor location may play a role, because EPCs may have greater access to the growing vasculature in subcutaneously implanted tumors than in brain tumors, given that the tight blood-brain barrier impedes their access [7•,10]. Also, the dependency on EPCs for tumor vessel growth may be intrinsic to the tumor type and its production of mobilization signals for EPCs [11•]. Apart from EPCs, hematopoietic (progenitor) cells expressing endothelial markers (such as Tie-2) may also contribute to the building of the tumor vasculature, and EPCs may, in fact, have a monocytic origin [8•,11•,12]. Mathematic models may be helpful in cal-

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Center for Transgene Technology and Gene Therapy, Flanders Interuniversity Institute for Biotechnology, Leuven, Belgium

Correspondence to P. Carmeliet, Center for Transgene Technology and Gene Therapy, Flanders Interuniversity Institute for Biotechnology, KULeuven, Campus Gasthuisberg, Herestraat 49, B-3000, Leuven, Belgium
Tel: +32 16 34 57 72; fax: +32 16 34 59 90; e-mail: peter.carmeliet@med.kuleuven.ac.be

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culating the contribution of EPCs to tumor vessel growth [9•]. Adverse metabolic stress factors in type 1 diabetes are associated with reduced EPC numbers, suggesting that EPC dysfunction may participate in the pathogenesis of diabetic vascular complications [13•]. EPC mobilization is also impaired in an age-dependent manner [14•]. The therapeutic potential of angiogenesis inhibitors such as vascular endothelial growth factor (VEGF) antibody or endostatin may, at least in part, also depend on their ability to suppress the recruitment of EPCs [15,16••]. Lymphatic endothelial progenitors have been also identified, but it remains unknown whether they contribute to growing lymphatic vessels in cancer [17•].

Artery or vein?

Arteries and veins are morphologically and functionally distinct vessels. Both vessel types are molecularly defined, and this molecular signature is acquired before they become functional and carry blood. Arterial endothelial cells (ECs) express EphrinB2 and the activin-like receptor-1, while venous ECs exclusively express EhpB4 [18–20]. The restricted expression of the activin-like receptor-1 in arteries suggests that the vessel defects in hemorrhagic telangiectasia-2, characterized by an activin-like receptor-1 mutation, may be arterial rather than venous in origin [19]. Recent gene profiling has further revealed that arterial and venous ECs express an entire set of distinct and specific genes [21•]. Additional studies, particularly in zebrafish embryos, have revealed a molecular cascade by which arterial identity is induced, at the expense of venous fate [22]. When Notch signaling is switched on, it represses venous cell fate—the latter being the default pathway [23,24]. Probably, gridlock acts as a downstream effector, because knockdown in zebrafish results in specific arterial—not venous—abnormalities [23,24]. Sonic hedgehog and VEGF (through phospholipase C γ -1 signaling) act further upstream from Notch/gridlock for arteriovenous specification [25•–27•,28]. VEGF also regulates the expression of Notch1 in arterial ECs [29]. These findings suggest that certain angiogenic molecules may selectively stimulate the growth of either arterial or venous ECs.

Lymphangiogenesis

The lymphatic vascular network drains extravasated fluid and proteins back into the venous circulation, plays a role in the immune response, and is an escape route for metastasizing cancer cells. The molecular basis of lymphangiogenesis has remained elusive for many years, in part because of a lack of molecular markers, cells, and models. The availability of procedures to isolate lymphatic EC cultures has now allowed the identification of vascular lineage-specific genes by transcriptional profiling of isolated blood vascular and lymphatic ECs and has confirmed the master role of the transcription factor Prox-1 in the transdifferentiation of blood vascular to

