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Surgical implications of therapeutic angiogenesis

Michael A. Zimmerman, MD, Craig H. Selzman, MD, *and* Alden H. Harken, MD, *Denver, Colo*

From the Department of Surgery, University of Colorado Health Sciences Center, Denver, Colo

IN 1966 FOLKMAN ET AL[1,2] observed that tumor cells could not grow beyond 2 to 3 mm without neovascularization of the tumor. This group postulated that paracrine release of mitogenic peptides was capable of stimulating new capillary networks.[3] Multiple laboratories have not only corroborated the importance of new blood vessel formation but have also identified locally synthesized proteins that may conspire to promote neovascularization. As such, much enthusiasm currently exists to understand the mechanisms involved with new blood vessel formation and to link these mechanisms to accessible clinical therapy. The purposes of this review are (1) to delineate mechanisms of new blood vessel formation, (2) to explore the role of new blood vessel formation in surgical disease, and (3) to examine the influence of clinical manipulation of neovascularization in treating both malignant and nonmalignant surgical disease.

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Reprint requests: Craig H. Selzman, MD, Department of Surgery, Campus Box C-320, University of Colorado Health Sciences Center, 4200 E Ninth Ave, Denver, CO 80262.

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CELLULAR BASIS OF NEOVASCULARIZATION

Blood vessels are formed by two separate, yet synergistic, processes, vasculogenesis and angiogenesis. During embryonal development, vasculogenesis is responsible for the original mesenchymal differentiation of hemangioblasts to create large capacity vessels such as the aorta and the posterior cardinal veins.[4] As a compensatory response to physiologic stress, vasculogenesis is also responsible for the transformation of preexisting arterioles into small muscular arteries. This latter process, termed *recapitulated vasculogenesis*, differs from true angiogenesis. Angiogenesis derives exclusively from preexisting vasculature. It is a dynamic process involving the active dissolution of the extracellular matrix and subsequent vascular endothelial cell proliferation, migration, and adherence into new luminal formation. Angiogenesis is less efficient in delivering bulk blood flow than vasculogenesis. Conversely, angiogenesis is a mechanism of regionally collateralizing ischemic tissues. Understanding the mechanisms of angiogenesis will allow for targeted therapy to manipulate this dynamic process in both neoplastic and atherosclerotic disease.

ANATOMY OF ANGIOGENESIS

Neovascularization is induced by physiologic stimuli including hypoxia, ischemia, mechanical

