

Angiogenesis and Current Antiangiogenic Strategies for the Treatment of Cancer



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Angiogenesis is a complex process critical for embryonic development and for survival. It is also a critical player in many pathologic processes, most notably in neoplasia. The cell signaling pathways involved in angiogenesis have become key targets for drug design, with more than 2,500 clinical trials currently under way. This review summarizes the essential features of angiogenesis and discusses therapeutic strategies that have been applied to specific diseases known to be associated with perturbation of normal angiogenic control.

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Abbreviations: bFGF = basic fibroblast growth factor, DLL4 = Notch ligand delta-like 4, ERK = extracellular signal-regulated kinase, FDA = Food and Drug Administration, HIF = hypoxia-inducible factor, MAPK = mitogen-activated protein kinase, MMP = matrix metalloproteinase, mRNA = messenger RNA, mTOR = mammalian target of rapamycin, PDGF = platelet-derived growth factor, PGF = placenta growth factor, PHD = prolyl hydroxylase, PI3K = phosphatidylinositol 3-kinase, RTK = receptor tyrosine kinase, S1P = sphingosine-1-phosphate, TGF = transforming growth factor, TK = tyrosine kinase, VEGF = vascular endothelial growth factor, VEGFR = vascular endothelial growth factor receptor, VHL = von Hippel-Lindau

THE blood vascular system develops from hemangioblasts in the mesoderm that differentiate into angioblasts (also called vasoformative cells). Proliferation of these cells gives rise to dense, syncytial masses that develop into the vascular plexus during the process known as vasculogenesis. Fluorescence imaging of zebrafish reveals that coalescing angioblasts form a single vascular cord that in turn becomes the first embryonic artery (the dorsal aorta). From this primordium a specific subset of angioblasts then sprout to form the first (ie, caudal) vein so that a common

precursor has given rise to two unconnected vessels—the first artery and vein. Further extensive development of these primitive networks then forms the arterial and venous systems in the process of angiogenesis—the formation of a continuous network of new blood vessels from an original, established vessel (1). Around the fifth week of embryonic development, lymph sacs form from venous endothelial cells. This is the first step in the process of lymphangiogenesis that involves sprouting of lymphatic endothelial cells from these sacs to form the peripheral lymphatic vascular network.

From this summary of the circulatory system, we proceed to consider the cellular basis of angiogenesis and the molecular pathways that are the regulators thereof. In particular, we focus on the vascular endothelial growth factor (VEGF) family and also on the impact of signaling by Notch and transforming growth factor (TGF)- β . We then consider the importance of hypoxia in regulating VEGF responses. This process establishes the molecular basis on which antiangiogenic agents have been developed, and those currently in clinical use, particularly for the treat-

ment of cancers, are discussed in turn. It was primarily the insight of Judah Folkman (2) approximately 40 years ago that initiated this field, and we conclude by discussing how much more fraught it has become than even the most pessimistic observer might have foreseen but that, nevertheless, there are grounds for therapeutic optimism.

STAGES IN ANGIOGENESIS

Angiogenesis is a complex process that involves the activation, proliferation, and directed migration of endothelial cells to form new capillaries from existing blood vessels. This sprouting of capillaries from preexisting vessels occurs during embryonic development but is almost absent in adult tissues except in wound healing (3). However, normal transient regulated angiogenesis occurs in adult tissues during the female reproductive cycle and during wound healing. Pathologic angiogenesis is characterized by the persistent proliferation of endothelial cells and is a prominent feature of a number of diseases, including rheumatoid arthritis, psoriasis, and proliferative retinopathy. Additionally, many tu-

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mors are able to attract blood vessels from neighboring tissues. The induction of new blood vessel growth is necessary if solid tumors are to grow beyond a minimal size, as in the example of relatively thin melanomas residing entirely above the basement membrane that are avascular and therefore rarely metastasize. In addition to promoting tumor growth and metastasis by supplying nutrients and oxygen, and removing waste products, angiogenesis also delivers immune cells, macrophages, and humoral factors to the vicinity of the tumor. The endothelial cells involved in tumor development dissolve their surrounding extracellular matrix, migrate toward the tumor, proliferate, and form a new vascular network (Fig 1). Extensive vascularization in early breast tumors appears to correlate with a poor prognosis, and the capacity to quantify angiogenesis and/or angiogenic growth factors may prove to be an important indicator for cancer therapies (4–6).

The relatively detailed picture of these events that we now have has, of course, been built up gradually, but there have arguably been two major landmarks in the story of angiogenesis. The first was contributed in 1971 by Judah Folkman (2) when he speculated, in view of the evidence that angiogenesis was essential for tumor development, if ways could be found to inhibit it, they might form “a powerful adjunct to other cancer therapies.” This notion was based on Folkman’s observation that, in the absence of neovascularization, most solid tumors become dormant—that is, they fail to grow beyond a diameter of approximately 2 mm—together with the isolation of a diffusible entity released by malignant tumor cells that he called tumor angiogenesis factor because it promoted the formation of new vasculature in solid tumors (2). Several earlier reports had also noted that tumors elicit the growth of capillary endothelium (7–9), and Tannock et al (10,11) determined that the probability of a tumor cell undergoing mitosis was inversely proportional to its distance from the nearest capillary. Even more remarkably, Warren and Shubik (7) used a transparent chamber to observe transplantable tumors in the hamster cheek pouch and recorded that, as

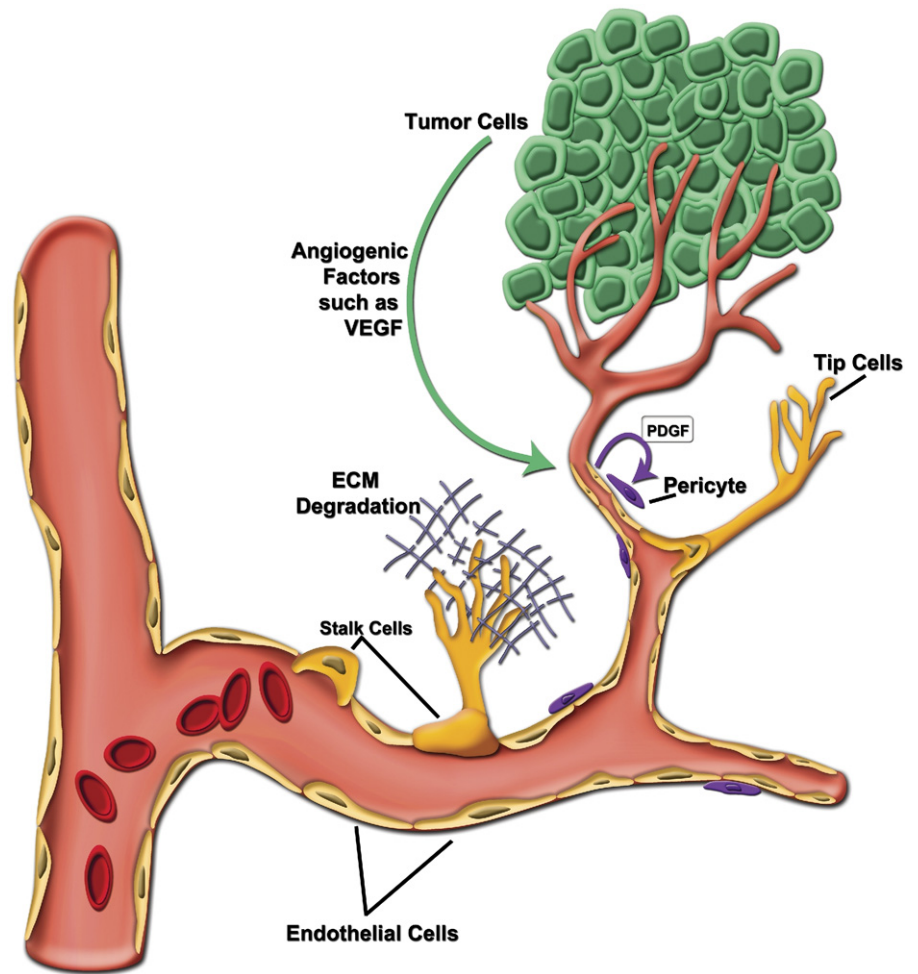


Figure 1. Angiogenic sprouting. In response to vascular endothelial growth factor A (VEGFA), endothelial cells release MMPs that degrade the extracellular matrix into which proliferating endothelial tip cells migrate. VEGFA also activates Notch signaling to inhibit proliferation of stalk endothelial cells. PDGF released from endothelial cells recruits smooth muscle cells (pericytes) that stabilize the neovasculature. MMP = matrix metalloproteinase, PDGF = platelet-derived growth factor. (Available in color online at www.jvir.org.)