lymphatic endothelium [30•,31•]. Recent gene inactivation studies have revealed the distinct role of neuropilin-2, podoplanin, angiopoietin-2, VEGF-C, and a hematopoietic signaling pathway via spleen tyrosine kinase and SH2 domain-containing leukocyte protein, molecular mass 76 in lymphangiogenesis [32–34,35••,36]. Together, these studies suggest that Prox-1 is necessary for venous endothelium to transdifferentiate into lymphatic endothelium, whereas VEGF-C and angiopoietin-2 regulate subsequent sprouting and remodeling into a more mature lymphatic vascular network. A recent knockdown study of VEGF-C in zebrafish reveals a role for VEGF-C in vasculogenesis and angiogenesis, but its role in lymphangiogenesis could not be studied because zebrafish lack well-developed lymphatic vessels [37]. Remarkably, only a few years after this field emerged, the first lymphangiogenic (gene) therapeutic strategies, using VEGF-C or VEGF-D, for lymphedema were developed and are currently being evaluated in clinical trials [38•,39–41]. Progress has been made, but no consensus reached, about the presence and functionality of lymphatic vessels in and around tumors and their role in tumor metastasis, although the expression levels of VEGF-C and VEGF-D generally seem to correlate with tumor progression and metastasis [4•,42–47].

Vascular bed specificity and endothelial heterogeneity

The distinct functional and molecular characteristics of arterial and venous ECs indicate that the endothelial population is heterogeneous. However, vascular beds in different organs also differ and exhibit specific characteristics, rendering therapeutic vessel formation or inhibition an even greater challenge. Indeed, endothelium in brain is different from that in endocrine glands, bone marrow, lungs, heart, or tumors. Not only have these vascular beds distinct molecular profiles, but we now know that certain tissues express vascular bed-specific angiogenic factors [3•,48–51]. For instance, endocrine gland-VEGF and Bv8 (also known as prokineticin-1 and -2, respectively) are specifically expressed in some endocrine glands, VEGF-C and VEGF-D mainly, but not exclusively, stimulate endothelial growth in the lymphatic system, and blood vessel/epicardial substance (bves) and fibulin-2 are present in the cardiac vasculature [2•,4•,49,52•,53,54]. In addition, signaling mechanisms may differ among different vascular beds. For example, the transcription factor Ets-1 is expressed in blood vascular but not lymphatic vessels [55]. The endothelium seems to acquire some of these tissue-specific characteristics through local cues. For instance, in the rodent brain, astrocytes help establish the blood-brain barrier by secreting src-suppressed C-kinase substrate, which alters the VEGF/angiopoietin balance and induces tight junction formation in neighboring ECs [56].

Neural guidance of angiogenic sprouts

The ECs divide and migrate when forming new sprouts, but the molecular basis of how they navigate to their targets was not much studied in the past. Elegant studies in the mouse retina have revealed that ECs at the leading edge of angiogenic sprouts (the “tip” cells) respond differently to gradients of angiogenic growth factors such as VEGF than those deeper in the sprout (the “stalk” cells). Whereas the latter proliferate, the former do not, but rather migrate and extend filopodia [57•]. These functional differences are likely attributable to different molecular mechanisms and expression patterns. For instance, only sprouting invasive ECs express reduced levels of an orphan 90-kDa membrane glycoprotein, which can be immunostained with the OX-43 antibody [58]. Interestingly, in the absence of WASP-verprolin homologous protein2, ECs failed to form lamellipodia, and knockout mice succumbed at embryonic day 10 because of defective capillary sprouting and branching [59]. WASP-verprolin homologous protein 2 is predominantly expressed in ECs during development and is crucial for Rac-induced membrane ruffling, a process necessary for cell motility. Neural guidance signals may also affect vessel guidance [60••]. At least four categories have been described: netrins and their receptors of the deleted in colorectal cancer (DCC) family (*ie*, DCC and neogenin; receptors of the UNC5 family; and the adenosine A2b receptor), semaphorins (the ligands for neuropilins/plexins), ephrins (and their corresponding Eph receptors), and Slits (interacting with Robo or roundabout receptors). Most of those neural guidance signals, except for netrins, have recently been endowed with a role in angiogenesis. For instance, ribozymes cleaving the adenosine A2b receptor block retinal neovascularization in mice [61]. Semaphorin3A affects vascular development as a chemorepulsive signal by mediating EC detachment from integrin binding sites, although mouse knock-in studies show that semaphorin3A/neuropilin-1 signaling is not critical for general vascular development [62••,63]. Plexin-D1 is expressed in the early embryonic mouse vasculature, suggesting a role in vascular development [64]. The role of the reciprocal Eph/Ephrin interactions in arteriovenous specification and boundary formation is now well established [2•,65]. Slit-Robo interactions mediate crosstalk between cancer cells (secreting Slit2) and Robo-1 expressing ECs, likely facilitating EC guidance into the tumor. Interestingly, Robo-1 acts as an EC attractant, whereas it generally functions as a repellent for neurons [66••]. Neurotransmitters such as neuropeptide Y may also have angiogenic properties, as evidenced by the reduced vascular growth in mice lacking the neuropeptide Y receptor Y₂ or treated with Y₂ antagonists [67–69].