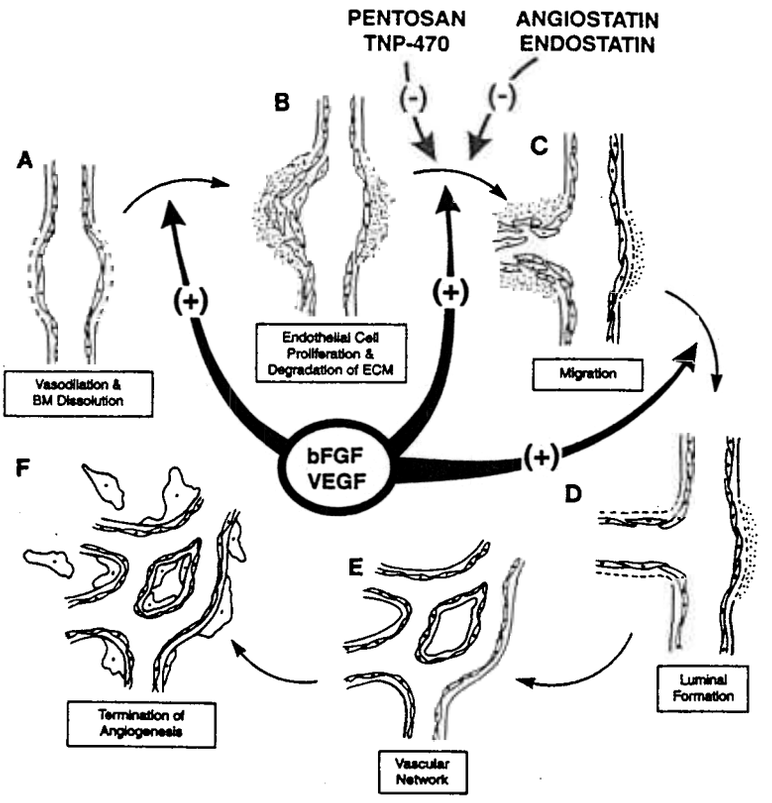

Fig 1. Cellular events of angiogenesis. Endogenous angiogenic stimuli interact with the vascular endothelium, resulting in vasodilation and the secretion of proteolytic enzymes from the microvasculature (**A**). This cell-mediated dissolution of the endothelial basement membrane is an important initial step in angiogenesis. As the formation of neomicrovasculature continues, the surrounding extracellular matrix is broken down (**B**). Concurrently, the vascular endothelial cell undergoes mitosis and proliferates. As the surrounding basement membrane and extracellular matrix are dismantled, the endothelial cells undergo chemotaxis toward the angiogenic stimulus (**C**). Migratory endothelial cells reform tubular structures with a patent lumen, buttressed by newly constructed basement membrane (**D**). Ultimately, an elaborate network of microvasculature is established (**E**). Termination of vascular morphogenesis is marked by the influx of pericytes that envelop the newly established microvasculature (**F**).

stretch, and inflammation.[5] The vascular endothelium secretes proteolytic enzymes, provoking dissolution of the vascular basement membrane (Fig 1, *A*). Plasminogen activator is the most extensively characterized proteolytic enzyme of endothelial origin. Urokinase plasminogen activator (uPA) is secreted as a proenzyme that binds to its receptor on the endothelial cell membrane uPA receptor (uPAR). Subsequently, the uPA-uPAR complex converts plasminogen to plasmin.[6] Plasmin directly degrades matrix proteins including fibrin, fibronectin, and laminin.[7] Plasmin also activates other proteolytic enzymes, including metalloproteases and elastase (Fig 1, *B*).[8,9]

With the degradation of the surrounding basement membrane, endothelial cells from adjacent established vessels begin to proliferate and migrate (Fig 1, *C*). Cellular migration relies on the expression of membrane adhesion molecules, termed *integrins*. Expressed on several cell types, integrins are transmembrane glycoproteins that mediate interactions between cells and the extracellular matrix. Integrins are classified according to the combination of noncovalently linked alpha-beta heterodimers. The β1 subfamily links the cell with the extracellular matrix dictating tissue organization, position, differentiation, inflammation, and growth.[10] The β2 subfamily shares a common β-chain (CD18), is restricted to leukocytes, and is central to firm leukocyte-endothelial adhesion. The leukocyte β2 integrins (CD11 and CD18) have been the target of several adult respiratory distress prevention protocols. The β3 subfamily consists of the platelet glycoprotein IIb/IIIa complex and the vitronectin receptor. Both the β1 and β3 families recognize their ligands through a tripeptide recog-

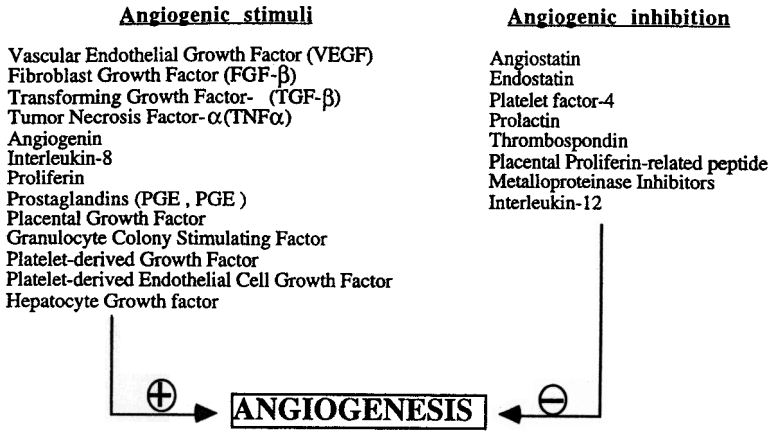

Fig 2. Positive and negative endogenous regulators of angiogenesis.