new blood vessels grew, they formed extremely tortuous patterns with many anastomoses and cross-linkages. Nevertheless, despite this apparent disorganization, it was possible to identify tumor types (eg, melanoma or mammary carcinoma) just from the vascular patterns generated by the tumor transplants (7,12,13). Indeed, it was Greenblatt and Shubik (14) in 1968 who first coined the term “tumor angiogenesis” to describe the vascularization associated with growing tumors.

Notwithstanding Folkman’s prescience, the avalanche of effort that was to be brought to the subject moved almost undetectably until the

second major angiogenesis event—the specific identification of proangiogenic agents. The first to be isolated in this quest were basic fibroblast growth factor (bFGF) (15) and VEGF, which was originally called “vascular permeability factor” (16,17). The significance of these discoveries is perhaps illustrated by the fact that, of nearly 70,000 published articles dealing with angiogenesis (68,703 to be precise), more than 96% have appeared in the 16 years since 1993.

For detailed summaries of the molecular biology of angiogenesis and lymphangiogenesis, readers are referred to numerous reviews, in particular those by Jain, Kerbel, Alitalo, Car-

meliet, and their respective colleagues (18–24).

ANGIOGENIC PROMOTERS

VEGF

The early identification of VEGF was significant because it has transpired that this family of cytokines—VEGFA, VEGFB, VEGFC, VEGFD (the gene encoding VEGFD is designated *FIGF* by the HUGO Gene Nomenclature Committee), VEGFE, and placenta growth factor (PGF, also known as PLGF)—are the most critical factors regulating the processes of vasculogenesis, angiogenesis, and lymphangiogenesis (Fig 2). Of this family, VEGFA is an essential regulator of vasculogenesis and angiogenesis, its effects being mainly on vascular endothelial cells, promoting cell division and migration and making the vasculature “leaky.” VEGFB acts predominantly as a survival factor for endothelial cells, vascular smooth muscle cells, and pericytes. VEGFC is essential for lymphangiogenesis and, in human tumors, its expression has been correlated with the development of lymph node metastases (25). VEGFD/FIGF (highly homologous to VEGFC) also promotes lymphatic metastasis in mouse tumor models. Its expression has been reported to be an independent indicator of poor prognosis for endometrial carcinoma (26) and also, together with VEGFC, for pancreatic cancer (27). PGF, like VEGFB, promotes the survival of endothelial cells and modulates the activity of VEGF signaling (28). Phenotypes of mice with modifications in VEGF family genes are provided in Table 1 (29–34).

VEGF Receptors

The major receptors to which the VEGF family of cytokines bind (VEGF receptors [VEGFRs] 1–3) belong to a subfamily of class III transmembrane receptor tyrosine kinases (TKs; RTKs) that are expressed at high levels in cells of the endothelial lineage. VEGFR1 is a kinase-defective RTK that negatively regulates angiogenesis by modulating VEGFR2 activity and acting as a decoy receptor. The decoy characteristic of VEGFR1 is required for normal development and for angioblast as-

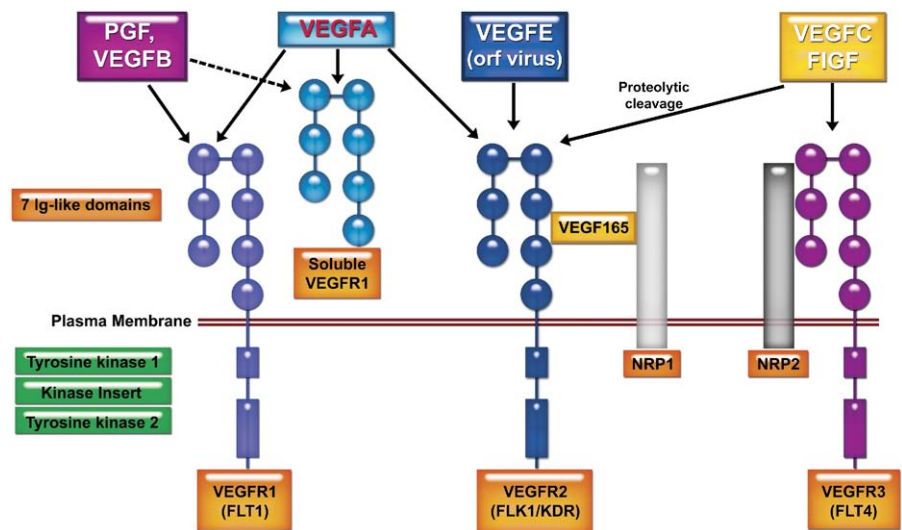


Figure 2. VEGF ligands, receptors, and coreceptors. The family of VEGFs (VEGFA, VEGFB, VEGFC, FOS-induced growth factor [FIGF; formerly VEGFD], and PGF/PLGF) bind as dimers with differing specificities to three types of receptor; binding promotes transphosphorylation of receptor dimers. Neuropilins (NRP1 and -2) are membrane-bound coreceptors for VEGFs and for semaphorins. There are five principal, alternatively spliced isoforms of VEGFA denoted by the number of amino acids (121/145/165/189/206). VEGF165 can be cleaved by some MMPs and by plasmin to yield VEGF110 or VEGF113. Gradients of VEGF isoforms regulate blood vessel branching (126). Proteolytic cleavage of VEGFC and VEGFD increases their affinity for VEGFR3 and for some VEGFR2s. An additional form, VEGFE, is encoded by parapoxvirus ovis (PPVO or Orf virus) and VEGFE, like VEGFA, binds with high affinity to VEGFR2 (127). The three isoforms of PGF activate VEGFR1, causing transphosphorylation of VEGFR2 and amplifying VEGF-driven angiogenesis. PGF2 also binds to NRP1. (Available in color online at www.jvir.org.)

sembly into blood vessels. Targeted deletion of *Vegfr-1* results in early embryonic lethality as a result of abnormal overgrowth of endothelial cells. Alternative splicing of VEGFR1 generates a soluble form of the receptor that inhibits VEGFA from binding to its cell surface receptor and is presumed therefore to regulate VEGF activity. VEGFR2 is mainly expressed in activated endothelial cells and their embryonic precursors and is required for angioblast differentiation (during vasculogenesis), whereas VEGFR3 is predominantly expressed in lymphatic endothelia. An engineered soluble form of VEGFR3 inhibits the activation of signalling by VEGFC and VEGFD and, when expressed in transgenic mice, causes inhibition of lymphangiogenesis and dissolution of preformed lymphatic vessels as embryogenesis proceeds (35).

Hematopoietic progenitor cells expressing VEGFR1 and endothelial precursor cells that express VEGFR2 are both involved in promoting the vascu-

larization of primary tumors. Remarkably, however, in a mouse model of metastasis, VEGFR1-expressing hematopoietic progenitors from the bone marrow respond to cytokines released from primary tumors by migrating to sites where fibronectin has been up-regulated to mark them as metastatic “niches” before the arrival of tumor cells (36).