Angiogenic factors: “organ”-izers with nonvascular effects?

A principal function of blood vessels is to supply oxygen and nutrients, but blood vessels may also play critical

roles in the development and homeostasis of organs, in part because angiogenic factors have direct effects on nonvascular cell types. Extending previous work that vasculogenic ECs and nascent vessels are critical for the earliest stages of liver and pancreas organogenesis, prior to blood vessel function, VEGF was also found to have a direct trophic effect on pulmonary epithelial cells during lung maturation before birth [3•,70•]. Moreover, by inducing ECs to release hepatocyte growth factor, selective activation of VEGF receptor-1 (VEGFR-1) provides angiogenesis-independent endothelial protection of the liver [71•]. VEGF also affects neural cells directly [60••]. For instance, VEGF stimulates neurogenesis, protects ischemic neural cells and diseased motoneurons, and affects ion channel function in neurons [72–76]. Remarkably, low VEGF levels in mice and humans predispose to motoneuron degeneration, which is reminiscent of the incurable disease amyotrophic lateral sclerosis [77••, 78••]. VEGF also plays a role in additional neurodegenerative disorders [79]. The fact that certain angiogenic molecules affect neural function cautions some warrant for careful design of antiangiogenic strategies in patients with neurologic deficits [77••,79].

Oxygen and angiogenesis

Hypoxia is an important stimulus of angiogenesis, both in health and in disease. Low oxygen levels activate type α hypoxia-inducible transcription factors (HIF-1 α , -2 α , -3 α), which bind as heterodimers with HIF-1 β , onto hypoxia-response elements in the promoter region of various genes encoding angiogenic factors. Because HIF-1 α up-regulates not only genes involved in endothelial growth (such as *VEGF*) but also those in smooth muscle cell recruitment (such as platelet-derived growth factor-B [*PDGF-B*]), it stimulates the growth of mature non-leaky vessels and is being considered for therapeutic revascularization of ischemic tissues [80–84]. Tumors are often hypoxic, and, by driving angiogenesis, HIFs are generally believed to promote tumor growth [85•]. Thus, inhibitors of HIFs or angiogenesis inhibitors dysregulating HIF (such as 2-methoxyestradiol) are being evaluated for cancer treatment [85•,86]. However, because HIFs may also have pleiotropic effects on various other processes, their precise role in cancer remains incompletely understood [87–89]. Moreover, the role of HIF-1 α in tumor growth is dependent on the tissue microenvironment, stimulating glioblastoma growth in the skin but inhibiting its progression in the brain [90•]. Recent studies also provided insight into how oxygen levels are sensed in cells and regulate HIF activity. By hydroxylating HIF in an oxygen-dependent manner, the prolyl hydroxylases (PH-1, -2, -3) target HIF to degradation, explaining why HIF protein levels are low in normoxia [91•,92•]. Conversely, in hypoxia, HIFs are not hydroxylated and thus are capable of activating angiogenic programs. By affecting the activity of PH, nitric oxide regu-

lates the stabilization of HIF-1 α during hypoxia [93]. An outstanding question is whether the various PHs have distinct functions and targets, as suggested by the recent finding that PH-2 is the most critical oxygen sensor [94]. These insights have prompted efforts to manipulate the expression or activity of PHs to inhibit or stimulate angiogenesis [95,96].