nition sequence in various matrix proteins termed RGD (arginine-glycine-aspartic acid). In particular, the αvβ3 integrin is a marker of angiogenesis.[11] When delivered to melanoma cells, monoclonal antibodies against the αvβ3 integrin result in a decrease in tumor-associated blood vessel density.[12] Ligand binding of the αvβ3 integrin signals cellular migration.[13] In addition, the αvβ3 integrin functions as the endothelial cell receptor for fibrin and fibronectin and therefore promotes extracellular matrix formation.

The final phase of neovascularization is luminal morphogenesis (Fig 1, *D*). A new basement membrane is constructed around the flattened, migrating endothelial cells, thus establishing an entire neovascular network (Fig 1, *E*). Angiogenesis terminates when an influx of pericytes envelops the newly established microvasculature. These specialized cells transform mature endothelium into a quiescent, nonproliferative state.[14]

MEDIATORS OF ANGIOGENESIS

More than 25 different endogenous regulators of angiogenesis have been identified (Fig 2). Two peptides, however, have received the most attention, basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF). Both bFGF and VEGF are synthesized and secreted by numerous tumor cell lines, healthy human peritoneal macrophages, and normal epithelial cells in the brain, ovary, kidney, and heart. VEGF and bFGF are similar in many of their effects on vascular endothelial cells. Both peptides influence proliferation, migration, and proteolytic activity by the target cell. Importantly, experimental inhibition of either peptide prevents neovascularization.[15,16]

Vascular endothelial growth factor. In 1983 a protein was isolated from guinea pig tumors that caused the accumulation of protein-rich ascitic fluid.[17] Initially called vascular permeability factor, it was later found to be identical to VEGF.[18] Thus far, 4 different homodimeric isoforms of VEGF, formed by alternative splicing of VEGF mRNA, have been identified, with the 165 amino acid variant being the most abundant. Whereas angiogenic peptide secretion can be directly stimulated by physiologic stressors such as hypoxia and mechanical stretch, expression also increases with stress-induced cytokines. VEGF appears to be a central paracrine mediator of angiogenesis. VEGF selectively activates arterial, venous, and lymphatic endothelium (Fig 1, *A* and *B*).[19] In addition to its mitogenic effects, VEGF is crucial to the initial dissolution of the extracellular matrix and chemotaxis of endothelial cells (Fig 1, *B*). VEGF up-regulates expression of tissue plasminogen activator, uPA, and uPAR and increases expression of the αvβ5 integrin that promotes both migration (Fig 1, *C*) and luminal formation (Fig 1, *D*).[20,21]

Fibroblast growth factor. Nine different members of the FGF family have been identified. Basic fibroblast growth factor is synthesized by many vascular cells including macrophages, monocytes, fibroblasts, endothelial cells, and vascular smooth muscle cells.[22] Four different FGF receptors have been characterized, each with a transmembrane domain linked to an intracellular tyrosine kinase. Receptor isoforms occur in a cell- and tissue-specific manner.[23] Ligand binding initiates sequential phosphorylation and activation of intracellular signals resulting in gene transcription and vascular cell proliferation. Irradiation or the exogenous influence of fibrin-split products results in local vascular FGF release.[24] Similar to VEGF, bFGF also up-regulates expression of uPA and the αvβ3 integrin,

facilitating endothelial migration (Fig 1, *C*) and luminal formation (Fig 1, *D*).[21,25]

ANGIOGENESIS AND SURGICAL DISEASE

Although neovascularization is often interpreted by angiographers as indicating a neoplastic process, stimulation of the angiogenic cascade to enhance vascular supply may be used therapeutically in ischemic diseases. As molecular insight into angiogenesis evolves, surgeons are uniquely positioned to access tissue, permitting us to tip the angiogenic scale in favor of either inhibition or promotion of new blood vessel formation.