Intracellular Signaling from VEGF RTKs

A striking characteristic of the different members of the RTK family is their capacity to activate a considerable number of intracellular pathways that are essentially ubiquitous in eukaryotic cells (Fig 3). The VEGFRs conform to this pattern and the phosphorylation of tyrosine residues in their cytoplasmic domains can activate multiple signaling pathways including, RAS/RAF1/extracellular signal-regulated kinase (ERK)–1 and -2 (regulating proliferation and differentiation),

Table 1
Phenotypes of Mice with Modifications in VEGF Family Genes (29–34)

Gene	Transgenic Genotype	Transgenic Phenotype	Comment	Reference(s)
<i>Vegf-a</i>	Heterozygous knockout	Embryonic lethal	Essential for vasculogenesis and angiogenesis	29
<i>Vegf-a</i>	Homozygous knockout	Embryonic lethal	—	29
<i>Vegf-b</i>	Homozygous knockout	Normal	Survival factor for endothelial cells, vascular smooth muscle cells and pericytes. Ablation of VEGFB activity inhibits neovascularization in various in vivo models	30,31
<i>Vegf-c</i>	Homozygous knockout	Embryonic lethal	Essential for lymphangiogenesis	32
<i>Pgf</i>	Homozygous knockout	Viable and fertile	Impaired pathological angiogenic response	33
<i>Pgf</i>	Overexpression	—	Induces vascularization and increases vascular permeability	34

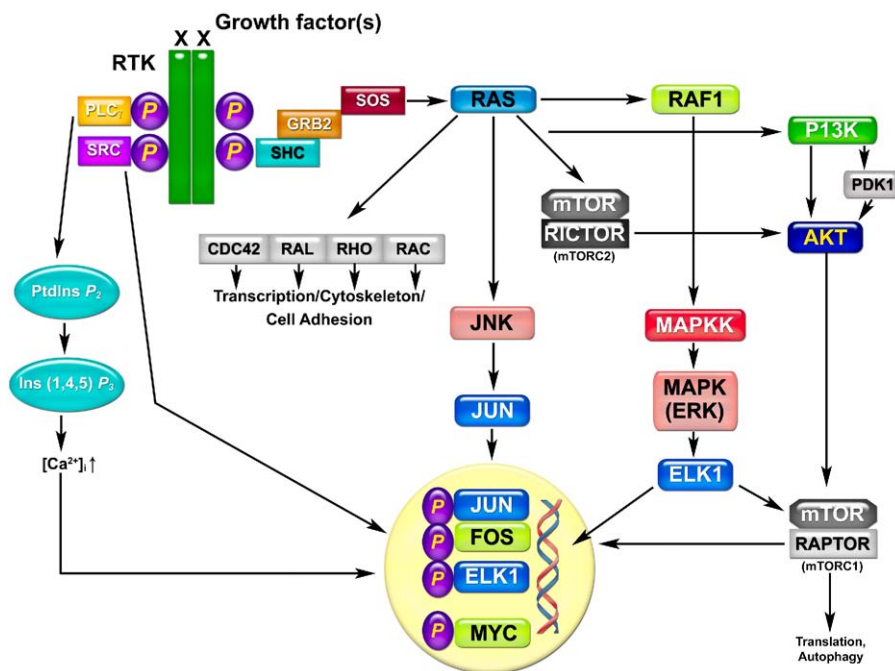


Figure 3. Major intracellular signaling pathways. The binding of growth factors to RTKs (eg, VEGFRs) activates multiple pathways. These control the activity of nuclear transcription factors (eg, JUN, FOS, ELK1, and MYC) by phosphorylation (P). The pathways also regulate the cytoskeleton and protein synthesis (translation) to determine the overall response of the cell. SHC, GRB1, and SOS are adapter proteins that switch on RAS and hence the pathways that diverge from RAS. mTOR can associate with RAPTOR or RICTOR, together with other proteins, to form mTORC1 or mTORC2, respectively. JNK = JUN N-terminal kinase; MAPK = mitogen-activated protein serine/threonine kinase or ERK; MAPKK = MAPK kinase; PDK1 = phosphoinositide-dependent protein kinase-1; PLC γ = phospholipase C γ ; PtdInP₂ = phosphatidylinositol 4,5-bisphosphate. (Available in color online at www.jvir.org.)

RAS/p38 mitogen-activated protein kinase (MAPK) and JUN N-terminal kinase 1–3 (regulating inflammation, apoptosis, proliferation, and differentiation), phosphoinositide-dependent protein kinase-1 and AKT (regulating

survival), and CDC42 and FAK (regulating the cytoskeleton and migration). These pathways are initiated by the binding of a variety of cytosolic proteins to the activated receptors (eg, GAB1, SHC, SRC, phosphatidylinosi-

tol 3-kinase [PI3K], and phospholipase C- γ). VEGFA/VEGFR2 signaling is RAS-independent but phospholipase C- γ -dependent, whereas VEGFC/VEGFR3 signaling activates RAS but not phospholipase C- γ . This bewildering network is presumably essential to give rise to a pattern of gene expression that ultimately manifests itself as the cell phenotype and that depends on the receptor (or receptors) that are activated and also on the specific ligand responsible. Therefore, for example, VEGFB or PGF acting via VEGFR1 produce distinct gene expression profiles. This clearly reflects temporal and tissue-specific differences in the expression levels of adapters and enzymes that interact with activated RTKs. However, the detailed molecular interactions that generate such exquisite specificity remain largely unresolved.

A recent advance in the context of the endothelium has come from the use of mutant forms of HRAS that selectively activate either the RAF-ERK1/2 or the PI3K pathway. Serban et al (37) have shown that the former drives angiogenesis but the latter is required for angiogenesis and vascular permeability. The diversity of PI3K function arises from there being four isoforms of this enzyme (α , β , δ , and γ), the first pair activating AKT and thereby promoting cell survival. A number of gain-of-function mutations in PI3K α have been identified in cancers, consistent with its role in an antiapoptotic pathway. In addition, Serban et al (37) showed that PI3K α/β also promoted the survival of endo-

thelial cells. However, PI3K δ/γ functions in an independent pathway driven by activated RAS (RAS-GTP) to induce vascular leakage. These observations, defining divergent pathways emanating from RAS, are consistent with transgenic data showing that mice deficient for PI3K α or PI3K β are nonviable as a result of defects in cell survival whereas PI3K γ -null mice are viable but have a reduced vascular permeability response to VEGF. They are also consistent with the observation that inhibiting the RAF-MAPK pathway decreases the growth, vascularization, and metastasis of orthotopic pancreatic tumors in mice (38).

The critical role of RAS in VEGF signaling is complemented by the action of oncogenic HRAS or KRAS or RAF in markedly upregulating VEGF expression in a variety of cell types, suggesting that RAS may contribute to the growth of solid tumors by indirectly promoting angiogenesis (39–41). It is notable that the critical signaling pathways differ with cell type. Therefore, in epithelial cells, PI3K is the major regulator, whereas in fibroblasts, it is the MAPK pathway (42). In immortalized endothelial cells, HRAS stimulates the expression of VEGF and of the matrix metalloproteinases (MMPs) MMP-2 and MMP-9 while reducing tissue inhibitor of metalloproteinase activity (43). This pattern indicates that the cells are potentially angiogenic. RAS-induced VEGF expression and MMP activity is dependent on the activity of PI3K but the suppression of tissue inhibitor of metalloproteinase activity is not. RAS also decreases the expression of the anti-angiogenic protein thrombospondin (44) that is also under the control of p53. Therefore, in fibroblasts from patients with Li-Fraumeni syndrome, the loss of both alleles of P53 promotes a decrease of 20-fold in thrombospondin secretion and a fourfold increase in VEGF secretion, whereas in 293 cells VEGF is down-regulated by normal p53 (45). In epithelial cells, RAS cooperates with TGF- β to induce the epithelial-to-mesenchymal transition. The expression of TGF- β is increased in the majority of tumors as it switches from acting as a tumor suppressor to a tumor promoter and oncogenic RAS synergizes with TGF- β to increase the transcription and the messenger RNA (mRNA) stability of a number of genes in-