The Id and homeobox transcription factors

Id proteins are helix-loop-helix transcription factors that regulate tumor angiogenesis, given that angiogenic defects in Id mutant mice inhibit the growth of tumor xenografts [97]. Gene profiling studies revealed that Id1 down-regulates thrombospondin-1, a potent inhibitor of angiogenesis, but up-regulates several proangiogenic factors, including the α_6 and β_4 integrins, matrix metalloproteinase (MMP)-2, and fibroblast growth factor (FGF) receptor-1 [98,99•]. Importantly, studies in Id-deficient mice revealed that there may be therapeutically relevant differences in angiogenic mechanisms between tumor-xenografts and autochthonous tumors [99•,100•]. The large family of homeobox transcription factors is involved in cellular differentiation, proliferation and migration. Not surprisingly, they have also been implicated in regulating the behavior of vascular cells during development and adulthood [101]. One of the initial steps in embryonic vessel formation is the induction of endothelial precursors (angioblasts) from the mesoderm. Whereas VEGF-receptor-2 (or Flk-1) is the first marker to be identified on those precursors, little if any information is available on upstream regulatory signals. Recently, HOXB5 was shown to colocalize with Flk-1 in embryoid bodies, and HOXB5 over-expression increased the number of Flk-1⁺ angioblasts [102]. *HOX* genes also play a role in adult blood vessel formation, some with inhibitory properties, others with stimulatory properties. Sustained expression of HoxD10 inhibits angiogenesis in the chick chorioallantoic membrane, whereas *HoxD3* gene transfer efficiently improved wound healing in diabetic db/db mice by up-regulating angiogenesis [103,104]. Growth arrest-specific homeobox, the expression of which is limited to the cardiovascular system in the adult, precluded the conversion of ECs to an angiogenic phenotype [105].

Extracellular matrix components

Tumstatin, a cleavage fragment of the α_3 chain of the basement membrane component type IV collagen (Col IV α_3), was originally discovered as an EC-specific inhibitor [106]. Recent studies have revealed an important role of this endogenous inhibitor in the control of pathologic angiogenesis [107•]. Indeed, mice with a genetic deletion of Col IV α_3 or MMP-9, which cleaves tumstatin from Col IV α_3 , show accelerated tumor growth and angiogenesis, whereas physiologic angiogenesis is unaffected. The suppressive effects of tumstatin require $\alpha_v\beta_3$ integrin expression on pathologic, but not on physi-

ologic, angiogenic blood vessels [107•]. The antiangiogenic activity of endostatin, another angiogenesis inhibitor derived from the α_1 chain of type XVIII collagen, is mediated by $\alpha_5\beta_1$ integrin [108]. Over the past few years, a new family of extracellular matrix-binding cysteine-rich proteins, the CCN family, has emerged with pleiotropic functions on vascular cells. Six members have been identified (CCN1-6) with differential involvement in developmental and adult angiogenesis. CCN1 (or cysteine-rich 61, CYR61), CCN2 (or connective tissue growth factor, CTGF), and CCN3 (or nephroblastoma overexpressed, NOV) stimulate angiogenesis *in vitro* and/or in the avascular cornea and tumors *in vivo* [109–111]. Moreover, loss of CCN1 resulted in abnormal blood vessel bifurcation in the placenta, and a deficiency of CCN2 yielded growth plate angiogenesis defects [112,113]. CCN5 (or Wnt-induced secreted protein-2, WISP-2) inhibits smooth muscle cell proliferation, and CCN6 (or WISP-3) is antiangiogenic in the chick aortic ring assay [114,115]. Their role in pathologic angiogenesis and their therapeutic potential remain to be further identified.