Wound healing. The phases of normal wound healing can be divided into 3 phases including inflammation, fibroplasia, and maturation. Provoked by liberated angiogenic factors, vessel-dense granulation tissue is central to the process of tissue repair. The formation of new blood vessels provides a route for oxygen and nutrient delivery, as well as a conduit for components of the inflammatory response. Experimental evidence indicates that bFGF is instrumental in wound healing.[26] Nissen et al[27] have documented peak levels of bFGF in surgical incisions. These elevated bFGF levels directly correlate with an increase in endothelial cell proliferative activity. Neutralization of bFGF results in a decrease in endothelial chemotaxis in vitro and angiogenic activity in vivo. Davidson and Broadley[28] recognized the constructive influence of recombinant human bFGF on healing wounds. Treatment of genetically diabetic mice with recombinant bFGF not only increased fibroblast and capillary density in the wound but also accelerated wound closure.[29] Despite encouraging experimental results to date, clinical application of recombinant human bFGF has fallen short of expectations. In a randomized double-blind placebo-controlled study, direct application of bFGF to nonhealing ulcers of diabetic patients did not accelerate wound healing.[30]

Peptic ulcer disease. Chronic duodenal ulcers contain inflammatory cells, necrotic debris, and granulation tissue. Endogenous bFGF has been detected in high concentrations in human gastric and duodenal mucosa. By administering an oral form of acid-stable bFGF, ulcer bed angiogenesis was significantly increased and ulcer healing was accelerated.[31] Folkman et al[32] demonstrated that aluminum sucrose octasulfate (sucralfate), a commonly used antiulcer agent, maintains a high binding affinity for bFGF. By binding endogenous bFGF and protecting it from acidic degradation, sucralfate may increase local levels of bFGF in the ulcer bed, indirectly stimulating angiogenesis and thus promoting ulcer healing.

Cardiovascular disease. The observation that intraplaque microvessels are associated with atheromatous lesions dates back to 1876.[33] Promoting collateral circulation by stimulating angiogenesis is an appealing therapeutic strategy for peripheral vascular occlusive disease. Delivery of both bFGF and VEGF has enhanced angiogenesis in the ischemic hindlimb of a rabbit.[34] Furthermore, combined administration of VEGF and bFGF has demonstrated a synergistic effect in vivo.[35] Bauters et al[36] have demonstrated both anatomic and physiologic evidence of collateral vessel formation via systemic administration of recombinant human VEGF in the rabbit ischemic hindlimb. Gene transfer techniques, using DNA encoding the VEGF gene, have induced collateral vessels and increased blood flow in the lower extremity of a 71-year-old patient.[37] Such promising reports have led to phase I clinical trials of percutaneous catheter-based delivery of the gene encoding VEGF into patients with chronic critical leg ischemia.[38] A single arterial bolus of recombinant human VEGF resulted in angiographic, hemodynamic, and physiologic evidence of augmented collateral arterialization.

The induction of collateral blood flow and tissue salvage are also applicable to coronary artery disease. In 1992, Yanagisawa-Miwa et al[39] reported improved cardiac systolic function, decreased infarct size, and increased capillary density in infarcted tissue after in vivo intracoronary injection of bFGF in dogs. Systemic administration of bFGF provoked enhanced collateral coronary blood flow in dogs after experimental coronary occlusion.[40] Conversely, Shou et al administered bFGF to animals with mature collateral vessels after coronary occlusion model and did not induce collateralization at 6 months. Thus ischemia may be required to synergize with growth factors to induce angiogenesis. These encouraging preclinical studies offer the foundation for current clinical trials in patients with coronary disease. Direct injection of recombinant human bFGF into the human heart, at the time of elective coronary artery bypass grafting, has induced newly formed capillary networks.[41] This neovascularization originates at the proximal artery and extends into the myocardium, bypassing areas of distal stenosis. Currently, however, the physiologic effects of such neocollateralization remain to be established.