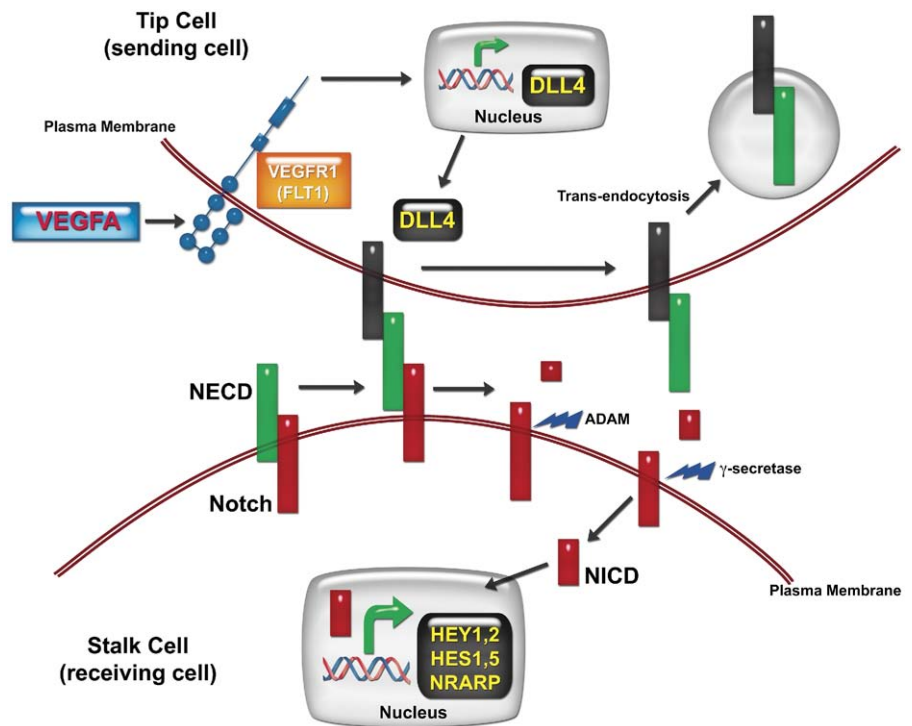


Figure 4. Notch signaling pathway. The Notch receptor is expressed on the cell surface as a heterodimeric receptor. The extracellular domain (Notch extracellular domain [NECD]) and the membrane-bound domain (Notch intracellular domain [NICD]) associate via noncovalent interactions. The DLL4 ligand promotes endocytosis and nonenzymatic dissociation of the Notch heterodimer. NECD is transendocytosed into the signal-sending cell, exposing Notch to ADAM and γ -secretase proteolysis for release of the NICD, which translocates to the nucleus to trigger transcriptional activation of Notch target genes that include *HEY1* and *-2*, *HES1* and *-5*, and *NRARP* (128,129). (Available in color online at www.jvir.org.)

involved in migration and angiogenesis, including *Vegf* (46).

Two families of proteins are known to act as negative regulators of RTK signaling: Sprad proteins, which are general inhibitors of ERK activation, and the family of four Sprouty proteins that inhibit VEGFA and bFGF activation of ERK. Therefore, in *Sprouty4*-null mice, angiogenesis and vascular permeability are increased in response to VEGFA but not VEGFC and there is an approximately sixfold increase in the rate of growth of implanted Lewis lung carcinoma cells (47). As Sprad and Sprouty expression is reduced in a number of human cancers, they may be regarded as tumor suppressor genes.

Notch

VEGFA and VEGFC play important roles in arterial/venous segregation in the embryo. VEGFA, signaling through VEGFR2, promotes sprouting of dorsal

intersegmental vessel tip cells. However, VEGFA also induces expression of the endothelium-specific Notch ligand delta-like 4 (DLL4): when DLL4 activates the Notch signaling pathway in adjacent cells, the effect is to inhibit dorsal sprouting. In contrast, the action of VEGFC is confined to vein morphogenesis by the restricted expression of its receptor VEGFR3. VEGFA also activates Ephrin-B2, the plasma membrane-spanning ligand for the EPH receptor B4 TK. This interaction can signal from outside to inside the cell and in the opposite direction. Ephrin-B2 is expressed on arterial progenitors and EPH receptor B4 on venous progenitors. This system therefore constitutes a boundary between venous and arterial angioblasts and permits directional control of sprouting by the two types of vessels (Fig 4) (1).

Notch signaling has also been directly implicated in tumor angiogenesis and in the process of activating

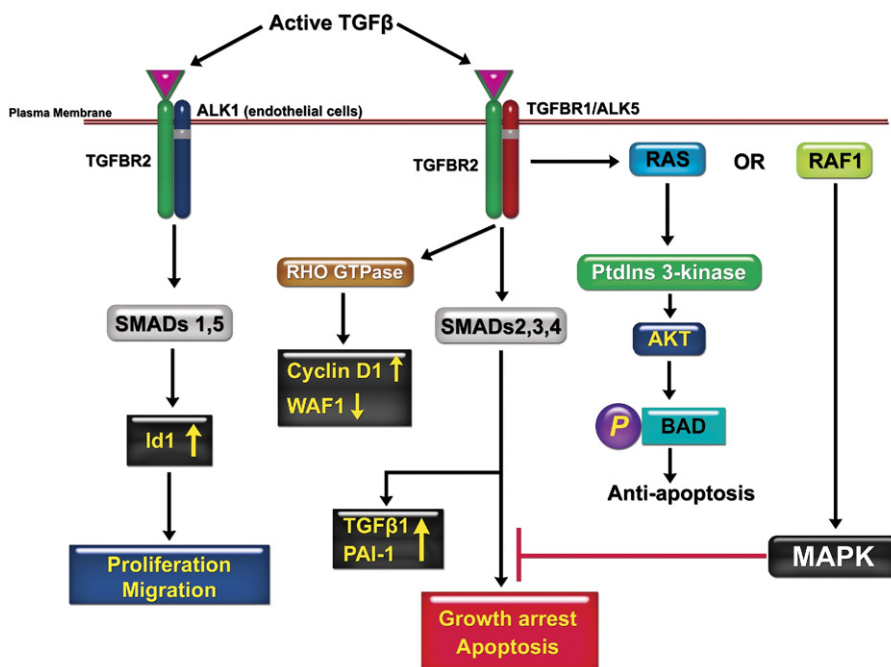


Figure 5. TGF- β signaling in endothelial cells. TGF- β binds to the constitutively active serine/threonine kinase TGFBR2, which then activates TGFBR1 and the canonical signaling pathway mediated by SMADs 2, 3, and 4 that causes cell cycle arrest and apoptosis in normal cells. In transformed cells, other pathways may be activated by TGF- β that inhibit apoptosis and promote cell migration and invasion. The ALK5 form of TGFBR1 is ubiquitously expressed. In endothelial cells, a second type 1 receptor is expressed, ALK1, that signals via SMADs 1 and 5 to promote proliferation and migration. (Available in color online at www.jvir.org.)

dormant tumors. When expressed in tumor cells DLL4 can activate Notch signaling in host stromal cells, thereby improving vascular function (48). Inhibition of DLL4-mediated Notch signaling promotes a hyperproliferative response in endothelial cells, a process that leads to an increase in angiogenic sprouting and branching. Despite this increase in vascularity, tumors are poorly perfused, hypoxia increases, and tumor growth is inhibited (49,50). Conversely, DLL4 expressed in endothelial cells acts via Notch 3 expressed on adjacent colorectal cancer cells or human T-cell acute lymphoblastic leukemia cells to promote the switch from dormancy to growth (51). These findings point to the Notch pathway as a potential therapeutic target.

TGF- β

In point of historical fact, angiogenic promoters had been isolated even before bFGF and VEGF without that property having been identified.