Matrix metalloproteinase-independent roles of tissue inhibitors of matrix metalloproteinases in angiogenesis

The involvement of proteolytic activity in angiogenesis is well established. The extracellular matrix surrounding ECs must be broken down to allow cell migration and proliferation. MMPs degrade virtually every component of the extracellular matrix and, in doing so, contribute to the angiogenic process [116]. Their action is tightly controlled by tissue inhibitors of MMPs (TIMPs). Numerous antiangiogenic cancer and rheumatoid arthritis trials have been conducted using compounds that inhibit the proteolytic activity of various MMPs—almost all with disappointing results, however. Two recent studies shed some light on why these trials might have been unsuccessful. First, TIMP-2 was shown to inhibit angiogenesis *in vitro* and *in vivo* by a mechanism independent of its MMP inhibitory activity, *ie*, by stimulating phosphatases to dephosphorylate, and thereby inactivate, FGF and VEGF receptors [117••,118]. Second, TIMP-3 could—again independently of its MMP inhibitory activity—halt angiogenesis by preventing VEGF from binding to Flk-1 [119••].

Genetic discovery of novel angiogenic molecules

The molecular basis of congenital vascular malformations and hemangiomas has remained largely enigmatic, in part because of the sporadic occurrence and mosaic body distribution of these lesions. Recently, one of the susceptibility genes predisposing to vascular malformations of the Klippel-Trenaunay syndrome was identified by analysis of a chromosomal translocation in an affected child. Enhanced transcription or gain-of-function muta-

tions of a novel angiogenic factor, VG5Q, were found to be responsible for these vascular malformations [120••]. Differences in proangiogenic and antiangiogenic gene expression levels may predict the different susceptibility of different mouse strains to an angiogenic response in the cornea or collateral vessel growth in the ischemic limb, and the identification of these angiogenic candidates is eagerly awaited [121•–123•]. Moreover, discovering novel angiogenic molecules will be relevant, because reliable surrogate markers to monitor the efficacy of proangiogenic and antiangiogenic treatments are urgently needed. Moreover, mapping the “angiogenome” of individuals may allow better selection of patients for optimal treatment. The rapidly increasing use of small animal models, such as zebrafish embryos, promises to be a powerful screening tool to discover novel angiogenic molecules or evaluate the efficacy of inhibitors or to study rapidly genetic interactions and pathways [22,25•, 26•,124,125,126•].

Crosstalk and synergism between angiogenic molecules: therapeutic implications

Angiogenesis is a complex process, requiring an interaction between multiple signals. Efficient therapeutic stimulation or inhibition of angiogenesis may thus require combinatorial delivery or inhibition of angiogenic molecules. Binding of growth factors such as PDGFs and VEGFs to their tyrosine kinase receptors induces dimerization, leading to phosphorylation of tyrosine residues, which serve as docking sites for downstream signaling [127]. In general, little is known about possible crosstalk of angiogenic growth factor receptor tyrosine kinases. VEGF binds VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1), whereas placental growth factor (PlGF), a homologue of VEGF, binds only Flt-1. In contrast to the established importance of Flk-1 signaling in response to VEGF, the roles of Flt-1 and its ligand PlGF have remained enigmatic for more than a decade. Recent studies have revealed that PlGF affects, in a pleiotropic manner, all known mechanisms of vessel growth in the adult: it stimulates collateral vessel growth (likely by recruiting and activating monocytes), which increases bulk flow to tissues; mobilizes EPCs from the bone marrow into growing vascular beds; and recruits smooth muscle cells to build mature durable vessels [128••–130••]. In addition, PlGF amplifies VEGF-driven angiogenesis in pathologic, but not physiologic, conditions via a novel receptor crosstalk. Indeed, activation of Flt-1 by PlGF induces a crosstalk between Flt-1 and Flk-1, resulting in transphosphorylation of Flk-1, which therefore becomes more active in signaling VEGF-driven angiogenesis [128••–130••]. When administered alone, PlGF and VEGF failed to stimulate ischemic myocardial revascularization in an angiogenesis-refractory urokinase-deficient mouse model. However, when delivered together, PlGF and VEGF were capable of inducing