Cancer. The importance of tumor neovascularization is illustrated by both histologic and angiographic inspection of surgical specimens. Weidner et al[42,43] demonstrated a positive correlation

between blood vessel density and the presence of either regional or distant metastatic disease in prostate and breast carcinoma. Malignant cells may induce angiogenesis by overexpression of angiogenic molecules or, conversely, by down-regulation of normally expressed inhibitors. Basic FGF overexpression has been localized to neoplastic tissue and in the cerebrospinal fluid, serum, and urine of patients with a wide variety of tumors.[44] Similarly, VEGF overexpression has been demonstrated in a large number of human tumors and is also positively correlated with regional lymph node and distant metastasis.[45,46] VEGF is primarily localized to ischemic portions of the tumor, near areas of necrosis.[47] Interestingly, VEGF mRNA expression was observed in tumor cells selectively. In contrast, the adjacent vascular endothelium lacked expression of VEGF mRNA, while overexpressing mRNA for the VEGF receptors *flt-1* and *KDR*.[46] These observations suggest that tumor cells preferentially secrete angiogenic factors that engage nearby endothelial cells apparently primed for growth factor stimulation. Finally, bFGF appears to stimulate VEGF synthesis in tumor cells, thus creating angiogenic synergy.[48]

Functional blockade of angiogenic peptides represents an attractive antineoplastic strategy. Therapy is directed at several levels: neutralization of the angiogenic stimulus, inhibition of endothelial cell activation (proliferation, migration, vascular morphogenesis), or inhibition of basement membrane turnover or synthesis (Fig 1).[49] Indeed, delivery of neutralizing antibodies to VEGF suppressed primary tumor growth, neovascularization, and the number and size of metastatic foci of human tumor cell lines injected into nude mice.[16] Similarly, bFGF-neutralizing antibodies suppressed neovascularization of several malignant glial tumors and inhibited primary tumor growth.[50] In addition, clinically accessible immunomodulators not only inhibit tumor cell proliferation but also down-regulate secretion of angiogenic mediators. Both interferon-α and interferon-β decrease bFGF production in several tumors including carcinoma of the kidney, bladder, colon, and breast.[51]

Ten antiangiogenesis agents are currently in clinical trials. Most agents act at the level of the vascular endothelial cell response to angiogenic growth factors. Pentosan, a direct inhibitor of vascular endothelial cell proliferation, was the first agent examined clinically. Although demonstrating experimental use, phase I trials of Pentosan have been plagued by multiple toxicities without measurable clinical efficacy.[52] TNP-470, an analog of the toxic fumagillin, has also shown laboratory promise. It directly inhibits endothelial activation in vitro (Fig 1, *A*) and has antiangiogenic activity in multiple in vivo assays.[53] Phase I antiangiogenesis trials are currently underway treating patients with androgen-independent prostate carcinoma and advanced squamous cell cancer of the cervix.[54,55] Granulocytopenia, muscle weakness, and dose-limiting central nervous system effects have been identifiable toxicities.

An alternate antineoplastic strategy is to augment delivery of natural antiangiogenic compounds. Angiostatin, a 38-kd internal fragment of plasminogen, was first isolated from the Lewis lung carcinoma.[56] Angiostatin inhibits tumor neovascularization by direct inhibition of endothelial cell proliferation. Systemic administration of angiostatin has arrested further growth of Lewis lung carcinoma.[57] Endostatin, a 20-kd peptide that is identical to a C-terminal fragment of collagen XVII, has recently been isolated from murine hemangioendotheliomas.[58] Systemic administration of endostatin inhibited primary tumor growth of the Lewis lung carcinoma by blocking tumor-associated angiogenesis. The isolation of these peptides suggests that endogenous inhibitors of angiogenesis, when delivered exogenously in supraphysiologic doses, may augment the host's natural antitumor response.

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