Most notable of these is TGF- β (Fig 5) (52), but it has taken many years for this most pleiotropic of cytokines to emerge as a strong challenger to the VEGF family as the most important angiogenic regulator. TGF- β is a ubiquitously expressed paracrine polypeptide (25 kDa); three highly homologous forms (TGF β 1 [gene, *TGFB1*], TGF β 2 [*TGFB2*], and TGF β 3 [*TGFB3*]) having been detected in humans and other mammals. TGF- β is synthesized in latent form as a zymogen (approximately 110 kDa); after secretion, a latency associated peptide is proteolytically cleaved to release active TGF- β . Tissue plasminogen activator cleaves plasminogen present in serum to release plasmin that activates latent TGF- β . TGF- β is a major regulator of tissue morphogenesis, each isoform inhibiting the growth of a wide range of normal and transformed cells (epithelial, endothelial, fibroblast, neuronal, lymphoid, and hematopoietic), with lung epithelial cells and keratinocytes being most susceptible. In some cells (eg, fibroblasts, osteoclasts) TGF-

β can act as a mitogen, probably by stimulating the release of autocrine factors (eg, platelet-derived growth factor [PDGF]). However, in general, TGF- β inhibits cell cycle progression by lengthening or arresting the G1 phase (53), although in vascular smooth muscle cells it greatly lengthens G2 with no significant effect on G1. TGF- β can also positively or negatively affect differentiation, for example, initiating growth arrest, which precedes differentiation of epithelial cells (54) or, under certain conditions, repressing MyoD and myogenin transcription and the differentiation of myoblasts (55). TGF- β 1 is a potent inhibitor of several differentiated functions of adrenal cells, an effect that can be reversed by treatment with antisense oligonucleotide complementary to *TGFB1* mRNA (56).

Active TGF- β homodimers signal by binding to constitutively active type 2 receptors (TGFBR2) to activate type 1 receptors (TGFBR1) in a heteromeric complex that controls transcription through the action of a family of SMAD proteins. Endothelial cells are unusual in that they express two forms of type 1 receptors, ALK1 (mainly expressed in endothelial cells) and ALK5 (ubiquitously expressed). ALK1 signals by promoting the phosphorylation of SMAD1 and SMAD5, and ALK5 similarly activates SMAD2 and SMAD3. The ALK1 pathway activates endothelial cell migration and proliferation whereas activated ALK5 is inhibitory. The activation of PAI-1 by ALK5/SMAD2/SMAD3 is important in angiogenesis because it promotes TGF- β -induced maturation of blood vessels (57). Endoglin (ENG, END, HHT1, CD105) is a proliferation-associated cell membrane glycoprotein antigen of endothelial cells and is strongly expressed on tumor-associated angiogenic vascular endothelium. Endoglin is essential for angiogenesis and, in endothelial cells, is the most abundant TGF- β -binding protein, associating with TGFBR1 or TGFBR2 in the presence of TGF- β and other members of the TGF- β superfamily (58).

The importance of TGF- β in angiogenesis is evident from the fact that mice defective for *Tgfb-1*, *Alk-1*, or *Alk-5* die in utero from similar vasculogenic defects (59). In humans, mutations in endoglin or ALK1 are responsible for the autosomal-dominant

disorder hereditary hemorrhagic telangiectasia that results in multisystemic vascular dysplasia and recurrent hemorrhage (60). Endoglin-null mice have defective vascular smooth muscle development and arrested endothelial remodelling (61). Abnormal activity of the TGF- β signaling system is widespread in cancer. Increased levels of circulating TGF- β s, principally TGF- β 1, have been identified in many human cancers and are associated with enhanced invasion and metastasis (62,63). Mutations in *TGFBR1*, *TGFBR2*, or SMADs that affect signaling have been identified in a number of cancers, notably ovarian, colorectal, pancreatic, and recurrent breast carcinomas. Single nucleotide polymorphisms in the *TGFB1* gene show association with the incidence of invasive breast cancer. Gene expression signatures associated with the TGF- β signaling pathway in human mammary carcinoma cells suggest that TGF- β mediates intrinsic, stromal-epithelial, and host-tumor interactions during breast cancer progression.

Like VEGF, TGF- β (TGF β 1) is a strong proangiogenic agent despite the fact that, in vitro, TGF- β causes not only growth arrest but apoptosis of endothelial cells. This apparently contradictory behavior may be explained by the fact that TGF- β activates the secretion of fibroblast growth factor 2, which acts as an autocrine signal to stimulate the expression of VEGF. VEGF in turn acts in an autocrine manner through its receptor VEGFR2 to activate the MAPK pathway (specifically p38MAPK). The combined action of VEGF and TGF- β promotes apoptosis, which can be prevented by blockade of VEGF action (by antibody) or inhibition of p38MAPK (by the inhibitor SB202190), whereas VEGF alone is a survival/proliferation signal for endothelial cells (64). TGF- β will reverse the protective action of VEGF, promoting apoptosis, which occurs in the pruning process, to form the final vascular network. However, surprisingly, a rapid burst of TGF- β /VEGF-mediated apoptosis is also essential in vivo (chicken embryo chorioallantoic membrane) to initiate angiogenesis (65). Endothelial cells subsequently become refractory to TGF- β -mediated apoptosis and TGF- β then directly promotes capillary lumen formation.

OTHER ANGIOGENIC REGULATORS

Since the recognition of the activities of bFGF, VEGF, and TGF- β , a wide variety of other factors have been shown to stimulate angiogenesis, some acting directly on endothelial cells, others stimulating adjacent inflammatory cells. Those acting on endothelial cells may cause migration but not division (eg, angiotropin, macrophage-derived factor, tumor necrosis factor- α) or stimulate proliferation (eg, VEGF, bFGF, acidic fibroblast growth factor, epidermal growth factor, TGF- α , platelet-derived endothelial cell growth factor). A number of angiogenic factors including platelet-derived endothelial cell growth factor, VEGF, acidic fibroblast growth factor, bFGF, and midkine bind heparin (66), and heparin itself can enhance or inhibit the actions of angiogenic factors, depending on the agent and receptor type involved as well as on the concentration of heparin or heparan sulfate (67).

Two other receptor-mediated signaling pathways are of particular importance in angiogenesis. The first of these is activated by the sphingolipid sphingosine-1-phosphate (S1P) binding to the G protein-coupled receptor S1PR1, highly expressed in endothelial cells, and thereby activating cAMP, RHO, and RAC GTPases and phosphatidylinositol signaling pathways (68). S1PR1 signaling is important in cardiovascular development and S1p1-null embryos die in utero from incomplete vascularization. The interdependence of diverse signaling pathways that contribute to the control of angiogenesis is emphasized by the fact that S1P receptors are also transactivated by RTKs (specifically the PDGF receptor). The importance of S1PR1 signaling in the initiation of angiogenesis has been highlighted by the demonstration that small interfering RNA-mediated silencing inhibits angiogenesis (69), and this has prompted efforts to develop inhibitors of this pathway. Thus far, FTY720, a S1P analogue, has been shown to inhibit vascular sprouting and tumor growth in mice (70), and CL2 is the first non-S1P analogue shown to inhibit angiogenesis in vitro and in two animal model systems (71). An alternative potential therapeutic target is suggested by the fact that another member of the

S1P receptor family, S1PR2, activates a pathway that inhibits RAC GTPase and hence cell migration (72). As S1PR2 is expressed mainly in endothelial cells and vascular smooth muscle cells, it would appear that it regulates negative feedback to the S1PR1 pathway. Accordingly, tumor growth is accelerated in S1PR2-deficient mice (73), opening the possibility that activators of S1PR2 signaling might function as antiangiogenic agents. Also of importance in angiogenesis are integrin signaling pathways. Integrins are cell surface receptors that respond to signals from the extracellular matrix to modulate cell shape, motility, and division. Integrins do not possess enzymatic activity, but when activated, they recruit cytosolic molecules and can transmit signals bidirectionally in the manner of ephrin. Integrins $\alpha v\beta 3$ and $\alpha v\beta 5$ are part of the angiogenic system and signal particularly through the RAS-RAF-ERK1/2 and PI3K pathways.