angiogenesis [129••]. The therapeutic implications of these findings are that PlGF treatment amplifies the activity of VEGF, endogenously up-regulated in ischemic tissues. As mentioned above, some of the contribution to tissue revascularization may come from hematopoietic (progenitor) cells [8•,11•]. PlGF may stimulate recruitment and activation of the latter during collateral growth, as also demonstrated in bone marrow ablation and inflammatory models [130••,131•,132••]. Moreover, the loss of VEGF in hematopoietic stem cells reduced their survival, whereas over-expression of VEGF and PlGF in hematopoietic stem cells increased their proliferation and survival *in vitro* [133••]. In addition, because most patients eligible for therapeutic angiogenesis are affected by conditions that impair angiogenesis (*ie*, atherosclerosis, hypertension, old age, diabetes) or receive medications with possible antiangiogenic activity, the administration of a single angiogenic compound may be insufficient and therapeutic success may thus require coadministration of both VEGF and PlGF [134]. A similar synergism between PDGF-BB and FGF-2 in stimulating revascularization of postischemic tissues was recently documented [135]. Recently, Flk-1 was also reported to interact with VEGFR-3 (Flt-4). Binding of VEGF-C (which has both angiogenic and lymphangiogenic activity) induced the formation of either Flt-4/Flt-4 homodimers or Flk-1/Flt-4 heterodimers; however, different tyrosine residues were phosphorylated on Flt-4, which may differentiate between the angiogenic and lymphangiogenic properties of VEGF-C [136•]. Interestingly, Flk-1 was also shown to crosstalk with other non-tyrosine kinase receptors like S1P₁ (a receptor for the bioactive phospholipid sphingosine-1 phosphate, or S1P) and the B2 receptor (a bradykinin receptor) [137,138]. Such genetic interactions may also be relevant to the development of therapeutic strategies to inhibit angiogenesis. Indeed, by targeting both ECs and pericytes, combinatorial inhibition of VEGF and PDGF receptors was more efficient in inhibiting growth of the often intractable late-stage solid tumor [139•]. Thus, therapeutic stimulation of new functional, mature, and durable vessels in ischemic tissues or inhibition of excess vessels in malignant or inflamed tissues may require and be more efficient by combinatorial delivery of multiple angiogenic compounds with synergistic activity or, alternatively, by delivery of compounds with pleiotropic activity.

Clinical translation of antiangiogenic strategies

Angiogenesis is essential for tumor growth and metastasis and is a promising target in the search for new anti-neoplastic agents [140]. However, because angiogenesis is a tightly regulated process dependent on the complex interplay of numerous molecules, identifying the key targets for drug development is challenging. Of the more than 4000 publications on angiogenesis in the past year,

more than 2500 reported a role for VEGF in angiogenesis, making VEGF the best-characterized angiogenic factor to date. Extending the initial discovery that the loss of even a single VEGF allele already causes fatal vascular defects in early embryogenesis, numerous subsequent studies in preclinical animal models have demonstrated the great potential of VEGF blockade to halt tumor progression [141–143]. VEGF blockade, alone or in combination with cytotoxic therapies, has thus been evaluated in several clinical cancer trials, but early results have been disappointing [144]. However, in the past year, the first successful phase 3 clinical trial using the VEGF-specific antibody bevacizumab (Avastin) in colorectal cancer patients was reported [145••]. Bevacizumab also prolonged the time to progression of disease in patients with metastatic renal cell cancer [146••]. Notably, bevacizumab decreased tumor perfusion, microvascular density, interstitial fluid pressure, and the number of circulating EPCs, and it increased the fraction of vessels with pericyte coverage in rectal carcinoma patients, all leading to tumor vessel normalization [16••]. This may retard the shedding of metastatic cells in the circulation, improve the delivery of therapeutic agents in tumors, and sensitize the endothelium to cytotoxic agents. Angiogenesis inhibitors are likely to be coadministered with cytotoxic or radiation therapy. Tumor ECs may be direct targets of such treatments. For instance, EC apoptosis, dependent on Raf-1 kinase signaling, determines the tumor response to radiotherapy [10,147]. When chemotherapeutic drugs are administered to tumor-bearing mice on a long-term basis at a metronomic dose, *ie*, according to a frequent schedule at doses substantially lower than the maximum tolerated dose, they cause antiangiogenic effects by targeting the ECs of newly growing tumor blood vessels. This antiangiogenic activity is lost in mice that lack the endogenous angiogenesis inhibitor thrombospondin-1, indicating that thrombospondin-1 mediates, at least in part, the antiangiogenic effects of some low-dose metronomic chemotherapy regimens [148].