Regulation of VEGF Expression: Hypoxia and Induction of Blood Vessel Growth

The transcription of the various VEGFs is selectively regulated by a variety of growth factors (eg, EGF and PDGF) and also by TGF- β (74,75) in a way that presumably reflects their distinct, although closely similar, functions. Therefore, for example, several MAPK pathways (p38, MEK1/2 and JUN N-terminal kinase) contribute to the full expression of VEGFA whereas p38 MAPK, JUN N-terminal kinase, and mammalian target of rapamycin (mTOR) promote VEGFC expression. Consistent with those data, rapamycin inhibits lymphatic vessel formation in a mouse xenograft model (76). However, the most potent signal regulating VEGFA expression is hypoxia together with other forms of stress (low pH and low glucose level) which stimulates transcription and increases mRNA stability, thereby elevating protein expression.

The induction of phenotypic responses to hypoxia is indirectly controlled by isoforms of prolyl hydroxylase (PHD; gene family is EGLN [egl nine homologue 3 (*Caenorhabditis elegans*)]) that regulate hypoxia-inducible factor-1 (HIF1), a transcription factor comprised of one of three subunits together with the constitutively expressed aryl hydrocarbon receptor

nuclear translocator (ARNT; also known as HIF1 β) subunit. Under normoxic conditions, prolyl residues in HIF α proteins are hydroxylated by PHDs in a reaction that uses molecular oxygen. Hydroxylation of HIF proteins permits their interaction with a multimeric complex within which the von Hippel-Lindau (VHL) tumor suppressor protein targets them for ubiquitin-mediated proteolysis. One HIF isoform (HIF2 α) is sensitive to the expression level of PHD2 so that, as the amount of PHD2 increases, the concentration of HIF2 α declines. HIF2 α in turn regulates expression of the genes encoding vascular endothelial cadherin (CDH5) and a soluble isoform of VEGFR1. When PHD2 is expressed in hypoxic regions of tumors, the result of its action via HIF2 α is to reduce the amounts of these two orchestrators of normal endothelium, resulting in the characteristic disorganized structure of tumor blood vessels. Remarkably, reduction in the level of PHD2 in heterozygous Phd2^{+/-} mice restores normal vessel structure within tumors (77).

VHL is therefore a critical regulator of normal cellular responses by promoting the degradation of HIF proteins. Reduction in the concentration of oxygen decreases the efficiency of this process: HIF proteins are stabilized and therefore become available to bind to hypoxia-response elements in the promoters of target genes, thereby activating transcription. Genes thus up-regulated include *VEGF*, *PFG*, *PDGF*, *VEGFR1*, *VEGFR2*, vascular endothelial cadherin (*CDH5*), and several MMPs (78). The synthesis of VEGF is indirectly controlled by VHL not only by its effects on transcription but also posttranscriptionally through the control of two proteins that modulate VEGF mRNA stability, HuR and TIS11B. HuR increases the mRNA half-life approximately fivefold from less than 1 h (79,80) whereas TIS11B decreases the half-life. HuR is down-regulated by VHL, leading to suppression of VEGF expression. High levels of HuR protein have been correlated with advanced disease and poor prognosis in patients with colorectal adenocarcinoma (81). The translation of TIS11B is inhibited under normoxic conditions by the micro-RNA miR-29b which binds to TIS11B mRNA and is itself posttranslationally regulated by

VHL. When the oxygen tension decreases, VHL mediates the upregulation of TIS11B expression (82). TIS11B is required for normal vascular development (83), consistent with it having a critical role in regulating VEGF expression that is mediated by VHL under normal and hypoxic conditions.

Consistent with the earlier observations on the role of ras, in colon cancer cells, oncogenic KRAS acts via PI3K to increase synthesis of HIF α protein and oncogenic BRAF acts via MAPK to enhance levels of HIF2 α (84). In either case the result is the upregulation of VEGF transcription.

ANGIOGENIC INHIBITORS

The activities of a variety of endogenous angiogenic inhibitors are also presumed to regulate tumor vascularization. These include thrombospondin-1, which is regulated by and is itself a major activator of TGF- β 1 in vivo (85).

One of the most notable developments in the context of inhibitors came again from the Folkman laboratory (86) via a mouse model in which micrometastases seeded from subcutaneous primary tumors failed to develop further but expanded rapidly after the primary tumor had been surgically removed. It was reasoned that the primary tumor itself was probably producing an antiangiogenic agent that inhibited vascular development in the micrometastases. After resection of the primary tumor the source of the anti-vascular agent was removed and the metastases therefore developed rapidly, consistent with reports of similar responses to human tumor resection going back many years. This was confirmed with the isolation of a protein called angiostatin from the urine of mice with primary tumors. Purified angiostatin given daily to mice after resection of the primary tumor completely prevented the development of micrometastases. Angiostatin was subsequently shown to be active against primary tumors established in mice from inoculated human tumor cells, and it also inhibits the proliferation of endothelial cells in culture (87). Angiostatin is a fragment of the protein plasminogen that occurs normally in the circulation, and the cleavage of plasminogen to produce angiostatin occurs in the tumor itself.

A number of other proteolytically activated antiangiogenic proteins have been isolated, notably endostatin derived from collagen XVIII, the gene for which is on chromosome 21 (88). There is a very low incidence of solid tumors in patients with Down syndrome, and if they are diabetic, they never develop diabetic retinopathy. These patients have elevated circulating levels of endostatin because of their extra copy of chromosome 21. Endostatin may therefore contribute to this remarkable protection, but two other chromosome 21 genes, *RCAN1* and *DYRK1A*, are induced by VEGF and, when overexpressed, inhibit capillary tube formation (89). An increased incidence of prostate cancer in patients with a specific polymorphism in endostatin has also been noted. As so often happens in cancer, clinical trials of angiostatin and endostatin were disappointing, and these agents have been superseded by other classes of angiogenesis inhibitor. Nevertheless, recent data show that plasmids expressing angiostatin, endostatin, or EPH receptor B4 TK can significantly inhibit tumor progression in a mouse melanoma model when they are combined with a melanoma vaccine (90), indicating that suppression of angiogenesis may enhance the efficacy of immunization.

ANGIOGENIC SWITCH

The identification of this range of endogenous regulators of angiogenesis—both positive and negative—led to the hypothesis that the homeostasis of normal, quiescent vasculature was a consequence of a balance between these activities, and that the development of new vasculature (eg, in an expanding tumor) requires a shift in the balance to provide a local excess of angiogenic factors (eg, VEGF) over antiangiogenic agents (eg, angiostatin). This represents the so-called angiogenic switch.

APPROACHES TO MODULATING DISEASE-ASSOCIATED ABNORMALITIES IN ANGIOGENESIS

One of the major advantages of targeting the endothelium for therapeutic purposes in conditions in which localized angiogenesis is anomalously activated is that the majority of endothe-

lial cells are quiescent. Therefore, the turnover time for normal endothelial cells has been estimated in the range of 47–23,000 days in most tissues (91). However, the estimate for tumor endothelium is 2.4–13 days. Therefore, not only do proliferating endothelial cells in tumors offer a target on account of their rapid cycling, but it is also assumed that, because the vasculature is host-derived, antiangiogenic and antivascular targeting might avoid the capacity of genetically unstable cancer cells to mutate rapidly, thereby neutralizing antagonists.

APPROVED ANTIANGIOGENESIS INHIBITORS

In 2004 the humanized version of a monoclonal antibody to VEGFA, bevacizumab (Avastin; Genentech, South San Francisco, California), became the first Food and Drug Administration (FDA)-approved antiangiogenic drug in the United States (92,93). It was approved as a first-line treatment agent for metastatic colorectal cancer, in combination with 5-fluorouracil (94), and was subsequently approved for treatment of unresectable, recurrent, or metastatic non-squamous-cell lung cancer, breast cancer, and glioblastoma multiforme (95). At least 300 clinical trials are currently under way for the evaluation of the efficacy of bevacizumab in many types of tumors including melanoma, ovarian carcinoma, renal cell carcinoma, gastric carcinoma, and prostate cancer. In addition, its role in treating age-related macular degeneration is also being evaluated in phase III trials, as recent phase II trials have shown improvement in vision in this group of patients (96). The antibody recognizes all isoforms of VEGFA and has a circulating half-life of as long as 21 days after intravenous infusion. Clinical trials have demonstrated that, although monotherapy with bevacizumab has been largely ineffective, bevacizumab combined with other drugs has a clinically significant effect. Although the mechanism of action is unknown, it is postulated that the anti-VEGF antibody may play a role in the “normalization” of the tumor vasculature, making it more susceptible to drugs administered subsequently (97).

Ranibizumab (Lucentis; Genentech),

another monoclonal antibody recognizing VEGFA, and pegaptanib (Macugen; Pfizer, New York, New York), a single-stranded nucleic acid aptamer that binds specifically to the heparin-binding domain of VEGFA165, are FDA-approved antiangiogenesis inhibitors in use for treating the “wet” type of age-related macular degeneration (96,98).

The FDA-approved drugs sorafenib and sunitinib are RTK inhibitors. Given that many growth factors produce their cellular effects via TK-mediated signalling cascades, it is no surprise that sorafenib, in addition to blocking VEGFR signaling, also blocks signalling from FLT3, PDGFRB, and KIT (99). Similarly, sunitinib blocks signaling from VEGFR1-3, FLT3, RET, PDGFRA, and PDGFRB (100). These two drugs have the advantage over other antiangiogenesis inhibitors in that they are administered by mouth. Sorafenib has been approved for unresectable hepatocellular carcinoma and advanced renal cell carcinoma, whereas sunitinib has been approved for gastrointestinal stromal tumors and metastatic renal cell carcinoma.

The human epidermal growth factor receptor (EGFR) comprises four closely related RTKs: EGFR/ERBB1, ERBB2/HER2, ERBB3/HER3, and ERBB4 (101). Homo- and heterodimerization can be promoted by approximately 20 ligands, of which TGF- α and epidermal growth factor are the most prominent. The EGFR and VEGFR families activate many of the same intracellular signalling pathways, which presumably partially accounts for the angiogenic effects of epidermal growth factor (102). Convergence of intracellular signaling of VEGF and EGFR partially explains resistance to anti-EGFR drugs and highlights the need for combination therapy that would inhibit VEGF and EGFR and thereby achieve efficacy. There are three FDA-approved EGFR inhibitors: cetuximab, panitumumab, and erlotinib (103–105). Cetuximab is a monoclonal antibody that blocks EGFR and is approved by FDA for use in head and neck cancers. The antibody binding blocks receptor dimerization and hence EGFR signaling, resulting in inhibition of tumor growth, invasion, and metastasis. It is used in combination with radiation therapy in locally advanced squamous cell carcinoma of the head and neck.

This combination of an EGFR inhibitor with radiation therapy has clinically demonstrated a significantly increased median survival compared with radiation therapy as the sole treatment (49 months vs 29.3 months) (106). Cetuximab is also approved for combination therapy with irinotecan in EGFR-expressing metastatic colorectal cancer. It is generally well tolerated by patients, with the most common side effect being an acneiform skin rash. Panitumumab is approved for treatment of EGFR-expressing metastatic colorectal cancer when treatment with fluoropyrimidine, oxaliplatin, and irinotecan combination chemotherapy has failed. Erlotinib is approved as a single-agent treatment for locally advanced or metastatic non-small-cell lung carcinoma in cases in which at least one chemotherapy treatment has failed and in combination with gemcitabine for locally advanced, unresectable, or metastatic pancreatic cancer.

Trastuzumab (Herceptin; Genentech) is a humanized monoclonal antibody to HER2 (107) that causes loss of receptor expression that in turn leads to reduced levels of the EGFR. It is FDA-approved for the treatment of metastatic breast cancers that overexpress HER2 in combination with paclitaxel as first-line therapy or as single-agent second-line therapy. It is also approved for early-stage breast cancer in combination with doxorubicin, cyclophosphamide, and paclitaxel. Trastuzumab is associated with cardiotoxic effects such as congestive heart failure, particularly when administered in combination with doxorubicin (108).

Inhibitors of mTOR represent a third, smaller category of antiangiogenic therapies, with only one currently approved agent. mTOR, a serine/threonine kinase regulated by RAS/PI3K-AKT signalling, exerts control over cell growth through its capacity to modulate metabolism, macromolecular synthesis, and autophagy. Temsirolimus is a small-molecule inhibitor of mTOR that is approved for the treatment of advanced renal cell carcinoma (109,110).

Thalidomide is a non-VEGF-based angiogenesis inhibitor with severe teratogenic effects (111). It is used in a wide range of disorders, some of which have been linked to abnormal angiogenesis, including Kaposi sar-

coma, multiple myeloma, rheumatoid arthritis, chronic tuberculosis, Behcet disease, Crohn disease, cutaneous lupus erythematosus, and cancers. In addition to its antiangiogenic effects, thalidomide also has immunomodulatory and antiinflammatory properties (112). For example, it can suppress graft-versus-host disease, inhibit nuclear factor- κ B signaling, and inhibit tumor necrosis factor- α , and has antiandrogenic activity. In glioma cells, thalidomide enhances autophagy induced by temozolomide. Lenalidomide is a more potent analogue of thalidomide with immunomodulatory and antiangiogenic properties recently approved by the FDA for patients with deletion 5q myelodysplastic syndromes and advanced multiple myeloma (113). Current antiangiogenic agents approved by the FDA for the treatment of cancer are listed and described in **Table 2**.

PROBLEMS AND PROSPECTS

The 12 agents that have received FDA approval clearly confer some benefits to at least some categories of patients. However, their efficacy has been relatively limited and, for cancers, there is at least anecdotal evidence that recurrence after antiangiogenic treatment may come in the form of more aggressive tumors (114–116). Recent studies in mice are beginning to illuminate this problem. Thus, by using intravenous injection of human metastatic breast cancer or melanoma cells into immunosuppressed mice as a model for metastatic seeding, Ebos et al (114,115) showed that treatment before or after tumor cell injection with any of the VEGFR inhibitors sorafenib, sunitinib, and SU10944 accelerated the formation of metastases, assayed by bioluminescence, by approximately 10-fold with a corresponding decrease in the median survival time. This occurred notwithstanding the fact that sunitinib, for example, strongly inhibits the growth of established primary tumors in mice (100). Pàez-Ribes et al (117) similarly showed that antiangiogenic drugs (anti-VEGFR2 antibody DC101) or tumor cell deletion of *Vegfa* may inhibit primary tumor growth but increase rates of invasion and metastasis. Similar findings have also come from targeting a different class of angiogenic promoter, namely integrins,

using, for example, cilengitide, which blocks α v integrins and is in phase III clinical trials. Low doses of such inhibitors stimulate angiogenesis and tumor growth in mice (118).

These findings in patients and mice illustrate what might be called the current angiogenesis conundrum. Bergers and Hanahan (116) have suggested two general responses by which the angiogenic system could resist the effect of inhibitors: (i) by evasion and (ii) by intrinsic nonresponsiveness.