Clinical translation of proangiogenic strategies

Many preclinical animal studies have demonstrated that revascularization of ischemic tissues can be successfully accomplished by the administration of growth factors [149]. During the past year or so, the results of several placebo-controlled clinical angiogenesis trials have been reported, with variable success rates. The recently completed vascular endothelial growth factor in ischemia for vascular angiogenesis trial, in which recombinant human (rh)VEGF protein was administered to patients with angina, reported no significant improvement over placebo in myocardial perfusion or angina symptoms 60 days after treatment; however, a trend toward reduced angina symptoms was observed with a higher dose of rhVEGF after 120 days [150]. Single-dose adenoviral *VEGF*₁₂₁

gene transfer in patients with peripheral arterial disease did not result in a substantial improvement over placebo in exercise time [151]. More encouraging results of significantly improved myocardial perfusion were obtained in the Kuopio angiogenesis trial at 6 months after intracoronary adenoviral *VEGF* gene transfer during angioplasty and stenting in patients with coronary heart disease [152•]. Also, in a small-scale placebo-controlled study that included 19 angina patients, intramyocardial injection of a plasmid encoding VEGF-2 resulted in a significant improvement in angina class, paving the way for initiating larger-scale trials [153•]. Similarly, trials with members of another growth factor family, the FGFs, have met with variable clinical benefit. The FIRST trial in angina patients (comparing a single intracoronary infusion of FGF-2 with placebo) gave significant symptomatic improvement at 90 days but no improvement of exercise time or myocardial perfusion after 6 months [154]. In patients with peripheral arterial disease, a single intraarterial infusion of FGF-2 improved exercise time at 90 days but not after 6 months *versus* placebo [155]. Finally, the intracoronary administration of Ad5FGF-4 (an FGF-4 expressing adenovirus) showed an encouraging trend in improved myocardial perfusion in angina patients [156•]. Overall, the results of these clinical therapeutic angiogenesis trials are rather modest in comparison with the promising results in preclinical models. Possible explanations relate to the significant placebo effect, the suboptimal dose, the duration and delivery method of the compound, the inclusion of angiogenesis-refractory patients (see above), and the monotherapy regimens with single molecules that efficiently stimulate the growth of capillaries but not of collateral vessels (and thus fail to substantially increase bulk flow to the ischemic tissue). Unfortunately, the risk for side effects has thus far precluded systemic or sustained delivery of sufficient amounts of these candidate factors. Future studies will have to sort out whether additional molecules with a desirable efficacy and acceptable safety profile will provide such improved therapeutic potential. At least, some new candidates that may fit this bill have emerged in recent years. For instance, PlGF stimulates collateral vessel growth, EPC recruitment, and capillary angiogenesis without inducing side effects [130••,131•] and HGF, which acts both on ECs and smooth muscle cells, may function as a common signal for many ets-1 responsive angiogenic genes through up-regulation of this transcription factor [157].

Conclusion

The characterization of the molecular basis of angiogenesis is progressing at a rapid pace. This has allowed the development of therapeutic strategies and compounds to stimulate or inhibit angiogenesis in ischemic or malignant disorders, respectively. The first success with a VEGF inhibitor in a phase 3 clinical cancer trial has demonstrated the potential of such strategies. In the near

future, more compounds will have to be developed to permit the efficient yet safe treatment of angiogenic disorders.

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