The evasion hypothesis reflects the fact that, as summarized earlier, multiple pathways emanating from several families of receptors constitute the angiogenesis signaling system. Blockade of one step by an inhibitor shifts the balance within these pathways without significantly impacting on the overall systemic response. The shift in balance may occur in many ways, including upregulation of alternative pathways, recruitment of proangiogenic cells from the bone marrow, increased pericyte recruitment for maintenance of tumor vasculature or an increased capacity of invading tumor cells to use established, normal vasculature. The evasion concept might perhaps be thought of as attempting to dam a stream with stones: no matter how carefully you place your inhibitory stones, the water will find a way around them and the overall flow rate will be undiminished. Supporting evidence for this model comes from studies showing that angiogenic inhibitors or transgenic knockout of VEGF receptors leads to enhanced expression of a variety of proangiogenic mediators (notably FGF1, FGF2, VEGFA, and PDGFA) (117,119,120). This sort of response is in contrast to the acquisition of mutations (eg, in chronic myeloid leukemia treated with imatinib when the target, BCR-ABL, mutates an amino acid at the drug binding site), and indeed there is no evidence that antiangiogenic agents induce mutations. However, it should be noted that mouse endothelial cells isolated from human melanoma and liposarcoma xenografts show heterogeneous aneuploidy (121). If the acquisition of cytogenetic abnormalities is a general phenomenon in tumor endothelium, it might imply that antiangiogenic treatments will need to be tailored to the genetic profile of specific tumors, an

emerging requirement for conventional chemotherapy.

The second hypothesis of Bergers and Hanahan (116), intrinsic nonresponsiveness, arises from the finding that angiogenic inhibitors (eg, bevacizumab, sorafenib, and sunitinib) have no detectable effects on a substantial cohort of patients (116,119), and the supposition is that the angiogenic system supporting tumors in those patients has already evolved one or more of the evasion characteristics summarized earlier. As noted earlier, bevacizumab has FDA approval for the treatment of late-stage metastatic colon, breast, and lung cancer only in combination with conventional chemotherapy. This may reflect the fact that patients are being treated with a drug that would be identifiable as useless if accepted markers for intrinsic refractoriness to antiangiogenic agents were available. However, another possibility is that VEGF inhibitors cause transient restructuring of the abnormal tumor vasculature, thereby reducing its permeability and improving blood flow and thus facilitating access of systemically administered drugs to tumor cells (97).

It is, of course, true that nonspecific effects of these inhibitors may have contributed to their effects in mice and human subjects. Nevertheless, the findings strongly suggest that VEGF inhibitors can promote signaling events that, for example, stimulate the release of bone marrow cells to act as markers for tumor cell adhesion in preconditioned niches. This in turn indicates that drug combinations and their administration regimes (eg, continuous versus discontinuous, neoadjuvant versus adjuvant) will be critical if effective strategies involving antiangiogenic agents are to evolve.

The drugs that have so far been approved, mainly for the treatment of cancers, have shown significant effects at least in subsets of patients. As the previous discussion highlights, these limited responses indicate the requirement for more sophisticated administration regimes, such as metronomic chemotherapy in combination with antiangiogenic agents (122). It may also be noted that the currently approved antiangiogenic agents very largely target VEGF or RTKs. That is, they have targeted the growth factors (by antibodies and soluble forms of

Table 2
Antiangiogenic Drugs with FDA Approval for the Treatment of Cancer

Drug	Trade Name (Manufacturer)	Mechanism of Action	Indication(s)
Bevacizumab	Avastin (Genentech)	Humanized MAb: binds to VEGF to inhibit activation of VEGFR1 and VEGFR2 receptor TK	In combination with 5-FU-based chemotherapy as first-line and second-line treatment of metastatic CRC; in combination with carboplatin and paclitaxel as first-line treatment of unresectable, locally advanced, recurrent, or metastatic nonsquamous NSCLC; in combination with paclitaxel as first-line treatment of locally recurrent or metastatic BCa
Temsirolimus	Torisel (Wyeth)	Small-molecule inhibitor of mTOR	Advanced RCC
Cetuximab	Erbix (Bristol-Myers Squibb)	Humanized MAb: EGFR TK inhibitor	In combination with irinotecan as second-line treatment for metastatic CRC refractory to irinotecan and single agent for metastatic CRC in patients who cannot tolerate irinotecan; in combination with radiation therapy for treatment of locally or regionally advanced SCC of the head and neck; approved as a single agent for recurrent or metastatic SCC of the head and neck after failed platinum agent-based therapy
Erlotinib hydrochloride	Tarceva (Genentech)	Small-molecule EGFR TK inhibitor: competitive inhibitor of ATP binding	Monotherapy for locally advanced or metastatic NSCLC after ≥ 1 chemotherapy regimen has failed; in combination with gemcitabine as first-line treatment of locally advanced, unresectable or metastatic pancreatic cancer
Sorafenib	Nexavar (Bayer/Onyx)	Small-molecule VEGFR1, VEGFR2, VEGFR3, PDGFRB inhibitor; also inhibits RAF1 and BRAF	Advanced RCC; unresectable HCC
Sunitinib	Sutent (Pfizer)	Small-molecule VEGFR1, VEGFR2, VEGFR3, PDGFRB and RET inhibitor	GIST after disease progression with, or intolerance to, imatinib mesylate
Trastuzumab	Herceptin (Genentech)	MAB: binds to HER2, modulates signaling, marks cells for immunologic attack	Adjuvant treatment of HER2-overexpressing, node-positive BCa in combination with doxorubicin, cyclophosphamide, and paclitaxel; single-agent, second-line therapy for HER-overexpressing metastatic BCa; monotherapy for locally advanced or metastatic NSCLC after 1 chemotherapy regimen has failed
Panitumumab	Vectibix (Amgen)	MAB: EGFR inhibitor	EGFR-expressing metastatic CRC with disease progression with or after fluoropyrimidine, oxaliplatin, and irinotecan chemotherapy regimens
Thalidomide	Thalomid (Celgene)	Multitarget drug: immunomodulatory, antiinflammatory, and antiangiogenic properties	Multiple myeloma, in combination with dexamethasone

Note.—BCa = breast cancer; CRC = colorectal cancer; 5-FU = 5-fluorouracil; GIST = gastrointestinal stromal tumor; HCC = hepatocellular carcinoma; MAB = monoclonal antibody; NSCLC = non-small-cell lung cancer; RCC = renal cell carcinoma; SCC = squamous cell carcinoma.

their receptors “ligand traps”), the ligand-binding domains of their receptors (by antibodies), and the activated

receptors (by kinase inhibitors) (123). As the complexity of the molecular biology is gradually unraveled, other

targets present themselves. Therefore, for example, HIF1 and PHD proteins, the micro-RNA/TIS11B circuit, and

the diverse functions of the PI3K isoforms have come to the fore as potential targets for the treatment of cancers and ischemic diseases. The major role of RAS in almost all RTK signaling, together with the lack of success with farnesyl transferase inhibitors, makes it perhaps a less attractive target for angiogenic therapy. However, it has recently been shown that nuclear factor- κ B signalling, promoted by RAS acting via RALGDS, RALB, and TBK1, is required for tumor formation in a mouse model of RAS-induced lung cancer (124). Inhibition of TBK1 selectively kills RAS-mutant cells in a synthetic lethal interaction (125).

It will be evident from the foregoing that antiangiogenic therapies have undergone a development strikingly similar to that of many of their predecessors in the history of cancer. As the vision of Folkman took hold, it seemed that the dependence of tumors on vasculature generated from host tissue offered a stationary drug target, unable to mount the evasive tactics facilitated by an unstable genome. Yet again, simplistic optimism has been overtaken by dawning comprehension of the extraordinary complexity and adaptability of tumors and their environment. Depressing though this saga may seem, there are some encouraging aspects. The fact that some antiangiogenic agents have beneficial effects on subgroups of patients indicates the need for a refined molecular analysis of individual tumors to determine appropriate treatments. The capacity for rapid whole-genome sequencing means that the technology for this development is at hand. In addition, as the present review has summarized, there is a substantial and growing list of new molecular targets. Drugs specific for those targets will gradually emerge and it still seems reasonable to hope, therefore, that antiangiogenic agents will come to occupy a significant position in the cancer chemotherapy repertoire.

The rapidly evolving field of interventional oncology has been pioneered by the interventional radiology community, with development of an armamentarium that includes percutaneous ablative techniques and transcatheter arterial embolization therapies with targeted chemoembolization or radioembolization. Inclusion of antiangiogenic compounds for the treat-

ment of various human cancers in treatment strategies may allow for more optimal outcomes as this exciting field of investigation continues to evolve.

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